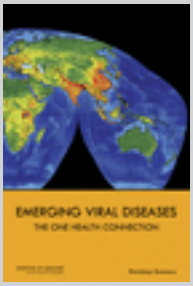

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Eileen R. Choffnes and Alison Mack, Rapporteurs; Forum on Microbial Threats; Board on Global Health; Institute of Medicine

EMERGING VIRAL DISEASES

THE ONE HEALTH CONNECTION

Workshop Summary

Eileen R. Choffnes and Alison Mack, *Rapporteurs*

Forum on Microbial Threats

Board on Global Health

INSTITUTE OF MEDICINE
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The serpent has been a symbol of long life, healing, and knowledge among almost all cultures and religions since the beginning of recorded history. The serpent adopted as a logotype by the Institute of Medicine is a relief carving from ancient Greece, now held by the Staatliche Museen in Berlin.

Cover image: Global hotspot map of emerging infectious diseases (EIDs). This map illustrates the relative risk of a zoonotic emerging infectious disease of wildlife origin spilling over into the human population. It was produced by analyzing with logistic regression the presence/absence of all known wildlife-origin EIDs since 1940 against a series of known drivers, including human population density, change in human population density, and wildlife diversity (mammalian species richness), gridded at 1km^2 resolution, and corrected for reporting bias by including a measure of the global distribution of infectious disease researchers. Map produced by EcoHealth Alliance and research funded by USAID-EPT PREDICT.

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"Knowing is not enough; we must apply.
Willing is not enough; we must do."
—Goethe

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[2] Staff member until January 31, 2015.

Reviewers

This workshop summary has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the National Research Council's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published workshop summary as sound as possible and to ensure that the workshop summary meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the process. We wish to thank the following individuals for their review of this workshop summary:

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JAMES M. HUGHES, Global Infectious Diseases Program, Emory University, Atlanta, Georgia
STANLEY M. LEMON, University of North Carolina, Chapel Hill, North Carolina
MICHAEL WOROBEY, University of Arizona, Tucson, Arizona

Although the reviewers listed above have provided many constructive comments and suggestions, they did not see the final draft of the workshop summary before its release. The review of this workshop summary was overseen by **Melvin Worth.** Appointed by the Institute of Medicine, he was responsible for making certain that an independent examination of this workshop summary was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this workshop summary rests entirely with the rapporteurs and the institution.

Acknowledgments

The Forum on Emerging Infections was created by the Institute of Medicine (IOM) in 1996 in response to a request from the Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH). The purpose of the Forum is to provide structured opportunities for leaders from government, academia, and industry to regularly meet and examine issues of shared concern regarding research, prevention, detection, and management of emerging, reemerging, and novel infectious diseases in humans, plants, and animals. In pursuing this task, the Forum provides a venue to foster the exchange of information and ideas, identify areas in need of greater attention, clarify policy issues by enhancing knowledge and identifying points of agreement, and inform decision makers about science and policy issues. The Forum seeks to illuminate issues rather than resolve them. For this reason, it does not provide advice or recommendations on any specific policy initiative pending before any agency or organization. Its value derives instead from the diversity of its membership and from the contributions that individual members make throughout the activities of the Forum. In September 2003, the Forum changed its name to the Forum on Microbial Threats.

The Forum on Microbial Threats, and the IOM, wish to express their sincere appreciation to the individuals and organizations who contributed their valuable time to provide information and advice to the Forum. Their participation in the planning and execution of this workshop made it greater than the sum of its parts. A full list of presenters, and their biographical information, may be found in Appendix E.

The Forum gratefully acknowledges the contributions of the members of the planning committee[1]: Peter Daszak (EcoHealth Alliance), Jeffrey Duchin (Public Health–Seattle & King County), Carole Heilman (National Institute of Allergy and Infectious Diseases, NIH), James M. Hughes (Emory University), Rima Khabbaz (CDC), David Swayne (U.S. Department of Agriculture), and John Watson (Public Health England).

The Forum is also indebted to the IOM staff who tirelessly contributed throughout the planning and execution of the workshop and the production of this workshop summary report. On behalf of the Forum, we unreservedly acknowledge these efforts led by Dr. Eileen Choffnes, Scholar and Director of the Forum; Katherine McClure,[2] Associate Program Officer; Rebekah Hutton,[3] Research Associate; Priyanka Nalamada, Senior Program Assistant; and Joanna Roberts,[4] Senior Program Assistant (Temp). Without the contributions and dedication of the staff to the work of the Forum in developing this workshop's agenda and for their thoughtful and insightful approach and skill in planning for the workshop and in translating the workshop's proceedings and discussion into this workshop summary report, this report would not have been possible. We would also like to thank the following IOM staff and consultants for their invaluable contributions to this activity: Laura Harbold DeStefano, Chelsea Frakes, Rosalind Gomes,[5] Greta Gorman, Faye Hillman, Sarah Kelley, Alison Mack, and Julie Wiltshire.

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[1] Institute of Medicine planning committees are solely responsible for organizing the workshop, identifying topics, and choosing speakers. The responsibility for the published workshop summary rests solely with the workshop rapporteurs and the institution.

[2] Staff member until December 31, 2014.

[3] Staff member until November 14, 2015.

[4] Staff member until March 31, 2014.

[5] Staff member until January 9, 2015.

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Workshop Overview[1]

EMERGING VIRAL DISEASES—THE ONE HEALTH CONNECTION

Viruses have caused some of the most dramatic and deadly disease pandemics in human history. Before it was declared to be eradicated in 1980, smallpox, a highly contagious human disease caused by the *Variola* virus, killed 300 to 500 million people worldwide in the 20th century alone (Koplow, 2003). The 1918–1919 "Spanish flu" pandemic infected roughly one-third of the world's human population and caused an estimated 50 to 100 million deaths. In the past half century, deadly disease outbreaks caused by novel viruses of animal origin—Nipah virus in Malaysia, Hendra virus in Australia, hantavirus in the United States, Ebola virus in Africa, along with HIV (human immunodeficiency virus), several influenza subtypes, and the SARS (severe acute respiratory syndrome) and MERS (Middle East respiratory syndrome) coronaviruses—have underscored the urgency of understanding factors influencing viral disease emergence and spread.

The world's current leading infectious killer, HIV, has caused an estimated 36 million deaths since the first cases were reported in 1981. In 2012, more than 2 million people were newly infected with the virus, and 1.6 million died of HIV/AIDS. In 2009, a novel swine-origin H1N1 strain of influenza A rapidly spread to over 213 countries in the first declared pandemic of the 21st century. And now, on

[1] The planning committee's role was limited to planning the workshop, and the workshop summary has been prepared by the workshop rapporteurs (with the assistance of Rebekah Hutton, Katherine McClure, and Priyanka Nalamada) as a factual summary of what occurred at the workshop. Statements, recommendations, and opinions expressed are those of individual presenters and participants and are not necessarily endorsed or verified by the Forum, the Institute of Medicine, or the National Research Council, and they should not be construed as reflecting any group consensus.

August 8, 2014, the World Health Organization (WHO) Director-General Margaret Chan declared the Ebola outbreak in West Africa a "public health emergency of international concern," triggering powers under the 2005 International Health Regulations (IHR). The IHR require countries to develop national preparedness capacities, including the duty to report internationally significant events, conduct surveillance, and exercise public health powers, while balancing human rights and international trade.

Emerging infectious diseases (EIDs) were both anticipated and studied by the late Joshua Lederberg, Nobel laureate and a founder of the Forum on Microbial Threats. He recognized microbes as humanity's competitors and appreciated their disregard for human sovereignty over Earth's creatures (Lederberg, 2000). The same wisdom, plus a dose of reality delivered by SARS and avian influenza A (H5N1), informed the 2005 revisions to the IHR. The IHR are legally binding regulations (forming international law) that aim to (1) assist countries to work together to save lives and livelihoods endangered by the spread of diseases and other health risks, and (2) avoid unnecessary interference with international trade and travel.

The purpose and scope of the IHR 2005 are to prevent, protect against, control, and provide a public health response to the international spread of disease in ways that are commensurate with and restricted to public health risks, and which avoid unnecessary interference with international traffic and trade (Art. 2, IHR 2005).

Statement of Task

Over the course of more than two decades, beginning with the landmark report *Microbial Threats to Health in the United States* (IOM, 1992), the Forum and its predecessors within the Institute of Medicine have examined the growing body of research on EIDs and the growing list of diseases that fit this description (IOM, 2003).[2]

In this tradition, on March 18 and 19, 2014, the Forum hosted a public workshop in Washington, DC, to examine factors driving the appearance, establishment, and spread of emerging, reemerging, and novel viral diseases; the global health and economic impacts of recently emerging and novel viral diseases in humans; and the scientific and policy approaches to improving domestic and international capacity to detect and respond to global outbreaks of infectious disease.

Organization of the Workshop Summary

This workshop summary was prepared by the rapporteurs for the Forum's members and includes a collection of individually authored papers and

[2] See http://www.iom.edu/Reports.aspx?Activity={C8EA50BF-D234-4E44-9E42-9636B7FC2D22} for a complete list of Forum workshop summary reports.

commentary. The contents of the unattributed sections of this summary report provide a context for the reader to appreciate the presentations and discussions that occurred over the 2 days of this workshop.

The summary is organized into sections as a topic-by-topic description of the presentations and discussions that took place at the workshop. Its purpose is to present information from relevant experience, to delineate a range of pivotal issues and their respective challenges, and to offer differing perspectives on the topic as discussed and described by the workshop participants. Manuscripts and reprinted articles submitted by workshop participants may be found, in alphabetical order by participant, in Appendix A.

Although this workshop summary provides a description of the individual presentations, it also reflects an important aspect of the Forum's philosophy. The workshop functions as a dialogue among representatives from different sectors and allows them to present their views about which areas, in their opinion, merit further study. This report only summarizes the statements of participants over the course of the workshop. This summary is not intended to be an exhaustive exploration of the subject matter, nor does it represent the findings, conclusions, or recommendations of a consensus committee process.

IMPACT OF EMERGING VIRAL DISEASES

In addition to causing nearly one in five human deaths worldwide, infectious diseases impose a heavy societal and economic burden on individuals, families, communities, and countries (Lozano et al., 2012; Murray et al., 2012). The appearance of new infectious diseases has been recognized for millennia, well before microbes were recognized as their causes (Morens and Fauci, 2013). EIDs comprise a substantial fraction of important human infections, and they have caused the deadliest pandemics in recorded human history, including the 14th-century Black Death (during which 75 to 200 million people in what is now Europe died of bubonic or pneumonic plague); the 1918–1919 Spanish influenza pandemic (at least 50 to 100 million deaths in a span of 18 months); and the ongoing HIV/AIDS pandemic, in which more than 35 million people have perished (Morens and Fauci, 2013).

Jones and coworkers described the emergence of 335 infectious diseases in the global human population between 1940 and 2004, of which nearly two-thirds originated in wildlife (Jones et al., 2008). These can be further characterized as either newly emerging or reemerging infectious diseases, that is, caused by pathogens infecting a new host species, or caused by pathogens that historically have infected the same host species, but continue to appear in new locations or in drug-resistant forms, or that reappear after apparent control or elimination (Fauci and Morens, 2012). Figure WO-1 illustrates the global distribution of key emerging and reemerging diseases including the anthrax-laced letters of fall 2001.

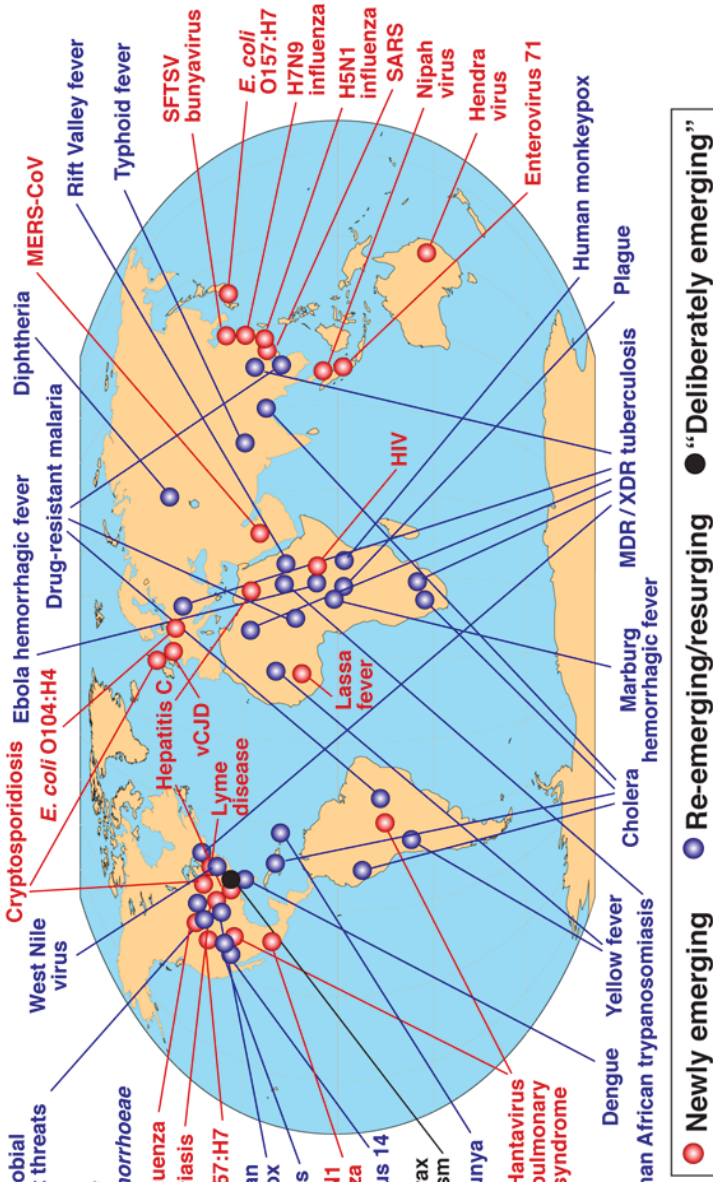

FIGURE WO-1 Global examples of emerging and reemerging infectious diseases.
SOURCE: Morens et al., 2004.

Zoonotic[3] viruses pose a particularly serious threat to human health as populations grow and expand geographically, increasing opportunities for contact with wildlife, disturbing habitat, and requiring intensified agriculture to meet increased demand for food; meanwhile, the precipitous rise in global travel and trade have vastly expanded transmission opportunities for emergent pathogens (Bean et al., 2013; IOM, 2014). As presented in Figure WO-2, a century of such global environmental change has produced a legacy of emerging viral diseases. HIV/AIDS is thought to have emerged a century ago through a complex transition from chimpanzees to humans, after which a combination of social and demographic factors eventually propelled it to pandemic status. Meanwhile, global environmental change allowed the formerly range-restricted dengue, chikungunya, and West Nile viruses to reemerge among major populations worldwide (Morens and Fauci, 2013, 2014).

Just as air travel has increased the variety of viruses to which humans are exposed, flying animals are particularly adept at dispersing viruses to new locations and hosts. As shown in Table WO-1, bats and wild birds predominate as primary hosts of important zoonotic viruses (Bean et al., 2013). This phenomenon was raised in several workshop presentations and discussions summarized in this overview, particularly with reference to Middle East respiratory syndrome coronavirus (MERS-CoV), for which bats appear to serve as a reservoir species, and for influenza A (H7N9), now largely limited to poultry, but with the potential to become a serious threat should it make the transition to wild birds.

Growing knowledge of the nature and severity of the threat posed by emerging viral diseases has spurred a range of responses from multiple sectors, described in several workshop presentations. Technical efforts to address emerging viral diseases encompass pandemic prediction; risk assessment; surveillance and detection; descriptive and analytic epidemiology; pathogen characterization; public health interventions; and drug and vaccine development. Legal and political means to reconcile the "borderless world" of microbes with the macroscopic structures of sovereignty continue to be developed and debated. All such work may be productively united under the One Health paradigm: "the collaborative effort of multiple disciplines—working locally, nationally, and globally—to attain optimal health for people, animals, and the environment" (AVMA, 2008).

Global Challenges and Trends in Emerging Viral Diseases

Keiji Fukuda, WHO's Assistant Director-General for Health Security, opened the workshop with a keynote address on global public health issues related to emerging infectious diseases, and more specifically, to the emerging viral diseases MERS-CoV and H7N9 influenza. At the time of this workshop, MERS-CoV and the H7N9 strain of avian influenza were under active surveillance for their

[3] Zoonotic diseases, or zoonoses, are diseases that can be transmitted from animals to humans.

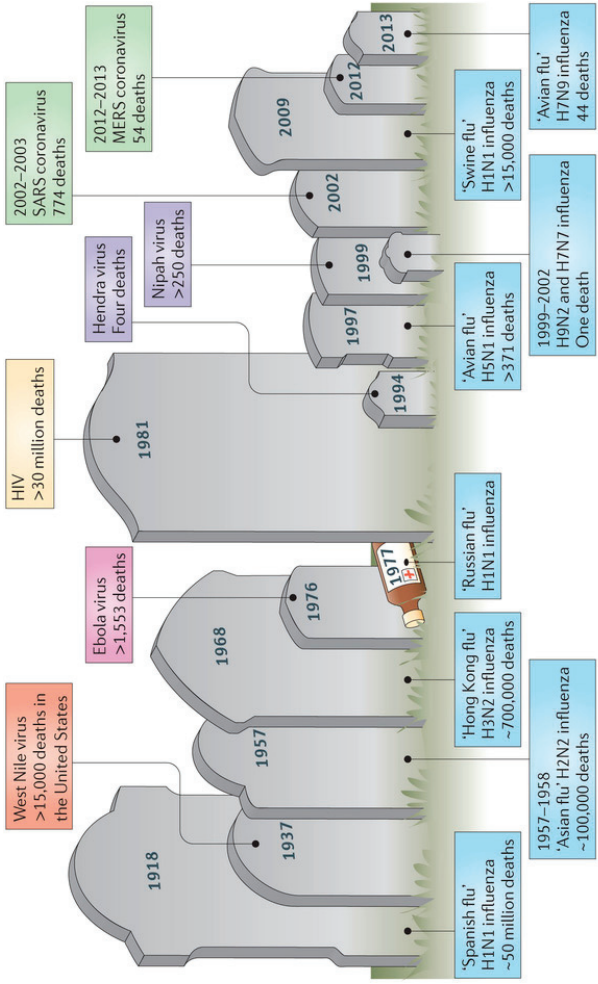

FIGURE WO-2 Emergence of zoonoses. Over the past century, humanity has witnessed the emergence of numerous zoonotic infections that have resulted in varying numbers of human fatalities. Influenza viruses that originate from birds account for an important proportion of these deaths, and recently many new zoonotic viruses that originate in bats, such as Hendra virus, Nipah virus, and the SARS coronavirus, have caused outbreaks with high mortality rates.

NOTE: As of June 2, 2014, the Centers for Disease Control and Prevention (CDC) reports that there were 39,557 cases of West Nile virus in the United States resulting in 1,668 deaths between 1999 and 2013. Source: http://www.cdc.gov/westnile/resources/pdfs/cummulative/99_2013_CasesAndDeathsClinicalPresentationHumanCases.pdf (accessed February 19, 2015).

SOURCE: Bean et al., 2013.

pandemic potential. Instead, the current outbreak of Ebola virus disease in West Africa has infected and killed more people than all previous outbreaks combined (Salaam-Blyther, 2014). According to the latest figures released by WHO on February 25, 2015, the total number of cases had risen to 23,694, with 9,589 deaths, in six West African countries—Guinea, Liberia, Mali, Nigeria, Senegal, and Sierra Leone—as well as one case in Spain and four cases in the United States (CDC, 2015).[4] Though the disease was identified in March, "more than 40 percent of the total number of cases have occurred within the past 21 days," according to WHO. "However, most cases are concentrated in only a few localities" (WHO, 2014e). The West African Ebola epidemic both epitomizes many of the concepts put forth by participants in the workshop, as well as highlights our current inability to successfully predict in almost any way what will next emerge.

Health, Fukuda noted, has reached a level of geopolitical significance that raises new challenges and opportunities for addressing infectious disease. While public health's traditional and effective focus has been disease prevention and control (through such measures as sanitation, immunization, and clinical care), a range of global trends now demands attention: climate and environmental change; population growth; urbanization; globalization (encompassing trade, travel, and migration); the predominance of poverty among the populations of middle-income countries leading to gaps in health care; and the deterioration of and declining investment in public health infrastructure (IOM, 2003, 2008, 2010, 2014).

As these trends have advanced so too have the global expectations for health care, Fukuda observed. "People have begun to take it for granted that food will be safe, water will be safe, that they will somehow be protected from epidemics and pandemics," he said. When that does not occur, the reaction—shaped and amplified by social communications—can be severe. Health issues therefore overlap with development, foreign policy, trade, sovereignty, and intellectual property—a phenomenon that is reflected in a policy transition from the Millennium Development Goals[5] to a "sustainable development agenda," a process that Fukuda identified as central to the future of global public health. "This is probably the single largest discussion in global public health taking place right now," he explained, and its broad scope includes health systems, determinants of health, sustainable development, environment, poverty reduction, and education. Much attention is focused on the implementation of universal health coverage that—while a topic of debate within the United States—is a widely accepted global goal.

[4] On September 30, 2014, the CDC confirmed the first laboratory-confirmed case of Ebola in the United States in Thomas Eric Duncan, a Liberian national who had traveled to Dallas, Texas. Mr. Duncan passed away on October 8, 2014.

[5] The eight Millennium Development Goals—which range from halving extreme poverty to halting the spread of HIV/AIDS and providing universal primary education, all by the target date of 2015—form a blueprint agreed to by all the world's countries and the entire world's leading development institutions. Source: http://www.un.org/millenniumgoals/bkgd.shtml (accessed February 19, 2015).

TABLE WO-1 Natural Reservoir Hosts and Susceptible Hosts Involved in Transmission of a Selection of Emerging Zoonotic Diseases[a]

Disease (virus)	Known reservoir hosts	Other susceptible hosts	Transmission host to humans	References
Avian influenza (H5N1, H7N9, H7N7, H9N2, H3N2, and others)	Waterfowl and wild birds	Bats, cats, dogs, ferrets, pigs, poultry (chickens, ducks, and turkeys), and marine mammals	Chickens	Centers for Disease Control and Prevention,[b] Zoonoses,[c] Animal Disease Information Summaries,[d] Reperant et al., 2012; Swenson et al., 2010; Tong et al., 2012
"Swine flu" strains (H1N1 and H3N2)	Pigs	Ferrets, foxes, cats, dogs, poultry (chickens, ducks, and turkeys), and marine mammals	Pigs	Centers for Disease Control and Prevention,[b] Zoonoses,[c] Animal Disease Information Summaries,[d] Reperant et al., 2012; Swenson et al., 2010; Tong et al., 2012
SARS (SARS coronavirus)	Bats	Civet cats	Civet cats	Centers for Disease Control and Prevention[b] and Shi and Hu, 2008
Dengue fever (dengue virus)	Primates	Unknown	Mosquitoes	Centers for Disease Control and Prevention[b] and Carver et al., 2009
Hendra (Hendra virus)	Bats	Horses and ferrets	Horses	Centers for Disease Control and Prevention[b] and Clayton et al., 2013
Rabies (rabies virus and other lyssaviruses)	Bats	Cats, cattle, coyotes, dogs, foxes, horses, mongooses, primates, raccoons, sheep, skunks, and wolves	Bats and dogs	Centers for Disease Control and Prevention,[b] Animal Disease Information Summaries,[d] Hatz et al., 2012; Rupprecht et al., 2011

Ebola viral haemorrhagic fever (Ebola virus)	Bats	Primates	Primates and bats	Centers for Disease Control and Prevention[b] and Zoonoses[c]
Crimean–Congo haemorrhagic fever (Crimean–Congo haemorrhagic fever virus)	Rodents, hares, hedgehogs, and ostriches	Cattle, goats, horses, pigs, and sheep	Ticks	Centers for Disease Control and Prevention[b] and Zoonoses[c]
Ross River fever (Ross River virus)	Kangaroos and wallabies	Bats, birds, cats, dogs, horses, and possums	Mosquitoes	Carver et al., 2009; Kay et al., 2007; Tong et al., 2008
Japanese encephalitis (Japanese encephalitis virus)	Pigs and wild birds	Horses	Mosquitoes	Centers for Disease Control and Prevention[b]
West Nile virus encephalitis (West Nile virus)	Domestic and wild birds	Bats, camels, horses, marine mammals, reptiles, and >30 vertebrate species	Mosquitoes and birds	Centers for Disease Control and Prevention[b] and Animal Disease Information Summaries[d]

NOTE: SARS = severe acute respiratory syndrome.

[a] Supplementary information S1 (table in Bean et al., 2013) lists numerous other zoonotic diseases, including bacterial, prion, parasitic, and other viral diseases.

[b] See the Centers for Disease Control and Prevention at http://www.cdc.gov.

[c] See Zoonoses on the World Health Organization website at http://www.who.int/zoonoses/diseases/en (accessed February 19, 2015).

[d] See Animal Disease Information Summaries on the World Organization for Animal Health website at http://www.oie.int/en/for-the-media/animal-diseases/animal-disease-information-summaries (accessed February 19, 2015).

SOURCE: Bean et al., 2013.

Having established the global context within which public health operates to address emerging infectious diseases, Fukuda proceeded to describe the direction of these efforts both in general and as applied to MERS-CoV and H7N9 influenza.

Lessons from Emerging Infectious Diseases

Global efforts to address emerging infectious diseases, as noted by Fukuda, have been shaped by experience in several ways:

- The consequences of slow response to threats such as HIV/AIDS has led countries to shift infectious disease efforts that once focused primarily on acquiring resources such as laboratories, physicians, and public health scientists to combat ongoing infectious diseases toward the goal of establishing active approaches and capabilities to identify and respond to outbreaks caused by emerging infectious diseases with epidemic or pandemic potential.
- The predominance of zoonoses among emerging infectious diseases illustrates the central role of the animal–human–ecosystem interface and informs the One Health paradigm. "Dealing with these kinds of diseases and responding to them, whether they are zoonoses or whether they are phenomena such as antimicrobial drug resistance can't be handled by single sectors anymore," Fukuda observed. "We live in a world where thinking about [infectious disease] . . . as a health issue alone has become outdated."
- Ongoing tensions involving the sharing of pathogen specimens and the benefits (e.g., vaccines) that result from the characterization of those pathogens must be balanced in global efforts to control emerging disease threats. If new technologies, vaccines, or countermeasures are derived from research on these samples, what is the appropriate quid pro quo? This dilemma sets up "a major balancing act, internationally," Fukuda stated.

The above considerations are reflected in major international agreements or frameworks governing responses to emerging infectious diseases, Fukuda continued. These include the 2005 revision to the IHR,[6] which was spurred by the emergence of both SARS and avian influenza H5N1 and was intended to accelerate

[6] See http://www.who.int/ihr/about/FAQ2009.pdf (accessed February 19, 2015).

the global response to "public health emergencies of international concern"[7] including emerging infectious disease threats. A rare, binding, treaty-level agreement among the 196 countries represented by WHO, the IHR were designed to facilitate the detection of emerging diseases of international concern, most but not all of which are infectious. "The IHR place a great deal of emphasis on detection and notification, verification and risk assessment," Fukuda explained, and they define a mechanism to coordinate the flow of information internationally during health emergencies. The IHR also attempt to avoid or reduce interference with international travel and trade. The regulations also specified the development of core capacities for health security (e.g., disease surveillance and laboratories) within each country by 2014—a deadline that fewer than 20 percent of the countries are on track to meet; this deadline has now been extended 2 years to 2016. "Right now there is a tremendous push to try to do whatever can be done to help countries attain those kinds of capacities," he stated. Similarly, the global Pandemic Influenza Preparedness Framework[8] has been developed, through "very difficult and long negotiations," to address concerns over equity in pathogen sample sharing and its resulting benefits, according to Fukuda (WHO, 2011a).

Another, less well-known agreement, the Convention on Biological Diversity,[9] has recently been adopted by environmental agencies within WHO countries. This convention, which was originally intended to promote sustainable development, will likely have major, unanticipated implications for health, in part because it establishes agreements for moving pathogen samples between countries, Fukuda added. How these agreements will affect the work of laboratories collaborating in response to emerging infectious disease outbreaks of international concern "is not very clear right now," he observed.

MERS and H7N9 Influenza

Fukuda provided a brief summary of the epidemiological findings on MERS and H7N9 influenza. As of mid-March 2014, the majority of MERS cases have

[7] On August 8, WHO Director-General Margaret Chan declared the West Africa Ebola crisis a "public health emergency of international concern," triggering powers under the 2005 International Health Regulations (IHR). The IHR requires countries to develop national preparedness capacities, including the duty to report internationally significant events, conduct surveillance, and exercise public health powers, while balancing human rights and international trade. Until last year, the director-general had declared only one such emergency—influenza A H1N1 (in 2009). Earlier this year (2014), she declared poliomyelitis a public health emergency of international concern and now again for Ebola, signaling perhaps a new era of potential WHO leadership in global health security (Gostin et al., 2014).

[8] The WHO Pandemic Influenza Preparedness (PIP) Framework, effective May 2011, is intended to improve and strengthen the sharing of influenza viruses with human pandemic potential, and to increase the access of developing countries to vaccines and other pandemic-related supplies. Source: http://www.who.int/influenza/pip/en (accessed February 19, 2015).

[9] Signed by 150 government leaders at the 1992 Rio Earth Summit, the Convention on Biological Diversity is dedicated to promoting sustainable development. Source: http://www.cbd.int/convention (accessed February 19, 2015).

been associated with Saudi Arabia, with a few additional cases reported in the region (updated information on MERS cases appears in the section, "Emergence of MERS-CoV"). Primary and secondary cases of the disease appeared distinct, with primary cases tending to be older and male, as compared with their secondary counterparts; fatalities had been higher in the primary cases than the secondary cases, who tend to be younger and healthier. People sickened by MERS tended to have significant underlying chronic diseases such as diabetes, heart disease, or hypertension, he noted. Fukuda added, however, that "the degree to which that reflects the background population versus some unusual predilection is not so clear yet."

Like SARS—and unlike H7N9 influenza—MERS case clusters have occurred within households and health care facilities; the latter comprised more than half of all secondary cases, Fukuda stated. "Most of the health care worker infections have generally been mild—some have been detected on contact tracing—but there have been deaths among them," he added. "We're not positively sure about what the mode of transmission from person to person is in these settings."

Recent efforts have attempted to identify possible animal reservoirs of MERS-CoV (see the section "Emergence of MERS-CoV"). "Much of the attention has been focusing on camels because of serologic, polymerase chain reaction (PCR), and virus studies identifying this virus in camels," Fukuda said. "There have been extensive efforts to look at whether there are other potential reservoirs, and so far nothing has really panned out."

As of mid-March 2014, 390 laboratory-confirmed human cases of H7N9 influenza had been reported, resulting in 121 deaths,[10] Fukuda said; most infected people who had been interviewed had been exposed to either live poultry or poultry markets. The cases occurred in two distinct waves that occurred in 2013, followed by a larger one in early 2014. Six small family clusters were associated with emergence, primarily in the second wave, he added.

First-wave cases occurred on China's eastern seaboard, Fukuda continued. During the second wave, the range expanded slightly to the north and significantly to the south, near the borders of Vietnam and Cambodia—a situation that is being carefully watched. H7N9 influenza cases encompassed a broad age range, but most involved middle-aged to older people, predominantly males, Fukuda reported; approximately 30 percent of cases were fatal.

Characterization of viral samples that revealed antigenic similarity among birds and humans led WHO to identify a recommended vaccine strain, Fukuda said. The viruses are uniformly resistant to one class of antiviral drugs, M2 inhibitors, and nearly all are sensitive to neuraminidase inhibitors, he said.

[10] As of the beginning of February 2015, 597 cases were reported, including 2 in Canada and 1 in Malaysia, with 207 deaths. Source: Hong Kong Centre for Health Protection: Avian Influenza Report. http://www.chp.gov.hk/files/pdf/2015_avian_influenza_report_vol11_wk07.pdf (accessed February 23, 2015).

Several similarities—beyond their near-simultaneous occurrence—unite the emergence of MERS-CoV and H7N9 influenza A, Fukuda observed:

- Both viruses were relatively limited in terms of their geographic spread. MERS-CoV cases in Europe, where the virus has failed to take hold, have clearly been imported or resulted from close contact with an imported case. At the time of this writing, while human infections had been reported from several countries both within and beyond the Middle East, those outside the region had recently traveled there (WHO, 2014d). No human cases of H7N9 influenza A had been reported outside China at the time of this writing (WHO, 2014c).
- Both viruses are zoonoses[11] with limited person-to-person transmission, resulting in sporadic cases and clusters, rather than community-wide spread.
- Future transmission patterns for either virus are uncertain. "Like all emerging infectious diseases, before something has actually happened, we are never quite sure what the potential is for these to change and escalate [or burn out] beyond the current patterns," Fukuda observed.

Several key features also separate MERS-CoV and H7N9 influenza, according to Fukuda:

- The viruses are not related and are geographically restricted to different regional locations.
- MERS-CoV is linked with camels and bats; H7N9 is linked with poultry.
- Clusters of MERS-CoV cases have primarily occurred in communities or health care settings, while H7N9 clusters have occurred primarily among people in contact with poultry, including families.
- Vaccine development against MERS-CoV is in the investigational stage, whereas production of an H7N9 influenza vaccine could be quickly launched if the need arose.
- Therapeutics for MERS-CoV are currently under investigation and include drugs and antisera; for H7N9 influenza, the value of antiviral drugs has been well established.

Fukuda elaborated on WHO's current priorities for MERS-CoV, beginning with regional surveillance. To better understand the risk factors associated with

[11] Zoonotic diseases are contagious diseases spread between animals and humans. These diseases are caused by bacteria, viruses, parasites, and fungi that are carried by animals and insects. Examples are anthrax, dengue, Ebola hemorrhagic fever, *Escherichia coli* infection, Lyme disease, malaria, plague, Rocky Mountain spotted fever, salmonellosis, and West Nile virus infection. Source: CDC factsheet: Zoonotic Disease: When Humans and Animals Intersect. http://www.cdc.gov/24-7/cdcfastfacts/zoonotic.html (accessed July 23, 2014).

human disease, an international case-control study is under discussion but has yet to be launched. In addition, there has been a great deal of discussion about the need for validating the serologic tests used to confirm exposure(s) to the MERS-CoV, but progress toward that goal has been slow. Therapeutics and vaccines for MERS-CoV are in the early stages of development. Finally, he said, controlling the threat of MERS-CoV will require coordination and discussion between human and animal health sectors, which so far has proven relatively difficult.

In the case of H7N9 influenza A, WHO's highest priorities include monitoring the regional spread in humans and animals, transmission patterns, and drug resistance; vaccine development and deployment planning; and developing strategies for preventing and controlling the spread of disease, Fukuda said. Unlike highly pathogenic H5N1 avian influenza, H7N9 influenza A is asymptomatic in poultry, making its spread among animals difficult to monitor, and increasing the need to anticipate its potential shift from its current status as a zoonotic infection to person-to-person transmission.

While vaccine development is well under way for H7N9, Fukuda noted that deployment planning is a concern, given the political, legal, and operational experiences with vaccine distribution during the H1N1 influenza pandemic of 2009.

Global Response to Emerging Infectious Diseases

As public health broadens its goals, perspectives, and connection to other sectors, a concomitant transition will change approaches to addressing emerging infectious diseases, Fukuda predicted. "We no longer have health discussions," he said. Today, global health deliberations encompass population growth, globalization, communications, economics, or social justice—all of which must be addressed in developing sustainable solutions to infectious threats. By providing "the accepted foundation for health security," the IHR represent one such solution, Fukuda observed. Likewise, the Global Influenza Surveillance and Response System[12] supports both virus detection and vaccine development worldwide. The acceptance of the One Health concept further extends global connections and demonstrates that "the need for intersectoral coordination is moving past the stage of rhetoric to actually being acted upon," he asserted.

More concretely, recent alliances among the Food and Agriculture Organization of the United Nations (FAO), World Organization for Animal Health (OIE),

[12] Global influenza virological surveillance has been conducted through WHO's Global Influenza Surveillance and Response System (GISRS) for over half a century. Formerly known as the Global Influenza Surveillance Network, the new name came into effect following the adoption of the PIP Framework (see above) in May 2011. GISRS monitors the evolution of influenza viruses and provides recommendations in areas including laboratory diagnostics, vaccines, antiviral susceptibility, and risk assessment. It also serves as a global alert mechanism for the emergence of influenza viruses with pandemic potential. Source: http://www.who.int/influenza/gisrs_laboratory/en (accessed February 19, 2015).

and WHO signal a "sea change in the degree to which the organizations work together on a functional basis," Fukuda observed. This collaboration (discussed in greater detail in the section "International and Domestic Responses to Emerging Viral Diseases") was initiated in response to economic and social concerns associated with emerging pathogens. It was formalized through a memorandum of understanding signed by the three directors-general of these organizations to (1) work together closely, (2) meet annually, and (3) strategically plan for the coming year together. In addition, he noted, FAO and WHO have agreed to collaborate on the next international conference on nutrition. Meanwhile, OIE and WHO are attempting to harmonize the ways by which they measure gaps in capacity.

Despite these indicators of progress toward global cooperation and coordination in addressing emerging infectious diseases, according to Fukuda significant challenges remain. These include the lack of core capacities for surveillance and detection in most countries, as previously noted—leading to incomplete information on the spread of emerging pathogens such as MERS-CoV. This is less the case with H7N9, he added, attesting to the fact "that we are at different stages in different parts of the world in implementing the concepts or the spirit of IHR."

There is no overriding reason for the gap in implementing the IHR, Fukuda explained during the discussion that followed his presentation. Some countries are simply too poor to build core capacity for infectious disease surveillance and detection, he observed, whereas others are "concerned that if they indicate that they have all core capacities, perhaps their funding or their support may go down." But most countries, he said "with a reasonable amount of support, are going to get there," at least over the long term. While the development of core capacities is the responsibility of national governments, he insisted, WHO can support them in implementing quality assurance and assessment.

While intersectoral collaboration has improved over recent years, it is not routine, particularly at the national level and below, Fukuda said. At the same time, the rapid pace of scientific development (e.g., high-throughput sequencing) has begun to strain existing frameworks and approaches, and even the concept of what constitutes a pathogen—a definition central to such frameworks as pandemic preparedness and disease eradication. "Right now we don't quite know how to handle . . . [such] questions," he acknowledged, noting that both MERS-CoV and H7N9 influenza are raising them anew. In particular, with regard to the Convention on Biological Diversity, Fukuda noted that its potential to inhibit sample sharing during a pandemic has been addressed in part through the ability to create special agreements, but he nonetheless predicted that its implementation would cause "a great deal of uncertainty and confusion" that would not be resolved without challenge (and resultant failure) during a major disease outbreak. "I don't think it's going to be orderly," he said of that transition.

Many of the same issues WHO confronts in addressing MERS-CoV and H7N9 are also relevant to antimicrobial resistance, which Fukuda characterized as a "super-emerging infectious disease." Although recognized by scientists

since penicillin's initial release in the mid-20th century, public awareness of this phenomenon has only recently become widespread. The impact of antimicrobial resistance extends well beyond the health of individuals, to development, foreign policy, and economics—a recognition that was articulated by President Obama's mention of antibiotic resistance in his 2014 State of the Union address.

"In the next year, there will be the development of a global action plan, which is an attempt to bring all of this together and to move this at a more coherent and coordinated level than is currently available," Fukuda announced. The plan will define the scope of the problem and functional issues to be tackled, describe a sustainable research base, and propose a new, sustainable and appropriate model for marketing antimicrobial drugs, he said. The latter innovation is important, he noted, given that "much [of the] effort is focused on how to stimulate research into new antibiotic modalities, and not on the overarching question of how risk will be distributed." The global plan should also, eventually, identify benchmarks for success and clarify actions that can be taken to achieve them, providing a blueprint that can be adopted, in whole or in part, by anyone in the world, he concluded.

When asked in the subsequent discussion to define the "hot button" issues the global action plan intends to address, Fukuda replied that an immediate concern is "future fights that you can almost predict" between poor and wealthy countries, and between the pharmaceutical industry and the public. "Probably the biggest sensitivity is the use of antibiotics for nonhealth (e.g., agricultural) uses," he observed, noting the paucity of relevant data on the risk of such uses, given their widespread occurrence. Further priorities include regulating antibiotic prescription practices, supporting antimicrobial research and development through marketing-independent mechanisms, and improving surveillance for antimicrobial resistance. "We can't think about antibiotics as products; we have to think about them as global goods," he insisted.

As Fukuda noted in response to questions from members of the Forum, the crafting of the global action plan to address antimicrobial resistance involves considerations of multiple sectors, including the private sector and the research community. "There will be a very concerted effort to bring in all of those viewpoints, including from the research community, but also from industry, from civil society, from a lot of the scientific groups that have been working on this for decades," he stated.

Relman noted a similar initiative, in February 2014, when the White House and the Secretary of Health and Human Services, together with WHO, FAO, and other organizations, announced a new global health security agenda intended to catalyze international action on this issue—for example, by completing the core capacity building specified by the IHR. Relman wondered how this effort might be directed to have maximum beneficial impact. Fukuda replied that that will happen if all of the involved parties move together in a coherent and coordinated way—a concept that is "very clear . . . [but] still challenging to implement."

Instead of a wasteful sector-by-sector approach, implementation should proceed in ways that link both countries and agencies, he urged. "This, in essence, is what things like the IHR were meant to try to get at," Fukuda observed. The global health security agenda strives for a new level of international coherence in responding to EIDs, he said, yet it remains to be determined how to reach that goal.

LESSONS LEARNED FROM THE 2009 H1N1 INFLUENZA A PANDEMIC

In his opening remarks to the workshop, Forum chair David Relman, of Stanford University, noted that EIDs—especially those caused by influenza viruses and β-coronaviruses—have greatly inspired expanding curiosity about and understanding of the origins and consequences of infectious diseases. If EIDs are teachers, pandemics provide a particularly intense educational experience. Keynote speaker Harvey Fineberg, president of the Institute of Medicine,[13] recounted lessons learned from the 2009 H1N1 influenza A pandemic—the first declared pandemic of the 21st century (Dr. Fineberg's contribution may be found on pages 152–165 in Appendix A). The international response to this pandemic was strongly influenced by the global experience with SARS.

Between November 2002 and July 2003, the introduction of SARS—primarily through international travel—wreaked havoc in 26 countries, resulting in more than 7,000 cases and more than 700 deaths, Fineberg recalled. While the epidemic was extinguished largely through the isolation and management of hospitalized patients, "It was a warning sign," he said. "MERS is another threat," Fineberg continued, "but among the many threats for pandemics, in terms of versatility, the persistence, the rapidity, and the possibility for extremely severe consequences, there's nothing that quite rivals influenza." These viruses, he noted, have caused some of the most catastrophic pandemics in history, most particularly the Spanish flu pandemic of 1918–1919. There are many lessons to be learned from the history of influenza and from the experiences of and with pandemics, he observed.

Fineberg took a global perspective in his analysis of the international response to the 2009 H1N1 influenza A pandemic (Fineberg, 2014). He focused on the role and actions of WHO, a topic he studied as the chair and member of the WHO Review Committee on the Functioning of the International Health Regulations (2005) and on Pandemic Influenza (H1N1) 2009 (WHO, 2011b).

[13] Dr. Fineberg's presidency ended on June 30, 2014. His current affiliation is with the Gordon and Betty Moore Foundation.

The Global Experience

History

As Fineberg described it, the 2009 H1N1 influenza A pandemic unfolded as follows:

> In late March 2009, the cause of flu outbreaks in Mexico was discovered to be a previously unrecognized H1N1 virus; it subsequently was associated with prior cases in California. By the end of April 2009, H1N1 had already been recognized in a number of states within the United States, as well as in Canada, New Zealand, Spain, the United Kingdom, Germany, and Israel. Invoking its authority under the 2005 IHR, WHO on April 25, 2009, declared a public health emergency of international concern and convened the emergency committee called for in the regulations. WHO also established a dedicated internal group to coordinate the international response to the widening outbreaks. (Fineberg, 2014)

By June 9, 2009, more than 70 countries had isolated more than 26,000 laboratory-confirmed cases. WHO declared on June 11, 2009, that a full-fledged pandemic was under way. This strain of influenza spread so rapidly, that by late July 2009, virtually every country in the world had identified and isolated laboratory-confirmed cases of H1N1 influenza.[14]

Burden of Disease

Two recent estimates (Dawood et al., 2012; Simonsen et al., 2013) suggest that between 100,000 and 400,000 deaths were attributable worldwide to the 2009 H1N1 influenza A pandemic, as compared with as many as 500,000 deaths in a typical interpandemic influenza season. Yet, the distribution of mortality was unlike that of seasonal flu, in which most illness and death occurs among the very young and the elderly. For H1N1, mortality among children, young adults, and pregnant women was especially high compared with a typical flu season. In considering the burden of disease in terms of years of life lost, according to Fineberg, the 2009 H1N1 influenza pandemic strain represented a more serious threat to global health than seasonal influenza.

[14] In March and early April 2009, 2009-H1N1 influenza A emerged in Mexico and the United States. During the first few weeks of surveillance, the virus spread worldwide to 30 countries by human-to-human transmission, causing the WHO to raise its pandemic alert to level 5 of 6. On June 11, 2009, WHO raised the worldwide pandemic alert level to level 6 in response to the ongoing global spread of the 2009-H1N1 influenza A virus. This virus has now become the first influenza pandemic of the 21st century. The third public health emergency of international concern (PHEIC) was declared on August 8, 2014, for the Ebola virus outbreak in Africa. In both cases, the scientific, public health, security, and policy communities are moving quickly to learn more about the nature and potential impact of these viral diseases on human and animal health.

Testing the 2005 IHR

To mount an effective response to an emerging and evolving pandemic public health authorities must act rapidly and authoritatively on incomplete knowledge of the disease they are attempting to address. This situation, which Gostin (2004) has aptly named "the public health paradox," lies at the heart of the ethical and legal response to pandemic disease. "There is no way to avoid the dilemmas posed by acting without full scientific knowledge," he writes. "The only safeguard is the adoption of ethical values in formulating and implementing public health decisions." Similar arguments have been made in favor of an ethical framework for decision making concerning biodefense and bioterrorism.

The IHR provides the legal framework for international cooperation on infectious disease surveillance. The IHR were adopted by the World Health Assembly in 1969, having evolved from the International Sanitary Regulations adopted in 1951 (which, themselves, were direct descendants of the international sanitary conventions adopted from the 1890s through the 1940s) (IOM, 2007). The IHR (1969) were intended to monitor and control six diseases—cholera, plague, smallpox, relapsing fever, typhus, and yellow fever—yet revisions in 1973 and 1981 resulted in only three reportable diseases—cholera, plague, and yellow fever—whose occurrence required WHO notification (WHO, 2009). In the mid-1990s it became clear that the IHR (1969) had become outdated given the vast number of global microbial threats that had emerged and reemerged, including those which were not deemed "notifiable" in the original set of guidelines (i.e., Ebola hemorrhagic fever) (IOM, 2007; WHO, 2009). There was also concern that the IHR's dependence on "official" country notification, along with a lack of a formal internationally coordinated mechanism to contain international disease spread, might prove problematic in effectively containing disease with pandemic potential (WHO, 2009). Several resolutions passed by the World Health Assembly (in 1995, 2001, 2003) encouraged revision of the IHR, with the final resolution WHA58.3 formally adopting the IHR (2005) on May 23, 2005.

When the revisions to the IHR came into force on June 15, 2007, member nations of WHO were required to report all new and reemerging diseases with epidemic or pandemic potential, irrespective of their origin or source (WHO, 2008). These revisions also stipulated that member nations were to assess their disease surveillance capacity and develop national action plans within 2 years, and meet the IHR requirements within 3 years regarding their national surveillance and response systems, as well as requirements at designated airports, ports, and ground crossings (although extensions may be obtained) (WHO, 2008).

The 2009 influenza A pandemic was the first real-world test of the IHR revisions (2005) in a PHEIC, and, Fineberg noted, it exposed vulnerabilities in public health capacity and response. The experience also revealed the limitations of available scientific knowledge in understanding and coping with pandemic disease in general. Decision making under these conditions of uncertainty, which inevitably occur during an evolving pandemic, was predictably difficult, he

observed. There were also many communications challenges between and within the many organizations involved in addressing the crisis.

A number of criticisms were leveled at decisions made by WHO in the course of the 2009 pandemic, Fineberg stated. Some of these criticisms were reasonable, and some were unjust, but it was difficult to discern between them until the consequences of the decisions in question were clear, he said; in some cases, that was not until the pandemic was long extinguished. To productively analyze these concerns, WHO convened the committee that Fineberg chaired; he described its findings to the workshop (Fineberg, 2014; WHO, 2011b).

Report of the Review Committee

In 2010, WHO formed an international committee to review the regulations of the IHR in their performance in the pandemic, and also the performance of WHO itself, Fineberg said. The committee consisted of 24 members, each from a different country. In response to criticism regarding WHO's secrecy in decision making during the pandemic, the committee held all fact-gathering sessions open to representatives from its member states, as well as to the public and the press, he reported. The committee did deliberate in closed sessions, he added, and there were subgroups of the committee that worked privately.

With its diverse makeup, the committee was often difficult to manage, according to Fineberg. At the same time, these challenges paid off since the recommendations of the committee were viewed as representing a broad spectrum of consensus opinions of WHO member states, and therefore readily endorsed at the World Health Assembly in 2011, stated Fineberg. The committee reached three key conclusions about the IHR, WHO operations, and global pandemic response(s)—which Fineberg discussed along with additional relevant findings, implications, and recommendations.

The IHR

Implementation of the IHR (2005), which were born of the 2003 SARS pandemic, clearly helped the world prepare to cope with the public health emergency presented in 2009 by the H1N1 influenza A pandemic, Fineberg stated. However, he noted, the committee found that core capacities specified by the IHR were not yet fully operational in many member states, and that many countries were not even on a path toward successful implementation of these capacities.

The IHR call for consistent communication and cooperation was in fact the case in many countries throughout the pandemic, Fineberg reflected. WHO provided needed and appreciated technical support to many countries. Provisions of the IHR that address the impact of public health emergencies beyond the health sector (e.g., social and economic impacts) "were very salutary," he observed.

These recent revisions to the IHR enabled more flexible, dynamic, and adaptive responses.

But for all of the virtues of the IHR (2005), there were a number of shortcomings, Fineberg continued. "In 2011, WHO surveyed all of its 194 member state signatories to assess where were they on the path towards successful implementation of the core capacities called for in the IHR," he stated; only 58 percent responded. Of them, only 11 percent had completed the core capacities called for by 2012. Moreover, as Fineberg noted, if a signatory to the IHR fails to comply with its provisions—for example, by taking unilateral action that interferes with travel and trade—that state must provide a rationale for its breach of regulations. Yet such a state faces *no consequences* if fails to do so, he said. "There are not provisions for any enforcement of these expectations and agreements," he observed.

Among the lessons learned regarding the IHR in the course of the pandemic is the recognition that the regulations, as they stand, are insufficient to ensure that countries will fully implement the required capacities for addressing public health emergencies of international concern. The case must be made that it is in the interest of each country to implement these capacities for the protection of their own citizens, as well as for the global good. Fineberg continued by stating that implementation of the IHR-required capacities must be made easier. "Mobilizing agencies willing to provide technical assistance to those countries that require it would be a help," he suggested, as would better-organized channels by which specialized resources could be shared internationally. Along these same lines, the committee recommended that WHO's information sites be geared to meet specific countries' needs under the IHR.

Finally, Fineberg noted that the requirements of the IHR must somehow be enforced, in order to encourage countries to support and comply with their effective implementation. "It is also going to be useful to try to clarify the effect and measure the consequences of various actions that may be taken in future public health emergencies," he concluded.

WHO Operations

The committee agreed that WHO achieved a number of successes as it led the international response to the 2009 pandemic, Fineberg stated. Be that as it may, systemic problems hampered the organization's performance, and the committee identified several operational shortcomings that occurred. During the pandemic, some had accused WHO of purposefully misleading countries and of making distorted decisions, Fineberg recalled. Based on their analysis of WHO's complete files on its actions, as well as interviews of all the relevant parties, they could find no evidence of malfeasance, only of error.

Overall, the committee determined that WHO provided very timely guidance in the face of the emerging pandemic to countries worldwide, according to Fineberg. "Preparedness plans specifically were in place in 74 percent of countries by

the time the pandemic emerged. Once the public health emergency was declared under the authority of the IHR, WHO immediately convened an emergency committee that was called for under the provisions," he reported. WHO provided rapid and appropriate field support as well as early recommendations on vaccine target groups and optimal dosages, he continued, and the organization gathered and disseminated information on laboratory-confirmed cases from countries around the world. WHO also strived to work with sister agencies of the United Nations and, in concert with national organizations, was largely successful in that effort, Fineberg observed.

Candidate vaccine strains of H1N1 were identified early—within weeks—and seed strains were quickly made available along with appropriate reagents, according to Fineberg, another success driven by WHO. Likewise, the organization oversaw the efficient deployment of antiviral drugs, a possible means of containing the pandemic, to high-risk populations in 72 countries.

"There's no substitute for WHO when it comes to capacity and role in managing a global emergency in the health sphere," Fineberg insisted. He also noted, however, that structural constraints within the organization led to some problems in its response to the 2009 pandemic. For example, reflecting its dual capacity as the moral voice for health equity in the world and as the servant of the member states, WHO encountered conflicts between the interests of individual member states and that of its global responsibilities.

Those responsibilities, Fineberg noted, vastly dwarf WHO's budget, which is only partially (25 percent) funded by its member states. Most of its funding is directed not by the organization, but by individual member states, or by foundations or other donors, he explained. "Organizationally, WHO is pretty well equipped to do two things," he said: (1) managing multiyear disease control programs (e.g., for malaria), and (2) mounting emergency responses to investigate emerging infectious disease outbreaks (e.g., of hemorrhagic fever in sub-Saharan Africa). The organization was not able to sustain the focused, global effort required to address a pandemic, Fineberg concluded.

Compounding these structural barriers within WHO was ambiguity about the definition of a pandemic, Fineberg reported, particularly in terms of severity. The pandemic preparedness plan in place when H1N1 arrived was designed to address H5N1, with its high fatality rate. But a pandemic is defined by its geographic spread, not its severity—which is arguably more important to determining an appropriate response, he stated, and as illustrated in Figure WO-3. "Failure to have a consistent, measurable, and explicable measure of severity was a real handicap throughout the [H1N1] pandemic," he observed.

WHO's six-level pandemic phase alert system was overly complex, Fineberg continued. Moreover, when WHO declared phase six, they ceased press conferences, which he deemed "a rather odd response to having now reached a full-fledged pandemic." Other practices raised public suspicions, he noted. For example, unlike standard 2-day WHO consultative committees, the emergency

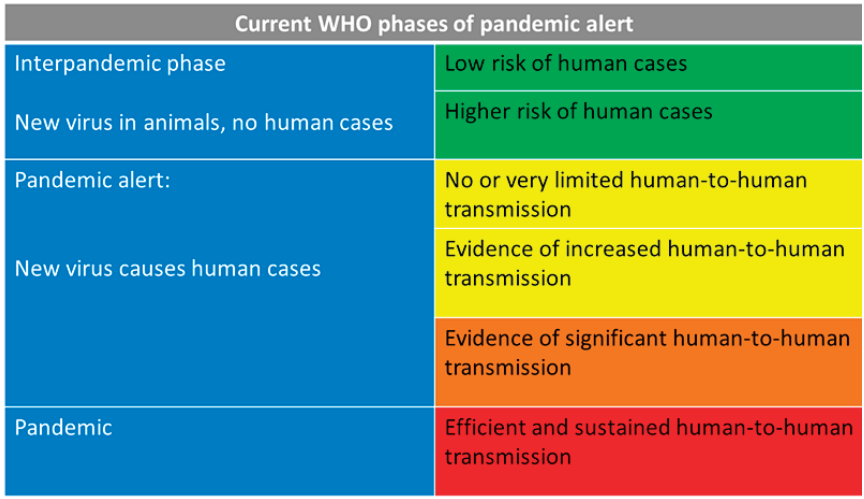

FIGURE WO-3 WHO phases of pandemic alert at the time of the H1N1 pandemic.
NOTE: As of April 18, 2007.
SOURCE: WHO, 2008.

committee invoked according to the IHR remained in place until the pandemic ended. When WHO convenes consultative committees, it does not publicize the identities of its members until their report is made public, in order to protect them from commercial or political pressure and obtain their best judgment—but this confidentiality strategy is only effective over short periods, he said. Also, the emergency committee shared little information with the public as to how it managed conflicts of interest among its members, he observed. For instance, it did not reveal whether any of its members were associated with pharmaceutical companies that were producing vaccines.

WHO generated tremendous amounts of guidance in response to the pandemic, but it lacked a system for collating, coordinating, and prioritizing that information, Fineberg stated, as well as a means to clearly communicate the pandemic's scope and severity. Delays in translation into all of WHO official languages rendered their guidance even less useful to national decision makers, he added.

Global Pandemic Response

The delayed distribution of vaccine represents a fundamental shortcoming in the otherwise strong global response to the 2009 H1N1 influenza A pandemic, according to Fineberg. In most countries, he observed, that delay would

have rendered the vaccine less useful had the pandemic been more severe. This shortcoming stems from the fact that vaccine production remains predominantly dependent on egg viral culture, he pointed out. Regulatory, legal, and logistical complexities in vaccine distribution further slowed its release. Most importantly, he added, arrangements with vaccine manufacturers were not in place when the pandemic struck, so negotiations began during the initial outbreak—a lesson in the importance of advance preparation for emerging infectious disease events.

Lessons for WHO

Fineberg described these recommendations by the review committee aimed at improving pandemic response by WHO:

- **Clarify and strengthen pandemic processes and guidelines.** This includes clarifying guidance around severity, simplifying the pandemic phase structure, and more flexibly responding to pandemics as they unfold, learning from and applying lessons learned from the experiences in different countries. There is also a need to develop practices to identify conflicts of interest and to encourage transparency and openness in the appointment of emergency committees.
- **Strengthen internal capacity.** A contingency plan for mobilizing and sustaining relevant expertise during a public health emergency needs to be developed, along with the increased financial support for WHO that would make such a plan possible.
- **Improve communications.** Sustain active communications throughout the emergency, and acknowledge mistakes appropriately, which strengthens refutations of unwarranted accusations. Track and archive changes in the web. Use social media to reach a wider public.
- **Encourage advance agreements.** Since the time of the 2009 influenza pandemic, a protocol has been adopted by many countries to support the solicitation of donated vaccine in advance of a pandemic. These PIP agreements preposition relevant vaccine, make seed strains widely available to manufacturers, and extend technology to developing countries, enabling them to produce their own vaccine. Such distributed capacity, Fineberg argued, is the only long-term solution to the vaccine-sharing dilemma that pits the interests of countries with manufacturing capacities against global needs when supplies are limited—as likely occur in a pandemic.

Lessons for the Global Pandemic Response

The overall conclusion of the review committee was that the world was ill prepared to respond to a severe influenza pandemic, or to any similarly global, sustained, and threatening public health emergency, Fineberg stated. Much

remains to be done, including building public health capacity and pursuing research to improve response to pandemic threats. "If we had a severe pandemic today, with the vaccine capacity that we have today, and the distribution of methodology and production that we have today, with a total global capacity for about one-third of the world's population, we could have tens of millions of people who would perish," he warned.

Fineberg described the following recommendations for global efforts to address future pandemics, derived from experience in the 2009 H1N1 influenza A pandemic:

- Mobilize during emergencies, and deploy to countries that need assistance.
- Create a contingency fund for public health emergencies.
- Prepare distribution agreements between industry, WHO, and countries.
- Make seed virus strains widely available to vaccine manufacturers.
- Require vaccine manufacturers to contribute vaccine to a global pool.
- Encourage countries to provide immunizations to high-risk populations.

Fineberg observed that research on the following key topics will enable even more significant improvements in pandemic preparedness:

- Detection, characterization, and monitoring of new viruses;
- Viral and host determinants of transmissibility and virulence;
- Point-of-care diagnostic tests;
- Accuracy and timeliness of modeling projections;
- More effective, safer, and long-lasting vaccines;
- Antiviral drugs;
- Protective equipment, personal hygiene, and social interventions; and
- Effectiveness and costs of border control measures.

The World Health Assembly endorsed the review committee's report and accepted their recommendations in May 2011. Within the next year, several WHO units incorporated recommendations from the report into their annual working plans, Fineberg stated. Even so, structural impediments and scientific shortcomings remain that can impair the world's ability to prepare for and respond effectively to the next pandemic, he concluded. For example, in discussion following his presentation, he observed that while WHO may have developed contingency plans to improve their capacity to mobilize response to a pandemic, he did not believe that they had been implemented to the extent that if confronted with another PHEIC, the organization would be more able than in 2009 to support sustained deployment of existing staff. This observation has been borne out by the current regional and global responses to the current Ebola outbreak in West Africa.

Recognizing that WHO's limited financial resources greatly hinder its ability to mobilize in a pandemic, Fineberg reported on the review committee's recommendation that hundreds of millions of dollars be made available to the

organization to support these efforts. Such funds, he suggested, could be authorized through the World Bank or International Monetary Fund as a line of credit conditioned upon the declaration of a public health emergency of international concern.

When asked whether the world was better prepared to respond to a pandemic today than in 2009, Fineberg answered yes, for such reasons as the advent of PIP agreements, and some (but not enough) progress toward implementation of the IHR (WHO, 2008). Nevertheless, the world could do far more to prepare but does not. Why?

"It is very difficult to invest in a possibility when you've got compelling alternative immediate demands," Fineberg observed. When people are actually dying of other diseases, it is difficult to shift limited resources away from those toward a possible disaster, he said. Thus, he concluded, the case for pandemic preparedness is a case for insurance, requiring an investment that not every country is ready or able to make.

OVERVIEW OF EMERGING VIRAL DISEASES

An early session of the workshop was devoted to four presentations that provide context for the subsequent discussion of the emerging MERS-CoV and influenza A viruses by exploring the ecology and immunology of emerging viral diseases; the political and social conditions that have facilitated their emergence, which in turn are affected and shaped by the consequences of infectious disease; and the research response to viral disease emergence.

Emerging Diseases in Wildlife

Once studied only for their role in regulating wildlife populations, infectious diseases of wildlife have recently gained attention as potential threats to domestic animal and human health, according to speaker Jonathan Sleeman, of the U.S. Geological Survey (USGS) National Wildlife Health Center. Indeed, three-quarters of known emerging infectious diseases are zoonotic, of which the majority are of wildlife origin, he noted, and disease agents infecting wildlife are twice as likely to become EIDs as those without wildlife hosts (Taylor et al., 2001) (Dr. Sleeman's contribution may be found on pages 248–262 in Appendix A).

Previously described global trends favoring disease emergence apply equally to diseases of wildlife, particularly ecological changes such as intensified farming, alterations in landscape and land use, human activity in formerly pristine areas, and climate change. These circumstances "are clearly leading to increased opportunities for spillover of pathogens from wildlife into domestic animal and human populations," Sleeman said. Moreover, he added, while public and animal health initiatives in wealthy countries have prevented or controlled many emerging viral diseases, comparable infrastructure to address wildlife disease and

health issues barely exists. Likewise, the health and economic consequences of emerging diseases of wildlife such as white-nose syndrome in bats, colony collapse disorder in bees, chytridiomycosis in amphibians, canine distemper virus in wild and domestic animals, and Ebola virus, while surely significant, have been largely unrecognized, he observed.

- **White-nose syndrome** is an emergent disease of hibernating bats that has spread from the northeastern to the central United States at an alarming rate. Since the winter of 2007–2008, millions of insect-eating bats in 25 states and 5 Canadian provinces have died from this devastating disease. The disease is named for the white fungus *Pseudogymnoascus destructans* that infects skin of the muzzle, ears, and wings of hibernating bats (Blehert, 2012; Blehert et al., 2009, 2011; USGS, 2014).
- **Colony collapse disorder** (CCD) is a serious problem threatening the health of honey bees and the economic stability of commercial beekeeping and pollination operations in the United States. Despite a number of claims in the general and scientific media, a cause or causes of CCD have not been identified by researchers (USDA/ARS, 2014).
- **Massive die-offs of amphibians are often caused by ranaviruses.** USGS scientists have isolated ranaviruses associated with die-offs in more than 25 states involving more than 20 species of turtles and amphibians in mortality events ranging from one to thousands of individuals affected. Some events may involve a single species; others may involve multiple species. Frogs and salamanders in the same pond, for example, may die from ranaviral infections at the same time (USGS, 2013).
- **Canine distemper virus** (CDV) is the second most common cause of infectious disease death in domestic dogs and is a significant viral disease of global importance in common and endangered wild carnivores. It is a multihost pathogen with abundant wildlife reservoir species, such as raccoon dogs (*Nyctereutes procyonoides*). Identification of positive tiger CDV cases suggests wide distribution for the Arctic-like CDV strain that infects and kills Amur tigers (Seimon et al., 2013).
- Repeated outbreaks of **Ebola** have had a devastating impact on humans, chimpanzees, and gorillas in central Africa over the last decade. There are particular fears for western gorillas (*Gorilla gorilla*). Although all apes and chimpanzees are threatened, these gorillas have a habitat ranging over a particularly small area, with the majority of the population found in parts of Cameroon, Gabon, and Republic of Congo.[15] It is estimated

[15] Western lowland gorillas are endangered, but they remain far more common than their relatives, the mountain gorillas. They live in heavy rain forests, and it is difficult for scientists to accurately estimate how many survive in Angola, Cameroon, Central African Republic, Congo, the Democratic Republic of Congo, Equatorial Guinea, and Gabon. National Geographic, Western Lowland Gorilla *Gorilla gorilla gorilla*, http://animals.nationalgeographic.com/animals/mammals/lowland-gorilla (accessed November 12, 2014).

> that one-third of the world's gorilla population living under protection in national parks have died in the past 15 years from this disease (Animal Research Info, 2014).

It is clear that emerging viral diseases—including ranavirus in amphibians, canine distemper in tigers, and Ebola hemorrhagic fever in gorillas—have caused major wildlife population declines since the 1990s, according to Sleeman. Whereas in the 1970s, wildlife diseases investigated by the USGS tended to cause large but localized die-offs, since the 1990s, the agency has encountered an ever-increasing number of novel diseases that are hard to predict, spread rapidly over large geographic areas, impact multiple species, and cause dramatic population declines and even extinctions, he reported—and outbreak investigations involving collaboration among multiple sectors and agencies.

In his presentation to the workshop, Sleeman chose to highlight the role of wild animals in the emergence of avian influenza and the resurgence of bluetongue and epizootic hemorrhagic disease, the phenomenon of disease transmission at the human–primate interface, and the significance of bats as reservoirs for numerous emerging viruses—including SARS and MERS-CoV, Nipah and Hendra, Ebola and Marburg—that cause human disease.

Emerging Avian Influenzas

Wild birds—primarily waterfowl, shore birds, and gulls—are the natural reservoirs for avian influenza (AI) viruses, Sleeman observed. Recently emerging AI strains, including H5N1, H7N9, H10N8, and H5N8, have threatened the poultry industry as well as public health. He noted that many of these strains originated in Southeast Asia, where the intensification of farming practices—including the co-mingling of domestic and wild species—and the growth of live markets[16] have fueled the spillover of viruses from wild to domestic birds, and then to humans.

The USGS genome studies have examined the genetic structure of AI viruses in Asia and North America, paying particular attention to continental edges in Alaska and along the eastern seaboard of Canada and Iceland, Sleeman stated. In such areas, the USGS found both Eurasian and North American strains, along with strains with mixed lineages—hot spots for the evolution of new viruses. "These are definitely areas of focus for surveillance," he observed.

The agency is also keeping a close eye on unusual events such as the 2011–2012 die-off of harbor seals in New England due to an H3N8 AI virus, Sleeman noted. This virus proved similar to one that was circulating in wild North American waterfowl, he added, but its sequence suggested its recent adaptation

[16] Variously known as live bird markets, live poultry markets, and wet markets, open marketplaces composed of stalls where live poultry (and often other live animals and fresh vegetables) are sold are found throughout China and many Southeast Asian countries. In this document, all such venues are denoted by the term "live market."

to mammalian hosts—in contradiction to the widely accepted "mixing vessel hypothesis,"[17] which defines a circuitous route of pandemic viral emergence from wild birds into domestic fowl, then pigs, before recombination with other mammalian viruses to create a pandemic viral strain. Instead, the H3N8 seal influenza virus appears to have jumped directly from wild birds into mammals through a yet-uncharacterized "direct pipeline."

Bluetongue and Epizootic Hemorrhagic Disease

Epizootic hemorrhagic disease is an acute, infectious, often fatal viral disease of some wild ruminants. This infection is characterized by extensive hemorrhages and has been responsible for significant epizootics in deer in the northern United States and southern Canada. A similar hemorrhagic disease called bluetongue also occurs throughout the United States and Canada. The two diseases are antigenically different (Howerth et al., 2001).

Bluetongue (BT) is a noncontagious viral disease affecting domestic and wild ruminants (primarily sheep, cattle, goats, buffalo, antelope, deer, elk, and camels) that is transmitted by insects, especially biting midges of the *Culicoides* species. BT has a significant global distribution in regions where this insect vector is present, including Africa, Asia, Australia, Europe, North America, and several islands in the tropics and subtropics. The virus that causes BT is identified as a member of the Reoviridae family (OIE, 2014a).

Severe infections of domestic and wild ruminants by these similar viruses have resulted in dramatic die-offs among deer and livestock, Sleeman reported. BT has been recognized for decades but is recently resurging. "There has been a dramatic extension of the [geographic range of the] virus into Northern Europe, in particular the UK and Scandinavia," he said. "Here in the United States we are seeing more severe, widespread outbreaks in wild ruminants. The disease has been found further north than it used to be, in states like Wisconsin and Michigan. It was found in New York for the first time several years ago."

BT's resurgence has been influenced by several drivers, Sleeman observed, including warmer temperatures in northern Europe that have allowed the disease vector to survive at ever-higher latitudes; higher summer and winter temperatures that have increased vector capacity and competence, causing more severe outbreaks; and novel viral serotypes that have appeared in North America, probably with the arrival of exotic game or illegally imported viremic cattle, he added.

[17] Due to the segmented nature of the influenza virus genome (eight individual segments of RNA), influenza viruses can undergo genetic reassortment to produce new variant strains of virus. Pigs are hypothesized to serve as the "mixing vessels" in which two influenza viruses co-infect and undergo reassortment. Source: Influenza as a zoonotic disease; zoonotic swine influenza, http://www.vetmed.wisc.edu/pbs/zoonoses/influenza/swineflu.html (accessed February 19, 2015).

Diseases at the Human–Primate Interface

The best-known pandemic disease of zoonotic origin, HIV/AIDS, is widely believed to have emerged from the spillover of the simian immunodeficiency virus through trade in bushmeat, Sleeman noted (Hahn et al., 2000; Smith et al., 2012). The international trade in bushmeat is known to be extensive, he said, and although it is difficult to precisely estimate the size of this market, about 10 tons of bushmeat are illegally imported annually into countries as small as Switzerland (Falk et al., 2013). Partnering with several U.S. government agencies and nonprofit organizations, the USGS examined bushmeat confiscated at U.S. borders by the Fish and Wildlife Service and found several novel retroviruses and herpesviruses, Sleeman reported, demonstrating bushmeat's potential as a pipeline for pathogen spread (Smith et al., 2012).

Human diseases can also spill over into great apes, Sleeman observed. "There is a lot of concern about the potential impact of human diseases on [the] Great apes, particularly in Africa," he said, where many Great ape populations live near densely populated human settlements with high burdens of disease. Gorillas and chimpanzees are often habituated to tourists, further increasing their vulnerability to exposures to infection with human pathogens from ecotourists, Sleeman stated. Recent research suggests that chimp die-offs have resulted from such human pathogens as respiratory syncytial virus and metapneumovirus, he said (Kondgen et al., 2008). Sleeman further observed that in her book *The Chimpanzees of Gombe* primatologist Jane Goodall described an outbreak of flaccid paralysis in chimpanzees that occurred simultaneously with a local outbreak of polio in the human populations around this animal reserve (Goodall, 1986).

Bats as Reservoirs of Emerging Viruses

Bats, as noted previously, have been identified as actual or potential sources of several important emerging human viral diseases, yet, New World bat populations have been severely decimated by introduced diseases including white-nose syndrome, a fungal infection that has killed at least 6 to 7 million North American bats since 2007/2008 (Bat Conservation International, 2014), Sleeman observed. Perhaps, some have reasoned, there is something unique about bats' biodiversity or immune system that allows them to harbor these viruses. In the case of white-nose syndrome, bats are vulnerable to fungal infection during hibernation, when their immune system is quiescent, and become ill when their awakening immune system hyper-responds to the pathogen, he said—a pattern that may underlie infection by other pathogens (Meteyer et al., 2012).

As illustrated in Figure WO-4, the 2007–2008 outbreaks of Marburg virus in Uganda were associated with caves. Caves were used by the local population for mining and also tourist attractions. African fruit bats were implicated as the reservoir for this filovirus. Bats were captured and sampled and peak viral

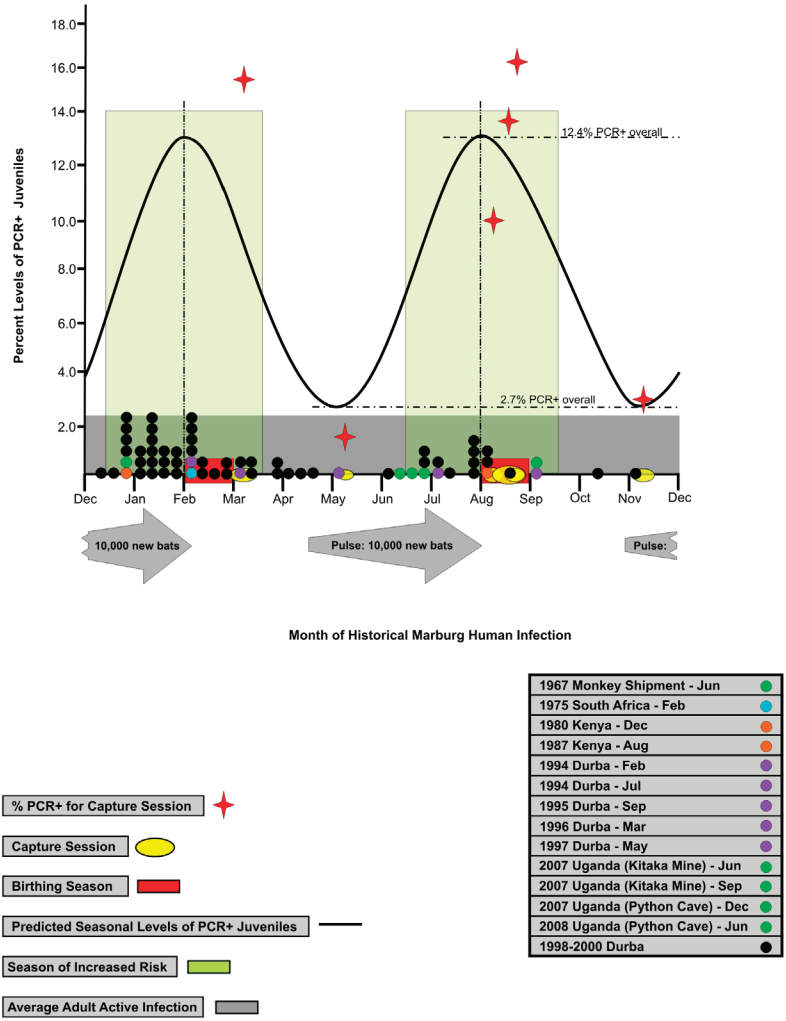

FIGURE WO-4 Increases in seasonal risk to human health. Historical spillover events (colored circles on *x* axis) compared to predicted seasonal levels of PCR+ juveniles (sinusoidal curve). The amplitude of the curve is based on average PCR+ juveniles experimentally determined during birthing (12.4%) and breeding (2.7%) seasons. Large light green vertical rectangles represent the proposed approximate 3-month seasons of increased risk based on the average level of juvenile infected bats at peak times of encompassing birthing (February and August) and breeding (May and November). Large gray arrows depict the twice yearly influx of newly autonomous juvenile bats born in the prior birthing season. The influx begins at the approximate time of the juvenile's independence from their mothers.
SOURCE: Amman et al., 2012.

transmission occurred twice yearly during breeding seasons. Eighty-three percent of spillover events to humans occurred during that time (Amman et al., 2012).

When local populations were informed of the results of this investigation they responded by killing off all of the cave bats—perhaps an understandable yet tragic reaction of the local populations to this threat in their midst (Amman et al., 2014). This study also points to opportunities for human–bat coexistence by allowing mining to occur while restricting human access to the caves during the periods of peak viral shedding. Although the risk may still be considered too high, this case study illustrates the types of investigations that need to be conducted in order for indigenous wildlife and humans to coexist.

Disease Management Strategies

Interventions to reduce spillover and transmission risks offer one route to managing infectious diseases in wildlife, according to Sleeman. This includes temporal and spatial separation of humans and wildlife during high-risk periods as well as farm biosecurity, which researchers have identified as key to preventing AI outbreaks (Olson et al., 2014). Additional management strategies include improving food security in communities currently reliant on bushmeat and providing basic sanitation for human populations in close proximity to Great apes.

According to Sleeman, the larger task of preventing, preparing for, responding to, and recovering from emerging infectious diseases of wildlife origin will require the following elements:

- Basic epidemiology to respond to wildlife disease events through field investigations, laboratory diagnostics, and molecular techniques for pathogen discovery, such as deep sequencing.
- Surveillance: Can we start detecting pathogens in wildlife populations before they spill over into humans or domestic animals?
- Risk analysis to determine exposure pathways and identify hot spots for disease emergence in order to enable optimal allocation of resources.
- Monitoring: Robust existing Earth and climate monitoring systems, as well as public health monitoring, could be expanded to collect sorely needed wildlife health data, he noted. “There’s no real system of sharing that data or integrating it with public health data or domestic animal data, nationally or internationally,” he added, nor is there standard terminology for describing wildlife diseases.
- Modeling to increase understanding of the drivers of emerging diseases of wildlife origin and development of predictive models for decision support, especially Bayesian models that allow the incorporation of uncertainty into model selection.
- Tools for managing wildlife diseases, including vaccines of similar effectiveness to the oral rabies vaccine. The USGS is currently pursuing a

vaccine to control sylvatic plague in prairie dogs—a significant problem in the western United States (Abbott et al., 2012).
- Infrastructure, which is especially weak for wildlife health.
- Collaborative, cooperative partnerships to address health and environmental issues of mutual concern. In the United States, authority to manage a particular wildlife species or disease is not always clear, Sleeman observed, and that confusion results in delayed responses to disease threats and outbreaks. In the case of white-nose syndrome—an introduced fungal disease decimating New World bat populations—4 years elapsed between disease detection and the development of a national response plan.

The One Health approach[18] provides a useful theoretical framework to address the interconnected health concerns of wild and domestic animals, humans, and the ecosystems they inhabit, Sleeman noted, but moving from this concept to implementation presents a major leadership challenge. In their recent study, *Making One Health a Reality—Crossing Bureaucratic Boundaries,* Sleeman and co-authors reviewed several case studies of projects involving multiple sectors in responses to such diseases as AI and anthrax in an attempt to identify factors that contributed to the success or failure of these projects (Rubin, 2014). They found that successful projects tended to include the following attributes:

- A sense of urgency and common purpose;
- A mandate or authority delegated to those who conduct the work;
- Oversight through an interagency steering committee or working group;
- A foundation of trust and a willingness to acknowledge all agencies' concerns and perspectives;
- Mutually agreed-upon, science-based goals;
- Clearly defined roles and responsibilities; and
- Leadership rotation among all involved sectors.

On a more philosophical level, Sleeman asked a series of rhetorical questions of those who would lead the implementation of One Health:

- What are the core values of One Health?
- Is wildlife a threat to human health, or is it something that we value?
- Is this a classic team-building challenge?
- Do we have the individual leadership skills to make this successful?
- Do we have the ability to think outside the boundaries of our own agencies and our own perspectives?

[18] One Health is the collaborative effort of multiple disciplines working locally, nationally, and globally to attain optimal health for people, animals, and our environment (AVMA, 2014).

- Do we have the ability to have influence when we have no real formal authority?
- Do we have the ability to compromise and reach consensus on issues?

The political, philosophical, and administrative challenges are every bit as daunting as the scientific hurdles that must be overcome in order to implement One Health, he concluded.

Eco-social Drivers of Viral Disease Emergence

Expanding on themes introduced by Fukuda and Sleeman, Dirk Pfeiffer of the Royal Veterinary College (UK) described six "global megatrends" that need to be considered when examining patterns of disease emergence (Anonymous, 2011):

- Rising living standards, as well as expectations for continued improvement in such amenities as food safety and quality will continue;
- Depletion of Earth's limited supplies of natural mineral, energy, and water resources, which, in turn, affects food supplies;
- Increasing biodiversity loss;
- Movement of the balance of economic power toward the East and South;
- Lengthening human life span; and
- Expanding connectivity via communications and trade.

He also emphasized that it is important to appreciate the medium-level importance of the risk of pandemic disease and antimicrobial resistance within the broader context of perceived threats to human welfare, as shown in Figure WO-5—a perspective that politicians will adopt when prioritizing the allocation of limited resources, he noted (Anonymous, 2014) (Dr. Pfeiffer's contributions may be found on pages 184–197, 197–209, 209–231, and 232–248 in Appendix A).

Briefly recapping points made by Fukuda and Sleeman, Pfeiffer noted that "eco-social changes"—phenomena such as urbanization, globalization, and land use changes—drive infectious disease emergence (see Figure WO-6). He paid particular attention to the intensification of agriculture in China, where a "food zone" in the northeast of 1,450 square kilometers[19] is devoted purely to raising livestock and agricultural products, and noted the biosecurity challenges emerging from such an intensive production scenario. "There will be situations like that occurring around the world, because that often is being seen as the only mechanism of securing sufficient food supply for urban communities," he

[19] The China Jilin (Singapore) Modern Agricultural Cooperation Food Zone [in Jilin China] is an ambitious project covering 1,450 square kilometers, or 560 square miles, twice the area of Singapore. Source: http://www.nytimes.com/2010/09/28/business/global/28iht-rbofsing.html?_r=0 (accessed November 11, 2014).

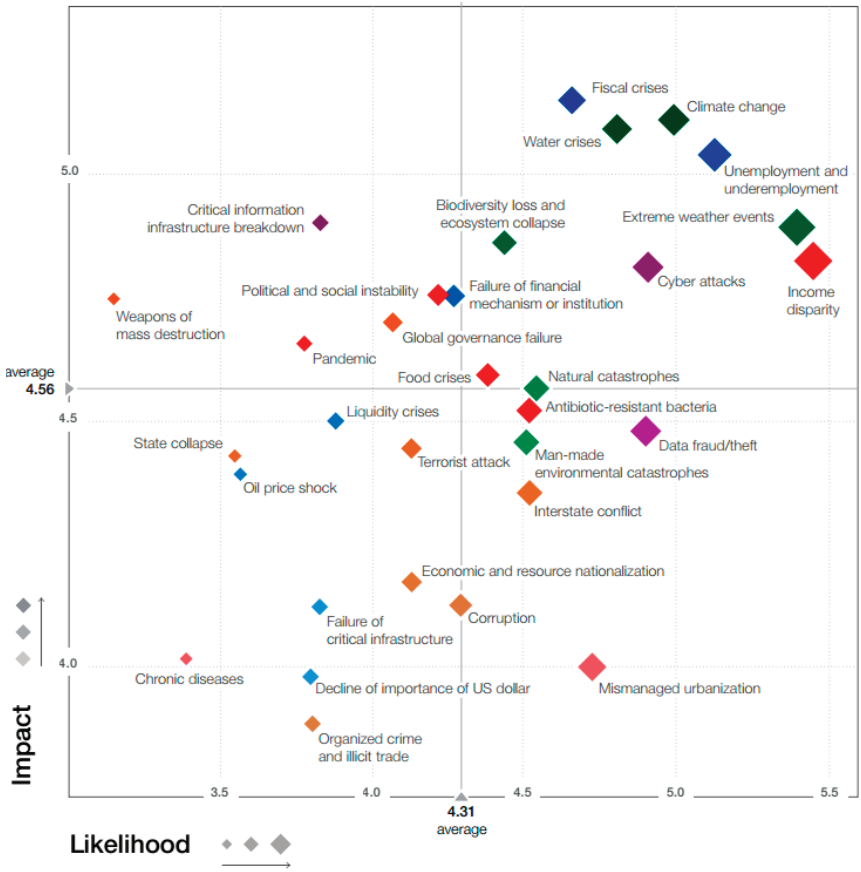

FIGURE WO-5 The global risks landscape, 2014. Blue diamonds represent economic risk (e.g., fiscal crises, oil price shock, decline of the U.S. dollar). Orange diamonds represent geopolitical risks (e.g., state collapse, corruption, interstate conflict, terrorist attack). Green diamonds represent environmental risks (e.g., extreme weather events, climate change, natural catastrophes). Red diamonds represent societal risks (e.g., food crises, chronic diseases, antibiotic-resistant bacteria). Purple diamonds represent technological risks (e.g., cyber attacks, data fraud/theft). Survey respondents were asked to assess the likelihood and impact of the individual risks on a scale of 1 to 7, 1 representing a risk that is not likely to happen or have impact, and 7 a risk very likely to occur and with massive and devastating impacts.
SOURCE: Global Risks 2014, World Economic Forum, Switzerland, 2014.

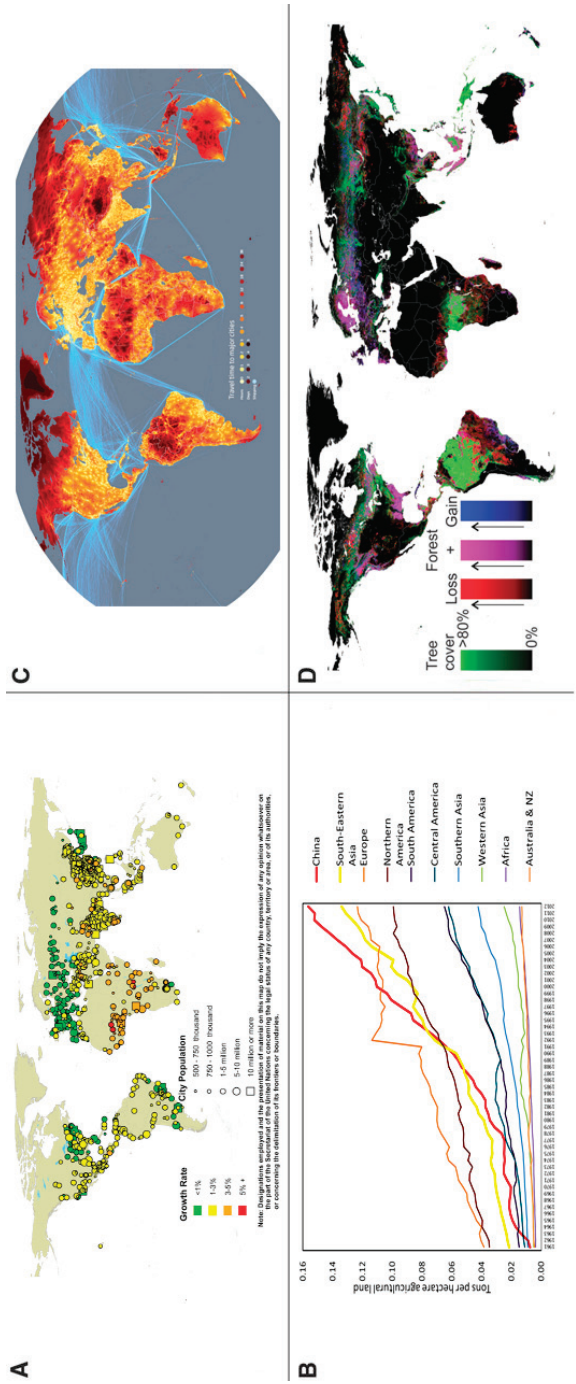

FIGURE WO-6 Urbanization, globalization, and land use changes. (A) Percentage of urban population and agglomerations by size class, 2025. (B) Global pattern of meat production density (1961–2012). (C) Travel time to major cities. (D) Global forest cover loss and gain between 2000 and 2012.
SOURCE: Pfeiffer presentation, 2014; (A) http://esa.un.org/unpd/wup/Maps/CityGrowth/CityGrowth.aspx (accessed February 19, 2015); (B) data from FAOSTAT database; (C) http://www.newscientist.com/data/images/ns/cms/mg20227041.500/mg20227041.500-1_1000.jpg (accessed February 19, 2015); (D) Hansen et al., 2013. Science 342:850–853.

warned. Noting the expanding numbers of countries and communities dependent on "global value chains" for food and other goods, he observed that nonlinear development of increased numbers of linkages within and between these populations had resulted in the theoretically predicted "connectivity avalanche" that will allow rapid dispersal of infectious diseases as already observed, for example, for food-borne illness (Appel et al., 2012; Ercsey-Ravasz et al., 2012).

Pfeiffer also emphasized the need for the global community to adopt the concept of "ecosystem services," which aims to recognize the vast benefits—including natural resources, food, climate regulation, and cultural riches—provided by the environment to human societies. Human use of natural resources threatens ecosystems worldwide, but such impacts are much greater in some places than in others, he noted (Haberl et al., 2007). As a consequence, some human populations are now exploiting distant ecosystem services to support their needs (Erb et al., 2009). The latter also contributes to increasing global connectivity.

Patterns of Emergence

The influence of eco-social change has played a role in many recent disease events, Pfeiffer emphasized. The rapid spread of foot-and-mouth disease throughout the United Kingdom in 2001, demonstrated the importance of livestock trade networks and within those particularly the role of markets as conduits for infectious disease spread. The recent expansion of viral diseases such as bluetongue and African swine fever[20] into new geographic areas, and the emergence of novel viral pathogens, like Schmallenberg virus[21] that was identified through an especially effective international collaboration, underscore the confluence of factors—including climate change, extreme weather events, transportation, and expanded international trade—that contribute to the expansion of these diseases into new hosts and environmental niches.

By contrast, Pfeiffer observed, most molecular clades of influenza A (H5N1) remain close to their apparent geographic origins in Southeast Asia and China—despite the availability of hosts such as migratory wild birds and exposure to global poultry and poultry-product trade networks (Pfeiffer et al., 2011). It

[20] African swine fever (ASF) is a highly contagious tick-borne hemorrhagic disease of pigs, warthogs, European wild boar, and American wild pigs. With high virulence forms of the virus, ASF is characterized by high fever, loss of appetite, hemorrhages in the skin and internal organs, and death in 2–10 days on average. Mortality rates may be as high as 100 percent. ASF is caused by a DNA virus of the Asfarviridae family. Source: http://www.oie.int/fileadmin/Home/eng/Media_Center/docs/pdf/Disease_cards/ASF-en.pdf (accessed February 19, 2015).

[21] In November 2011, scientists in Germany identified novel viral sequences in serum from cattle affected by a febrile syndrome that was reported during August–September 2011 in Germany and the Netherlands. Clinical signs included decreased milk production and diarrhea. The virus, named Schmallenberg virus (SBV), was isolated from blood of affected cattle, and similar clinical manifestations were observed in experimentally infected calves. In the Netherlands, SBV was detected retrospectively in serum from affected cattle in December 2011 (Reusken et al., 2012).

suggests that the characteristics of the eco-social systems in that part of the world provide more effective transmission opportunities than elsewhere. What factors underlie the apparent hot spot for AI evolution in Southeast Asia and China? "We are not absolutely certain about what the exact mechanisms are, despite all the research that has gone on," he admitted. It is most likely the interaction between several factors, such as relatively intensive duck and chicken production traded across complex live bird marketing networks (Pfeiffer et al., 2013).

The Risk Governance Framework for Disease Management

The ecohealth or One Health approach to managing emerging viral diseases requires a systems perspective that takes into account the various drivers of emergence and places them within the context of institutions and societal goals, Pfeiffer explained. Thus, he said, traditional, linear, technocratic models of policy development solely based on translating scientific findings into policy as a "one way" street are being replaced by more interactive, cyclical models such as the "risk governance framework" depicted in Figure WO-7 (Pfeiffer, 2014). By identifying stakeholders and defining what is important to them, assessing risk with clear questions, using qualitative as well as quantitative analytical approaches, and evaluating the findings in the context of stakeholder priorities, policy makers using this framework can identify more effectively "what is to be done and how," he said.

At the same time, Pfeiffer observed, researchers need to "embrace systems thinking and integrated research approaches . . . [and to] link biological and environmental [sciences] with [the] social sciences" in order to address the complex and dynamic impacts of global eco-social changes.

Studying Zoonoses in Their Natural Hosts

Bats, as illustrated by the flying fox in Figure WO-8, have been identified as the reservoirs of several recent emerging viral diseases (Calisher et al., 2006). As such, according to speaker John Lowenthal of the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Biosecurity Flagship, bats deserve focused study (Dr. Lowenthal's contribution may be found on pages 166–180 in Appendix A). Bats are the most abundant mammal on Earth. The members of the more than 1,200 bat species comprise about one-quarter of all mammals, he noted, yet many important questions about these mammals remain to be answered.

For example, Lowenthal observed, very little is known about bats' response to disease. They are apparently asymptomatic carriers of viruses that threaten other animals and humans, including SARS, Hendra, rabies and other lyssaviruses, and Ebola, among more than 60 different viruses (and counting) identified to date, he said. Lowenthal remarked that it is only a matter of time until the next

FIGURE WO-7 Risk-governance framework.
SOURCE: Adapted from Renn, 2005.

novel virus emerges from bats—perhaps a new influenza A virus, as was recently detected in a bat species native to Central and South America (Tong et al., 2012).

Alternatives to Mouse Models

The containment of emerging infectious outbreaks is very challenging due to their unpredictable nature and the absence of effective medical countermeasures, such as vaccines and antivirals. The dearth of medical countermeasures is largely due to a lack of essential knowledge of the immune responses induced by zoonotic viruses, particularly the responses that are attributable to protection, Lowenthal noted. While mice have provided a useful and convenient model for understanding fundamental immune responses to infection—due to their ease of handling and rapid generation time—they may not always adequately model the behaviors of emerging infectious diseases in human hosts, he observed.

Mice "usually don't accurately replicate the disease that is seen in the human," Lowenthal explained. "They may allow viral replication, but a lot of the pathology and symptoms and responses are not directly relevant or related, and that creates a lot of problems. There are often differences in the symptoms of

FIGURE WO-8 Bats and emerging viruses. Bats are the most populous mammal—with more than 1,200 species representing approximately 25 percent of all classified mammal species—and are found in all regions of the world except for the North and South poles and some remote islands. Although they carry a number of zoonotic viruses without symptoms (e.g., SARS, Hendra, Nipah, and Ebola), little is known about their response to disease. SOURCE: ©2014 EcoHealth Alliance/J. Epstein.

disease when you compare the disease in the natural host versus the transmission host versus what you see in the human," he continued. For example, he observed, many zoonotic diseases "are asymptomatic and nonlethal in the natural host, but when they spill over into a transmission host or to a human, you can see potentially lethal effects and sometimes up to 100 percent mortality."

Thus, nontraditional animal models—such as bats, chickens, and ferrets—are increasingly being used to study disease pathogenesis, host–pathogen relationships, and the nature of the immune responses to particular diseases, Lowenthal said. Studying immunology in nontraditional animal species might provide insights into improving the control of emerging diseases and suggest preparations for future pandemics or potential medical countermeasures against bioweapons, he added.

Ultimately, Lowenthal and fellow researchers question the use of animals other than the natural host in such studies, if that species or a close likeness is

available. For example, he noted, "Chickens have been used for a long time to study immune system development. They are a very good model for avian influenza. Bats are a host of very many different diseases. Ferrets are becoming the preferred model, and have been for a while now, for human influenza . . . [and] the woodchuck [serves] as a model for hepatitis B in humans."

Chicken models of avian influenza Water birds, particularly ducks, are known to be the natural hosts of H5N1 influenza, which they generally carry without exhibiting symptoms, Lowenthal reported. "Infections in chickens and humans, on the other hand, can lead to very severe inflammation and very high levels of cytokine production (a phenomenon called the 'cytokine storm')", he continued. "The big question is, is this aberrant immune response responsible for the high levels of mortality?"

To examine this question, one must first understand immunological differences among the species it infects. As illustrated in Figure WO-9, a duck infected with a highly pathogenic influenza strain is asymptomatic, with viral replication typically limited to the heart or to muscle tissue, coupled with a relatively low level of cytokine production, he said. The immune system has been shown to recognize the virus, but its response to it is mild. By contrast, he continued, the same virus causes a more severe reaction, involving the lung and possibly the brain, in intermediate hosts such as the pig, ferret, or mouse. Cytokine levels are elevated in these cases, but "nothing too serious," he noted. In more susceptible hosts such as humans and chickens, however, infection is severe, spreads rapidly, and produces a cytokine storm (Bean et al., 2013).

The mechanisms underlying these differences could reveal novel approaches to mitigating influenza's effects, Lowenthal observed. "It would be very useful to compare what is happening in terms of immunological responses in the natural host, which is resistant to the transmission host, and try to identify what the key differences and what the underlying differences are," he explained. "If we can identify [the] factors that trigger an inappropriate inflammatory response that might inform us of therapeutic intervention strategies for human disease." It has already been demonstrated that humans can be protected from infectious disease by treating transmission hosts to prevent them from spreading pathogens to humans, he reported, as was accomplished with an equine vaccine against Hendra virus that protected horses, and thus humans, from infection. (See Lowenthal original manuscript on pages 166–180.) This strategy could potentially be used to prevent human infection by other zoonotic viruses, he concluded.

Nontraditional Host Studies at the Australian Animal Health Laboratory

"There are lots of restraints and certain requirements in working with nontraditional animal species, particularly for highly pathogenic agents," Lowenthal observed. Special high biocontainment facilities and trained staff are necessary, and a variety of animal husbandry and welfare issues relevant to the capture,

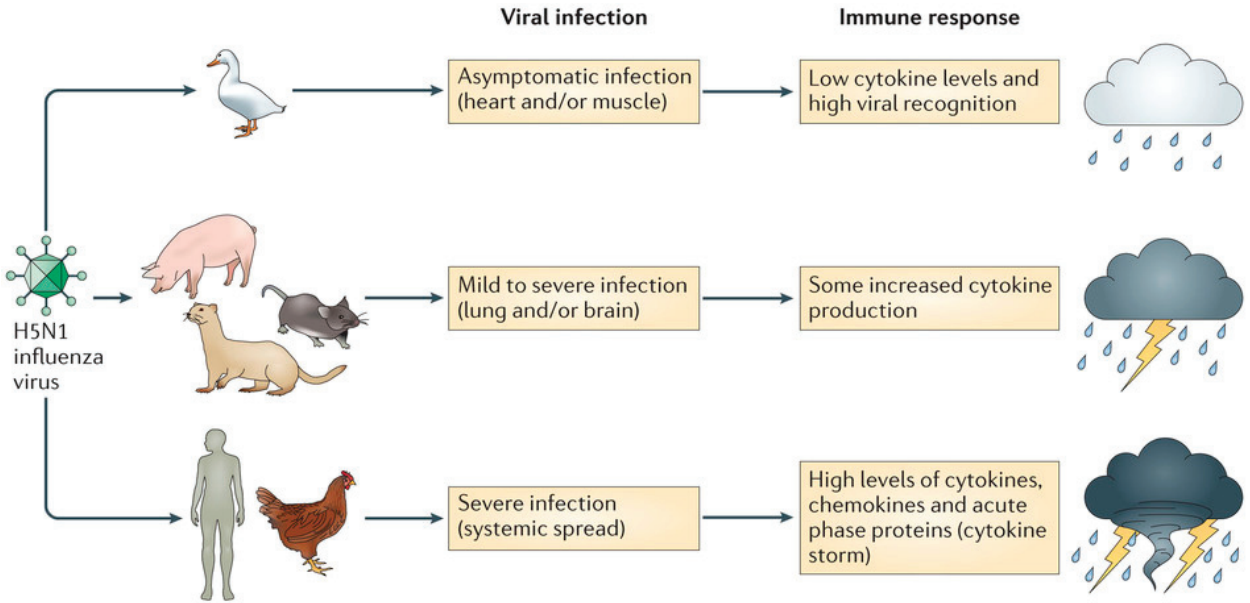

FIGURE WO-9 The host immune response to an infection influences the disease outcome. Infection with H5N1 influenza virus can cause very different disease outcomes in different reservoir and spillover host species. Waterfowl, such as wild ducks, are the natural host for this virus and develop a limited inflammatory response that is associated with low levels of cytokine expression. Intermediate hosts, including mice, pigs, and ferrets, are often used to study this infection and display mild to severe disease symptoms (depending on the H5N1 virus strain used) that are associated with increased levels of proinflammatory cytokines. By contrast, spillover hosts such as chickens and humans display a rapid and strong inflammatory response, often referred to as hypercytokinemia (or cytokine storm) and the infection becomes systemic, causing severe disease symptoms and high mortality rates.
SOURCE: Bean et al., 2013.

acclimatization, and breeding of wild animals must be addressed, he explained. In addition, immunological reagents for many nontraditional species are either scarce or nonexistent. Many of these hurdles have been overcome at the CSIRO Australian Animal Health Laboratory (AAHL), one of the few laboratories in the world that offers a wide range of different nontraditional animal species, including those of large animals, in expansive, sophisticated containment facilities, he said. "We have the whole farmyard covered," he quipped.

In the AAHL's Biosecure Immunology Laboratory, researchers can undertake comparative immunology across a spectrum of nontraditional species under biosafety level (BSL) 3 and 4 conditions,[22] using state-of-the-art cell sorting and flow cytometry, Lowenthal stated. "We're developing an immunological toolbox and cell lines for a number of these different species," he continued; this will enable the use of high-throughput gene silencing to knock out individual immune genes, so as to measure their effects on the immune response to specific viruses in both natural and susceptible hosts. "We would love to be able to get a knockout bat or a knockout ferret," he said. "I don't think we're too far away from doing that."

Studying the immune response to a zoonotic pathogen in its natural reservoir species and comparing that with the response in spillover or transmission hosts will identify key processes and factors in disease susceptibility and transmission, Lowenthal stated. "Together with new technologies, such as gene knockout technology, we can identify new strategies to prevent and minimize the impact of emerging infectious diseases and enhance our pandemic preparedness," he concluded.

[22] BSL 3 is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents, and must be supervised by scientists competent in handling infectious agents and associated procedures. All procedures involving the manipulation of infectious materials must be conducted within biological safety cabinets or other physical containment devices. A BSL-3 laboratory has special engineering and design features.

BSL 4 is required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that is frequently fatal, for which there are no vaccines or treatments, or a related agent with unknown risk of transmission. Agents with a close or identical antigenic relationship to agents requiring BSL-4 containment must be handled at this level until sufficient data are obtained either to confirm continued work at this level, or redesignate the level. Laboratory staff must have specific and thorough training in handling extremely hazardous infectious agents. Laboratory staff must understand the primary and secondary containment functions of standard and special practices, containment equipment, and laboratory design characteristics. All laboratory staff and supervisors must be competent in handling agents and procedures requiring BSL-4 containment. The laboratory supervisor in accordance with institutional policies controls access to the laboratory. For further details of the BSL-3 and BSL-4 requirements please see the Biosafety in Microbiological and Biomedical Laboratories 5th Edition, http://www.cdc.gov/biosafety/publications/bmbl5 (accessed February 19, 2015). Source: HHS et al., 2009.

The NIAID Response to Emerging Viral Diseases

The National Institute of Allergy and Infectious Diseases (NIAID) funds a significant proportion of the nation's efforts to address emerging and reemerging viral diseases, according to speaker Anthony Fauci, who directs the institute, and who described those efforts in his workshop presentation (Dr. Fauci's contributions may be found on pages 133–136, 136–143, and 144–152 in Appendix A). NIAID undertakes a dual mandate unmatched by other National Institutes of Health (NIH) agencies, he noted: It must maintain and grow a portfolio of basic and applied research on EIDs, and rapidly respond to new and emerging threats.

Over the course of human history, extraordinary progress has been made in the control of infectious diseases, Fauci observed, noting the following key advances:

- The recognition that infections are caused by microbes;
- Improvements in sanitation, hygiene, and vector control;
- The discovery and implementation of antimicrobials;
- The development of vaccines and vaccination programs; and
- The development of diagnostics, enabling disease detection and monitoring.

With these breakthroughs, however, came a sense of complacency, illustrated by the following quote from the 1963 book *The Evolution and Eradication of Infectious Diseases*, by Aidan Cockburn:

> We can look forward with confidence to a considerable degree of freedom from infectious diseases at a time not too far in the future. Indeed . . . it seems reasonable to anticipate that within some measurable time . . . all the major infections will have disappeared. (Cockburn, 1963)

This quote,[23] Fauci said, also reflects "the extraordinary provincialism that we have in the developed world to have the temerity to say that we're going to be free of infectious diseases when you have malaria, tuberculosis, and diseases that cause millions of deaths throughout the world."

HIV: From Emerging Disease to Established Infection

NIAID had been studying emergent diseases for many years before HIV. Its efforts accelerated in 1981 with "the mother of all emerging and reemerging infectious diseases—HIV/AIDS," Fauci recalled. "As devastating as this pandemic

[23] About this quote, Fauci also noted that for many years, he and others had instead quoted U.S. Surgeon General William Stewart, who is claimed to have stated in 1967 or 1969, depending on the source, "It is time to close the book on infectious diseases, and declare the war against pestilence won" (Spellberg, 2008, p. 1). However, despite concerted efforts of Fauci's scientific staff and many other individuals, including historians of public health, no primary source for this quote has been identified.

has been, it is really an extraordinary model for what happens when the public health and research communities mobilize with extraordinary resources to address an emerging public health threat."

In the ensuing years, some 70 million people have been infected with HIV, resulting in 36 million deaths; more than 35 million people are currently living with the disease, Fauci reported. At the same time, collaboration between fundamental basic researchers, industry, clinical researchers in clinical trials, grantees, and contractors have produced more than 30 antiretroviral drugs that have transformed the lives of HIV-infected individuals, rendering an acute infectious disease that once killed an infected individual in less than a year into a chronic, manageable, illness. "Right now, if you go to any reasonable clinic in the developed world, and even in the developing world, and someone comes in who is 20-plus years old, recently infected with HIV, and you put them on triple combination,[24] you could predict with accuracy and look them in the eye and honestly say, 'If you take your medicine, you [are likely to] . . . live an additional 50 years,'" he said (Samji et al., 2013).

Implementing these interventions represents "another extraordinary accomplishment," Fauci continued. Supported by the President's Emergency Plan for AIDS Relief,[25] the Global Fund,[26] various philanthropies, and the governments of affected countries, deaths due to AIDS have declined by 30 percent since 2005, he said. Much of this improvement is due to a suite of nonvaccine preventive measures, including condom use, needle exchange, medical interventions to prevent mother-to-child transmission, circumcision, and prevention as treatment, he noted. Even so, he added, "It is far too soon for a victory lap. We have much to do in implementation, and also in discovery, such as with a vaccine or a cure."

From SARS to MERS

The short-lived SARS pandemic, which consisted of approximately 8,000 cases resulting in 800 deaths before it was extinguished by "simple 19th-century public health measures," introduced a new era of vaccine development, according to Fauci. Rapid sequencing platforms sped the characterization of SARS-CoV, showing that a safe and effective vaccine (which in the end was not needed) could be developed against a novel pathogen within a year of its discovery, he said.

"No sooner did we deal with one coronavirus . . . than we had another," Fauci said, introducing the multipronged NIAID response to MERS, launched at a meeting in June 2013 (NIAID, 2013). The institute's research response to the MERS-CoV is a rapid response capability similar to that applied to SARS, he explained, and focused on basic research; on developing animal models, vaccines,

[24] Any antiretroviral regimen composed of three agents from at least two drug classes used to manage HIV infection, also known as highly active antiretroviral therapy (Henkel, 1998).

[25] See http://www.pepfar.gov.

[26] See http://www.theglobalfund.org/en.

and therapeutics; and on identifying animal reservoirs. For example, he noted, a NIAID-supported animal model has been used to demonstrate that interferon-α and ribavirin are effective against MERS-CoV (Zhao et al., 2014)—a good start, he said, but "we likely need to do better than that."

Similarly, NIAID supported the recent isolation of MERS-CoV from a large number of camels identical to viruses isolated from MERS patients—very much identical to the coronavirus of MERS, Fauci noted. However, it is still unclear whether camels have infected humans or vice versa, he added—a question NIAID researchers continue to investigate.

Reemerging Viruses

Among the many reemerging or resurging viral diseases, Fauci identified dengue[27]—a leading cause of illness and death in the tropics and subtropics—as being particularly important. Cases have increased sharply over the past 60 years, and the disease reemerged in the Caribbean islands and southern Florida;[28] locally acquired cases had been absent in Florida since 1934. NIAID's research approach to dengue encompasses fundamental basic research, vector biology, sharing research resources with the scientific community, and pursuing vaccines, therapeutics, and diagnostics, he said.

Another reemerging vector-borne virus, chikungunya, is also transmitted by more than one species of *Aedes* mosquitoes. Outbreaks of this debilitating, usually nonfatal disease have significant public health impact, and there are as yet no licensed vaccines or specific treatments for it, Fauci observed. Since crossing the Atlantic Ocean in December 2013, chikungunya has spread to more than 10 countries in the Americas and produced more than 780,000 suspected and 15,000

[27] Dengue is caused by any one of four related viruses transmitted by mosquitoes, which infect as many as 400 million people each year. There are not yet any vaccines to prevent infection with dengue virus; the most effective protective measures are those that avoid mosquito bites. Early recognition of infection and prompt supportive treatment can substantially lower the risk of medical complications and death. Dengue emerged as a worldwide problem beginning in the 1950s. To date it has rarely occurred in the continental United States, but it is endemic in Puerto Rico and in Latin America, Southeast Asia, and the Pacific islands. Source: http://www.cdc.gov/dengue (accessed September 4, 2014).

[28] Between 1946 and 1980, there were no reported cases of dengue acquired in the continental United States, and, according to the CDC, there hasn't been an outbreak in Florida since 1934. However, in 2009, the first locally acquired case of dengue in the continental United States (other than those associated with outbreaks on the Texas–Mexico border) was detected in Key West, Florida. This outbreak was followed by several additional local cases (CDC, 2010). By the end of 2009, 27 cases of dengue infection had been confirmed in Key West residents. As of the end of June 2010, an additional 12 cases of locally acquired dengue had been reported in Key West and surrounding areas (Preidt, 2010). According to Dr. Harold Margolis, chief of the dengue branch at the U.S. Centers for Disease Control and Prevention, "[t]hese cases (in Key West) represent the re-emergence of dengue fever in Florida and elsewhere in the United States after 75 years. These people had not traveled outside of Florida, so we need to determine if these cases are an isolated occurrence or if dengue has once again become endemic in the continental United States" (Preidt, 2010).

laboratory-confirmed cases (CDC, 2014; Morens and Fauci, 2014). NIAID has pursued vaccine development to address this threat, he reported, and in November 2013, it completed a phase I trial of a candidate vaccine that appears to be both safe and immunogenic (Chang et al., 2014).

Prospects for a Universal Influenza Vaccine

Influenza is both a reemerging, due to its seasonality, and an emerging infectious disease, because novel influenza viruses with pandemic potential have arisen several times in the past century, Fauci observed. Human cases of AI H5N1 were first detected in 1997. Further spread was halted at the time through the mass slaughter of poultry in Hong Kong. Since 2003, the disease has smoldered in China when NIAID began tracking cases. Similarly, transmission of AI H7N9 continues within the live markets of China following detection of the first human cases in March 2013.

A series of challenges complicates efforts to address the threat of influenza, Fauci noted:

- Neither infection nor vaccination results in lifelong immunity.
- Seasonal strains inevitably exhibit genetic drift, necessitating a "timetable" approach to vaccine development.
- Seasonal strains for vaccine production must be predicted well in advance, providing a "best guess" rather than a precise match,
- The annual cost of preparing seasonal influenza vaccines is $2–$4 billion.
- Vaccines cannot be stockpiled for years ahead of time.
- Pandemic emergence is an ongoing threat.

Given these concerns, he asked, "Should we put on a full-court press to develop a universal influenza vaccine?"

Such a possibility has only recently become viable, thanks to advances in deep sequencing, structural biology, and crystallography, Fauci said. All of these technologies are being brought to bear in order to design antibodies that target the shared stem region of otherwise diverse influenza hemagglutinin molecules, as illustrated in Figure WO-10. "If you get an antibody to the stem, the antibody can find the stem, but the stem is not exposed enough to induce a very good antibody," he explained. Researchers have now discovered multiple ways to "show" the stem to the immune system, thereby inducing potent antibodies against it—an advance Fauci called "the first good step towards developing a universal influenza vaccine."

In this effort, as in all of its work on infectious diseases, NIAID focuses on three main goals, Fauci said:

- Supporting fundamental basic research;
- Producing resources to advance research; and
- Translating basic findings into clinical research with the ultimate goal of developing diagnostics, therapeutics, and vaccines.

In the case of emerging and reemerging infectious diseases, the microbes by their nature ensure that this will be "a perpetual and never-ending challenge," he concluded.

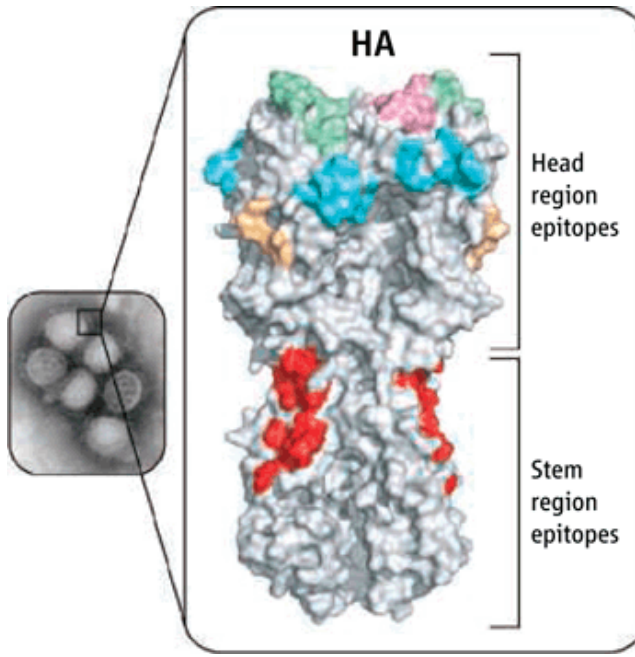

FIGURE WO-10 Antibody recognition. Most antibodies against influenza A virus (inset shows the 2009 H1N1 strain) bind to the highly variable part of the hemagglutinin (HA) glycoprotein at the surface of the virus particle (head region). In the H1 subtype, these antibodies recognize four major sites (Sa, Sb, Ca, and Cb are shown in green, pink, cyan, and yellow, respectively). The HA structure of the 2009 H1N1 virus is shown (PDB code 3LZG). Antibodies that neutralize multiple strains both within a virus subtype and from different subtypes bind to a highly conserved region (red) in the stem region of HA.
SOURCE: CDC (Inset); D. Ekiert, I. Wilson in Doms, 2010.

EMERGENCE OF MERS-COV

At the time of the workshop, in mid-March 2014 (and also at the time of this publication, nearly 1 year later) many more questions had been posed than answered about MERS-CoV in the 2 years since its initial identification as the cause of a novel viral respiratory disease syndrome.[29] Globally, 1,026 laboratory-confirmed cases of infection with MERS-CoV including at least 376 related deaths have officially been reported to WHO as of February 23, 2015. About two-thirds of these cases are male, and the median age is 49 years old (9 months to 94 years old) (WHO, 2014f).

A β-coronavirus, MERS-CoV is a member of the large viral family that includes the SARS coronavirus as well as viruses that cause the common cold (WHO, 2014b). MERS-CoV appears to be circulating widely throughout the Arabian Peninsula, where all primary cases to date apparently became infected. While some secondary cases of MERS—including several large hospital outbreaks[30]—have arisen, the virus does not appear to be readily transmissible (WHO, 2014b). Intensive screening of MERS-CoV contacts revealed very few instances of household transmission (WHO, 2014d). Secondary cases tend to present with a milder disease than primary cases, and many of the recently reported secondary cases have been mild, or were people whose tests were positive for MERS-CoV but were asymptomatic.

According to WHO, a typical MERS patient presents with fever, cough, and shortness of breath, and is often found to have pneumonia. Some patients also experience gastrointestinal symptoms, including diarrhea. Severe illness can cause respiratory failure that requires mechanical ventilation and support in an intensive care unit. Some patients have had organ failure, especially of the kidneys, or septic shock. Nearly one-third of patients with laboratory-confirmed MERS-CoV have died. The virus appears to cause more severe disease in people with weakened immune systems, older people, and those with chronic comorbidities including diabetes, cancer, and chronic lung disease (WHO, 2014b).

How people become infected with MERS-CoV has yet to be determined. As is discussed below, strains of MERS-CoV that match human strains and antibodies to MERS-CoV have been isolated from camels in Africa and the Middle East, and strong similarity has found between viruses isolated from humans, camels, and bats (Haagmans et al., 2013; Memish et al., 2013; WHO, 2014b). Although camels are suspected to be the primary source of infection for humans, the routes of direct or indirect transmission remain unknown and investigations are ongoing.

[29] A syndrome, in medicine and psychology, is the collection of signs and symptoms that are observed in, and characteristic of, a single condition.

[30] In May 2014, WHO reported that the number of laboratory-confirmed MERS-CoV infections had risen sharply since mid-March, largely due to health care–associated outbreaks that occurred in Saudi Arabia and in the United Arab Emirates (WHO, 2014d).

"Further epidemiological investigations are urgently needed to confirm or refute these hypotheses," according to WHO (2014d).

The five workshop presentations, summarized below, add considerable and intriguing detail to the current state of knowledge about MERS-CoV. They also reveal a multitude of pressing questions to be pursued, such as this list compiled by speaker Linda Saif, of Ohio State University:

- How was MERS-CoV transmitted to humans?
- What circumstances promote interspecies transmission of the virus?
- Are bats the only persistent animal reservoir for MERS-CoV?
- What is the role of camels: intermediate host or reservoir community?
- Are there subclinical MERS-CoV infections in humans?
- Why is there enhanced disease severity in people with comorbidities and the elderly?
- What is the role of cofactors and treatments (e.g., co-infections, antibiotics, corticosteroids) in enhancing the severity of MERS in people with comorbidities?
- Are there superspreading events as per SARS?
- What is the role of nonrespiratory viral shedding routes (feces, urine) in the transmission and pathogenesis of MERS-CoV (in both humans and camels)?

Human Coronavirus Emergence and Cross-Species Adaptation

Ralph Baric, of the University of North Carolina, opened the workshop session with a general description of coronavirus structure, as illustrated in Figure WO-11. Focusing on the spike (S) glycoprotein, which is embedded in the lipid bilayer surrounding the nucleocapsid, he noted that it mediates viral binding to host receptors and encodes critical determinants for cross-species transmission. The S glycoproteins of SARS-CoV and of another human coronavirus, known as HCoV-NL63, bind angiotensin I converting enzyme 2 (ACE2) on the surface of host cells for docking and entry; their more distant relative MERS-CoV recognizes instead dipeptidyl peptidase 4 (DPP4). "The spike glycoprotein is the major target for vaccine development because it encodes neutralizing epitopes, as well as T-cell epitopes, and it encodes the major component of protective immunity," he explained.

MERS-CoV is the sixth animal coronavirus to have emerged in humans, according to Baric. Two other human coronaviruses, the closely related[31] HCoV-OC43 and HCoV-HKU1 strains, likely originated in mice and cattle, he said. Baric went on to observe that SARS-CoV, along with two additional closely related strains, HCoV-NL63 and HCoV-229E, are believed to be of bat origin, and

[31] Based on spike protein sequence.

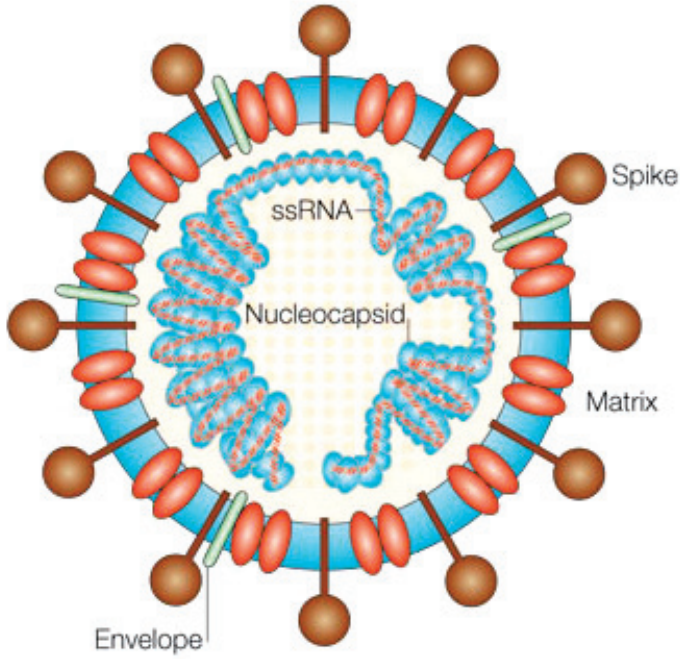

FIGURE WO-11 SARS-CoV virion—ssRNA, single-stranded RNA.
SOURCE: Dandekar and Perlman, 2005.

the closest known relatives to MERS-CoV are two viruses that have been isolated from bats. The emergence of MERS-CoV continues an accelerating pattern of cross-species transmission and emergence among coronaviruses: while the first human coronavirus appears to have emerged from bats more than 500 years ago, the vast majority of human strains either emerged suddenly (SARS-CoV, MERS-CoV) or were identified within the past 12 years (HCoV-NL63, HCoV HKU1), according to Baric (Graham et al., 2013; Huynh et al., 2012). In parallel, a number of new animal Nidovirales emerged over the past 30+ years, including porcine epidemic diarrhea virus, porcine respiratory coronavirus, porcine reproductive and respiratory disease virus, and bovine respiratory coronavirus (Graham et al., 2013), providing additional support for the hypothesis that cross-species transmission events are frequent among the Coronaviridae.

Mechanisms of Interspecies Transmission

Two distinct mechanisms enable the interspecies transmission (also known as "spillover") of coronaviruses, Baric explained. The first is the ability of some

coronavirus S glycoproteins to bind analogous receptor proteins (receptor orthologs) in species other than their primary host (Bolles et al., 2011). These viruses, which include SARS-CoV and MERS-CoV, are capable of replicating in multiple hosts, typically clusters of species. Other coronaviruses with an even broader host range possess mutations in the S glycoprotein gene that render them (a) capable of recognizing receptor orthologs, (b) "preprogrammed" to fuse a variety of host cell proteins (Graham and Baric, 2010), or (c) easily mutatable, in the course of cell culture, to recognize heparin sulfate as a receptor for docking and entry to host cells, which vastly extends their host range (de Haan et al., 2005). These latter two mechanisms of host range expansion have only been identified using in vitro models, although the potential exists for similar mutants to emerge naturally in nature. Finally, several coronaviruses can use sugar moieties as receptors or co-receptors for entry, providing an alternative strategy for rapid trans-species movement (Li, 2013).

Many believe that the 2003 SARS epidemic resulted from the emergence of a bat-like coronavirus that also recognized the ACE2 receptor from civets, Baric recalled. Then, through a second rare mutational step, the civet-adapted virus acquired the ability to use human ACE2 for docking and entry. The resulting human-adapted strain was then thought to have circulated back and forth between civets and humans to mediate the expanding outbreak. The civet as an amplifying host in the open markets was clearly associated with the expanding epidemic. However, he continued, another, perhaps more likely, explanation for the initial emergence event was that bat SARS-like coronaviruses are naturally capable of recognizing ACE2 receptors from multiple species, including humans, civets, and a subset of other species (Graham et al., 2013). Once these generalists infect across species, additional mutations arose that permitted efficient cross-transmission between humans and civets as well as more efficient human-to-human transmission.

This scenario raises the prospect that additional coronaviruses will follow this path to emergence in humans—or in the reverse direction. "Can bat SARS-like coronaviruses use human ACE2 for docking and entry into human cells?" Baric wondered. Conversely, he asked, can human coronaviruses, such as epidemic SARS-CoV and HCoV-NL63, recognize bat ACE2 molecules? Interestingly, HCoV-NL63 is capable of replicating efficiently in select bat cell lines from North America raising the possibility of cross-species movement between human strains and bat species (Huynh et al., 2012). Researchers recently identified a cluster of SARS-like viruses in Chinese horseshoe bats, including two that were more than 99 percent homologous across the majority of their genome sequences with SARS-CoV, but only 90 to 95 percent homologous within their S glycoprotein sequence (Ge et al., 2013). "Our results provide the strongest evidence to date that Chinese horseshoe bats are natural reservoirs of SARS-CoV, and that intermediate hosts may not be necessary for direct human infection by some bat SL-CoVs [SARS-like coronaviruses]," the authors wrote.

Ge and coworkers (2013) were able to isolate one of these SL-CoVs and show that it could bind to human, civet, and bat ACE2 molecules. Baric and coworkers then replaced S protein in a molecular clone of SARS-CoV with S glycoproteins synthesized from two bat SL-CoV sequences and found that the resulting hybrid viruses could also replicate efficiently in cultured primary human airway epithelial lung cells to high titer (unpublished observation). "In essence, these two bat coronavirus spike glycoproteins—that, as far as we can tell, have never circulated through human populations—allow for extremely efficient replication in one of the primary targets for SARS coronavirus replication in humans," he observed. "Both of these [viruses] are, in essence, poised to emerge in human populations." There are 1,200 to 1,300 different bat species, each of which encodes its own versions of the major coronavirus receptor-proteins, including aminopeptidase N, ACE2, and DPP4—and each host species can support an estimated 7 to 10 different coronaviruses, he continued. All of this adds up, he concluded, to "a heck of a lot of epidemic potential."

Routes to Emergence

Investigators in Baric's lab sought to characterize bat coronaviruses in North America by surveying about 500 different bat species. One of the viruses, when isolated and sequenced, proved to be the closest known relative of HCoV-NL63 (Huynh et al., 2012). They also determined that both HCoV-NL63 and, less efficiently, SARS-CoV and a civet coronavirus, HCSC6103, were capable of infecting and replicating in these North American primary bat lung cells, and therefore probably recognize ACE2-like molecules from a range of mammalian hosts. "These observations support the hypothesis that human coronaviruses are capable of establishing zoonotic–reverse zoonotic transmission cycles that may allow some coronaviruses to readily circulate and exchange genetic material between strains found in bats and other mammals, including humans," the authors concluded (Huynh et al., 2012).

In what Baric termed the "classic model" of viral emergence, zoonotic RNA viruses—which have high error frequencies—produce a "quasispecies" or a random swarm of heterologous mutants and some encode mutations that enable host range expansion when another appropriate warm-blooded host (such as a human) comes into contact with their primary host species. If more mutations arise within the new host that increase transmission efficiency within that species, a disease outbreak may be more likely. However, the possibility that some zoonotic coronaviruses are "programmed" to infect other species, as previously described and as suggested by the work of Huynh et al. (2012) and Ge et al. (2013), streamlines the route to viral emergence. "You don't need a random mutation," Baric observed. "They can immediately jump into different species, like humans, and move back and forth—after which, of course, [an additional few mutation(s)] might be required . . . to [establish] severe disease and transmission and pandemic potential."

In addition to error-prone transcription typical of RNA viruses, coronaviruses encode two unique genetic factors that further promote variation, Baric noted. The first is a replication strategy that produces high rates of RNA recombination during mixed infections. "If you infect the cell with two different but closely related coronaviruses, up to one-third of the progeny that come out of those cells may be recombinants containing genome material from both parents," he stated. The second variation-enhancing feature is an enzymatic proofreading activity called ExoN, which functions as a novel RNA fidelity proof-reading complex that is unique to coronaviruses (Denison et al., 2011; Graham et al., 2012). Baric speculated that this proof-reading function was probably under tight regulatory control, allowing for fluctuations in fidelity. Thus, the newly emerged virus could adapt quickly by relaxing fidelity control and then tightening fidelity control to stabilize these adaptive mutations in the new host species.

Host age has also been identified as a factor in regulating the cross-species transmission of coronaviruses, Baric added. In mice infected with SARS-CoV, Baric noted that the virus will replicate efficiently but not cause disease. A mouse-adapted virus—with mutations in the S and membrane (M) glycoproteins and the viral replicase resembling those that arose in humans during the 2003 SARS epidemic—may be created by passaging[32] the virus multiple times through mice. Baric explained that this adaptation process requires 15–25 passages at 2-day intervals in young mice, which then develop mild alveolitis[33] and bronchiolitis.[34] In 1-year-old mice, only three to four passages are required for an adapted virus with S protein mutations to induce an acute, lethal, respiratory distress syndrome and end-stage lung disease (unpublished observation). About 6–9 or 1 mutation is needed for increased virulence in young and aged animals, respectively. This pattern, according to Baric, strongly resembles the progress of SARS and MERS in humans, suggesting that host age not only influences pathogenesis, but could also enhance animal-to-human cross-species transmission. It is possible that sufficient human-to-human and animal-to-human transmission events have occurred in the Middle East to model the role of aging in MERS-CoV transmission.

Characterizing MERS-CoV

Given the pandemic potential of coronaviruses, the emergence of a novel human virus, MERS-CoV, within a decade of the SARS epidemic is not surprising. Like SARS, MERS is a β-coronavirus that belongs to a phylogenetic group that includes a large number of bat viruses. That MERS-CoV can infect *Pipistrellus* bats and camels, as well as humans—via DPP4—raises an important question

[32] Transferring some or all cells from a previous culture to a fresh growth medium. Subculture is used to prolong the life and/or expand the member of cells or microorganisms in culture. Source: cell passage; www.ruf.rice.edu (accessed February 26, 2015).

[33] An inflammation of the alveoli of the lungs caused by the inhalation of an allergen.

[34] An acute viral infection of the small air passages of the lungs called the bronchioles.

for vaccine development, Baric noted: Would a vaccine that was effective against MERS-CoV protect against other viruses that recognized DPP4? It was recently reported by two groups that another group 2c β-coronavirus, BtCoV HKU4, can also use human and bat DPP4 as receptors for docking and entry, but that efficient entry into human cells is limited by the availability of downstream S glycoprotein proteolytic processing, which is needed for virion fusion and entry (Wang et al., 2014; Yang et al., 2014).

To examine the antigenic variation among coronaviruses, investigators in Baric's laboratory expressed S glycoproteins from the three known group 2c coronaviruses and most of the reported group 2b coronaviruses. Antisera against MERS-CoV S glycoprotein were capable of neutralizing two different human MERS-CoV strains. However, antisera against the group 2c MERS-like bat viruses HKU4 and HKU5 could not neutralize MERS-CoV or SARS-CoV. Likewise, antisera against closely related group 2b bat coronaviruses could not neutralize SARS-CoV or MERS-CoV (Agnihothram et al., 2014). These data led the investigators to conclude that sufficient "antigenic space" exists within the group 2b and 2c gene clusters to allow for three or more antigenically unique coronaviruses to emerge, he said. It is, therefore, not surprising that the previously mentioned pair of novel SARS-like bat viruses isolated by Ge et al. (2013) evade vaccines and immunotherapeutics that were developed against SARS-CoV, especially in highly vulnerable aged animals, he added.

In another effort to characterize MERS-CoV, Baric and coworkers synthesized a full-length cDNA clone of the coronavirus and used it to reconstitute virus that functioned similarly to the wild-type isolate (Scobey et al., 2013). Using this recombinant virus, tagged with a fluorescent protein, they demonstrated that MERS-CoV replicates preferentially in differentiated primary lung cells, like nonciliated bronchial epithelial cells, type II pneumocytes, and endothelial cells, and, therefore, shows much broader tissue tropism than SARS-CoV.

Unfortunately, Baric observed, MERS-CoV does not replicate in mice, ferrets, or guinea pigs, all of which are frequently used as small animal models for immunological studies. As he and his collaborators discovered, this incompatibility results from differences in the receptor interface between MERS-CoV and DPP4 among these species, which ultimately hinge on 1 or 2 amino acid differences and the presence of a glycosylation site in the small animal DPP4 interface sequence (Cockrell et al., 2014). These seemingly subtle distinctions mean that it will be difficult (but not impossible) to make a mouse-adapted strain of MERS-CoV, he said, and only slightly less difficult to use the guinea pig instead.

To get around this obstacle, Zhao and coworkers (2014) made an adenovirus gene therapy vector that encoded the human DPP4 receptor, transduced the lung of mice, then infected with MERS coronavirus, which yielded a viral replication model without serious disease. Taking a different approach, Baric and coworkers created a surrogate model for MERS-CoV that replicates in mice (Zhao et al., 2014). To do this, they synthesized the full-length genome of the MERS-like bat coronavirus HKU5—that can replicate in human cells, but cannot spread between

them—and then replaced its S glycoprotein with that of SARS-CoV. This recombinant virus was able to replicate well in the same tissues as SARS-CoV, he noted. The recombinant virus also produces severe disease, to which older animals are much more vulnerable. He anticipated using this surrogate MERS-CoV to test both therapeutics and vaccines targeting non-S glycoprotein antigens.

This is important, he explained, because NIH spent nearly $40 million on two killed SARS vaccines early in the epidemic that induced a strong immune response that protected in young and to a lesser extent in aged animals. Unfortunately, a Th2 immune response to the nucleocapsid protein enhanced immune pathology and eosinophilia, which may result in enhanced disease in some vaccines. These types of immune complications are often revealed in a robust animal model that recapitulates the human disease phenotypes. Curiously, Baric added, "when you do this experiment using the HKU5 challenge virus with animals vaccinated against either the HKU5 or the MERS nucleocapsid protein, there's no increase in the number of eosinophils." Perhaps, then, the pathology associated with the SARS vaccines might not recur with MERS-based vaccines, he suggested.

Pathogenic Potential of Coronaviruses

"Coronaviruses do two novel things to innate sensing and signaling programs to promote their disease potential," Baric stated, reflecting on comparisons of human lung cell responses to SARS- and MERS-CoV, and to H1N1 and H5N1 influenza viruses. After infecting airway epithelial cells with these viruses, the researchers monitored interferon-stimulated genes that are important in establishing cell-intrinsic immunity and antiviral defense and discovered that approximately 150 interferon-stimulated genes (ISGs) are quickly turned on in human airway cells. Most of these ISGs are also strongly induced within 3 to 7 hours in airway cells treated with low-pathology H1N1 influenza virus, he added, whereas high-pathology H5N1 influenza turns on only a subset of these ISGs, and turns off many others. This suggests that H5N1 "has some additional trick to downregulate cell-intrinsic immune responses to allow for more efficient replication," he speculated.

Even more intriguing, "If you infect [airway] cells with SARS, for the first 12 to 24 hours, you see no measurable ISG response," Baric observed; some ISGs are activated at 24 hours, and most, but not all, follow. Meanwhile, however, SARS reaches peak titers between 24 and 30 hours, "so by the time the cell-intrinsic defense mechanism gets turned on, coronaviruses are done with the cell," he concluded. "MERS does the same thing. It has this huge delay in cell intrinsic immune recognition and ISG induction. It also downregulates a subset of these ISGs, just like H5N1, so that they never get turned on after infection. The mechanism underlying this response is probably epigenetically regulated," he added, and it allows both the SARS-CoV and high-pathology H5N1 influenza virus to manipulate host cell intrinsic response, thereby increasing disease severity.

Lessons from Animal Coronaviruses

Both α- and β-coronaviruses are known to infect a range of species, and some—such as the β-coronavirus MERS-CoV—appear to be host range mutants that evolved through interspecies transmission and adaptation, as Baric described. Known species of the other two genera, gamma- and deltacoronaviruses, are largely avian viruses. Saif provided further context for understanding MERS-CoV through her discussion of coronaviruses known to cause disease in domestic livestock.

The α-coronaviruses—transmissible gastroenteritis epidemic virus (TGEV) and porcine epidemic diarrhea virus (PEDV), along with the feline infectious peritonitis virus (FIPV) and the canine coronavirus (CCoV)—appear to have evolved from a common ancestor (Le Poder, 2011), Saif noted. Such closely related viruses may have altered cell or tissue tropisms, enabling interspecies recombination events that drive genomic modification (e.g., of the spike glycoprotein gene). As summarized in Table WO-2, coronavirus variants have arisen through diverse mutational routes, which suggests that they have multiple ways of adapting to infect new species or tissues, she added.

Emerging Porcine Coronaviruses

Saif described three emerging α-coronaviruses of swine: TGEV, PEDV, and porcine respiratory coronavirus (PRCV) (see Table WO-3). TGEV and PEDV circulate in U.S. herds, causing intestinal infections and high mortality in seronegative piglets. PRCV, a spike glycoprotein gene deletion mutant of TGEV, instead infects the upper and lower respiratory tract, where it causes an atypical pneumonia that resembles SARS.

PEDV, TGEV, and PRCV all share the same aminopeptidase N receptor, but she explained that the loss of a sialic acid-binding spike region in PRCV apparently disabled it from binding to mucins associated with binding of TGEV strains in the gut. In laboratory studies, PRCV was found to induce partial immunity to TGEV, but when PRCV circulates as an endemic among swine populations, it induces repeat infections and the development of widespread herd immunity to TGEV, she noted. Although both are α-coronaviruses, PEDV and TGEV do not induce cross-neutralizing antibodies and do not cross-protect.

In April 2013, a virulent PEDV strain emerged in the United States as a highly fatal diarrheal disease in baby pigs, as illustrated in Figure WO-12. According to Saif, this epidemic is ongoing and still spreading among U.S. swine herds. Based on available genome sequence data, the virus has continued to evolve, including variants with insertions and deletions in the spike glycoprotein gene (S INDEL strains) that have been associated with milder disease (Vlasova et al., 2014), she reported. It remains to be determined whether these attenuated variants were introduced into the United States along with virulent PEDV, or if their mutations arose here.

TABLE WO-2 Coronaviruses That Emerged as a Result of Interspecies Transmission or Tissue Tropism Changes and Suggested Associated Genomic Modifications

Resulting CoV/host	Suspected original CoV/host	Genomic modification	References
TGEV/pig	CCoV-II/dog	ORF3 insertion	Decaro et al., 2007
CCoV-II/dog	TGEV/pig	Recombination in the 5′ end of the spike gene	Decaro et al., 2009
CCoV-II/dog	CCoV-I/dog and unknown CoV	Recombinant spike gene	Lorusso et al., unpublished
FIPV/cat	FCoV/cat and CCoV/dog	Substitutions in M and ORF7b genes and FCoV-CCoV recombinations in spike and pol genes	Brown et al., 2009
PRCV/pig	TGEV/pig	621-681-nt deletion in the 5′ end of the spike gene; deletions in ORF3	Wesley et al., 1991
HCoV-OC43/human	BCoV/cow	290-nt deletion (corresponding to the absence of BCoV nsp 4.9 kDa and nsp 4.8 kDa)	Vijgen et al., 2005
HECV-4408/human	BCoV/cow	Not known	
GiCoV/giraffe	BCoV/cow	Deletion in the S1 subunit (amino acid 543-547) of the spike protein	Hasoksuz et al., 2007
SARS-CoV/human	Bat and civet SARS-CoV/ horseshoe bat and civet cats	29-nt deletion in ORF8 and substitutions in spike gene and ORF3	Lau et al., 2005

SOURCE: Vlasova and Saif, 2013.

This is but one of a list of unresolved questions associated with the emergence of PEDV in swine, Saif observed. Also unknown are the following questions:

- What is the host reservoir from which PEDV emerged in European swine in the 1970s (a bat virus is the closest relative)?
- Why did PEDV outbreaks cease in Europe in the late 1990s without implementation of immunization against the virus, much as SARS disappeared from China while its bat host remains? Did both viruses emerge, and then disappear, from secondary hosts to which they were not well adapted?

TABLE WO-3 Emerging Porcine Coronaviruses—Target Tissues and Diseases

			Disease/Infection Site			
Genus	Virus	Host	Respiratory	Enteric	Other	Year
Alpha	TGEV	Pig	x	x		1946
	PRCV	Pig	x			1989
	PEDV	Pig		x		2013
	PEDV S	Pig		x (mild?)		2013
Beta	HEV	Pig		x	CNS	1962
Delta	PDCoV	Pig		x?		2014

NOTE: CNS, central nervous system.
SOURCE: Saif Presentation, 2014.

- Why did a more virulent form of PEDV emerge in China in 2010—did the use of live, partially attenuated vaccines in swine select for this variant?
- What is the origin of PEDV in U.S. swine? Was it imported from China, as sequence similarities suggest?
- Will spike-variant S INDEL PEDV strains associated with milder disease moderate PEDV's impact here as well?

In February 2014, porcine delta-coronavirus was first identified as a cause of diarrheal disease in U.S. swine (Li et al., 2014a). Outbreaks in herds infected with this virus resembled those associated with PEDV and TGEV, but were less severe, Saif said. It remains to be determined whether this virus actually causes diarrhea in swine, she noted. This association is of interest because it suggests an expanded disease potential among δ-coronaviruses, which previously had been confined mainly to avian species. In this case, she observed, a virus of the same species that infects sparrows also infects swine and another mammals, including the Asian leopard cat, signaling possible spillover events.

Evidence of Interspecies Transmission

Over the past two decades, Saif's group has amassed evidence showing that some (but not all) coronaviruses have broad host ranges; this body of research is illustrated in Figure WO-13. Early experiments involved inoculating immunologically naïve germfree calves with enteric coronaviruses isolated from disease outbreaks in captive wild ruminants such as sambar, white-tailed deer, and waterbuck, as well as a human enteric coronavirus isolate. Bovine β-coronaviruses share sequence identity with coronaviruses from these and other animal species. "We put all these [viruses] into our calves, and all the calves got diarrhea, they

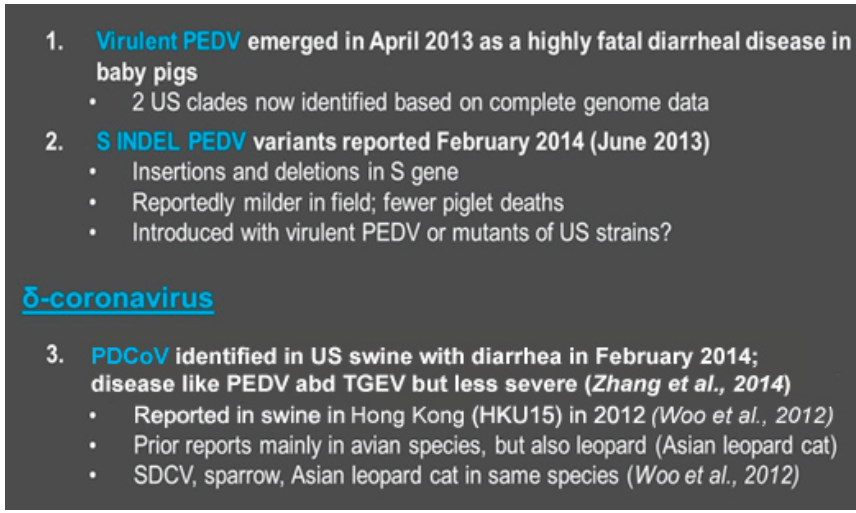

FIGURE WO-12 Emerging CoVs in U.S. swine (2013 to present).
SOURCE: Saif presentation, 2014.

all shed the virus, and they all seroconverted with neutralizing antibodies to bovine coronavirus," Saif reported (Han et al., 2006 Tsunemitsu and Saif, 1995; Tsunemitsu et al., 1995). "This was early evidence that coronavirus from wild ruminants or humans can experimentally infect young calves." Such studies were among the earliest to challenge the dogma that coronaviruses are highly species-specific by demonstrating the potential for coronaviruses to cause interspecies infections.

Both SARS- and MERS-CoV have proven to be promiscuous with regard to host, Saif noted. SARS-CoV has been found either naturally or experimentally to infect humans, civet cats, raccoon dogs, horseshoe bats, swine, nonhuman primates, ferrets, cats, mice, guinea pigs, and hamsters. MERS-CoV can infect humans, bats, camels, and rhesus macaques, as well as monkey and pig cells in culture.

Among circumstances that favor spillover at interfaces between wildlife, domestic animals, and humans, Saif expressed particular concern regarding fecal contamination of animal food sources, against which few protections exist. For example, grain destined for livestock feed is often stored in open areas, where many species of birds can readily eat and defecate on it, as shown in Figure WO-14. "We talk about food safety from the human perspective," she observed, "but maybe we should talk about food safety for animals, too."

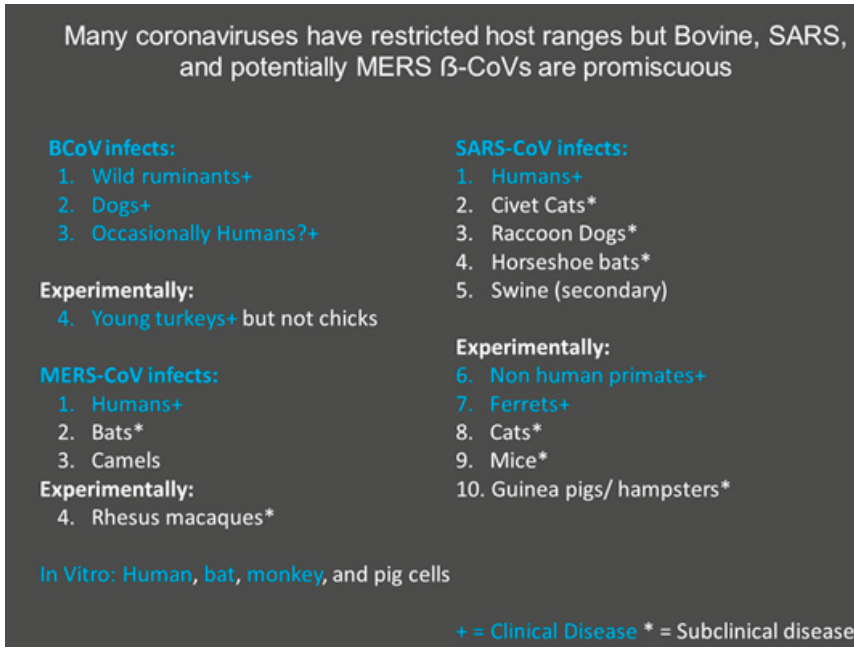

FIGURE WO-13 Coronaviruses with broad host ranges.
SOURCE: Saif presentation, 2014.

FIGURE WO-14 Birds consume and contaminate livestock feed.
SOURCE: USDA, 2010.

A One Health Challenge

Discovering whether bats are the sole animal reservoir for SARS-CoV or whether it also persists in an intermediate host or community reservoir is an important step in determining the likelihood that the virus will reemerge in humans, according to Saif. Equally pressing is the need to identify the host reservoir of MERS, as she and several workshop speakers noted throughout this workshop. Efforts to pursue this question are discussed in the section, "Ecology and Animal Origins of MERS-CoV." More generally, Saif noted, two key potential reservoir animals for coronaviruses—birds (for δ- and γ-coronaviruses) and bats (for α- and β-coronaviruses)—include migratory populations that congregate in high densities, often as multiple species—conditions that further favor the interspecies viral transmission of coronaviruses, which are already genetically predisposed to adapt to new hosts.

The emergence of SARS, MERS, and coronavirus diseases of domestic animals such as those Saif described, combined with accumulating knowledge of the potential for interspecies transmission among coronaviruses, suggests that coronavirus spillover presents an ongoing threat to animal and human health. It will therefore be important to survey diverse coronavirus strains from wild and domestic animals and to study their pathogenesis in the natural host, she advised. Such a goal exemplifies the One Health approach to addressing zoonotic diseases, and one best pursued by multidisciplinary teams that combine the expertise and efforts of medical and veterinary scientists.

Tracing the Origins of MERS-CoV

Soon after the first MERS case was recognized and the novel virus identified, the EcoHealth Alliance in partnership with the Center for Infection and Immunity at Columbia University, and the Kingdom of Saudi Arabia's Ministry of Health, became involved in efforts to identify animal reservoirs of MERS-CoV. Presentations by researchers Jonathan Epstein and Kevin Olival described the multipronged approach taken by the team that involved simultaneous epidemiological, immunological, ecological, and evolutionary investigations (Drs. Epstein and Olival's contribution may be found on pages 119–133 in Appendix A).

Epidemiology and Immunology

"Early on, as the genetic code was being analyzed for this new virus and there were linkages being made to other coronaviruses—namely, bat coronaviruses that were linked to [bats] *Pipistrellus* and *Tylonycteris* species from Hong Kong—we also had some insight into the potential bat reservoirs based on some

work we were doing under the USAID-funded PREDICT program,"[35] Epstein noted. To catalog viral diversity among bats, rodents, and primates that inhabit so-called hot spots for infectious disease emergence (Jones et al., 2008), PREDICT researchers created a genomics library that included novel bat coronaviruses, some of which proved to be closely related to MERS-CoV.

The team began its work in Bisha, where the first human MERS case was identified. The index case was a 60-year-old man who died from respiratory disease, and who had no reported history of animal contact or underlying disease, Epstein said. To better understand how this patient might have been exposed to and infected by this virus, they met with and interviewed his family and toured his several properties, where they found domestic animals, including pet camels. While few people recognized that there were bats in the vicinity, the researchers observed several in flight on a single evening and ultimately discovered bats roosting in abandoned buildings in downtown Bisha, as well as a colony of about 500 bats just outside of town. There they set up a mobile laboratory and collected samples of saliva, feces, urine, and blood from seven different bat species to search for MERS-CoV, which they found in the Egyptian tomb bat, *Taphozous perforatus*—depicted in Figure WO-15 (Memish et al., 2013).

At the same time, several research groups investigating MERS-CoV in camels and dromedaries found indications of the widespread presence of MERS-CoV and closely related viruses (Haagmans et al., 2013; Hemida et al., 2013; Meyer et al., 2014; Perera et al., 2013; Reusken et al., 2013). Other livestock species have been sampled, including sheep and goats, but to date all have proven negative for both serology and molecular evidence for MERS-CoV infection, Epstein stated (Alagaili et al., 2014).

When Columbia University and EcoHealth Alliance, in collaboration with scientists from King Saud University, conducted a survey of camels throughout Saudi Arabia for evidence of MERS-CoV exposure, including archived and fresh camel blood samples dating back to 1992, and nasal and rectal swabs, they found PCR-positive camels across Saudi Arabia, as well as antibodies from past infection (Alagaili et al., 2014). About 35 percent of juvenile camels and 15 percent of adult camels were PCR-positive for the virus, he reported. The majority of these positives were detected in nasal swabs from camels in western Saudi Arabia, near Taif and Jeddah. They detected four different clades of MERS-CoV, suggesting that diverse strains of the virus are circulating among camels, either as a result of camel movement or of multiple spillover events from a reservoir host (Alagaili et al., 2014).

[35] The U.S. Agency for International Development (USAID) funds an Emerging Pandemic Threats program composed of four projects: PREDICT, RESPOND, IDENTIFY, and PREVENT. PREDICT project partners, including the EcoHealth Alliance, conduct research to identify novel infectious diseases that could become a threat to human health, focusing on wildlife species that inhabit geographic hot spots for infectious disease emergence. Source: http://www.ecohealthalliance.org/programs/28-predict_program (accessed February 19, 2015).

FIGURE WO-15 Egyptian tomb bat. The Egyptian tomb bat (*Taphozous perforatus*) is a species of sac-winged bat in the family Emballonuridae (Mickleburgh et al., 2004). It is a medium- to large-sized bat with a mass of approximately 30 g (1.1 oz) (Monadjem et al., 2010). It is an aerial insectivore, foraging in open space (Monadjem et al., 2010). Based on individuals captured in Ethiopia, it is thought to feed predominantly on Lepidoptera, but is also known to feed on Isoptera, Coleoptera, and Orthoptera (Skinner and Chimimba, 2005). Viral RNA matching MERS-CoV was found in an Egyptian tomb bat near the victim's home in Saudi Arabia.
SOURCE: Photo courtesy of Jonathan Epstein, EcoHealth Alliance © 2014.

Data on camel imports and exports compiled by the FAO reveal that Saudi Arabia is predominantly an importer of camels, mainly since 1993, when an urban development boom may have increased demand, Epstein said. Although this date coincides with their earliest serological finding of MERS-CoV antibodies in camels, he added, "that's not to say that there wasn't MERS prior to 1992." More importantly, Epstein noted, researchers should be looking for MERS-CoV in camels and humans in those countries that export the majority of camels to Saudi Arabia: Somalia, Oman, United Arab Emirates (UAE), Qatar, Djibouti, and Sudan (Oman, UAE, and Qatar have reported human cases). For example, he said, about 232,000 camels were imported by Saudi Arabia in 2005. Assuming an infection rate among camels of 25 percent (reflecting their PCR survey results), about 58,000 infected camels entered the country that year.

Epstein hypothesized that a relatively small proportion of the male population of Saudi Arabia, which totals 13 million, that includes camel herders, owners, and traders, would have the most frequent contact with camels and therefore the highest risk of coming into contact with an infected animal. And because camel

importation and evidence for camel infection predates 2012 by at least 20 years, human MERS infections have likely occurred in Saudi Arabia (and elsewhere) prior to 2012. He cautioned, however, that additional epidemiological data were still needed to determine whether and how camel-to-human transmission of MERS-CoV actually occurs. In addition, further studies of MERS-CoV in bats are needed to support the initial finding that Egyptian tomb bats are a reservoir, and to determine whether MERS coronavirus is circulating in other bats or other wildlife species, and whether transmission from bats to camels, or to humans, occurs.

Ecological and Evolutionary Approaches

Olival described ecological and evolutionary approaches to understanding the frequency, timing, and geographic "footprint" of MERS-CoV transmission among wildlife, livestock, and humans, and thereby predicting future spillover events. He noted that EcoHealth Alliance had conducted similar work to understand the emergence of Nipah virus in Malaysia (Epstein et al., 2006)—a threat he likened to MERS-CoV as another bat-borne viral disease likely driven to emerge by human-precipitated ecological changes. In the case of Nipah in Malaysia, bats shed the virus into pigpens through their waste products along with partially chewed fruit that pigs subsequently consume. Olival wondered, What comparable interactions might take place between bats and camels—or bats and humans—that could result in spillovers of MERS-CoV from their animal host(s) to humans?

Bats cohabit with humans far more often and more easily than we appreciate, Olival observed. While detailed ecological surveys are needed to better describe bat–livestock and bat–human interfaces, a few detailed initial investigations would provide significant preliminary data, he said. Thus he and coworkers mapped bat species richness—and by association, coronavirus richness—in Saudi Arabia. They also examined viral diversity within a single bat species, in which they detected 7 to 10 different coronaviruses (Memish et al., 2013). On the basis of these results, they estimate that in some regions of Saudi Arabia, hundreds of coronaviruses could be circulating in bat communities, he concluded.

Bats in Western Europe have been shown to shed virus seasonally, coincident with periods of birthing and lactation, Olival reported (Drexler et al., 2010). Presumably this holds true elsewhere, he said, but this premise should be tested through longitudinal studies in bat host populations for MERS-CoV.

Phylogenetic studies of MERS-CoV isolates suggest that either camels transmitted the virus to humans through multiple spillover events, or that viral diversity is being maintained in both camel and human populations, Olival said. His own analysis suggests generally strong cophylogeny between β-coronavirus species and their bat hosts, but there are some cases of crossover. He and coworkers believe that *T. perforatus* is the reservoir host for MERS-CoV, but because

bat-to-bat spillover of the virus may have occurred, he advised investigation of other bat species is needed. "There are probably a lot of [MERS-CoV] related coronaviruses out there in bat populations, and if you believe my cophylogeny, these include the genus *Taphozous*," he added.

As illustrated in Figure WO-16, bat species richness varies across the landscape of the Arabian Penninsula. Some bat "hot spots" occur in pockets across the known geographic range of human MERS-CoV cases, as well as in Africa and South Asia, Olival reported. Fourteen species of *Taphozous* are found across a broad swath of Africa, South Asia, and Australia—including a substantial portion of the geographic range for camels. The import and export of camels between countries could further have facilitated localized spillover. Therefore, he concluded, it is entirely possible that MERS-CoV emerged outside of the Middle East.

Given this possibility, surveillance for MERS-CoV virus should not be confined solely to the Middle East, Olival advised. "We need more global surveillance in both livestock and presumptive wildlife reservoirs," he insisted, and noted that EcoHealth Alliance is pursuing active viral surveillance in bats on a global scale. Moreover, he said, the demonstrated ability of MERS-CoV to replicate in cell lines from multiple species (Eckerle et al., 2014) suggests that "we should be casting a wider net when we do our animal surveillance." Returning to the human experience with MERS-CoV, Olival urged workshop participants to consider its ecological context. Why did the disease first become noticeable in the Middle East? Did it truly emerge there? The answers to these questions likely involve ecology, he suggested.

MERS Epidemiology and Pandemic Potential

Early Epidemiology of a Novel Disease

Trish Perl of Johns Hopkins University began her discussion with a detailed account of the index case mentioned by Epstein: a 60-year-old man admitted to the Dr. Soliman Fakeeh Hospital in Jeddah, Saudi Arabia, in June 2012 (Zaki et al., 2012) (Dr. Perl's contribution may be found on pages 181–184 in Appendix A). He had suffered fever, productive cough, and shortness of breath for the prior week. He had no history of cardiopulmonary or renal disease, took no regular medications, and did not smoke. Treated initially as a case of influenza, he received the antiviral medication oseltamivir, as well as antibiotics and antifungals to treat apparent opportunistic infections. His condition continued to deteriorate, and he subsequently suffered renal failure and died within 2 weeks.

The virologist at this hospital, Ali Moh Zaki, is "one of the heroes in this particular story," according to Perl. Zaki applied tracheal aspirate from the index patient to two monkey cell lines, recognized that they became infected with a coronavirus, and sent samples of the virus to the Erasmus Medical Center in the

FIGURE WO-16 Arabian bat species richness. While some areas have low bat richness and bat densities, some areas are very rich in bat diversity. Good locations to target for understanding the baseline of viruses "zoonotic pool" in these hosts. Some Arabian bat hot spots include Southwest KSA and Yemen; Iraq and Iran; Jordan and Israel; Egypt along the Nile; and parts of Sudan, Eritrea, and Ethiopia.
SOURCE: Permission provided by EcoHealth Alliance, 2014.

Netherlands for sequencing, where its novelty and phylogeny was verified, she explained. Perl noted that the discovery, announced on ProMED[36]—another hero in this story—alerted clinicians in Qatar's National Health Service who were treating a patient with similar symptoms; this patient was later identified as the second case. Thirteen close contacts of this case with mild self-limiting respiratory illnesses were tested for the virus, but none were found to be infected with it, she reported. Within 2 weeks, the novel virus was identified in a patient in the United Kingdom who had recently returned from travel in the Middle East.

An earlier MERS case cluster was recognized to have occurred in Jordan in April 2012, based on retrospective testing of specimens from two deceased patients in a hospital intensive care unit and their contacts (Hijawi et al., 2013). Most of those found to be positive for the virus were health care workers, much as had occurred a decade earlier during the SARS epidemic, Perl pointed out. She then described an intrahospital MERS case cluster also reminiscent of SARS, which occurred in several Al Hasa facilities in April 2013.

A rural governorate comprising about one million people, Al Hasa is located in eastern Saudi Arabia. "Initially, it appeared that this cluster was located in two dialysis units and several of the intensive care units in this hospital," Perl recalled. "We went in to do chart review and investigate the hospital outbreak." The initial case was recognized on April 8, 2013, by an infection control practitioner investigating multiple deaths from pneumonia in one hospital. As a result, infection control measures were put in place, and expanded about 1 week later. Once effective measures were put in place in all three local institutions, she said, no additional MERS cases were reported. A total of 21 confirmed and 2 probable cases were acquired by person-to-person transmission in dialysis units, intensive care units, or in-patient units. Among 217 household contacts and more than 200 health care worker contacts, MERS-CoV infection developed in only 5 family members and 2 health care workers (Assiri et al., 2013). There were additional health care workers who had febrile illnesses at that time who were not tested and are now suspected to have had MERS as well, she added.

Using the epidemiological information they collected, Perl and coworkers traced the path of infection through multiple contacts, as shown in Figure WO-17. One patient clearly transmitted the virus to multiple people in various settings, she stated, although she was reluctant to call him a "superspreader." "Were these people more vulnerable, for some reason?" she wondered. "We don't have a case-control study to tell us. But there was something about this patient."

Perl's team of investigators was also able to estimate the incubation period for MERS—5.2 days—and the serial interval (the time between successive cases

[36] ProMED—the Program for Monitoring Emerging Diseases—is an Internet-based reporting system dedicated to the rapid global dissemination of information on outbreaks of infectious diseases and acute exposures to toxins that affect human health, including those in animals and in plants grown for food or animal feed.

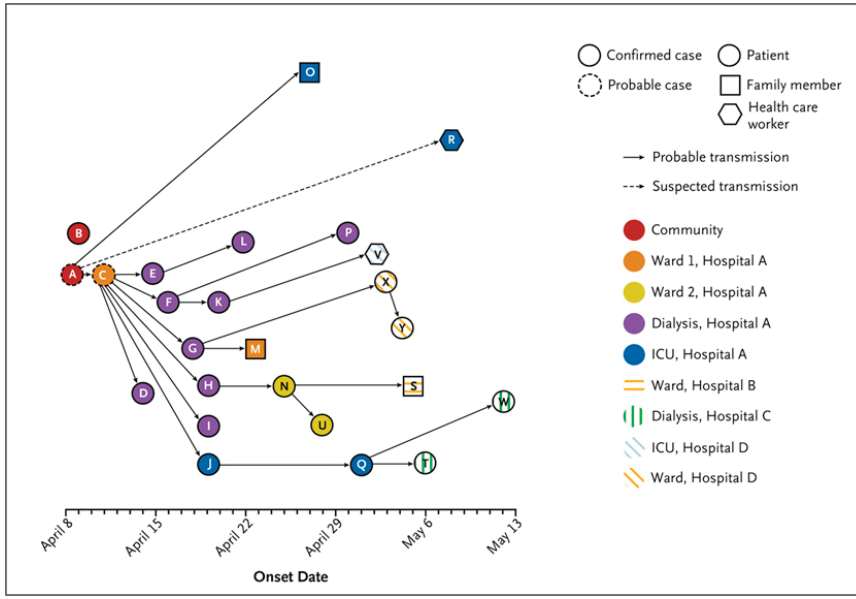

FIGURE WO-17 Transmission map of outbreak of MERS-CoV infection. All confirmed cases and the two probable cases linked to transmission events are shown. Putative transmissions are indicated, as well as the date of onset of illness and the settings. The letters within the symbols are the patient identifiers.
SOURCE: Assiri et al., 2014.

in a chain of transmission) at about 7.6 days (Assiri et al., 2013). Both the incubation period and the serial interval closely resembled those for SARS (4 days and 8.6 days, respectively), Perl reported.

Genetic mapping of Al Hasa and other MERS-CoV isolates was consistent with the epidemiological findings, suggesting that the Al Hasa cases were closely related and distinct from cases from other locations, Perl said (Cotten et al., 2013). Based on genetic information on the Al Hasa strains, minor changes were made to the transmission map and to the incubation and serial interval estimates, which were initially derived from epidemiological findings, she noted.

Assessing Pandemic Potential

Using data from the Al Hasa outbreak, Perl and coworkers estimated the reproductive number (the average number of secondary infections attributable to a single infectious individual in a susceptible population, or R_0) for MERS to be about 0.6. Other estimates have ranged from 0.69 (Breban et al., 2013) to up to 1.3 (Cauchemez et al., 2014); she noted for comparison that the R_0 for SARS

in the early days of that epidemic was calculated to be 0.8. Because the MERS estimates are based on scant information, and therefore uncertain, she suggested that a more salient current indicator of MERS' pandemic potential is the relatively small sizes of case clusters, and the fact that person-to-person transmission has so far successfully been controlled using standard interventions against infectious diseases. Analysis of the epidemic curve and of genetic sequences of various MERS isolates "indicates a slowly growing epidemic either in humans or in an animal reservoir," she asserted.

Analysis of epidemiological parameters by Cauchemez and coworkers (2014) suggests that sustained transmission of MERS could be possible if the characteristics of the current animal and human environments remain relatively stable, Perl noted. Thus, where infection or animal control measures are lacking, there is significant potential for self-sustaining MERS transmission, she concluded. Treatments for the disease are needed, and clinical communities should collaborate in order to systematically assess the use of agents such as interferon or ribavirin that have shown some promise against MERS, she advised. "I don't think any of us want to be caught like we were with SARS, where we were giving people steroids," she said, referring to their use, which remains controversial in the absence of assessment by clinical trial (Gomersall, 2004). Similarly, she observed, diagnostic strategies for MERS have not been established, although deep and multiple sampling appears crucial.

Perl also urged efforts to identify factors favoring MERS transmission, which appears primarily to occur person to person or animal to person. "There [are] some data suggesting that this organism survives in the environment better than influenza," she noted; therefore, fomite transmission may be an important route of exposure (van Doremalen et al., 2013). "Analysis of individual time course of transmissibility could really help us in determining and prioritizing interventions," she continued. Much might be learned about seasonality of transmission by comparing epidemic curves of virus shedding in bats and humans. Finally, she cautioned, asymptomatic MERS transmission has been very poorly characterized. "The fact that we think that there are asymptomatics out there is going to be an albatross in terms of control measures, if we don't figure that out," she declared.

MERS' pandemic potential should not be discounted, Perl concluded—and it should prompt the creation of better theoretical models (e.g., through the synthesis of genetic and incidence data) to guide ongoing research, as well as the sharing of data on a global scale in order to expedite the development of appropriate interventions.

Mass Gatherings and the International Spread of MERS

Proclaim the pilgrimage to all people. They will come to you on foot and every kind of swift mount, emerging from every deep mountain pass.

—Qur'an Chapter 22, verse 27

With this passage from Qur'an, Kamran Khan of the University of Toronto reminded workshop participants that the ancient ritual of pilgrimage—and particularly that of Muslims' required journey to Mecca known as the Hajj—brings vast numbers of people from disparate locations together in close proximity, after which they return to their communities. As the world's population grew, and particularly as modern transportation developed, the "swift mount" of choice shifted from camels to airplanes, which transport increasing numbers of pilgrims from around the globe to Mecca (see Figure WO-18). In 2012, about three million pilgrims performed the Hajj, about 60 percent of whom came from outside Saudi Arabia.

Although the Hajj passed in 2012 and 2013 without triggering the international spread of MERS, this and the Umrah—another annual pilgrimage to Mecca that peaks during Ramadan—are annual events, "so we're going to be facing this issue again," Khan cautioned. "We really can't get too complacent, unless, of course, MERS disappears in the next 4 months or so." And although the number of pilgrims performing Hajj declined in 2013, likely due to the threat of MERS, that trajectory is certain to continue upward, he insisted.

Mass Gatherings and Infectious Disease

Khan compared the global transportation network created by commercial air travel to an organism, with passengers flowing through its "arteries" creating patterns akin to physiological states (see Figure WO-19).

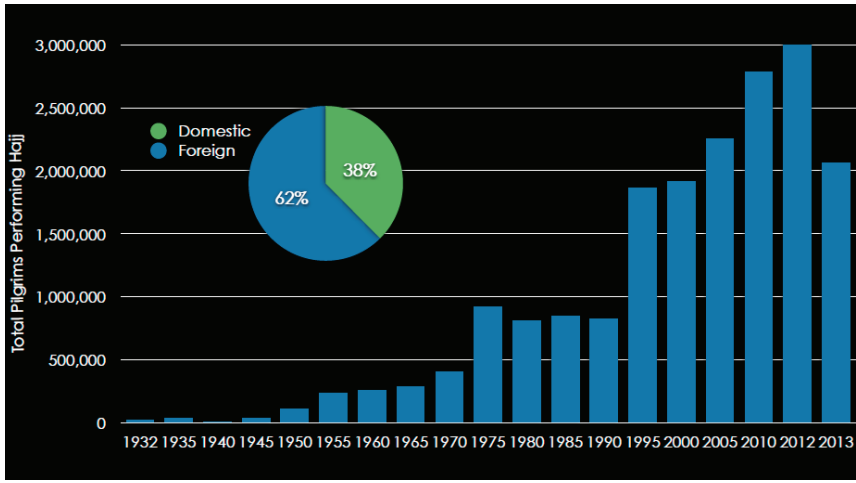

FIGURE WO-18 Pilgrims performing Hajj—1932 to 2013.
SOURCE: Khan presentation, 2014.

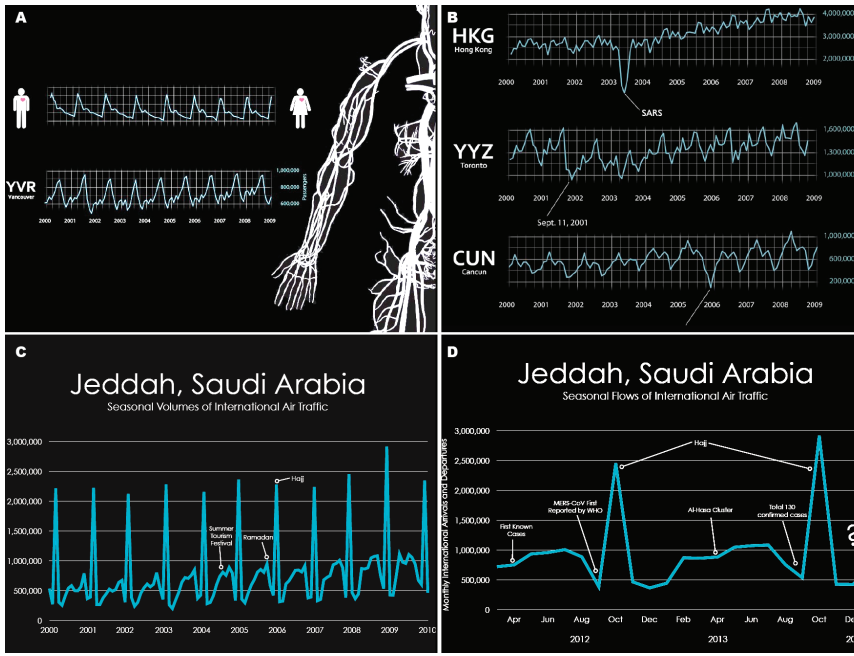

FIGURE WO-19 Seasonal passenger and air traffic volume. (A) Seasonal air passenger volume in Vancouver, Canada, from 2000 to 2009. (B) Seasonal air passenger volume in Hong Kong; Toronto, Canada; and Cancun, Mexico. Major public health and extreme weather events can be seen to have an impact on travel. (C) Seasonal volumes of international air traffic in Jeddah, Saudi Arabia. (D) Seasonal flows of international air traffic by month from April 2012 to December 2014.
SOURCE: Khan presentation, 2014.

"Each one of these particular flight lines, in Figure WO-19 (a) has its physiology, its own pattern. Understanding that pattern is particularly important since this is a major conduit for the international spread of infectious diseases. As illustrated in Figure WO-19 (b) this system can get sick, almost like an individual can, from things like viruses, where we can see a change in the normal physiologic pattern, if you will, things like terrorism even affecting a city like Toronto, which wasn't directly affected by the attacks of September 11, 2001, and even things like natural disasters, Hurricane Katrina having a big impact on global population movements. There is some evidence of how behavior can be changed negatively, where travel can decrease. The opposite may also occur in association with mass gatherings. As depicted in Figure WO-19 (c), the seasonal pattern of aircraft going into Jeddah, the city that is closest to Mecca where pilgrims tend to arrive prior to going by road for an hour or so over to Mecca, shows a very, very large

spike in terms of population movements into this particular city."[37] Turning to Figure WO-19 (d), Khan reported, "If we look at some of the events that occurred [in 2012], this is where MERS CoV is first being reported . . . some of the [9 or 10] initial cases were thought to occur, back in April."

As has occurred in response to other health threats and disasters (e.g., the terrorist attacks of September 11, 2001, the SARS epidemic, and Hurricane Katrina), air passenger transit through Saudi Arabia declined in 2013, as awareness of MERS began to spread globally. But this temporary disruption occurs against the backdrop of globalization, the context that enables mass gatherings to amplify and disperse disease worldwide, Khan pointed out. This can occur when infected individuals travel to a mass gathering, where they transmit disease to other international travelers who then introduce the disease back to their home countries upon their return, or when—as is possible in the case of MERS—travelers to a mass gathering site expedite the international spread of a formerly local epidemic. By their nature, pilgrimages such as the Hajj involve crowded conditions, Khan observed, further facilitating the spread of communicable diseases.

To anticipate and prepare for the possible spread of MERS following a future Hajj, Khan and coworkers sought clues to where exported cases would likely appear, and how quickly specific nations will be able to detect, identify, and effectively respond to such an event. By gathering and integrating voluminous and detailed information on the geographic origins of pilgrims, on air traffic patterns in the Arabian Peninsula, and on the public health capacity of nations to which infected pilgrims might return (see Figure WO-20), they learned that nearly two-thirds of air travelers leaving the Arabian Peninsula during this period returned to low-income or lower-middle-income countries, he reported (Khan et al., 2013). This finding does not augur well for the timely detection of and response to international MERS transmission, he noted. Indeed, it suggests that the Hajj presents a greater challenge to global disease control than the Olympic Games, which attracts wealthier travelers with better access to health care.

Pandemic Risk and Epidemiologic Blind Spots

Why did MERS not spread internationally after the 2013 Hajj? "There may be a variety of reasons why," Khan explained: chance, enhanced infection control measures undertaken within Saudi Arabia in response to MERS, or the reduction by one million in the number of pilgrims in Mecca as compared with previous years. But given such containment of MERS, it is surprising that international

[37] Hajj/October; there is an earlier bump in population due to the summer tourism festival . . . every year in Jeddah. It is mainly a domestic event, but there are people traveling into Saudi Arabia from the neighboring countries. . . . Right after that, you have this period where Ramadan is occurring and you have large numbers of pilgrims performing Umrah [lesser pilgrimage] . . . large spike here is the Hajj, . . . [with] very, very large numbers of people coming into the country in a short period of time and performing this particular event.

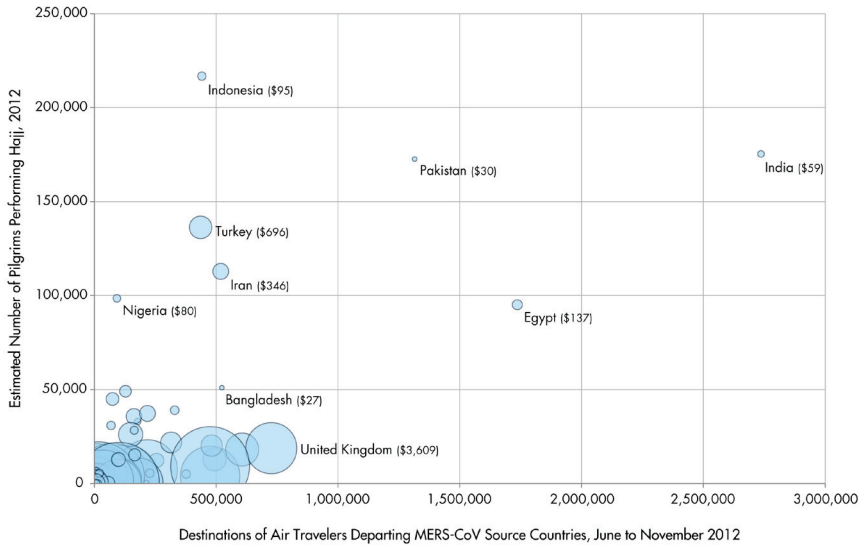

FIGURE WO-20 Country-level destinations of air travelers departing MERS-CoV source countries,* origins of Hajj pilgrims,† and health care expenditures per capita.‡

* Final destinations of air travelers departing Saudi Arabia, Jordan, Qatar, and the United Arab Emirates via commercial flights between June and November 2012.

† Estimated for 2012.

‡ Sizes of the circles are proportionate with health care expenditures per capita as estimated by the World Bank, 2011.

SOURCE: Khan et al., 2013.

cases arose prior to the 2013 Hajj in Western Europe and North Africa. Meanwhile, no imported cases were reported in travelers from South Asia and Afghanistan, Pakistan, India, Nepal, and Bangladesh, which together comprise almost 30 percent of international air passengers out of the Arabian Peninsula, and about 25 percent of Hajj pilgrims, Khan stated. "It's possible, certainly with the spectrum of an illness ranging from subclinical to minimally clinical, that there were undetected cases that may have moved into those [more likely] areas," he pointed out.

Additional countries vulnerable to MERS spread include Egypt and Indonesia, Khan said. "Cairo has the strongest ties of any city in the world to [Saudi Arabia]. . . . About 10 percent of all the international air traffic winds up in Egypt, and about 5.5 percent of all the pilgrims." However, he added, "We didn't observe any imported cases of MERS there either." Similarly, no cases have been reported in Indonesia, the world's most populous Muslim country and home to more than 12 percent of all Hajj pilgrims, he said. Conversely, MERS cases were introduced through travel to the United Kingdom, France, Italy, and Tunisia, which together

account for about 7 percent of all the international air travelers and 2 percent of all the Hajj pilgrims, he noted.

Even with the most sophisticated mathematical modeling, according to Khan, it remains difficult to predict the international spread of an infectious disease resulting from a mass gathering. Factors including the amount and activity of the disease, the volume of travel leaving the particular region, and public health measures within the country complicate patterns of disease transmission, he noted. Nevertheless, Khan added, in both the SARS and H1N1 influenza epidemics, the association between geographic and temporal patterns of global travel and those of disease spread were clear. Thus, it is fair to wonder whether the unexpected distribution of international MERS cases results from actual transmission, or from "epidemiologic blind spots," where cases have gone undetected in resource-limited settings.

Clearly, many more questions have been raised about MERS than have been answered and, as Khan observed, their pursuit is crucial to the mitigation and prevention of this and other emerging infectious diseases.

MERS in Context

In the discussion that concluded this session, the topic of immunization strategy led to an exchange that illustrates how difficult it remains to gauge the threat MERS presents, and therefore to address it appropriately. Saif raised the possibility that if camels were determined to be an important or intermediate host for MERS-CoV, it might make sense to vaccinate them (once a vaccine is developed) in order to break the chain of transmission to humans.

William Karesh of the EcoHealth Alliance noted that despite abundant opportunities for camel-to-human transmission of MERS-CoV in Saudi Arabia, there had been very few human infections:

> If you go to Saudi Arabia . . . and you go to an abattoir there where they are slaughtering camels . . . [you'll see that] these animals are bled out on the floor, and somebody is standing there with a hose, spraying. All this blood is being aerosolized. No one has a mask or a glove on. . . . [Yet] in Egypt, where they tested abattoir workers, all are seronegative, but the camels are seropositive. . . . There are hundreds of thousands of camels [imported] to Saudi Arabia annually. . . . Most of those are being slaughtered. . . . And we have only had 40 or 50 or 60 people that seem to be primary cases.

Given these odds, he wondered, why is MERS-CoV so feared? "We're thinking maybe we should put $40 million[38] into vaccine development for camels, and 55,000 people die of rabies every year?" he asked. "We have a great rabies vaccine that you could give to dogs and prevent human rabies. But we can't seem to

[38] The cost of developing a SARS vaccine, which was never used.

muster that together, and tens of thousands of people are dying every year, when we're thinking about a MERS vaccine for camels," he said.

Perl noted that a case-control study and additional "shoe-leather epi"[39] would go a long way toward defining the threat posed by MERS-CoV without costing $40 million. Baric observed that four human coronaviruses "have solved all the problems in terms of transmission and disease," he said, and thus have considerable pandemic potential. Despite the adoption of robust approaches to prevent the spread of infectious diseases, novel α-coronaviruses, including PEDV, TGEV, and PRCV are able to circumvent those transmission barriers and produce significant disease in swine.

"To some extent, the SARS epidemic was an example of the public health success story that prevented an expanding outbreak," Baric noted. "But what if the virus had evolved mutations that allowed it to transmit 36 hours earlier or 24 hours earlier, before symptomatic disease? It would have been a very different story. The window is actually quite small. With MERS," Baric continued, "we have a virus that has a 40 percent mortality rate. We have a virus family that is able to solve fundamental problems in transmission and to produce high mortality rates, both in animals and in humans." He argued, therefore, that "another equally good question is: would we be responsible stewards if we did nothing? I think the answer is, we should do something."

Saif emphasized that coronaviruses cause devastating diseases in livestock. Millions of pigs have died in Asian outbreaks, and they have also occurred in the United States, where "they have not been able to keep this PEDV coronavirus out, even with the most stringent high-security measures," she warned. "It is a concern."

EMERGENCE OF INFLUENZA A VIRUSES IN ASIA

Avian Influenzas A (H7N9) and A (H5N1)

Ruben Donis, of the CDC,[40] provided an introduction to two important type A avian influenzas that recently emerged in humans: H7N9 (in 2013) and H5N1 (in 1997). Neither virus has yet adapted sufficiently to humans to support sustained person-to-person transmission, he noted. "Who knows if they ever will?" he added. Even so, he said, "We need to be prepared for that."

[39] The term *shoe-leather epidemiology* is often synonymous with field epidemiology or intervention epidemiology. All three terms imply investigations initiated in response to urgent public health problems and for which the investigative team does much of its work in the field (i.e., outside the office or laboratory) (Koo and Thacker, 2010).

[40] Donis presented for Robert Webster, of St. Jude Research Hospital.

H7N9 in Context

Concern about the severity and pandemic potential of H7N9 has prompted comparisons with its predecessor, H5N1. That virus, which is highly pathogenic in birds, first infected humans during a poultry outbreak in Hong Kong in 1997 (WHO, 2014a). It was controlled, but later reemerged in 2003, spreading from Asia to Europe and Africa. Since then, it has caused more than 650 human cases, of which nearly 400 were fatal. H5N1 has become endemic in poultry in some countries, where it has had a severe economic impact.

For each of the 22 human cases of H5N1 reported between September 2013 and March 2014, H7N9 caused more than 10 cases in China alone, Donis reported. As Fukuda noted, most people infected with H7N9 have had direct contact with poultry through urban live bird markets. By contrast, most H5N1 cases have been linked to domestic "backyard" poultry rearing. "This difference in exposure settings we think is more a reflection of the circulation of the virus in different parts of the chain," he said. "I don't think that there is a fundamental difference in the way that these viruses are transmitted to people."

In response to the 1997 emergence of H5N1, Hong Kong closed its live bird markets temporarily, which drastically reduced infections in both humans and poultry. However, as Donis pointed out, this could only be a temporary solution, given the importance of such markets to the economy and culture of this and many other Asian countries.[41] After a series of less stringent solutions failed to suppress transmission, the authorities required bird market vendors to slaughter all birds at the end of each day and disinfect the premises, which halted the previously simmering outbreak.

Chinese officials also temporarily closed live markets in response to each of two waves of human H7N9 cases, in April 2013 and February 2014, resulting in a drastic drop in case incidence, Donis reported.

Origins of H7N9

H7N9 is a novel, reassortant influenza A virus, and genetic analyses have provided the clearest clues to its origins. Phylogenetic analyses of 100 closely related sequences for each viral gene suggest that H7N9 is derived from at least four viral strains with distinct origins: "duck origin for HA, duck (probably also wild bird) origin for NA, and at least two H9N2 chicken viruses for the internal genes" (see Figure WO-21) (Cohen, 2013; Liu et al., 2013). Researchers hypothesize that the H7N9 NA gene originated in viruses carried by wild birds, and that wild ducks probably transferred the viruses to domesticated ducks. Later, H7N9 began to circulate with low pathogenicity in chicken populations (Liu et al., 2013).

[41] Donis observed that by closing live markets for extended periods, "you could potentially make the [transmission] problem worse by creating alternative opaque channels of distribution. . . . At least if you have open channels you can regulate, you can inspect, you can promote better biosecurity."

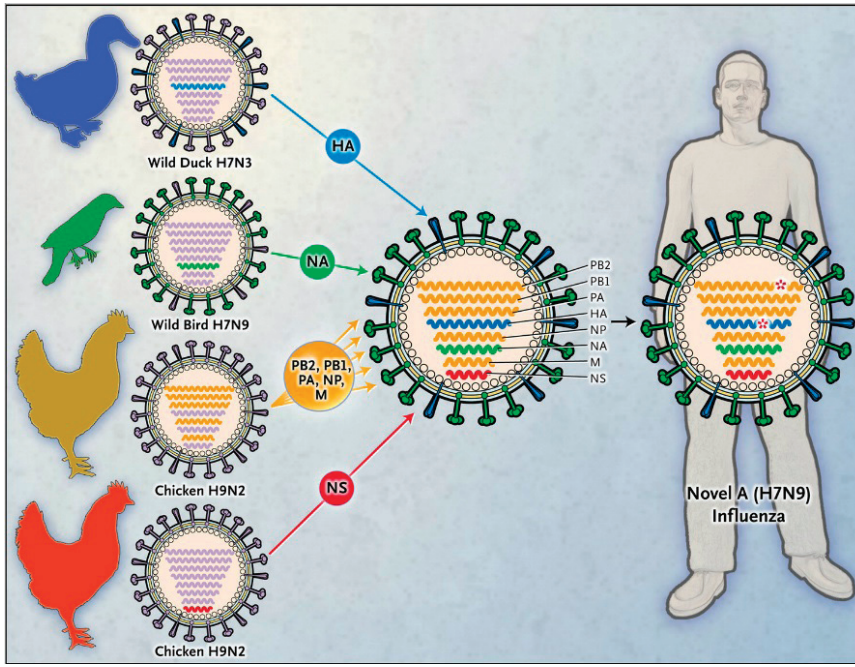

FIGURE WO-21 Origin of the novel avian influenza A H7N9 virus. On the basis of published sequences and phylogenetic analyses, it has been hypothesized that the novel avian H7N9 influenza virus is a reassortant virus containing gene segments derived from four separate avian influenza viruses, including two different wild-bird viruses contributing the H7 hemagglutinin (HA) (closest match to wild-duck virus) and N9 neuraminidase (NA) (closest match to a wild-bird isolate) gene segments, and two different domestic poultry–derived H9N2 viruses contributing the other six "internal" genes (polymerase PB2, PB1, and PA genes), the nucleoprotein (NP) gene, and the matrix (M) and nonstructural (NS) genes. The avian origin of each of the eight H7N9 gene segments is coded by color. SOURCE: Morens et al., 2013. ©2013 Massachusetts Medical Society. Reprinted with permission.

The amino acid composition of the H7N9 cleavage site resembles that of human seasonal influenzas and renders it less severe in birds, which develop asymptomatic infections. By contrast, the multi-basic cleavage site of H5N1 is recognized by proteases present in all tissues of its avian host, facilitating systemic, often fatal infection. Poultry die-offs may signal outbreaks that threaten human populations. Lacking this "warning system," H7N9 outbreaks in humans have served as a sentinel for detection of the virus in birds, Donis observed.

When H7N9 is detected in China, consumers tend quickly to avoid buying poultry, according to Donis. "This causes tremendous economic loss to the

industry, to the whole value chain," he said. So far, the Chinese economy has lost about $16 billion as a result of H7N9 emergence, he reported. "H7N9 today is low pathogenic in birds, but it could change," Donis observed. "These subtypes of the hemagglutinin, the H5 and the H7, are the only two ones that we know have consistently shifted from low pathogenic phenotypes to the high pathogenic phenotype upon multiple rounds of replication in chickens," he stated.

Transmission

When human influenza viruses are inoculated into ferrets that are placed in close proximity to—but not in direct contact with—naïve animals, transmission occurs through respiratory droplets, and infection is successful nearly 100 percent of the time, Donis stated. By contrast, H7N9 does not often transmit via droplets, and H5N1 never does under normal circumstances. This happens because H7N9 and H5N1 do not efficiently recognize receptors in the upper respiratory tract. H7N9 has evolved to recognize so-called partially human-like receptors present in the upper airway, nasal turbinates, and trachea. The two mutations responsible for this ability arose, separately, in the 1957 (H2N2) and 1968 (H3N2) pandemic influenza strains, Donis observed. This change is completely unprecedented for the H7 subtypes, he said, and has never been detected in chickens—a situation he deemed "a reason for concern."

Ecology

Aquatic and gallinaceous[42] birds (poultry) are reservoir species for the H5N1 virus, which has been transmitted into and dispersed by wildlife into pigs and to other mammalian species. A few human-to-human transmission events have been reported, but none were sustained. By comparison, H7N9 appears to be restricted to its reservoir in gallinaceous birds, occasionally infecting aquatic birds, and, rarely, humans. "If H7N9 becomes transmissible in wild migratory birds, it will likely spread to many countries," Donis warned, as did H5N1 after it became adapted to wildlife hosts. And if H7N9 reached the frozen lakes of Siberia, a major breeding ground for migratory birds, that would be even worse, he added. The virus could be widely dispersed, and it would be difficult to eradicate from this frigid environment, where infectious virus can persist. To date, Donis reported, neither H5N1 nor H7N9 persistence has been detected in this location, which is being closely monitored.

[42] Gallinaceous birds, or galliforms, belong to an order (*Galliformes*) of heavy-bodied ground-feeding birds that includes the turkey, grouse, chicken, New and Old World quail, ptarmigan, partridge, and pheasant.

Influenza A (H7N9) in Poultry

As Donis made clear, the low pathogenicity of H7N9 in birds—in contrast to its severe consequences for humans—creates significant challenges for detecting the virus and preventing human exposure. Speaker David Swayne, of the U.S. Department of Agriculture, further explored this question by describing the distribution and behavior of the virus among chickens and other species sold as poultry in China's live markets, which have been strongly associated with human cases of H7N9 influenza (Dr. Swayne's contribution may be found on pages 263–284 in Appendix A).

Poultry Production in China

Today, amid a period of rapid agricultural modernization, China's poultry production system comprises two equal and independent tracks that serve the industrial market and live markets, Swayne explained. Industrial producers raise breeds collectively known as white chickens. These are birds that grow rapidly and/or readily produce eggs. Farmers who supply live markets raise "yellow" chickens, which grow more slowly but are preferred by customers. Live markets also feature other bird species: ducks and geese, quail and other gallinaceous birds, pigeons, and captive-reared wild waterfowl, he noted.

The Chinese industrial chicken meat production system is highly integrated, Swayne said: production companies control the supply of chicks, feed, veterinary care, and processing. Contract growers' farms must meet the company's biosecurity standards, as well as stringent government standards and high consumer expectations. Meat production cycles are short and farms are large (generally 20,000 to 200,000 chickens). Most industrial chickens are processed and purchased as fresh or frozen meat by urban grocery stores and chain restaurants, which largely serve China's young, two-career families.

The chicken production system for live markets is increasingly integrated, as its companies consolidate their resources, according to Swayne. Thus, he noted, farmers in this system may buy chicks from a broker, feed from a feed mill, and medicines from another source—or all of those things from a single source. While most growers are independent, some are under contract, as in the industrial system; in either case, chickens are raised indoors. There still are chickens in the village setting that are raised for local consumption, he added, but those that are transported to live markets are raised on larger farms. Figure WO-22 is a schematic of the production system supplying retail live markets via wholesale markets; large cities in China often have several live markets.

Regulatory and consumer oversight of the live market production system is lax in comparison to industrial production, Swayne observed. Yellow chickens could be raised at high density and may be moved over long distances to the live markets, he noted. Yellow chicken producers use large amounts of vaccines and antibiotics, he said. Largely due to the conditions under which chickens are

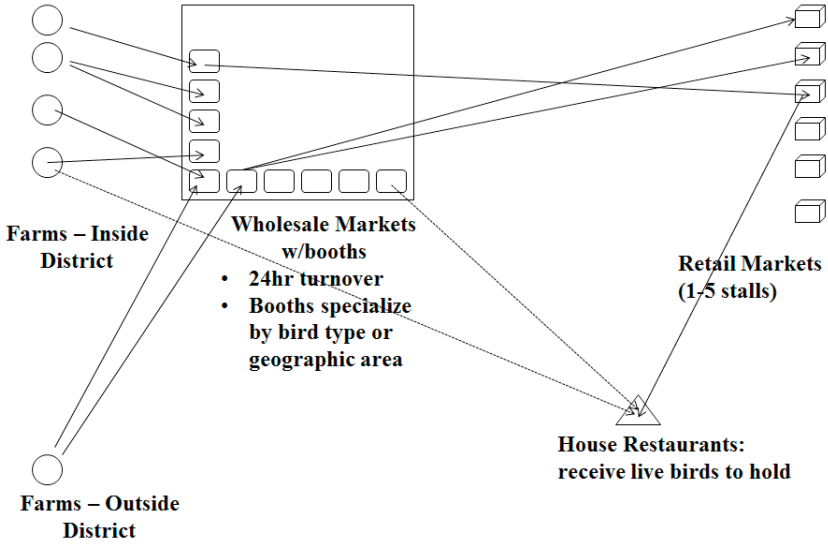

FIGURE WO-22 Live poultry market system.
SOURCE: Swayne presentation, 2014.

raised, he continued, biosecurity is significantly lower in this sector than in industrial production. In addition, the chickens live three times longer before being consumed as compared to industrial white chickens.

Response to H7N9 Emergence in Humans

Soon after the first human cases of H7N9 were reported, China quickly undertook active surveillance for the virus by expanding its existing H5N1 surveillance program. In 2003, more than 1 million serum and swab samples were taken from poultry; only 83 were determined to be positive for the virus, Swayne reported. However, because the H5N1 program was directed toward industrial chicken production, he noted, the samples were skewed to that population, and so did not accurately represent viral prevalence in the live poultry markets or farms that supply them. Based on the first 24 human cases that could be traced back to specific live markets, 22 markets had H7N9-positive birds or environments, he said—suggesting that the contaminated environment, and not individual birds, was the major driver of H7N9 persistence and transmission. In response to this finding, half a million birds were culled from live markets, the markets were closed, and movement restrictions were placed on poultry. The markets reopened within 3 months under new sanitary standards and monitoring practices.

Subsequent investigations determined that live bird markets were heavily contaminated with H7N9, and that they had no protocol for daily closure to allow cleaning and disinfection, Swayne said. In fact, he and coworkers suggested that environmental samples better predicted the presence of H7N9 in a given market than swab samples from individual birds. The actual poultry species that served as the source of the virus that infected humans remains unknown, but, based on sampling and field epidemiology, it has been strongly associated with yellow chickens—and the fact that no new human infections arose during the temporary closure of live markets further supports yellow chickens in live markets as the source, he observed.

Between Two Waves

In addition to the range expansion of H7N9 cases between the 2013 and 2014 waves mentioned by Fukuda, Swayne noted that cases also spread beyond live market exposures to include poultry workers and farmers in the second wave. The results of poultry testing also reflected geographic range expansion between the two waves, from a few provinces to a large region of the country. "We don't know where the affected farms are, but it appears that the locations of affected farms have spread, and we have [affected] farms in more distant provinces than we had last year, which means the problem is growing and not declining," he said. Thus, while transmission control measures directed at live markets have proven effective in limiting human cases in the short term, the more challenging and equally necessary work of identifying viral reservoir populations remains to be realized.

Pathogenesis in Poultry

To better understand the environmental context of human H7N9 cases, Swayne and coworkers sought to answer the following questions about avian infection (Pantin-Jackwood et al., 2014):

- **What bird species are susceptible to infection by H7N9?** By inoculating various poultry species across a range of viral titers, the researchers produced infection in all species tested.
- **Is clinical disease associated with infection in birds?** The only evidence of infection was reduced weight gain.
- **Do infected poultry shed large amounts of virus?** Chickens and quail were found to shed large amounts of virus for up to 11 days following inoculation. Muscovy duck had similar shedding behaviors but represent only a small percentage of birds in live markets, which are dominated by chickens (and to a lesser extent, quail).
- **Does the virus become systemic in birds, thereby facilitating foodborne transmission to humans?** H7N9 is generally limited to the upper

respiratory tract with little evidence of systemic spread. In all bird species tested, virus was detected at much higher levels in oral swabs, i.e., respiratory tract replication, than cloacal swabs, i.e., digestive tract replication.

Eradication Strategies

Experience with the H5N1 influenza A virus provides a basis for developing eradication strategies for H7N9, Swayne observed. When he and coworkers compared the effectiveness in eradicating H5N1 on a national basis, against a range of economic indicators, they found no statistical association between rapid eradication and wealth alone, he reported (Pavade et al., 2011). They did, however, find that countries belonging to the Organisation for Economic Co-operation and Development—which are not only wealthy, but also maintain strong principles of governance—had shorter and significantly fewer avian outbreaks, quicker eradication times, and lower mortality rates, he added. Further analysis by the OIE (see the section "The OIE Perspective") found that countries with "core competencies"[43] in national, provincial, and local veterinary services and practice were most effective in limiting H5N1 spread. "Without strong veterinary services you cannot control and eradicate [zoonotic] diseases," Swayne concluded.

These strategies appear all the more important given the evolutionary history of H5N1 and H7N9, as Swayne briefly explained during the discussion that concluded his workshop presentation. Both viruses acquired their internal genes from influenza A (H9N2), a low-pathogenicity virus found throughout Asia and the Middle East, he said. Epidemiological surveys of poultry in China frequently find H9N2 infections in as many as 10 percent of the birds tested. H9N2 is endemic among poultry raised in many countries, often in conditions that expose them to wild birds, he added. Additional reassortant viruses could easily continue to evolve and emerge much as H5N1 and H7N9 did. As long as H9N2 moves freely through the environment, "We are going to continually have this emerging disease issue in humans from avian influenza virus, because we still have the donors out there, the donors of the internal genes," he explained.

Influenza A (H7N9) in Humans

While not the first influenza A virus of the subtype H7 to emerge causing illness in humans, H7N9 is the first of its subtype to cause an extensive number of infections in humans, according to speaker Dan Jernigan of the CDC. Other H7 infections had been confirmed in people who had direct contact with infected birds, often during outbreaks in poultry. The severe symptoms of H7N9 in

[43] Veterinary core competencies include staffing of veterinarians and para-veterinarians, professional competencies and continuing education of the veterans, emergency funding, veterinary laboratory diagnosis, epidemiological surveillance, availability of veterinary medicines and biologicals, transparency, disease prevention, control, and eradication.

humans are also unusual, compared with previously reported H7 infections; these were generally mild, causing conjunctivitis and influenza-like illness (Belser et al., 2009). Although H7N9 caused severe illness in humans, it caused little to no disease in poultry. H7N9 is truly something new, he observed.

To illustrate this point, Jernigan described two recent H7 influenza outbreaks in humans. In 2003, an outbreak of highly pathogenic influenza H7N7 in the Netherlands resulted in the culling of more than 30 million birds (Fouchier et al., 2004). The virus was also detected in 86 people who had been directly exposed to infected poultry, and in three of their family members, he said. Among them, 91 percent had conjunctivitis, and one person—a 57-year-old veterinarian with underlying conditions—died. A 2012 outbreak of highly pathogenic H7N3 influenza in Mexico, in which at least 3.8 million birds were culled, resulted in two cases of conjunctivitis without fever or respiratory symptoms in persons exposed to poultry, he added (CDC, 2012).

On March 31, 2013, the China Health and Family Planning Commission notified WHO of three cases of human infection with influenza A H7N9 in Shanghai Municipality (CDC, 2013; WHO, 2013): an elderly man, 87 years old, who had visited poultry markets; a 27-year-old worker in a live market; and a 35-year-old housewife from Guangxi. All three suffered from respiratory tract infection that progressed to fatal pneumonia, he stated. As there was no coincident poultry die-off, the disease had not previously been recognized in poultry in the area where human cases emerged.

Jernigan identified some oft-cited factors in infectious disease emergence with particular relevance to H7N9: crowded conditions[44] in the region where the first cases were reported and high-density areas within that region such as live poultry markets and airports; interconnectivity between these regions and the rest of the world via international travel and trade; and an expanding animal–human interface coincident with increasing meat consumption.

International Response

As shown in Figure WO-23, and as previously noted, two waves of human H7N9 cases had occurred at the time of the workshop. Because they occurred in a "post-SARS, post-H1N1 world," Jernigan said, the response to H7N9 was much swifter and more effective than in past crises such as SARS. The CDC has a long history of public health collaboration with China at multiple levels, including cooperative agreements, laboratory training, and supporting the Chinese National Influenza Center, and the WHO Collaborating Centre for Reference and Research on Influenza established in 2010, he noted. The preparation in China allowed for

[44] This region includes about 575 million people, which is 45 percent of the population of China and 8 percent of the population of the world. Approximately 131 million people, 241 million domestic chickens, and millions of ducks and pigs live within 50 kilometers of the first 60 H7N9 cases, according to Jernigan.

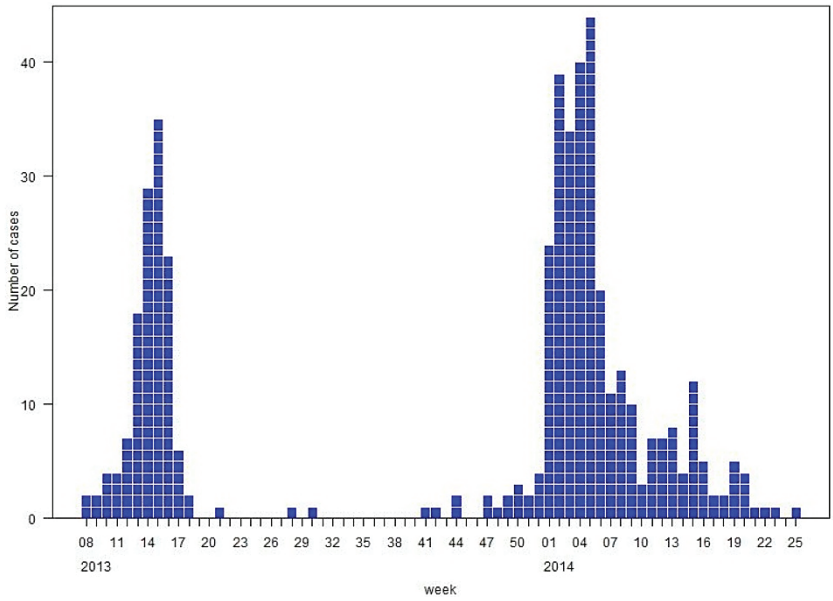

FIGURE WO-23 Number of confirmed human H7N9 cases by week as of July 14, 2014. SOURCE: WHO, 2014c.

a rapid response to H7N9 emergence. At the global level, WHO's GISRS played an important role in international influenza coordination, and virus sharing was organized under a global agreement known as the Pandemic Influenza Preparedness Framework. The CDC developed, manufactured, and oversaw the global distribution of H7N9 PCR testing kits and developed an H7N9 candidate vaccine virus, now completing clinical trials.

Three different surveillance systems, in place when H7N9 emerged, contributed to monitoring the virus in China. The Chinese National Influenza-Like Illness Surveillance Network of more than 900 sentinel facilities submitted about 60,000 swabs from laboratories and influenza-like illness patients throughout the country between March and April 2013, of which only six samples from known affected provinces were found to be positive for the virus by PCR (Xu et al., 2013). Specimens collected by the Severe Acute Respiratory Infection and Pneumonia of Unknown Etiology (PUE) surveillance systems were also screened for H7N9 (Xiang et al., 2013); PUE surveillance was the most common means by which cases were identified, Jernigan said.

Epidemiology

At the time of this writing, as at the time of the workshop, no human cases of H7N9 influenza had been reported outside China. "It's surprising that we have not seen more of these [exported cases]," Jernigan remarked. "I would have expected to have a couple of more of these than we've actually seen."

Peak H7N9 case numbers have coincided with those of seasonal influenza. At the time of this writing, confirmed cases and deaths from H7N9 in China during the most recent wave—from late 2013 through early spring 2014—are shown in Figure WO-24. The focus of disease has moved southward, and now affects Guizhou and Guangxi provinces on the border with Vietnam, Jernigan noted. This is troubling, he said, because Vietnam does not have China's resources for controlling viral transmission.

Using geospatial mapping coupled with surveillance data to develop risk maps for H7N9 spread in Asia, Fuller and coworkers (2014) identified northern Vietnam as a likely site of disease emergence. "That's an area where we do need to spend a lot of focus and effort," Jernigan insisted. He noted that the CDC has worked with the government of Vietnam to improve symptomatic surveillance for humans along the border with China, and also to conduct environmental testing in live poultry markets in order to detect the presence of H7N9. However, he added, H7N9 may have already arrived in Vietnam, as illegal poultry movement between China and Vietnam is common.

At the time of the workshop, Jernigan reported that nearly 400 cases of H7N9 had been reported and eight confirmed clusters had been identified. Since then, as compiled in Table WO-4, 98 percent of confirmed cases were hospitalized, with a 32 percent case fatality. "This is very different than the previous H7 cases in humans—something that is very substantial and has to be addressed," he observed.

About 70 percent of H7N9 cases to date are males, most of whom live in urban areas and have had direct contact with poultry in live markets, according to Jernigan. Among 139 cases and more than 2,600 contact evaluations they reviewed from the first epidemic wave, Li and coworkers discovered four family disease clusters in which person-to-person transmission could not be ruled out; however, among the large numbers of close contacts, only 28 developed respiratory symptoms, and none tested positive (by PCR) for the virus (Li et al., 2014b). Commenting on this demonstrated lack of efficient and sustained person-to-person transmission of H7N9, Jernigan remarked that the possibility of an emerging pandemic is "clearly something that we have to follow closely."

The incubation period for H7N9 influenza has been calculated at 3.3 days (Yu et al., 2014), as compared with about 2 days for seasonal influenza, Jernigan reported. As the case contact study suggests, the reproductive number (R_0) for H7N9 is quite low: 0.1, as calculated by Chowell et al. (2014). This compares with novel influenzas as a whole ($R_0 = 0.34$) and seasonal flu ($R_0 = 1.28$).

Among the first 111 cases, 61 percent had an underlying condition (Gao et al., 2013). In the previously mentioned study of 139 cases, about 73 percent had

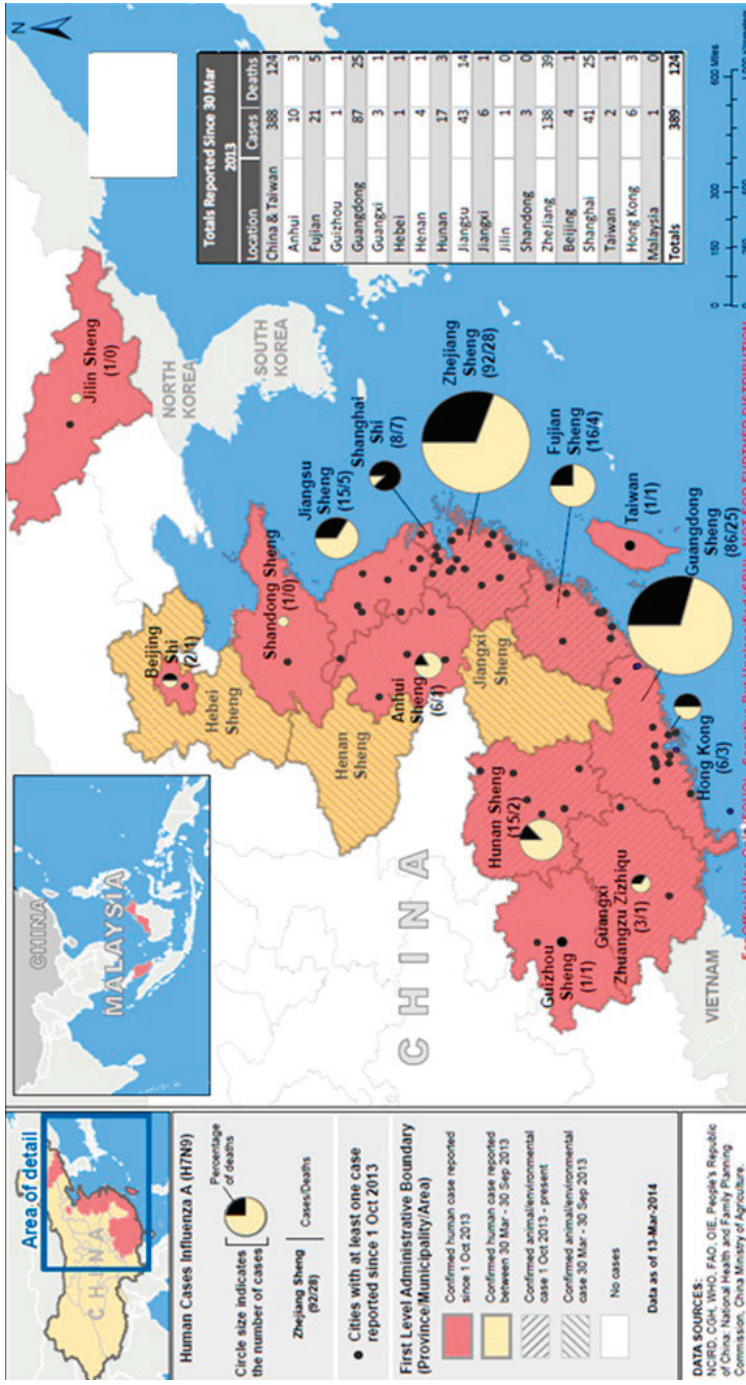

FIGURE WO-24 Confirmed cases and deaths from avian influenza A (H7N9) from October 1, 2013, to mid-March 2014.
SOURCE: Jernigan presentation, 2014.

TABLE WO-4 Avian Influenza A (H7N9) Update: March 17, 2014

Cumulative counts by report date	30 Mar–30 Sept, 2013	1 Oct 2013–Present	Total
Countries affected	China	China, Malaysia	China, Malaysia
Number of confirmed cases[a]	135	257	392
Number of confirmed cases hospitalized	131	256	387 (98%)
Number of fatal confirmed cases	45	80	125 (32%)
Cases of confirmed human-to-human transmission[b]	0	0	0
Number of confirmed clusters[c]	5	3	1
Number of asymptomatic infections	1	0	1

[a] Confirmed cases include persons with laboratory confirmation of H7N9 infection through report from China CDC or Provincial CDC.

[b] Represents transmission between confirmed cases.

[c] Confirmed clusters are two or more confirmed cases of H7N9 that are close contacts of one another.

SOURCE: Jernigan presentation, CDC, 2014.

underlying disease (Li et al., 2014b). Hypertension was by far the most common, followed by diabetes. Jernigan described the typical H7N9 influenza case—a member of the target group for intervention—as an older man with chronic heart disease that frequents live markets. Nearly all confirmed H7N9 cases analyzed by Gao and colleagues (2013) developed pneumonia; 71 percent developed acute respiratory distress syndrome (Murray et al., 2012), and 76 percent were admitted to intensive care. "These are tremendously high numbers, showing a very severe infection," Jernigan remarked. At illness onset, 71 percent of cases presented with cough, which was higher than among human cases of H5N1, he noted (Cowling et al., 2013). The median age of H7N9 influenza cases in the first wave was calculated at 61 years, as compared with 26 years for H5N1 (Cowling et al., 2013; Li et al., 2014b). Clearly, Jernigan concluded, the epidemiology of H7N9 differs markedly from that of H5N1, its likeliest comparator.

Is H7N9 New?

Exploring the possibility that H7N9 is not a novel virus, but merely newly recognized, several studies have produced somewhat conflicting results, Jernigan

observed (Bai et al., 2013; Lebarbenchon et al., 2013; Yang et al., 2013), but overall, the preponderance of data lead to the conclusion that H7N9 has emerged relatively recently and is not merely an artifact of improved detection. Moreover, he added, phylogenetic studies suggest little to no dissemination of H7N9 by waterfowl, as was alluded to previously. It is, therefore, not surprising that serologic studies conducted by the CDC demonstrate that the U.S. population lacks cross-reactive antibodies to H7N9.

Role and Repercussions of Live Markets

Of the 139 cases described by Li and coworkers, more than 80 percent had exposure to animals, which, in nearly every case, included chickens (Li et al., 2014b). In 65 percent of those instances, that exposure came through visiting a poultry market, "So this [poultry markets] is the risk factor of interest," Jernigan emphasized.

In addition to the significant toll H7N9 influenza has already exacted on human life and health and the attendant costs of medical care, the H7N9 influenza epidemic has also led to major economic losses to the poultry industry in China, Jernigan reported (Qi et al., 2014; Wu and Gao, 2013). The figures associated with these economic effects vary wildly, he noted, but their implications are clear. The poultry industry losses amounted to $1.24 billion in 10 affected provinces and $0.59 billion in 8 nonaffected adjacent provinces (Qi et al., 2014). Economic loss associated with live poultry market closures was in excess of $8 billion in one report (Wu and Gao, 2013).

The closure of live markets dramatically reduced the mean daily number of H7N9 infections in four cities, according to a study by Yu and co-authors (2014). An editorial that accompanied this publication in *Lancet* also credited the media with informing people about how to avoid infection, Jernigan said. Live market closures have been sporadic in many areas, he noted. "Honestly," he said, "it has been very difficult to find out exactly what places have been closed and for how long. . . . Clearly there are places that have been implementing measures." On the other hand, he noted, some traders in Shanghai reportedly sidestepped live market closures by selling poultry online.

What's Next?

Is H7N9 here to stay? Recalling their recent experience with the H3N2 swine influenza virus, which has caused several human infections since August 2011, Jernigan observed that he and his colleagues at the CDC expected the H3N2 epidemic to last several years. Instead, "We had huge numbers of cases in 2012, and then very few last year. Is that going to happen for H7N9?" Jernigan continued. "I don't think that's the case at all."

"All of the factors really point towards this being an intransigent problem that we will have a hard time detecting, and so it may be a new H5N1 that will be harder to monitor," Jernigan surmised. He, therefore, argued for improvements in the active surveillance of live markets for the detection of influenza A viruses, "because it's hard to know exactly where the H7 is, and that may help us to know what to do with human health measures." Meanwhile, he added, surveillance for H7N9 in humans will unfortunately be a sentinel for animal disease.

A vaccine has been developed against H7N9 and may be stockpiled, Jernigan stated. "The Chinese government is also supporting a vaccine being developed," he reported, which may become available to the CDC as well. Vaccination of animals may also become an option, albeit a controversial one, he noted.

Ultimately, response to H7N9 must be coordinated on a global basis, Jernigan said, echoing remarks by several other speakers. "We at the CDC will maintain our stocks of diagnostic reagents and other things to help manufacturers, and we will maintain readiness ourselves in working very closely with the Chinese government," he stated.

INTERNATIONAL AND DOMESTIC RESPONSES TO EMERGING VIRAL DISEASES

A series of workshop presentations described two distinct approaches to addressing the threat posed by emerging viral diseases: first, efforts to control, mitigate, and study recent and ongoing epidemics caused by influenza A (H5N1 and H7N9) and SARS- and MERS-CoV; second, research directed toward predicting the pandemic potential of viruses such as MERS-CoV and H7N9 that are identified during the early stages of their emergence into human populations.

H7N9 Emergence: The Big Picture

The OIE Perspective

Responding to the H7N9 epidemic, the World Organisation for Animal Health (OIE)[45] offered assistance to China, and upon being invited to do so, coordinated with the FAO[46] and WHO to help that country create a national strategy to address the crisis, according to speaker Alex Thiermann, of the OIE. Several problems hindered this process, he noted: live bird markets were closed too late and infected birds were not sampled on time; a lack of coordination among the many national laboratories involved with the response; and limited sharing of reagents, which were of variable quality.

[45] World Organisation for Animal Health.

[46] Food and Agriculture Organization of the United Nations.

These difficulties could arise anywhere, Thiermann emphasized. To improve participation by most countries in detecting and reporting these zoonotic disease outbreaks, countries must have the proper animal health infrastructure, he noted. The epidemiological characteristics of the next pandemic may be entirely different from those of outbreaks of the past, so we must be prepared to detect and respond to any emerging pathogen. To ensure that this happens, every country must have adequate capacity for disease detection and control—an underlying principle of the IHR, and also of the OIE international animal health standards for veterinary services. From the point of view of the OIE, the most effective route to reduce the burden of known and emerging diseases is through assistance and capacity building activities, provided by the OIE, at member country's request, Thiermann explained. His presentation focused on an evaluation tool developed by the OIE to evaluate and strengthen national veterinary services in their ability to comply with the OIE standards (OIE, 2014c).

Evaluating the performance of veterinary services The OIE Tool for the Evaluation of Performance of Veterinary Services (PVS Pathway) is a continuous process intended to assist countries in evaluating the performance of their veterinary services against 47 critical elements; identifying gaps and weaknesses in their ability to comply with OIE international standards; and to determine a path to improvement and sustainable efficiency, Thiermann stated (OIE, 2014c). Figure WO-25 diagrams this process, which he compared to the course of diagnosing and treating illness. PVS also supports the implementation of international standards, as is the case under the IHR for public health; however, he noted, unlike the WHO, the OIE provides assistance, training and resources to countries to conduct the PVS and follow-up activities, funded through the OIE Animal Health and Welfare Fund.

Thiermann briefly described each step in the PVS Pathway: evaluation; gap analysis; capacity building activities on legislation, laboratories, etc., as well as, follow-up evaluation missions.

Evaluation assesses four categories of critical competencies based on the OIE standards: human, physical, and financial resources; technical capability and legislative authority; interaction with interested parties; and market access. The Evaluation help to raise awareness and to improve the understanding across sectors of the requirements for the effective functioning of the veterinary services, this step results in the creation of a detailed, reliable document for analysis by national authorities. As of June 2014, 129 of the OIE's 178 member countries had requested PVS evaluations, of which 117 evaluations were completed, 86 reports have been finalized, and 19 are publicly available.

Gap analysis facilitates the identification of priorities for strategic action to address the gaps and improve compliance of veterinary services with the OIE

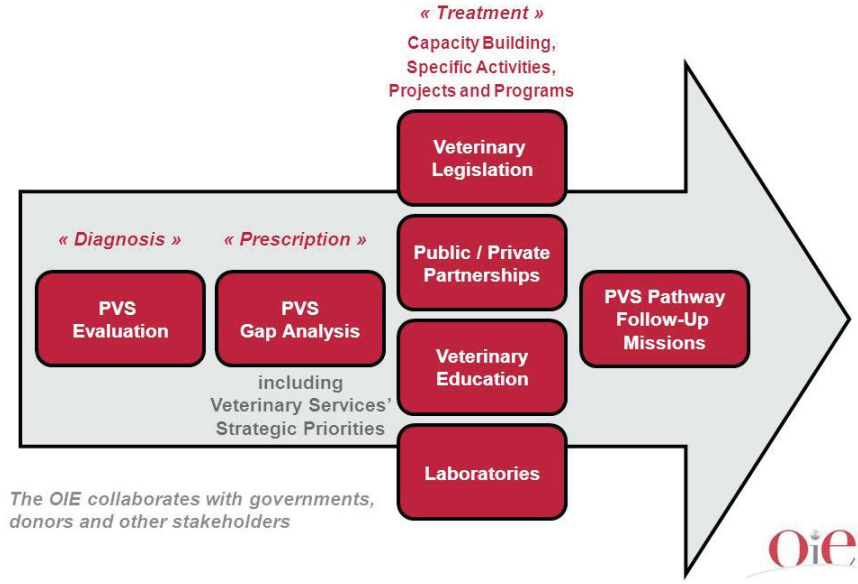

FIGURE WO-25 The PVS Pathway.
SOURCE: OIE, 2014b; Courtesy of the World Organisation for Animal Health. Available at: http://www.oie.int/en/support-to-oie-members/pvs-pathway (accessed June 12, 2014).

international standards and pursue national goals over the next 5 years. In June 2014, the OIE had received 95, and completed 73, requests for gap analyses. This generated 42 completed reports, 11 of which are publicly available.

Capacity building supports the improvements of identified needs through gap analysis. The PVS Pathway Laboratory Mission also helps national veterinary services identify and allocate appropriate resources to the various areas, including the national animal health laboratory system. The missions on Legislation Support offer advice in examining and modernizing national laws and regulations pertaining to veterinary services.

Follow-up missions are conducted every three to five years to measure progress toward the implementation of the PVS-defined strategy to improve compliance with OIE standards.

Many large countries, including China, have not yet participated in the PVS evaluation process, Thiermann reported. However, the OIE, at China's request, trained 1,000 Chinese veterinarians to become familiar with the PVS Pathway, and apply the same concept at a national level. The European Union is also opting

for internal evaluations, he said. "I've been part of discussions between the US, Canada, New Zealand, and Australia on this subject," he added.

In the discussion that followed Thiermann's prepared remarks, he noted that countries derive trade benefits from engaging in the PVS process, which produces trusted impartial evaluations by the OIE trained experts. In addition, these evaluations can assist member countries in the process by which the OIE officially recognises countries free of certain animal diseases, creating additional incentives for participation. In order to maintain disease-free status, countries must conduct and share the results of their ongoing disease surveillance activities. This, he noted, allows member countries to focus on diseases and routes to eradication of national importance, providing incentives to maintain a surveillance network that could eventually detect emerging infectious diseases.

The OIE and WHO As global institutions responsible for animal and human health, intergovernmental standards, and strengthening infectious disease surveillance, detection, reporting and response capacity, the OIE and WHO have great potential to work synergistically to advance a One Health agenda, Thiermann stated. As previously noted, the OIE has much greater leverage in influencing member states to comply with its standards than does WHO to enforce the IHR. Now, with support from the World Bank, the OIE and WHO are examining the possible harmonization of national animal and public health capacities for assessing zoonotic disease response, he announced.

Three countries are serving as pilots for the OIE-WHO harmonization effort: Costa Rica, Kenya, and Thailand. The OIE and WHO have been mapping shared outcomes and critical elements, and the OIE provides resources such as costing tools to WHO and encouraging collaboration between animal and human health sectors, Thiermann said. They also plan to implement a joint OIE-WHO workshop at a regional level.

The OIE is also conducting research aimed at determining the cost of controlling or preventing specific diseases, and eventually hopes to demonstrate that this can be best accomplished through collaboration between public health and veterinary services, Thiermann reported. "They don't have to be merged into a common agency," he added. "The issue is they need to learn how to work together." He also noted that the potential for creating joint animal and public health laboratory facilities had been discussed, and countries such as Canada provide good examples for such synergistic arrangements.

The USAID Perspective

Dennis Carroll, director of USAID's Pandemic Influenza and Other Emerging Threats Unit, discussed research in countries neighboring China that may be at risk for introduction of the H7N9 influenza A virus. As he began his presentation, he noted that it was often said about SARS: "If only we had known what

we could have done to have disrupted and prevented this situation from becoming a global situation." Ten years later, having detected H7N9 at a much earlier stage—while the disease is still zoonotic, and also geographically limited—we are faced with the challenge of deciding what to do, he observed.

Efforts over the past decade to study the periodic emergence of new H5N1 clades and subclades and their spread within Asia have been very useful in considering how H7N9 may spread geographically, Carroll noted. Thanks to these studies, the role of value chains and the marketing dynamics that move poultry (and disease) throughout Asia is better understood, and the resulting routes and flows that spread disease have been mapped, he explained. Analysis of farm-to-market dynamics—as illustrated in Figure WO-26—reveal how a virus in Shanghai might spread to Guangdong, and from there to Guangxi, and onward to the border of north Vietnam. While not perfect, such "first-order" understanding of these routes is viewed by USAID as an opportunity to target surveillance to monitor for the presence and plan interventions to control the spread of H7N9.

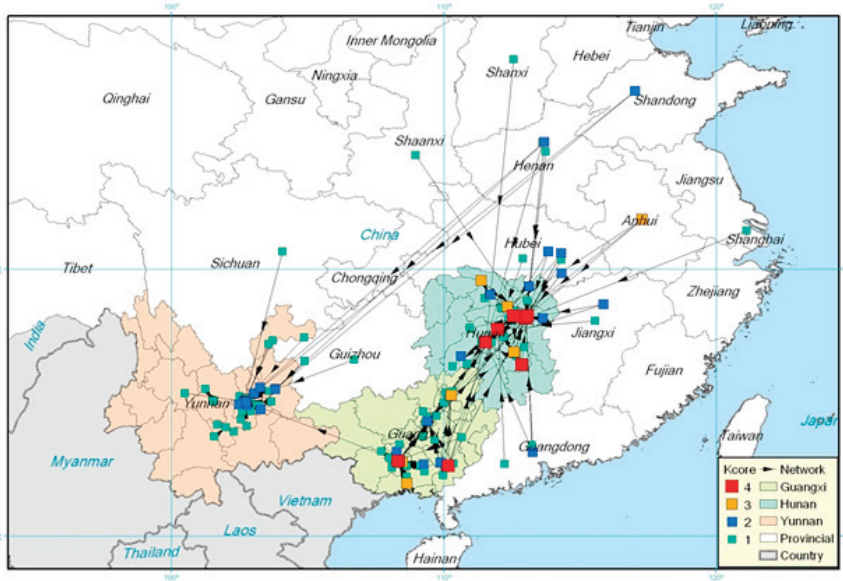

FIGURE WO-26 Live bird market (LBM) networks in Guangxi, Yunnan, and Hunan. Representation of the two-mode "market–source node" network of poultry movement in southern China according to the *k*-core value. The *k*-core is a network parameter that measures the centrality of a node within a network. Some LBMs have a higher *k*-core than others, especially in Hunan and Guangxi provinces, where some LBMs displayed a maximum *k*-core value of 4 and could play a greater role in highly pathogenic avian influenza virus (HPAIV) maintenance.
SOURCE: Martin et al., 2011.

Implications of early detection Currently, with H7N9 still largely contained within animal reservoirs and limited in geographic distribution, we may be able to disrupt its further spread, and potentially preempt its emergence as a virus with efficient human to human transmission, Carroll stated—something that has never been done before. However, he cautioned, pursuing this goal demands strategies and approaches that fully exploit early detection in ways that do not unnecessarily trigger an emergency response which results in the disruption of ongoing public health and veterinary services. As advances in technology increasingly allow zoonotic pathogens to be detected at a stage before they can move efficiently from person to person, we need to make an effort to distinguish these circumstances from emergencies, Carroll observed. That, he noted, will require coordination and cooperation between ministries of health and agriculture.

Exploiting early detection USAID's strategy for H7N9 is to attempt to disrupt its spread at the point of introduction, while human disease prevalence is low and the affected poultry population is limited, Carroll stated. Since May 2013, the agency has partnered with FAO, WHO, and the CDC, and eight countries in Asia (see Figure WO-27) to establish capacities for early detection of the virus in both poultry and human, and rapid control of the virus at the point of introduction, he reported. At the same time, as part of its support for operations led by FAO and WHO in China, USAID established H7N9 surveillance activities in border provinces such as Guangxi and Yunnan.

Based primarily on knowledge of regional poultry trade dynamics, the eight Asian countries were categorized in terms of the risk for H7N9 introduction, Carroll explained. In high-risk areas, USAID supports surveillance of live bird markets and human populations. In all countries, the agency is supporting the strengthening clinical care practices for H7N9; disseminating communications to educate political leaders, market owners, traders, and consumers about the virus;[47] and supporting the development of a disease-control "tool kit" of interventions (live market closures, cleaning, depopulation, movement control) to contain the virus should it be detected. The agency also cohosted a series of planning and review sessions in China, Myanmar, Rome, and Thailand that brought together representatives from Ministries of Health and Agriculture with technical experts, with the goal of educating ministries on H7N9, and how to use that knowledge to create preparedness plans and recognize needs; these functions continued at subsequent national planning sessions.

[47] The communications package, targeting local authorities as well as high-risk groups in the general public, is in part intended to garner support for possible market closures or culling of apparently healthy but infected birds, Carroll explained—and thereby, reduce the chance that if such methods are used, they will not result in market shocks or the unmonitored movement of poultry (and virus). The importance of creating a supportive environment for disease control is a lesson learned from the global experience with H5N1 influenza, he noted.

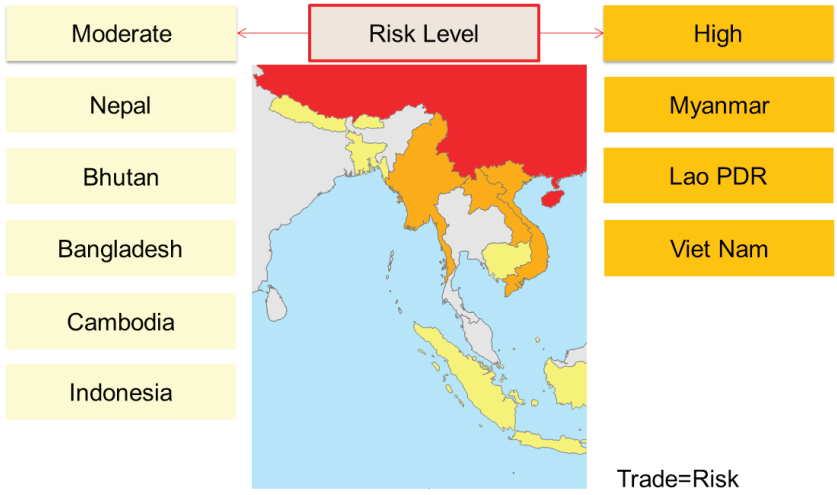

FIGURE WO-27 Stratifying risk.
SOURCE: Carroll presentation, 2014. Adapted from FAO.

As previously noted, low-pathogenicity H7N9 infection is difficult to detect in all but humans. Thus, Carroll said, it is important for Ministries of Health to coordinate their monitoring for human infection with the Ministries of Agriculture, as knowledge of poultry production would inform determinations of vulnerable "points of entry" for H7N9 into their countries. Guidance documents were distributed to encourage standardized sample collection and diagnostic approaches, along with a group of recommended contingency control measures, should the virus be detected.

Operations As previously illustrated in Figure WO-27, USAID ranked the three countries sharing a border and direct commercial trade with China—Laos, Myanmar, and Vietnam—as having a "high risk" for H7N9 introduction, and five others— Bangladesh, Bhutan, Cambodia, Indonesia, and Nepal—as having a "moderate risk." USAID support in each country reflects its relative risk.

High-risk entry points have been identified (and illustrated in Figure WO-28) within Laos and Myanmar where joint planning between the Ministry of Health and Ministry of Agriculture has focused on surveillance of live bird markets, along with influenza-like illness and severe acute respiratory illness surveillance in people living near market sites, as previously described by Jernigan. "This is a work in progress," Carroll observed. In Laos, poultry and human surveillance has been aligned in the most high-risk provinces, and in several locations in

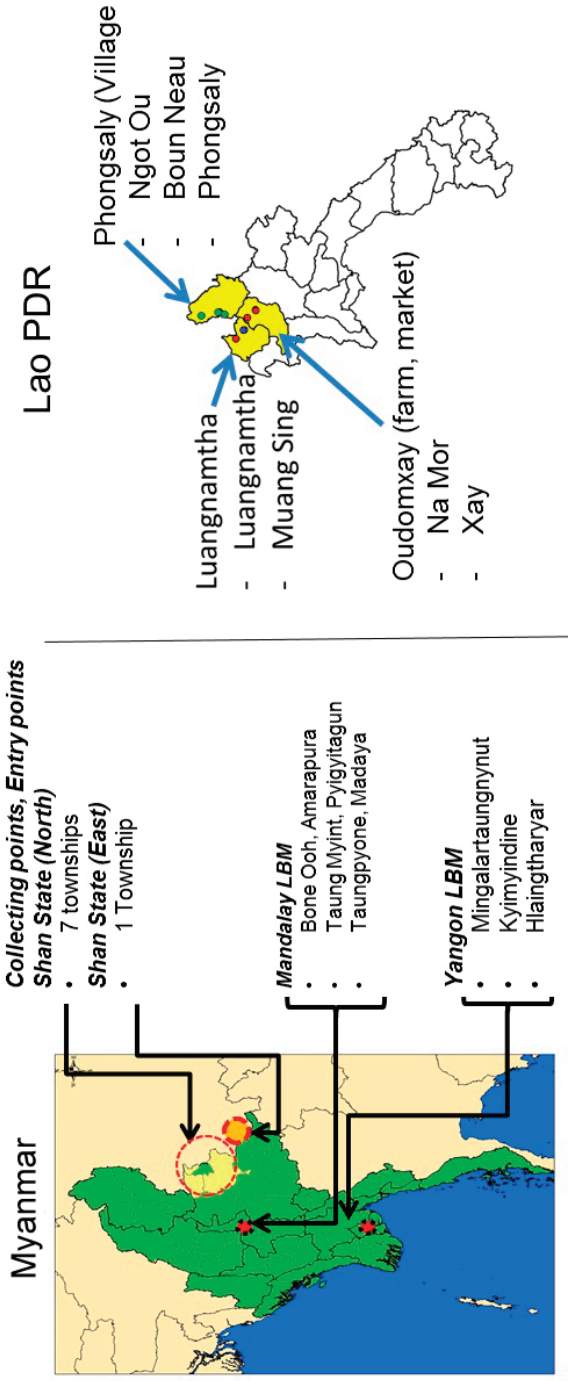

FIGURE WO-28 Ongoing surveillance for H7N9: Laos and Myanmar. Joint planning between ministries of health and ministries of agriculture has increased coordinated monitoring for H7N9 in targeted live bird markets and adjacent health facilities.
SOURCE: Carroll presentation, 2014. Adapted from FAO.

Myanmar, "but they're still working out the details of further alignment within both of these countries," he reported. In Vietnam, more than 20,000 samples obtained from 70 live bird markets in 12 northern provinces were all negative, according to Carroll. Surveillance is also ongoing in live bird markets deemed high risk for H7N9 introduction in Bangladesh, Bhutan, Cambodia, Indonesia, and Nepal, he said.

In China, analysis of initial data on market closure strategies does not clearly demonstrate that it is effective in breaking the chain of transmission, Carroll reported. USAID and FAO plan to gather additional evidence on the effects of market closures and cleanings on influenza rates over the long term. More generally, he noted, the emergence of H7N9 provides an opportunity to evaluate the feasibility of infectious disease disruption through a combination of early detection and rapid control measures instituted during the "introductory phase" of emergence.

Disruption as a long-term strategy Can we develop strategies that exploit the earlier and earlier identification of emergent zoonoses? Can appropriate interventions be developed to minimize the spread of such a pathogen, thereby disrupting its ability to adapt to humans? If so, Carroll said, this would represent a long-term strategy for disease control, particularly in areas of Asia where multiple strains of avian influenza viruses are cocirculating (e.g., H5N1, -N2, and -N8; H6N1; H7N1; H9N2; and H10N8—in addition to H7N9). Southeast Asia is "a rich cauldron for new viral emergence," he observed; those viruses that infect poultry will move along regional value chains. This presents the possibility of "normalizing" strategies for early detection and control of emergent viruses through existing surveillance platforms and interventions focused on live animal markets, he suggested.

Even so, Carroll emphasized, disrupting the adaptation of emergent viruses to human hosts is not the same as preempting the emergence of zoonotic threats. Strategies for disruption are not a solution; they are a response to problems that arise from current systems of livestock production and marketing. These factors continue to raise the risk for pandemic influenza and other pathogens that can only be reduced through measures that truly improve biosecurity on farms and in markets, he concluded.

β-CoV Challenges in Health Care Facilities

Having played a central role in responding to and describing the SARS outbreak in Toronto, speaker Allison McGeer, of Mt. Sinai Hospital, confessed to finding her task of health care issues associated with emerging viruses "a little bit depressing"—not because of the memories it rekindled, but because there has been relatively little progress on these issues in the ensuing years (Raboud et al., 2010) (Dr. McGeer's contribution may be found on pages 181–184 in Appendix A). To illustrate this point, she described the scenario of one of the

last smallpox outbreaks in the United Kingdom, which occurred in Bradford, England, in 1962. In much the same way that smallpox spread to 13 contacts of the index case within a hospital, coronaviruses and other emerging diseases could spark a nosocomial outbreak.

Several things about emerging diseases spell trouble for hospitals, according to McGeer: in the case of smallpox, an infectious period that extended late into the disease (not common among bacterial or viral pathogens); an unrecognized disease; open waiting areas and emergency department bays, along with multibed rooms; lack of standard practices adequate to control the transmission of infectious disease; and hospitals that are inadequate to contain spread of communicable pathogens. "The only thing that has changed in the last 50 years is that we have eradicated smallpox, and we have better vaccination programs at a hospital level," she observed.

SARS vs. MERS

Unlike MERS, SARS spread rapidly and widely, McGeer recalled. More than three-quarters of SARS cases in Hong Kong and in Toronto were hospital associated, she reported; about a third of those were health care workers, and the remaining two-thirds were visitors and patients in the hospital. The fatality rate of MERS at first appeared much higher than that of SARS, she said, "but in fact this difference is driven almost entirely by the difference in infected populations." About 40 percent of people who were infected in SARS outbreaks outside of China were health care workers, between the ages of 25 and 40, without underlying illness, she explained. By contrast, most people who have been infected with MERS have been hospital patients with primary infections, who are much older and more likely to have underlying illness. In Toronto, for example, the case fatality rate in 60-year-olds was 54 percent, and in patients with nosocomial infections, it was 50 percent. "That looks a lot like MERS," she observed.

Indeed, she continued, "The longer we've been watching MERS evolve, the more closely it resembles SARS." Both are primarily pulmonary diseases, she noted, which have shown similar times from onset of symptoms to hospitalization (4 days for MERS; 3 for SARS), as well as incubation times (5.2 days for MERS; 4.6 for SARS) and serial intervals (7.6 days for MERS; 8.4 for SARS), she reported. With its slightly longer incubation period and slightly shorter serial interval, MERS patients can transmit the disease earlier in their infection than could SARS patients, she observed. "What saved us in SARS was that people were not infectious until they were really sick in the hospital," she said. That does not appear to be true of MERS, and if that is the case, it is a significant difference, she concluded.

While primary MERS cases had been predominantly male, health care–associated cases were 80 percent female, McGeer reported. This percentage is roughly equivalent to the gender ratio of that population, she stated—supporting

Fukuda's earlier observation that the demographics of primary MERS cases largely reflect exposure, rather than specific vulnerability. Nevertheless, McGeer continued, "There are still some mysteries about what goes on [with MERS] in hospitals." In the Al-Musa Hospital outbreak, previously described by Perl, about 100 health care workers were thought to have been exposed to the virus, including 43 patients on dialysis, of whom 17 were confirmed probable cases. However, she added, of 18 full-time staff in that unit, only 1 presented with fever for 2 days and was not tested for MERS. The other health care workers remained apparently healthy—a dramatically different outcome compared with what happened during SARS. "I don't yet have serology to know whether there was a substantial number of asymptomatic infections [among hospital staff at Al-Musa]," she acknowledged. However, she added, it is mystifying that so much transmission occurred there between patients without the development of illness among staff members.

It is also notable that MERS does not resemble other viral respiratory diseases. Influenza, for example, produces more cases of mild illness, fewer people with severe disease, and a much lower case fatality rate, McGeer said. This behavior resembles meningococcal meningitis, she observed: "You either don't get sick, or you get really sick and you have a high case fatality rate." Clearly, she concluded, this is "different from what we're used to seeing, and because of that, significant."

The current case fatality rate of MERS in Saudi Arabia of 41 percent suggests that it either causes a more severe disease in young healthy people than SARS, or that only a small fraction of infections in health care workers are being detected, according to McGeer. "Either way, it's bad news, because either we're looking at a disease that has a 10 percent case fatality rate in health care workers . . . or we're looking at a disease that is much more transmissible to health care workers than we're recognizing in Saudi Arabia," she said.

McGeer noted two important differences between SARS and MERS: the length of the period of infectiousness, and the reproductive ratio (R_0). The number of secondary cases from every index case of SARS ranged from 2.2 to 3.9 in various locations, she stated. Even in the outbreak in Al Hasa, the R_0 for MERS was found to be 0.5, and it lies between 0.4 and 0.6 for other MERS cases to date, she reported. However, she added, "It doesn't seem like there is necessarily going to be a big jump for MERS to become more transmissible and more like SARS."

MERS in Health Care

There are several reasons to be concerned about the impact of coronaviruses such as SARS and MERS in health care settings, according to McGeer, including the fact that health care–-associated cases represent a significant proportion of disease with these coronaviruses (WHO MERS-CoV Research Group, 2013); their high case fatality rates; and the inherent difficulties involved in diagnosis and prevention of transmission.

On its face, preventing coronavirus transmission is simple, McGeer observed: put people in private rooms and wear barriers when you take care of them—that is, follow so-called droplet contact precautions. But how can you tell you are dealing with SARS or MERS? "With coronavirus, as [with] other viral respiratory illnesses . . . you are dreaming in Technicolor if you think you can distinguish one cause of respiratory illness from another," she quipped. "It cannot be done, and in fact many older people who have viral respiratory illnesses present with complications of those illnesses rather than the illnesses themselves, so what seems like something that is really simple turns out to be really difficult." These circumstances raise the following important challenges for health care institutions.

Evaluating basic practices Results from several studies analyzing health care worker protection during the SARS epidemic suggest that basic practices such as hand hygiene are helpful in controlling the spread of coronaviruses, McGeer concluded. However, research is ongoing to identify factors that influence how viruses are transmitted in health care, as well as what can be done to control it, she said. Past efforts toward this goal have to some extent improved our ability to prevent infectious disease transmission, but much remains to be done.

Infection control education during the SARS outbreak was associated with a reduction in the risk of infection, McGeer reported. "The better we train our health care workers, the better we as health care workers understand how to implement prevention, the better off we will be," she declared. She also acknowledged, however, that it is hard to persuade health care workers to change. "It took us 2 months in the middle of [the SARS] outbreak to persuade health care workers that they needed to be adhering to precautions against infection," she recalled. "So you can imagine how hard it is to do in a much lower risk situation" such as MERS. "It will require a revolution in the provision of care in our hospitals to manage the kind of change that we need [in order to] to protect people from emerging viruses," she predicted.

Changing the built environment "If we didn't have open bays in our emergency department, we would not have had the SARS outbreak in Toronto, and that would have saved us about $1.2 billion," McGeer stated. "We could have built closed rooms in every emergency department in the country for that price." Efforts to change any hospital's built environment to reduce infectious disease transmission will require careful, appropriate analysis of cost effectiveness, she added.

Recognizing disease Thanks to ongoing progress in point-of-care diagnostics, patients with coronavirus infections such as SARS and MERS may someday be rapidly identified, McGeer predicted. "Recognition of disease is a critical element of managing these cases, and our abilities to do that is within reach now," she

said. "We need to accelerate our ability to diagnose disease and, in particular, diagnose communicable disease in hospitals."

Therapeutic prospects β-coronavirus diseases are "begging for therapy," McGeer observed, and with MERS, as with SARS, it is likely to be difficult—if not impossible—to develop new drugs in short periods of time, let alone discover them. An old methodology, convalescent plasma therapy—which apparently reduced mortality when used during the 1918 influenza pandemic—might be worth investigating as a stopgap measure, she suggested (Hung et al., 2013; Luke et al., 2006). "It seems to me a really important, if perhaps relatively small, intervention that might help in the future emergence of disease in hospitals," she concluded.

Health care workers' expectations "Health care workers in truth are at very low risk of occupational disease or injury in any circumstance, but we have become accustomed to thinking that we are safe," McGeer observed. Indeed, coronavirus infections are only some of the risks faced by health care workers, which also include higher rates of influenza and antimicrobial resistance, she reported. Moreover, she added, occupational risk associated with emerging infections is not limited to health care workers—but the nature of these additional occupations, the risks involved, and how to mitigate them, remain largely to be determined.

Predicting Pandemic Potential of Zoonotic Influenza Viruses

While the emergence of a pandemic strain of H5N1 or H7N9 influenza appears unlikely to happen, it could be disastrous if it did. Since the emergence of H5N1 in Hong Kong in 1997, the question of how easily this virus could evolve to transmit readily among humans has preoccupied many researchers and policy makers; now it is being asked about H7N9 as well. "Many of the best flu labs in the world for over 10 years were working on trying to figure out whether or not such viruses could go airborne among mammals," observed speaker Derek Smith, of Cambridge University.

In 2012, after two groups of scientists separately showed that H5N1 viruses could be genetically engineered through so-called gain-of-function experiments (Herfst et al., 2012; Imai et al., 2012), Smith and coworkers demonstrated that the likelihood of these changes occurring naturally was sufficient to present a "potentially serious threat" (Russell et al., 2012). In his presentation to the workshop, Smith discussed the state of research and policy on zoonotic threats in light of these discoveries.

Predicting Transmissibility of Influenza Viruses in the Ferret Model System

"It's absolutely clear what we should do next," Smith argued: test naturally occurring influenza viruses to see if they possess the functional equivalent of the

substitutions determined by experiment to confer transmissibility between mammals; choose those that are closest to making this transition and test them for their ability to transmit between mammalian animal models (e.g., ferrets); and then experimentally determine which substitutions make this possible. Using this method, he and coworkers discovered a 2006 German isolate of H5N1 that he deemed "closest to transmissible" (Herfst et al., 2012).

"There is an enormous amount that we can know about what the emergence potential of this particular virus is," Smith stated. Moreover, researchers can gain information from such discoveries to refine predictions about which viruses are more likely to transmit, or to require the fewest adaptations to transmit, and continue to test those predictions, he said. "We can learn this for H5 based on what we know, and we can apply it to other influenza threats as well," he added.

However, Smith continued, such experiments are not being conducted in a systematic way, for reasons that are understandable. A comprehensive program to discover preemergent viral threats would require a major commitment of resources, he noted, and it would constitute "dual-use research of concern" as its results could be misused to pose a biologic threat to public health and/or national security.[48] As predicted a decade ago in the influential report *Biotechnology Research in an Age of Terrorism* (NRC, 2004), the need for biosecurity has had a chilling effect on efforts to identify preemergent influenza viruses, he observed.

Addressing Dual-Use Concerns

For many of the experiments Smith and colleagues have conceived to explore influenza transmission it is not easy to determine whether the risk they pose for dual use outweighs their potential benefit, he said. A robust, consensus process needs to be developed that involves both scientists and national security experts in making such decisions, he argued—and soon; otherwise, scientists will simply stop doing work that supports such decisions for lack of funding and trained personnel. "If we don't come together on this . . . we do run the risk that we will lose . . . the scientific partners because it's just too hard to do work in the area. What's really critical is that the people on the science [and health care] side, and the people on the national security side, need to be around the same table, because neither . . . are experts in the other domain," he insisted.

Both do, however, understand the concept of risk, and this should be the basis for their deliberations, according to Smith. While acknowledging that "it's very easy to overestimate risks, and it's very easy to underestimate risks," he suggested

[48] Dual-use research of concern is life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, materiel, or national security. Source: http://osp.od.nih.gov/office-biotechnology-activities/biosecurity/dual-use-research-concern (accessed June 12, 2014).

that these extremes could be balanced in much the same way as an actuary sets the price of insurance.

On the other hand, Smith added, these decisions should not only reflect careful estimates of risk, but also human judgments that should be applied in a quantitative, transparent way whenever possible—perhaps guided by peer review. "If these decisions are taken in a way where this normal scientific process can't apply to at least some of the calculations, then I think we really do run the risk of driving the scientists away," he warned. Fortunately, he said, efforts are under way to better connect academic research with national security efforts.

"We know so little about H7N9 compared to what we could know about it," Smith observed. Barring introduction of a universal influenza vaccine—as previously described by Fauci, and which would obviate the need to predict pandemic potential—there is no alternative to gain-of-function experimentation, he insisted.

A Pandemic Risk Assessment Framework for Animal Influenza Viruses

Speaking this time about one of his own CDC projects, Donis reminded workshop participants that real-time PCR diagnosis of influenza, which first came into widespread use in U.S. hospitals and laboratories in 2009, vastly increased detection and comprehension of many influenza subtypes, most notably zoonotic strains. Expressing hope that this technology, now becoming common in Europe, will eventually gain a foothold in Asia as well, he observed, "The more we use these molecular diagnostic tools, the more we're going to find what novel virus is causing sporadic infections" such as human cases of H5N1 and H7N9, he said.

A Basis for Comparison

But this expanding catalog of novel influenza viruses presents a challenge: how to identify those likeliest to develop the capacity for human-to-human transmission? "We have a number of viruses that are being detected in zoonotic infections, and we have to have a mechanism to understand their relative importance," Donis stated. To meet this need, he and coworkers have developed the Influenza Risk Assessment Tool (IRAT) to identify, define, and assess risk associated with a specific viral subtype relative to others, providing actionable information to risk management programs (Trock et al., 2012). As such, it could be considered an instrument of the "risk governance" model advocated by Pfeiffer (see the section "The Risk Governance Framework for Disease Management").

A risk-scoring algorithm informed by expert observation, Donis explained that the IRAT was designed to answer two key questions: What is the risk of a given virus emerging as a pandemic? And, if it does so, how severe would the pandemic be? IRAT's developers determined that three categories of factors contributed to pandemic risk: the properties of the virus, the attributes of the population, and the ecology and epidemiology of the virus. Each category contains several risk elements. For example, "transmission in animal models"

is an element of the category, "properties of the virus"; "disease severity" is an "attribute of the population"; and "global distribution" is an element of "ecology and epidemiology." Each risk element is defined precisely in terms of what constitutes low, moderate, or high risk, he said, and each risk level is assigned a numerical score. A subject-matter expert—such as a researcher knowledgeable about a particular virus—assigns the various scores for that virus, which incorporate both range and confidence level.

Once calculated, the total scores for several viruses can then be compared to each other, in order to answer the two key questions: the relative risk of emergence, and of high public health impact, Donis said. However, the risk elements composing those scores must first be weighted to reflect the question being posed, as different factors favor pandemic potential and severity of disease, he noted.

Using IRAT

The IRAT offers researchers and policy makers a consistent approach to evaluate risk, Donis observed; it reduces bias in comparisons among viruses and documents information used in decision making. It is a tool that is useful not only for the comparisons it facilitates, but for the facility by which it allows information to be shared. IRAT is also readily modifiable, he pointed out.

"The use of the IRAT has also been humbling in some ways, because many times we have very little data to perform a score," Donis acknowledged. "Most of the viruses of concern are those that we know very little about, and especially when there are a large number of cases. H7N9 was a perfect example of that," he observed, noting Jernigan's description of the onset of severe human disease from a virus that formerly was associated with conjunctivitis. "Now, it's a totally different virus," he observed. When they attempted to compute an IRAT score for H7N9, Donis and coworkers found only a few H7N9 sequences in the database. "We know a lot about Eurasian H7s, but this is a totally different beast. This has H9N2 internal genes. So you're faced with a lot of gaps, huge gaps of knowledge," he explained. "I think many times we're forced to compare apples with pineapples."

Next Steps

The more IRAT is used, the more useful it will become, according to Donis. He hopes to put it in the hands of anyone who can benefit from it, and particularly WHO, in order to support GISRS.[49] "Please go and find novel viruses and score them and put the information on the table for everybody to discuss," he urged, because that will create incentive for even greater exploration and information sharing.

[49] See http://www.who.int/influenza/gisrs_laboratory/en (accessed June 12, 2014).

In this way, IRAT could foster a global effort in sample collection and analysis extending to the creation of standardized methods for virus assessment such as transmission studies or measures of human population immunity, Donis observed. "It is hoped this will lead us to better databases, better data, better reporting, and ultimately, to better public health," he concluded.

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Contributed Manuscripts

A1

ANIMAL RESERVOIRS OF MIDDLE EAST RESPIRATORY SYNDROME CORONAVIRUS

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Introduction

Middle East respiratory syndrome coronavirus (MERS-CoV) is a newly recognized group C β-coronavirus within the family Coronaviridae that was first isolated from a Saudi patient suffering from severe respiratory disease in June 2012 (Zaki et al., 2012). A phylogenetic analysis of the complete viral genome indicated that it was related to severe acute respiratory syndrome (SARS) coronavirus, making it the second β-coronavirus to be identified in humans, although it was of a different lineage (van Boheemen et al., 2012). As of July 2014, MERS-CoV (formerly hCoV-EMC) has caused more than 837 laboratory-confirmed cases of human infection in 21 countries with an overall mortality rate of approximately 35 percent (WHO, 2014). The majority of cases have occurred in Saudi Arabia, and human-to-human transmission has resulted in several clusters of cases, some of which have included mild or asymptomatic infections (Milne-Price et al., 2014).

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Epidemiological studies have identified hospital-based infections as responsible for several clusters of cases; however, there were many cases that did not report contact with other MERS-CoV patients, and the source of their infection was unknown. The genetic relationship to SARS and other bat-associated CoVs lead to early suspicion that this, too, was a bat coronavirus, yet it was unclear which species it was associated with and whether patients had had direct exposure to bats or whether another animal host may have been involved in human infections (van Boheemen et al., 2012). Here we review current evidence for the animal origins of MERS-CoV and the involvement of a domestic animal reservoir, dromedary camels, in human infection.

Early Evidence for a Bat Reservoir for MERS-CoV

Since the discovery of as SARS-like CoV in *Rhinolophus* bat species in China and Hong Kong (Lau et al., 2005; Li et al., 2005) in 2004, there has been a huge proliferation of coronavirus studies in bats worldwide. Subsequent studies have described other SARS-like CoVs in bats in Asia, Africa, and Europe (Rihtaric et al., 2010; Tong et al., 2009; Yuan et al., 2010), as well as a diversity of other coronaviruses in bats around the world (Anthony et al., 2013; August et al., 2012; Falcon et al., 2011; Osborne et al., 2011; Shirato et al., 2012; Tao et al., 2012). Phylogenetic analyses of coronaviruses from bats from both the Old and New World, humans, and other known mammalian and avian coronaviruses have shown a greater diversity of viral species compared to other taxonomic host groups, and support the hypothesis that major groups within the family *Coronaviridae* originated in bats (Drexler et al., 2014; Huynh et al., 2012).

Evidence of Host Range from Receptor Binding Studies

Characterizing receptor binding for a given coronavirus can provide insights into the range of potential host species and tissue types that the virus may infect (Graham and Baric, 2010). SARS coronavirus requires the angiotensin-converting enzyme 2 (ACE2) receptor to enter cells (Eickmann et al., 2003). In humans, ACE2 receptors are found in lung and small intestine epithelial tissue, which was where SARS-CoV replication primarily occurs resulting in severe lower respiratory tract and gastrointestinal tract infection (Hamming et al., 2004; Ksiazek et al., 2003; Nicholls et al., 2003). The SARS-like coronavirus strains first identified in horseshoe bats were closely related to SARS-CoV (88 to 92 percent nucleotide homology across the full genome), but they did not enter cells expressing human or civet ACE2 receptors, nor could SARS-CoV infect cells expressing bat ACE2, leaving doubt as to whether they were the direct progenitor of SARS-CoV (Ren et al., 2008). Recently, a SARS-like virus with 95 percent homology to SARS-CoV and that does use the human ACE2 receptor was isolated from *Rhinolophus sinicus*, providing the most convincing evidence to date that

bats are the natural reservoir for SARS-CoV and that direct zoonotic transmission from bats is possible (Ge et al., 2013). Early genetic characterization led to the recognition that MERS-CoV was related to SARS-CoV and to two other group C β-coronaviruses, HKU4 and HKU5, which generated the hypothesis that MERS-CoV also had a bat reservoir (Woo et al., 2012; Zaki et al., 2012). In vitro infection of various mammalian cell lines with MERS-CoV (then called hCoV-EMC) showed that the virus did not use the ACE2 receptor and that it was able to replicate in a variety of animal cell lines, suggesting the possibility of a broad mammalian host range including nonhuman primates, pigs, and multiple species of bats (Muller et al., 2012). Similarly, Eckerle et al. used cell lines from common Arabian livestock and other mammal species to show that MERS-CoV replicates efficiently in goat, camel, bat, human, and African green monkey cells, but less efficiently in cow, sheep, bank vole, and shrew cell lines (Eckerle et al., 2014).

Although BtCoV-HKU4 and BtCoV-HKU5 coronaviruses, which were identified in two species of vespertilionid bats (*Tylonycterus pachypus* and *Pipistrellis abramus,* respectively), were closely related to MERS-CoV, phylogenetic analysis revealed that these bats were unlikely its natural reservoir (Lau et al., 2013; Woo et al., 2012). An in vitro study using virus surface spike proteins from HKU4 and HKU5 demonstrated that HKU4 binds to the dipeptidyl peptidase 4 (DPP4) receptor, but HKU5 does not (Yang et al., 2014). Yang et al. further showed that HKU4 had a stronger affinity to bat cells over human cells; the opposite pattern was observed in MERS-CoV.

Shortly after the first human case of MERS-COV was identified in 2012, coronaviruses closely related to MERS were found in bat species in Mexico, Thailand, Europe, and Africa, illustrating the wide geographic and bat family range of viruses similar to MERS-CoV and adding support to the hypothesis that MERS-CoV had originated in bats; however, the potential host species in Saudi Arabia could not yet be deduced (Annan et al., 2013; Anthony et al., 2013; Ithete et al., 2013; Wacharapluesadee et al., 2013).

The Search for the Natural Reservoir in Saudi Arabia

The initial investigation of the natural reservoir for MERS-CoV began in October 2012 in Bisha, Saudi Arabia, the town where the index patient had lived (Figure A1-1). Despite the initial hypothesis that MERS-CoV had bat origins, there was no information available from the medical records of the first patient that described any contact with bats or other animals. Furthermore, there was little known about the bat fauna or distribution of other wildlife in this area. It was also unknown at the time whether MERS-CoV was capable of infecting other species, including domestic livestock. The investigation included a visit to the patient's households and place of business to observe whether bats and domestic animals were present. Interviews with the patient's surviving family members and an absence of evidence of bats in the households (he had multiple residences)

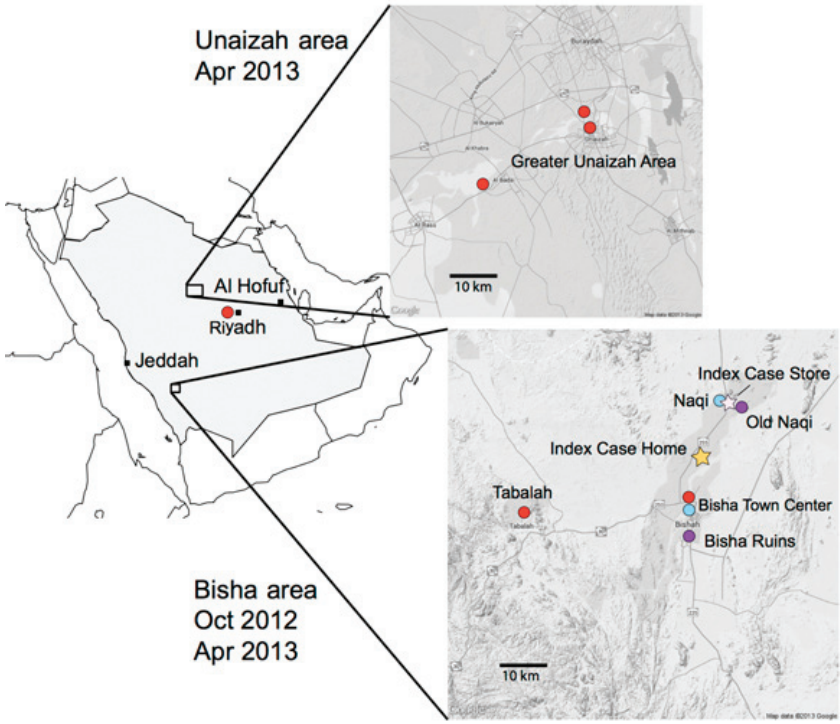

FIGURE A1-1 Map of the initial investigation of bats as a reservoir for MERS-CoV in Bisha and Unaizah.
SOURCE: Memish et al., 2013.

suggested that direct contact with bats was unlikely. The patient was a 60-year-old business man who owned four camels, kept as companion animals in a paddock next to his house, and a flock of sheep and goats kept at his business about 15 km north of Bisha. His business was a hardware shop with a large warehouse, and although bats were observed foraging in a palm grove behind the warehouse, there was no evidence of bats roosting inside the building, which again suggested direct contact with bats or their excreta inside his home or business was unlikely. Seven different species of bats representing four families (*Rhinopoma hardwickii*, *Rhinopoma microphyllum*, *Taphozous perforatus*, *Pipistrellus kuhlii*, *Eptesicus bottae*, *Eidolon helvum*, and *Rosettus aegyptiacus*) were captured and sampled either near his business or in Bisha on two separate field investigations (Figure A1-1). In April 2013, in addition to the Bisha area, additional bat specimens were collected from other geographic areas with human cases including Unaizah and Riyadh (Figure A1-1). Insectivorous bats were primarily found

roosting in abandoned buildings, one occasionally inhabited building, and captured during evening emergence from the roost, while frugivorous bats (*Rousettus* and *Eidolon*) were captured while foraging. Fecal samples, blood, and oropharyngeal swabs were collected from each bat, and fecal samples were also collected by laying out plastic sheets beneath roosts. Samples were screened at the Center for Infection and Immunity at Columbia University using pan coronavirus and MERS-CoV-specific polymerase chain reaction (PCR) assays (Memish et al., 2013). A short 190nt fragment of viral RNA that was 100 percent identical to MERS-CoV in the *RdRp* region was detected in a fecal sample from an Egyptian tomb bat (*Taphozous perforatus*) captured in Bisha (Memish et al., 2013). One of 29 tomb bats was positive, indicating a prevalence of 3.5 percent (95% CI 0–20%). Although similar coronaviruses had been identified in other bat species from other regions, this represented the first finding of RNA matching MERS-CoV in a bat in Saudi Arabia. Although this finding provided a valuable indication that *Taphozous perforatus* may be a reservoir for MERS-CoV, a broader survey is needed to confirm the finding and potentially identify other bat species that may be involved as reservoirs for MERS-CoV or related viruses on the Arabian Peninsula. For example, HKU10 CoV (an α-coronavirus) was found to naturally infect two bat species from distinct families (*Pteropodidae* and *Hipposideridae*) that had ecological overlap (Lau et al., 2012). It remains unknown how the index patient was infected with MERS-CoV, and possibilities include infection by another MERS case or zoonotic transmission. The finding of MERS-CoV in a bat did not rule out the potential involvement of other animal hosts as being involved in human infection, as transmission directly from bats to humans seemed unlikely given the limited opportunities for exposure to bat excreta.

Potential Livestock Hosts

The first two cases of MERS-CoV had no information regarding possible exposure to animals in their clinical history. As the numbers of cases increased, and information was reported to WHO and the international community, there continued to be little or no information about animal exposure. The four camels as well as the sheep that were owned by the index patient from Bisha all tested negative for MERS-CoV (Alagaili et al., 2014). The cellular receptor used by MERS-CoV was later identified as the DPP4 receptor, which is conserved across many mammalian species and found in a variety of tissue types including lung and kidney epithelium (Raj et al., 2013). As previously noted, in vitro studies of host range using cell lines suggested a breadth of potential hosts. Muller et al. and Eckerle et al. determined that MERS-CoV could infect bat cell lines derived from six species as well as pig, camel, sheep, nonhuman primate, and human cell lines (Eckerle et al., 2014; Muller et al., 2012).

By August 2013, human cases had been reported from several countries including Jordan, Qatar, and the United Arab Emirates, though most were from

Saudi Arabia, and most reported infections were the result of human-to-human transmission (Assiri et al., 2013a,b). However, human-to-human transmission was limited ($R_0 \sim 0.69$), indicating MERS-CoV was not easily transmitted among people, and supporting the hypothesis that repeated spillover from an animal reservoir may be occurring (Breban et al., 2013). The first study to provide evidence of a domestic animal host found IgG antibodies specific to MERS-CoV in dromedary camel herds in Oman and the Canary Islands (Reusken et al., 2013b). One hundred percent of the camels tested in Oman (n = 50) and 14 percent (n = 105) of Spanish camels were positive for MERS-CoV antibodies. Several species of domestic animals in various countries including Oman, Egypt, Jordan, and Saudi Arabia were screened for antibodies against MERS-CoV, including sheep, goats, cattle, and buffalo, but all were negative (Alagaili et al., 2014; Hemida et al., 2013; Perera et al., 2013; Reusken et al., 2013a). Six of 126 sheep were positive for MERS-CoV reactive antibodies in Jordan; however, none of the six had neutralizing antibodies, and it was suspected that there was cross-reactivity with the antigen used for the initial screening assay (Reusken et al., 2013a). Anti-MERS-CoV antibodies have subsequently been found in camels in Egypt, Qatar, and the United Arab Emirates at high prevalence (Alexandersen et al., 2014; Chu et al., 2014; Meyer et al., 2014; Nowotny and Kolodziejek, 2014). A study of dromedary camels in Saudi Arabia examined sera from 2013 and dating back to 1993 found antibodies to MERS-CoV, indicating that it had been circulating in camels in Saudi Arabia for at least 20 years (Alagaili et al., 2014). The same group detected MERS-CoV RNA in nasal swabs from adult and juvenile camels, and isolates were obtained from nasal swabs from camels in Saudi Arabia in 2014 (Briese et al., 2014). Sequences from camels were 99 percent identical to human full-genome sequences, yet individual camels were found to be infected by multiple, closely related strains of MERS-CoV, or viral quasispecies (Briese et al., 2014). The observed genetic diversity of MERS-CoV in camels, which was greater than in humans, and the sequence homology between human and camel strains, suggested that multiple introductions from camels may be occurring and that there may be some bottleneck selection if only certain strains are being transmitted from camels to humans (Briese et al., 2014; Cotten et al., 2014). These findings did not specifically provide direct evidence for zoonotic transmission from camels; however, support for this hypothesis is provided by two other studies. In October 2013, two patients with laboratory confirmed MERS-CoV infection had a history of contact with camels on their farm. An investigation found MERS-CoV RNA in nasal swabs from 5 of 14 camels tested within a week of detection of the first human case. Sequences from the camels and two patients were closely related (Haagmans et al., 2014). In a second investigation, MERS-CoV was isolated from a patient in Jeddah, Saudi Arabia, who became ill after having had contact with nasal discharge while treating several of his camels that were ill. MERS-CoV was also isolated from one of his camels, and the sequence matched that of the isolate from the patient (Azhar et al., 2014). It is

unclear whether camels experience severe pathology or disease from MERS-CoV infection, although the infected camels in Jeddah were reported to have had nasal discharge (Azhar et al., 2014). MERS-CoV antibodies and viral RNA have been detected in camel milk in Qatar, and experimentally the virus has been shown to be stable in camel milk, though it is currently unknown whether consumption of raw milk has led to human infections (Reusken et al., 2014a; van Doremalen et al., 2014). Definitive proof of transmission from camels to humans, or of a potential mechanism for transmission, has not yet been characterized, although there is a preponderance of serological evidence now that MERS-CoV is circulating widely in camels both in the Middle East and in parts of Africa (Chu et al., 2014; Corman et al., 2014a; Perera et al., 2013; Reusken et al., 2014b).

Camel Trade as a Driver of MERS-CoV Emergence

Antibodies to MERS-CoV have been identified in dromedary camel serum samples in both Saudi Arabia and Kenya dating as far back as 1992, indicating that MERS-CoV has been present in camel populations for more than 20 years in both Africa and Saudi Arabia (Alagaili et al., 2014; Corman et al., 2014a). The majority of the world's camels (82.2 percent) produced between 1992 and 2013 have come from northern Africa, with Somalia and Sudan being the top two producers (Figure A1-2) (Food and Agriculture Organization of the United Nations Statistics Division, 2014). The Kingdom of Saudi Arabia imports most of its camels from Africa, and it is the top camel importer in the world, with an average of 60,900 camels imported per year between 1992 and 2011 (Food and Agriculture Organization of the United Nations Statistics Division, 2014). Given the apparent ubiquity of anti-MERS-CoV IgG found in camels in several African countries, including Ethiopia, Kenya, Tunisia, Egypt, and Nigeria, it is likely that MERS-CoV-infected camels have been imported into Saudi Arabia multiple times over the past two decades. It is also possible that MERS-CoV was introduced to Saudi Arabia from Africa via the camel trade, which presents an alternate hypothesis to one proposed by Memish et al. (2013) that spillover of MERS-CoV from its original bat host occurred in Saudi Arabia. Indeed, one of the MERS-CoV isolates obtained by Briese et al. in Saudi Arabia came from a juvenile (< 1 year old) camel of African origin (Briese et al., 2014). While multiple bat species could potentially be involved in the ecology and zoonotic spillover of MERS-CoV, if we look at just the distribution of *Taphozous* spp. bats we see that their species ranges overlap with dromedary camel distribution in parts of northern Africa, Arabia, and even Australia (Figure A1-3). In Saudi Arabia, bats were observed roosting in abandoned structures used to occasionally house camels and other livestock species (Epstein and Olival, pers. comm.), and thus the ecological potential for spillover exists although more studies are needed to better quantify this overlap. The wide distribution of anti-MERS-CoV antibodies in dromedary camels in Africa also suggests that zoonotic transmission has occurred there, and

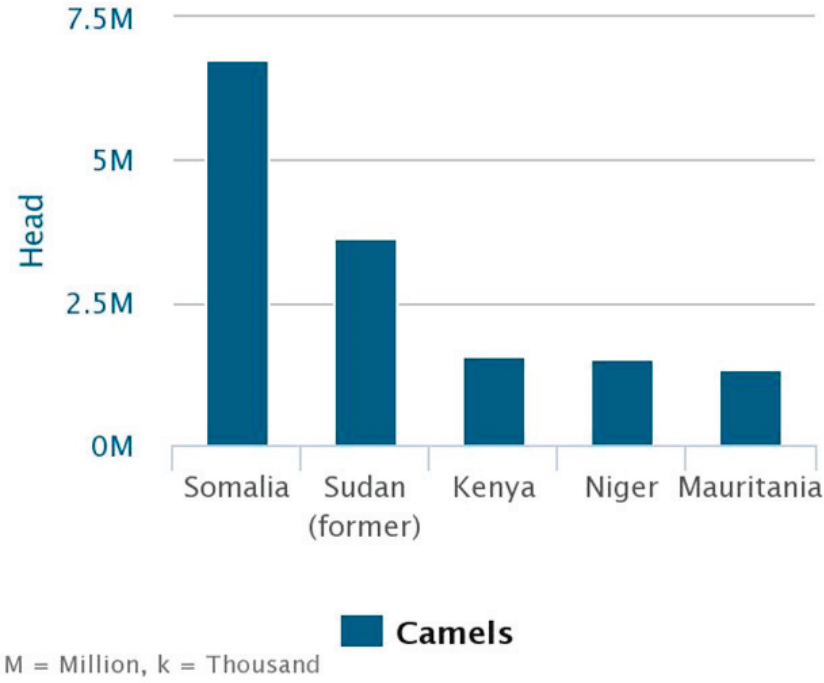

FIGURE A1-2 The top five camel-producing countries (1992–2013).
SOURCE: Data from FAO.

further studies are warranted to determine whether MERS-CoV is circulating in human populations in Africa.

Discussion and Future Research Needs

While there has been an abundance of data collected that suggests an apparent ubiquity of MERS-CoV infection in camels both in the Middle East and Africa, little is understood about potential mechanisms of zoonotic transmission or the frequency of zoonotic transmission. To date, no case-control study has identified high-risk exposures in human MERS cases, other than contact with another MERS case. Nor have there been sufficient epidemiological studies that might identify human MERS cases in Africa. Information about animal exposure has been absent or vague in the majority of reported cases from Saudi Arabia. The outbreak investigation in Qatar of two cases of MERS identifies a history of contact between the cases and sick camels, as well as confirmed MERS-CoV infection in the camels, which provides the best evidence to date that camels may be involved in MERS-CoV transmission to people. Phylogenetic analyses

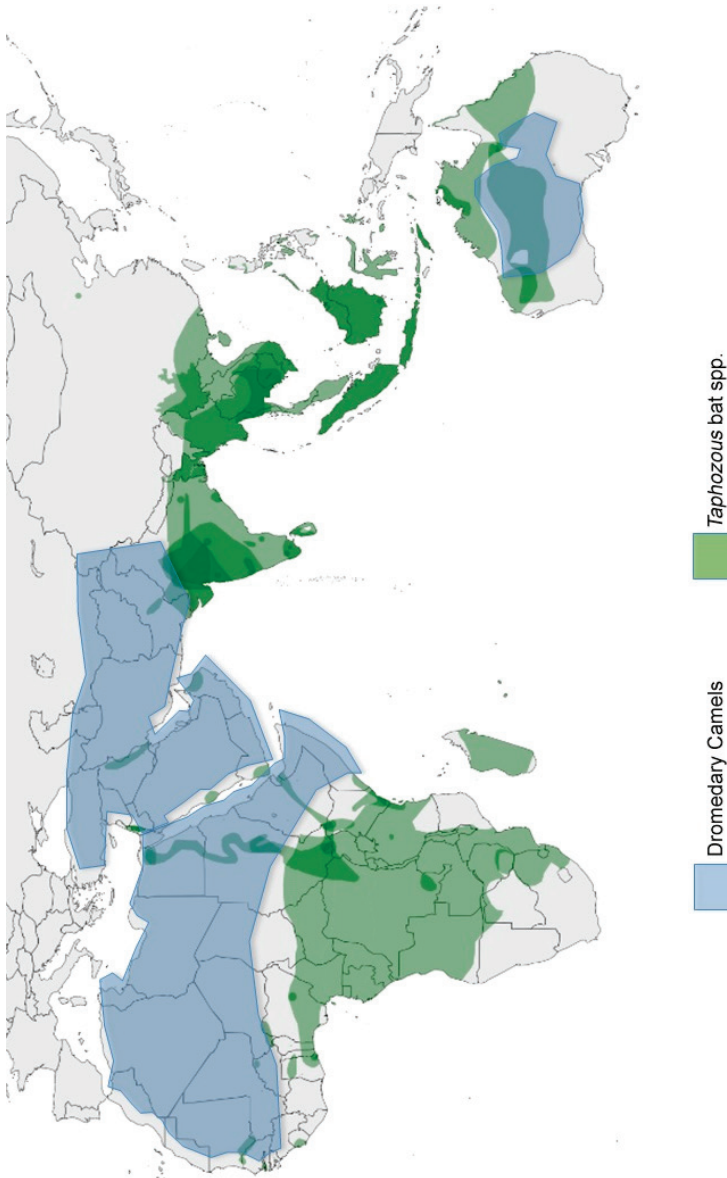

FIGURE A1-3 Geographic range of 14 species in the bat genus *Taphozous* (green; data from International Union for the Conservation of Nature [IUCN]) and overlap with approximate geographic range of dromedary camels (blue).
SOURCE: Data from FAO.

of MERS-CoV and MERS-related β-coronaviruses in bats support the hypothesis that bats are the natural reservoir for MERS-CoV; however, broader studies are needed to identify which bat species (and it may that more than one is involved) may be responsible for camel and/or human infections either in the Middle East, or more likely, in Africa. There is anecdotal evidence for bat–livestock and bat–human contact in Saudi Arabia, but detailed ecological investigations are needed to quantify the level of contact and identify the specific interfaces that may increase risk of spillover of MERS-CoV and MERS-related CoVs in Arabia and northern Africa. MERS-related CoVs have been identified in bat species in Africa (Annan et al., 2013; Ithete et al., 2013), although more extensive surveys of bats in countries where camels are produced, as well as identification of specific bat–camel interfaces, would provide valuable data on the potential for spillover from bats to camels or people. It will also be important to rule out the involvement of other wildlife species in MERS circulation. MERS-related coronaviruses have been identified in European hedgehogs (*Erinaceus europaeus*) (Corman et al., 2014b). A related species of hedgehog, *Paraechinus aethiopicus*, is commonly found across the Middle East and parts of northern Africa; however, to date there have been no epizootiological studies in wildlife species other than bats.

Multidisciplinary, ecological, and virological studies of MERS coronavirus will help further elucidate possible wildlife origins; mechanisms for primary human infections; risk factors for human infection; and the mechanisms and frequency of spillover from bats to camels or other domestic livestock, all of which would help answer the question of whether MERS-CoV is a recently emerging virus in humans or one which has simply escaped detection until 2012. As with SARS-CoV in China or Nipah virus in Bangladesh, the risk of human infection may be reduced when the proximal source of infection (e.g., pteropid bats and date palm sap for Nipah virus) is identified and transmission is interrupted (Nahar et al., 2010). However, without knowing the wildlife reservoir, the risk of reintroduction into animal or human populations cannot be managed. For nearly 10 years following the discovery that SARS-like CoVs were carried by Rhinolophid bats, it was assumed that SARS required intermediary animal hosts such as civets to become transmissible to people. The recent discovery of a strain of SARS-CoV in the same bats that uses the ACE2 receptor provided evidence that direct bat-to-human transmission was possible, and underscored the importance of reducing bat–human exposure. Opportunities for direct contact with bats or their excreta appear to be limited in Saudi Arabia, but more studies are needed to quantify and characterize this. Camel trade is likely the major driver of primary human infection in Saudi Arabia and other Gulf countries, where the majority of camels are imported from Africa. Expanded human and camel surveillance; educational outreach and public health initiatives designed to reduce exposure to camel bodily fluids, particularly nasal secretions; and improved infection control practices in health care settings will be instrumental in reducing the incidence of MERS in human populations.

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A2

CHIKUNGUNYA AT THE DOOR—DÉJÀ VU ALL OVER AGAIN?[2]

David M. Morens[3] *and Anthony S.* ***Fauci***[3]

In 2008, we noted that the global reemergence of dengue fever threatened U.S. residents (Morens and Fauci, 2008). An outbreak of locally acquired dengue subsequently occurred in Florida, and the risk of U.S. dengue outbreaks will probably continue indefinitely.

We now face a new threat posed by the unrelated chikungunya virus, which causes a disease clinically similar to dengue in a similar epidemiologic pattern, which is transmitted by the same mosquito vectors, and for which we also lack vaccines and specific treatments.

In December 2013, an outbreak of chikungunya fever appeared in the French sector of Saint-Martin/Sint Maarten and spread epidemically throughout the French West Indies to other Caribbean islands and contiguous Central and South American countries. By July 11, 2014, the Pan American Health Organization had reported more than 355,000 suspected and confirmed cases of chikungunya fever from more than 20 countries or jurisdictions in the Americas, with continuing local transmission and epidemic spread.

In 2014 in the continental United States, 232 imported cases of chikungunya fever had been reported as of July 15, according to the National Center for Emerging and Zoonotic Infectious Diseases at the Centers for Disease Control and Prevention (CDC); many of these cases occurred in the 14 or more states that harbor the classic mosquito vector, *Aedes aegypti,* that is capable of supporting

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local chikungunya transmission. Moreover, an even more tenacious vector mosquito, *Ae. albopictus,* has established itself in at least 32 states over the past three decades.

Chikungunya is an arbovirus (arthropod-borne virus) first described during a 1952 outbreak in southern Tanganyika (now Tanzania). It is an RNA virus within the alphavirus genus of the Togaviridae family. The name "chikungunya" derives from a word in the Kimakonde language meaning "to become contorted" or "to walk bent over," an apt description of the appearance of some infected people with arthralgias.

Typically, chikungunya disease manifests as acute onset of fever and prostration, muscle and joint pains, lymphopenia (as in many arboviral diseases), and frequently a nonspecific maculopapular rash that can be difficult to identify in dark-skinned patients. Symmetric arthralgias are usually prominent in phalanges, wrists, and ankles; after 1 year, at least 20% of patients still have severe recurrent joint pains. The case fatality ratio is about 1 per 1000, with most deaths occurring among newborns, the elderly, and the debilitated. The differential diagnosis for chikungunya includes dengue and other arboviral infections, as well as influenza. Chikungunya is distinguished from dengue clinically by persistent or recurring polyarthralgias, which are uncommon in dengue, and epidemiologically by a low rate of asymptomatic infection (as low as about 4%, vs. 50% or more with dengue).

Patterns of chikungunya emergence and reemergence are complex and incompletely understood. Mosquito-borne arboviruses typically exist in locale-specific enzootic cycles involving nonhuman vertebrates and one or more mosquito vectors. They may occasionally break out of their ecologic niches to infect humans, but human outbreaks are usually constrained by proximity to enzootic foci. For example, both St. Louis encephalitis virus and West Nile virus are flaviviruses that circulate in enzootic mosquito–bird–mosquito cycles. Spillover cases and outbreaks in humans are temporally, climatically, and geographically restricted by human exposure to these enzootic cycles, which leads to a pattern of sporadic and unpredictable, but local, reemergences.

Rarely, however, an arbovirus may evolve to permanently escape its enzooticity and become established as a human disease that is spread in a mosquito–human–mosquito cycle. Only three important arboviruses are known to have achieved this feat: the flaviviruses yellow fever and dengue, and chikungunya. Despite their phylogenetic distances, each of these viruses has become adapted to transmission by *Ae. aegypti,* the vector now provisionally implicated in the Caribbean chikungunya outbreaks (Weaver, 2014).

The epidemiology of chikungunya, like that of dengue and yellow fever, is related not only to mosquitoes and their environments, but also to human behavior. The warming and drying of North Africa about 5,000 years ago probably forced ancestral arboreal mosquitoes (*Ae. aegypti* formosus) to adapt to new environments in which humans were increasingly storing precious water (Powel and Tabachnick, 2013). This adaptation provided access to new mosquito breeding

sites, leading *Ae. aegypti* to develop as a subspecies closely tied to human habitation and creating a new ecologic niche into which evolving viruses could move. European involvement in West African slave trading, which began about 500 years ago, spread *Ae. aegypti* mosquitoes and their viruses around the world. The emergence of yellow fever, dengue, and chikungunya is thus a story of human behavior driving vector adaptation, which in turn has driven viral adaptation.

Over the past five centuries, these three diseases have caused periodic epidemics in tropical regions. Phylogenetic evidence suggests that chikungunya may have emerged and evolved in Africa, developing into distinct, distantly related West African and East Central South African (ECSA) clades. According to historians, chikungunya fever arrived in Asia and the Americas two or more centuries ago to cause, among other outbreaks, a notable epidemic in Batavia (now Jakarta) in 1779 (Carey, 1971) and a pandemic involving parts of the Western Hemisphere in the 1820s. These outbreaks were at the time called dengue, since chikungunya was not fully distinguished from dengue until the 1950s (Carey, 1971). Chikungunya may thereafter have left Asia and the Americas, only to return to Asia in the mid-1900s in the form of a new ECSA lineage (Weaver et al., 2012) transmitted by *Ae. aegypti*.

In 2004, another ECSA chikungunya virus spread pandemically from East Africa across the Indian Ocean, causing epidemics and seeding outbreaks as far away as Italy, France, and Southeast Asia. In the process, the virus acquired envelope gene mutations, giving rise to an ECSA Indian Ocean Lineage (IOL). These mutations significantly increased viral transmission by a different mosquito vector, *Ae. albopictus*—the vector that has been spreading globally and causing dengue outbreaks in the United States and elsewhere. There was little or no concomitant reduction in transmissibility by *Ae. Aegypti* (Weaver et al., 2012). Thus the new IOL chikungunya lineage could now be efficiently transmitted by each of the two mosquito vectors.

Surprisingly, preliminary viral genetic data from the Caribbean implicate not this current pandemic IOL strain but the older Asian chikungunya strain, which is so far unadapted to explosive *Ae. albopictus* transmission (Weaver et al., 2012); indeed, Caribbean data so far suggest that *Ae. aegypti* is the principal vector (Weaver, 2014). It appears that an epistatic mutation in the Asian–Caribbean chikungunya lineage may restrict transmission by *Ae. albopictus,* one of the two important potential Western Hemisphere vectors (Weaver et al., 2012).

Nevertheless, the course of the burgeoning epidemic in populations not previously exposed to alphaviruses offers little room for optimism: the growing number of imported chikungunya cases in the Americas raises concerns about possible future local transmission. The possibility that the Western Hemisphere may actually have experienced a chikungunya pandemic in the past (Cary, 1971) is hardly reassuring. The potential for chikungunya to become established in the Western Hemisphere, either in an urban mosquito–human–mosquito transmission cycle or in an enzootic cycle involving other vertebrates, must be considered.

Antiviral agents and monoclonal antibody treatments for chikungunya are in early stages of testing. Several chikungunya vaccines are in development (Weaver et al., 2012), including a viruslike-particle vaccine that appeared to be immunogenic, safe, and well tolerated in a recent phase 1 clinical trial at the National Institutes of Health; however, licensure is not imminent for any vaccine. Even when there is a vaccine, public health officials will face a significant challenge in determining whom and when to vaccinate, since chikungunya appears unpredictably and proceeds so explosively that epidemic catch-up vaccination is impractical. Thus, the current chikungunya threat to the United States must be met primarily with standard public health approaches such as mosquito control and avoidance. In addition, there is an important role for astute clinicians in diagnosing and reporting the disease when it occurs. In the meantime, we can only keep our fingers crossed—painful as that would be for many people infected with chikungunya—that the Caribbean epidemic will decline and the virus will depart from the Western Hemisphere, as it may have done nearly two centuries ago.

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A3

EMERGING INFECTIOUS DISEASES: THREATS TO HUMAN HEALTH AND GLOBAL STABILITY[4]

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The inevitable, but unpredictable, appearance of new infectious diseases has been recognized for millennia, well before the discovery of causative infectious

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agents. Today, however, despite extraordinary advances in development of countermeasures (diagnostics, therapeutics, and vaccines), the ease of world travel and increased global interdependence have added layers of complexity to containing these infectious diseases that affect not only the health but the economic stability of societies. HIV/AIDS, severe acute respiratory syndrome (SARS), and the most recent 2009 pandemic H1N1 influenza are only a few of many examples of emerging infectious diseases in the modern world (Fauci and Morens, 2012); each of these diseases has caused global societal and economic impact related to unexpected illnesses and deaths, as well as interference with travel, business, and many normal life activities. Other emerging infections are less catastrophic than these examples; however, they nonetheless may take a significant human toll as well as cause public fear, economic loss, and other adverse outcomes.

Determinants of Emergence and Reemergence

Historical information as well as microbial sequencing and phylogenetic constructions make it clear that infectious diseases have been emerging and reemerging over millennia, and that such emergences are driven by numerous factors (Table A3-1). Notably, 60 to 80 percent of new human infections likely originated in animals, disproportionately rodents and bats, as shown by the examples of hantavirus pulmonary syndrome, Lassa fever, and Nipah virus encephalitis (Committee on Microbial Threats to Health, 1992; Karesh et al., 2004; Morse, 2004).

TABLE A3-1 Some Major Factors That Underlie Disease Emergence and Reemergence

The Microbial Agent	The Human Host	The Human Environment
Genetic adaptation and change	Human susceptibility to infection	Climate and weather
Poly microbial diseases	Human demographics and behavior	Changing ecosystems
	International trade and travel	Economic development and land use
	Intent to harm (bioterrorism)	Technology and industry
	Occupational exposures	Poverty and social inequality
	Inappropriate use of antibiotics	Lack of public health services
		Animal populations
		War and famine
		Lack of political will

SOURCES: Committee on Microbial Threats to Health, 1992; Morens et al., 2004.

Most other emerging/reemerging diseases result from human-adapted infectious agents that genetically acquire heightened transmission and/or pathogenic characteristics. Examples of such diseases include multidrug-resistant and extensively drug-resistant (MDR and XDR) tuberculosis, toxin-producing *Staphylococcus aureus* causing toxic shock syndrome, and pandemic influenza (Committee on Microbial Threats to Health, 1992; Fauci and Folkers, 2012; Fauci and Morens, 2012; Karesh et al., 2012; Morens and Fauci, 2012; Morens et al., 2004, 2008; Morse, 2004; Morse et al., 2012; WHO, 2013).

Although precise figures are lacking, emerging infectious diseases comprise a substantial fraction of all consequential human infections. They have caused the deadliest pandemics in recorded human history, including the Black Death pandemic (bubonic/pneumonic plague; 25–40 million deaths) in the fourteenth century, the 1918 influenza pandemic (50 million deaths), and the HIV/AIDS pandemic (35 million deaths so far) (Fauci and Folkers, 2012; Morens et al., 2008).

Definition and Concepts

Two major categories of emerging infections—*newly emerging* and *reemerging* infectious diseases—can be defined, respectively, as diseases that are recognized in the human host for the first time; and diseases that historically have infected humans, but continue to appear in new locations or in drug-resistant forms, or that reappear after apparent control or elimination (Fauci and Morens, 2012). Emerging/reemerging infections may exhibit successive stages of emergence. These stages include adaptation to a new host (Parrish et al., 2008), an epidemic/pathogenic stage, an endemic stage, and a fully adapted stage in which the organism may become nonpathogenic and potentially even beneficial to the new host (e.g., the human gut microbiome) or stably integrated into the host genome (e.g., as endogenous retroviruses). Although these successive stages characterize the evolution of certain microbial agents more than others, they nevertheless can provide a useful framework for understanding many of the dynamic relationships between microorganisms, human hosts, and the environment. It is also worth noting that the dynamic and complicated nature of many emerging infections often leaves distinctions between emerging and reemerging infections open to question, leading various experts to classify them differently. For example, we describe as "reemerging" new or more severe diseases associated with acquisition of new genes by an existing microbe, e.g., antibiotic resistance genes, even when mutations cause entirely new diseases with unique clinical epidemiologic features, e.g., Brazilian purpuric fever (Papazisi et al., 2010). Similarly, we refer to SARS as an emerging disease a decade after it disappeared, and apply the same term to the related MERS (Middle East respiratory syndrome) β coronavirus which appeared in Saudi Arabia in late 2012 (van Boheemen et al., 2012).

Examples of Newly Emerging Infectious Diseases

The most salient modern example of an emerging infectious disease is HIV/AIDS, which likely emerged a century ago after multiple independent events in which the virus jumped from one primate host to another (chimpanzees to humans) and subsequently, as a result of a complex array of social and demographic factors, spread readily within the human population. AIDS was not recognized as a distinct entity until 1981 (Fauci and Folkers, 2012; Morens et al., 2008), after its initial detection among certain risk groups, such as men who have sex with men, recipients of blood products, and injection drug users. It was soon apparent, however, that the disease was not restricted to these groups, and indeed, the bulk of HIV infections globally has resulted from heterosexual transmission that has been heavily weighted within the developing world, particularly sub-Saharan Africa where a number of factors were responsible for this rapid spread; chief among these were human movement along truck routes accompanied by a high level of commercial sex work, inadequate public health infrastructures, poverty, and social inequality.

Other examples of disease emergences (Committee on Microbial Threats to Health, 1992; Fauci and Folkers, 2012; Fauci and Morens, 2012; Karesh et al., 2012; Morens and Fauci, 2012; Morens et al., 2004, 2008; Morse, 2004; Morse et al., 2012; WHO, 2013) include SARS, which emerged from bats and spread into humans first by person-to-person transmission in confined spaces, then within hospitals, and finally by human movement between international air hubs. Nipah virus also emerged from bats and caused an epizootic in herds of intensively bred pigs, which in turn served as the animal reservoir from which the virus was passed on to humans. The 2009 H1N1 pandemic influenza virus emerged from pigs as well, but only after complex exchanges of human, swine, and avian influenza genes (Morens et al., 2009). H5N1 influenza emerged from wild birds to cause epizootics that amplified virus transmission in domestic poultry, precipitating dead-end viral transmission to poultry-exposed humans. Additional examples are many (Committee on Microbial Threats to Health, 1992; Fauci and Folkers, 2012; Fauci and Morens, 2012; Karesh et al., 2012; Morens and Fauci, 2012; Morens et al., 2004, 2008; Morse, 2004; Morse et al., 2012; WHO, 2013); however, the variables associated with emergences are unique for each and typically complex.

Examples of Reemerging Infectious Diseases

Most of the important reemerging infectious disease agents first appeared long ago, but have survived and persisted by adapting to changing human populations and to environments that have been altered by humans. Dengue virus and West Nile virus (WNV), distantly related flaviviruses, serve as good examples. They have been spread by geographic movement of humans in association with the mosquito vectors for the diseases. For example, dengue came to the Americas

in association with the slave trade of earlier centuries. In this regard, slaves infected by mosquitoes in Africa presumably brought the infection to the Americas by seeding the mosquito population upon arrival (Laughlin et al., 2012). Similarly, WNV came to the United States in 1999 when an infected human, bird, or mosquito came by air travel from the Middle East to the Western Hemisphere, providing a source for introduction of infection to New World mosquitoes and birds. Pathogenic strains of dengue have also spread back from Southeast Asia to the Western Hemisphere, as has a major mosquito vector, *Aedes albopictus*. Unlike most arboviruses, which are partly or completely host-restricted, WNV has become adapted to multiple mosquito and avian species, a major factor in increasing its opportunity to infect humans. The lack of additional hosts undoubtedly drove the mosquitoes that are the vectors of dengue and the dengue virus itself to favor adapting to humans and to their behaviors and environments. The association of dengue with *Aedes* mosquitoes that live in and around human habitations mean that crowding, poor sanitation, and poverty provide ideal environments for transmission to humans (Laughlin et al., 2012). Host immunity factors are also thought to be involved in the severe/fatal form of dengue known as dengue shock syndrome (Laughlin et al., 2012).

Other non-arboviral examples of emerging infections abound. For example, cholera has repeatedly reemerged over more than two centuries in association with global travel, changing seasons, war, natural disasters, and conditions that lead to inadequate sanitation, poverty, and social disruption. Emergences of disease caused by community- and hospital-acquired *Clostridium difficile* and methicillin-resistant *Staphylococcus aureus* (MRSA) have been driven by increased and/or inappropriate use of antibiotics, and some hospital-acquired organisms such as MRSA have now moved into community transmission. The global emergence of plasmid-spread NDM-1 (New Delhi β-lactamase) Gram-negative pan-resistant organisms, linked to global antibiotic use and inadequate antibiotic stewardship, medical tourism, economic globalization, and other aspects of modern life, has prompted calls for development of international control mechanisms (Walsh and Toleman, 2012) that are applicable to a number of emerging bacterial diseases in the developing and developed world. Drug resistance mutations have also caused the reemergences of certain pathogens such as multidrug-resistant and extensively drug-resistant tuberculosis, drug-resistant malaria, and numerous bacterial diseases such as vancomycin-resistant enterococci. Fungi have made significant contributions to disease emergence as well. In Africa, cryptococcal disease has already surpassed tuberculosis as a leading cause of death (Park et al., 2009). Other examples of fungal emergence include comorbidities in HIV-infected individuals (17), *Cryptococcus gattii* epidemics in predominantly healthy persons in the U.S. (D'Souza et al., 2011; Perfect, 2012), and a 2012 U.S. nationwide epidemic of *Exserohilum rostratum* infections associated with contaminated pharmaceutical products (CDC, 2012).

Will We Ever Eliminate Emerging Infectious Diseases?

While it has become possible to eradicate certain infectious diseases (smallpox and the veterinary disease rinderpest), and to significantly control many others (dracunculiasis, measles, and polio, among others), it seems unlikely that we will eliminate most emerging infectious diseases in the foreseeable future. Pathogenic microorganisms can undergo rapid genetic changes, leading to new phenotypic properties that take advantage of changing host and environmental opportunities. Influenza viruses serve as a good example of emerging and reemerging infectious agents in their ability to rapidly evolve in response to changing host and environmental circumstances via multiple genetic mechanisms. New "founder" influenza viruses (Taubenberger et al., 2012) appear periodically, cause a pandemic, raise widespread population immunity, and then, in response to human immune pressures, evolve and persist for decades using multiple genetic evolutionary mechanisms to sustain continual immune escape. The 1918 influenza pandemic virus is one example: over the past 95 years, its descendants have evolved continually by antigenic drift, intra-subtypic reassortment, and antigenic shift, the latter producing new pandemics in 1957 and 1968 (Morens et al., 2009). Even the genetically complex 2009 pandemic H1N1 influenza virus is a descendant of the 1918 virus (Morens et al., 2009). Such continuous genetic hyper-evolution forces us to develop new influenza vaccines containing new antigens on an annual basis.

In the meantime, new human diseases keep emerging. As noted, in late 2012 the novel MERS coronavirus emerged in Saudi Arabia (van Boheemen et al., 2012), and in early 2013 a new H7N9 avian influenza virus became epizootic in Eastern China, causing 132 spillover infections of humans (as of June 7, 2013), with 28 percent case fatality (Li et al., 2013; WHO, 2013). Its pandemic potential, if any, remains to be determined. Whether or not such outbreaks become more widespread, they nonetheless attract global attention and require significant international effort to monitor and contain. Microbial advantages can be met and overcome only by aggressive vigilance, ongoing dedicated research, and rapid development and deployment of such countermeasures as surveillance tools, diagnostics, drugs, and vaccines.

We appear to be entering a new era in which several important emerging, reemerging, and stable infectious diseases are becoming better controlled (e.g., hepatitis B, rabies, *Haemophilus influenzae* type B, and even to some extent HIV/AIDS). However, our success in stopping the many new emerging diseases that will inevitably appear is not assured. We have many tools in our armamentarium, including preparedness plans and stockpiles of drugs and vaccines. But each new disease brings unique challenges, forcing us to continually adapt to ever-shifting threats (Committee on Microbial Threats to Health, 1992; Fauci and Folkers, 2012; Fauci and Morens, 2012; Karesh et al., 2012; Kilpatrick and Randolph, 2012; Morens and Fauci, 2012; Morens et al., 2004, 2008; Morse, 2004; Morse et al., 2012; WHO, 2013). The battle against emerging infectious diseases is a

continual process; winning does not mean stamping out every last disease, but rather getting out ahead of the next one.

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EMERGING INFECTIOUS DISEASES IN 2012: 20 YEARS AFTER THE INSTITUTE OF MEDICINE REPORT[6]

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Abstract

Twenty years ago (1992), a landmark Institute of Medicine report entitled *Emerging Infections: Microbial Threats to Health in the United States* underscored the important but often underappreciated concept of emerging infectious diseases (EIDs). A review of the progress made and setbacks experienced over the past 2 decades suggests that even though many new diseases have emerged, such as SARS (severe acute respiratory syndrome) and the 2009 pandemic influenza, significant advances have occurred in EID control, prevention, and treatment. Among many elements of the increase in the capacity to control EIDs are genomics-associated advances in microbial detection and treatment, improved disease surveillance, and greater awareness of EIDs and the complicated variables that underlie emergence. In looking back over the past 20 years, it is apparent that we are in a time of great change in which both the challenge of EIDs and our responses to them are being transformed. Recent advances support guarded optimism that further breakthroughs lie ahead.

Introduction

Twenty years ago (1992), a landmark Institute of Medicine (IOM) report entitled *Emerging Infections: Microbial Threats to Health in the United States* underscored the important but often underappreciated concept of emerging infectious diseases (EIDs) (Committee on Microbial Threats to Health, 1992). Although the IOM report was influential in thrusting the issue of EIDs squarely into scientific and public discourse, the awareness that diseases periodically emerge and reemerge actually goes back millennia (Krause, 1992; Morens et al., 2008a). For example, ancient Greek, Roman, and Persian writers documented the emergence of many new epidemics. During and after the 14th-century "Black Death"

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pandemic of bubonic/pneumonic plague, European city officials quarantined arriving ships to prevent its importation and set up quarantine stations to isolate and care for patients. In 1685, the scientist Robert Boyle presciently observed that "there are ever new forms of epidemic diseases appearing…among [them] the emergent variety of exotick and hurtful…" (Boyle, 1685; Creighton, 1894).

By the mid-19th century, the discovery of microbes as causative agents of infectious diseases led to the development of preventive countermeasures such as passive immunotherapy, vaccines, and drugs against infective agents (Fauci and Morens, 2012). These advances spurred optimistic predictions that infections would soon be conquered (Deming, 1894), and physicians and public health workers began to lose sight of the possibility of the emergence of new and previously unrecognized infectious diseases. To a large extent, it was the shock of the recognition of HIV/AIDS in the early 1980s, followed by the IOM report of 1992, that rekindled awareness of, and interest in, EIDs. Two decades after the IOM report, it is appropriate to ask what has been learned about EIDs, where have we succeeded or failed in our efforts to fight them, and what challenges remain.

The Perpetual Threat of Emerging and Re-Emerging Infectious Diseases

As predicted in 1992 (Committee on Microbial Threats to Health, 1992), previously unrecognized infectious diseases have continued to emerge, including variable Creutzfeldt-Jakob disease/bovine spongiform encephalopathy (vCJD/BSE), severe acute respiratory syndrome (SARS), and 2009 pandemic H1N1 influenza, and others have reemerged, e.g., disease caused by multiple-drug-resistant *Staphylococcus aureus* (MRSA), multiple-drug-resistant and extensively drug-resistant (MDR and XDR) tuberculosis, cholera, and dengue.

The recent EID with the greatest global impact has been HIV/AIDS. Over the past 3 decades, humankind has witnessed the unexpected emergence of, and then the relentless devastation resulting from, one of history's deadliest pandemics (Fauci and Folkers, 2012). At the same time, modern research tools have helped us to understand how, where, and when HIV emerged; to understand its pathogenesis and natural history; and to develop life-saving treatment and prevention modalities that have put the control of the HIV/AIDS pandemic within reach. Surely, future generations will look back on the era of HIV/AIDS as one of the most remarkable periods in the history of human disease, in which civilization was challenged by a devastating pandemic EID and aggressively addressed it from a scientific and global health standpoint, leading to the real possibility of effective control in a relatively timely manner.

Greater Awareness of EIDs Is Itself an Important Countermeasure

The term *EID* and the concepts of newly emerging and reemerging infectious diseases have recently become much more widely appreciated. The 1992 IOM report led to rapid and heightened awareness of this issue in the scientific, public health, medical, and lay communities. For example, both the United States Centers for Disease Control and Prevention (CDC) and the National Institute of Allergy and Infectious Diseases of the National Institutes of Health released EID research and response plans (CDC, 1994; NIAID NIH, 1994). In 1995, the CDC established an EID-oriented scientific journal, *Emerging Infectious Diseases*. Now in its 18th year, the journal has published nearly 10,000 articles and has become standard reading for many in the disciplines of microbiology, clinical infectious diseases, public health, and allied medical fields. Other microbiology and general medical journals emphasizing EIDs have been established, e.g., *PLoS Pathogens*, or expanded their coverage of EIDs, e.g., the *Journal of Infectious Diseases* and *Vaccine*, while *mBio* and other journals published by the American Society for Microbiology (ASM) have remained leaders in publishing important EID-related research.

Internet resources devoted to EIDs also have flourished. For example, ProMED was launched in 1994 as a grass roots effort by the Federation of American Scientists and has been continued by the International Society for Infectious Diseases. Today, ProMED's 60,000-plus subscribers from 185 countries can read—openly, online, and in real time—about virtually all important EIDs occurring anywhere in the world. This creates immediate awareness of epidemics not only for scientists but also for the public and the media. ProMED has made it extremely difficult for cautious governments to suppress outbreak information and has greatly enhanced the capacity of public health systems to control infectious disease outbreaks (Chan et al., 2010).

CDC has expanded the MMWR (*Morbidity and Mortality Weekly Report*), which is now abstracted in medical journals such as the *Journal of the American Medical Association*, so that every week practitioners around the world can get the latest information about EIDs. Such heightened EID awareness has been transformational and catalytic. It has become clear that the five or six EIDs emerging annually (on average) over the past 8 decades have disproportionately emerged from perturbed ecological niches, especially those in tropical areas with vector-borne enzootic diseases (Jones et al., 2008; Morens et al., 2004).

Genomics/Proteomics Facilitate Diagnosis, Prevention, and Treatment of EIDs

Since 1992, high-throughput genetics techniques have led to the sequencing of thousands of microorganisms, their vectors, and many of their hosts. Genomics and proteomics have helped in the discovery of new infectious diseases and in acquiring a better understanding of the pathogenesis of existing ones; have

substantially improved surveillance, diagnosis, and drug and vaccine design; and promise to help elucidate host susceptibility factors and host responses to treatment of infections. For example, by 2003, the genomes of the human species, the mosquito *Anopheles gambiae*, and the malaria parasite *Plasmodium falciparum* all had been sequenced, representing the first time that all the major actors in the drama of an important emerging/reemerging infectious disease had been characterized at the molecular genetic level (Greenwood and Owusu-Agyei, 2012; Morens et al., 2004). These breakthroughs are important additions to our continuing efforts to control malaria, which have had recent successes but still require new countermeasures. These genomic data are contributing to vaccine and drug development and are elucidating the pathogenesis of and human resistance and susceptibility to malaria (Greenwood and Owusu-Agyei, 2012).

Scientific Advances Have Redefined the Concept of EIDs

Genomics techniques, like PCR and high-throughput deep and whole-genome sequencing, that now greatly facilitate the discovery of EIDs (e.g., the etiologic agents of Hantavirus pulmonary syndrome and Kaposi sarcoma) also reveal previously unimagined genomic diversity among microbes. This diversity includes complex and evolving viral quasispecies and microbes that have undergone considerable interbacterial horizontal gene transfer, creating new phenotypic properties of virulence and drug resistance.

Given these and other advances in science and technology, it is now possible to perceive, as Dawkins argued decades ago (Dawkins, 2006), that the evolution and natural selection of human diseases are not simply a struggle between microbes and hosts. Rather, it is fought out at a more basic level of gene-to-gene competition, pitting the genomes of microbes against those of their hosts (many of whose genomes contain genetic evidence of past microbial encounters). Dawkins contended that the visible evidence of genomic survival is an organism's expressed phenotype, its "survival machine," which is akin to a simple virus being protected by its external protein coat; however, Dawkins proposed that we should think of natural selection as operating at the level of the gene, not the organism it encodes.

This picture becomes more complex when we consider the human microbiome. Specifically, our gut flora represents a complex "external" organ system comprising at least three different "enterotypes" that have coevolved with us over millennia and appear to affect our health, including by preventing and modifying infection (Kuss et al., 2011; Walter and Ley, 2011). Indeed, fecal transplantation is now a novel treatment for *Clostridium difficile* colitis (a potentially fatal EID) (Borody and Khoruts, 2011). Infants who start life with or develop "reduced" flora (e.g., via pre- or postnatal antibiotics) may be at increased risk of IDs and EIDs. Variations in the microbiome may also affect the occurrence of certain chronic diseases, allergies, and malnutrition (Blaser, 2011). In this newer view,

humans are not just static victims of virulent microbes but hubs of gene flow in which pathogens not only "seek" to survive environmental barriers and natural and acquired immunity but also compete with other microbes on the playing field that we think of as "us."

Additional conceptual advances in EIDs include the realization that many chronic diseases have a direct or indirect infectious basis, e.g., cervical, hepatic, and gastric cancers; gastroduodenal ulcers; hemolytic-uremic syndrome; and possibly some types of tics and obsessive-compulsive disorders (Fauci and Morens, 2012; Morens et al., 2004). We also have become aware of the critical role of microbial coinfections in the pathogenesis of certain infectious diseases (e.g., HIV and numerous opportunistic infections; influenza and measles in association with secondary bacterial pneumonias) and of nutrition, e.g., the link between vitamin A and measles (Morens et al., 2008b; Randolph and Rogers, 2010).

The "one-health" concept, which emphasizes understanding and studying the unity of human and animal infectious diseases (Coker et al., 2011), reflects growing awareness that the majority of human EIDs, probably more than 60 per cent (Chan et al., 2010), are of animal origin (zoonotic), a realization that has implications not only for disease surveillance but also for understanding pathogenesis and controlling disease. For example, HIV/AIDS, influenza, Lyme disease, tuberculosis, measles, plague, smallpox, and possibly even leprosy are directly or primarily of animal origin. Viral host switching, in some cases associated with rapid and complicated microbial comutations (Meyer et al., 2012), has become an important research topic (Meyer et al., 2012; Parrish et al., 2008) for both newer EIDs, such as SARS, and reemerging ones, such as influenza. The processes by which animal-adapted microorganisms leave their hosts and adapt to new species, such as humans, are largely unknown and represent an important challenge in the study of EIDs.

Moreover, host-switching is not just a one-way street from other animals to humans. For example, Ebola virus, a devastating disease for humans, has decimated African gorilla populations; in the United States, suburban expansion associated with deforestation has driven raccoons into the suburbs, increasing rabies transmission to and from them; and a human strain of *Staphylococcus aureus* has adapted to chickens, spread globally, and developed new mutations enhancing avian virulence (Lowder et al., 2009; Pedersen and Davies, 2009). These examples remind us that ecosystem dynamism in which humans play a critical role is a key variable in EID occurrence and prevention (Fauci and Morens, 2012; Morens et al., 2004).

The Past Is Prologue in the Study of EIDs

Since 1992, enormous strides have also been made in understanding the history of EIDs, most notably by genetic sequencing of historically preserved microbial DNA and RNA. Perhaps the most significant example is the 1918

pandemic influenza virus, which caused the deadliest single disease event in recorded human history (Morens et al., 2009). Although that pandemic occurred 15 years before influenza viruses were first identified, recent sequencing efforts from RNA in preserved tissues allowed full reconstruction of the 1918 pandemic viral genome, leading to a remarkable body of ongoing research and a greater understanding of how influenza viruses continue to emerge among humans and other animal species (Morens et al., 2009; Taubenberger et al., 2012).

Of the several thousand microorganisms already sequenced, those of historical importance include smallpox virus strains, the plague bacillus (*Yersinia pestis*) (Bos et al., 2011), and ancient tuberculosis organisms. Strikingly, paleovirus oncogenes have even been resurrected and studied in infectivity assays to find the original cellular receptors to which they had become adapted millions of years ago (Soll et al., 2010). Both traditional historical research and study of phylogenetic trees derived from gene sequencing of modern organisms have added significantly to these efforts, leading, for example, to the discovery that the initial jump of what became known as HIV from nonhuman primates to humans probably occurred nearly a century ago with multiple independent host-switching events that ultimately led to the pandemic that was first recognized in 1981 (Sharp and Hahn, 2011). Understanding the history and evolution of emerging microbes allows us to predict more accurately what their potential pandemic impact will be, and to understand how we can best prevent and control them.

Growing Optimism About the Control of EIDs

It is now becoming accepted that disease eradication has a legitimate place in the armamentarium of responses to EIDs (Fauci and Morens, 2012). Smallpox, a devastating reemerging disease for millennia, was eradicated in 1980, and the epizootic morbillivirus (measles-related) disease rinderpest was eradicated in 2011 (Breman et al., 2011; Morens et al., 2011). With dracunculiasis and polio disease close to eradication, with measles on the path to eradication, and with significant strides in controlling such diseases as hepatitis B and even malaria and HIV infection being made, it is now possible to realistically consider eradication as an ultimate means of controlling certain EIDs.

Even though antibiotic resistance has accelerated alarmingly, new generations of antibiotics have kept pace (albeit, barely), and vaccines against some of the most important diseases have been developed or improved, such as those against *Haemophilus influenzae* type B, pneumococci, and cancer-causing human papillomavirus strains. The development of antivirals and antiviral combination therapies has led to a historic breakthrough in helping to control HIV/AIDS (Morens et al., 2004) and major strides in curing chronic hepatitis C virus infection. Future directions in research and drug development likely will include better antibacterial and antiviral combination therapies as well as the development and

use of more narrow-spectrum drugs against infective agents, which are less likely to cause polymicrobial resistance.

In the 20 years since the IOM report on EIDs, remarkable progress has been made in understanding and controlling them. In 1992, HIV infection was considered a death sentence for most patients. In 2012, after the tragedy of more than 35 million AIDS deaths, persons treated early with combination antiretroviral therapy, although not "cured" of their viral infection, can expect to live normal life spans with only a low risk of transmitting infection to others. In 1992, at least a million children died annually of measles. In 2012, fewer than 100,000 are expected to die, and measles eradication based upon an already-available effective vaccine is a realistic near-term goal. In 1992, it was possible to enter villages in many developing countries to monitor poliovirus circulation by conducting childhood "lameness surveys." In 2012, most lame individuals are adults whose children are largely free of the threat of polio and probably will live to see it eradicated (poliovirus type 2 has already been extinguished).

Despite extraordinary progress during the past 2 decades, infectious diseases still kill 15 million people each year (Fauci and Morens, 2012), and new and deadly diseases continue to emerge and reemerge. The perpetual nature of the emergence of infectious diseases poses a continuing challenge, which is volatile and ever-changing. This challenge includes a need for constant surveillance and prompt, efficient diagnosis; a need to develop and deploy new vaccines and drugs to combat new diseases; and a need for ongoing research not only in developing countermeasures but also in understanding the basic biology of new organisms and our susceptibilities to them. The future is ever uncertain, because unimagined new diseases surely lie in wait, ready to emerge unexpectedly; however, our ability to detect and identify them, our armamentarium of treatment and prevention options, our capacity to undertake and maintain basic and applied research, and our commitment to eradicating certain EIDs have never been greater. We have made far-reaching advances in the past 20 years since the original IOM report, and scientists are guardedly optimistic that further breakthroughs lie ahead.

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A5

PANDEMIC PREPAREDNESS AND RESPONSE—LESSONS FROM THE H1N1 INFLUENZA OF 2009[8]

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A number of viruses have pandemic potential. For example, the coronavirus responsible for the severe acute respiratory syndrome (SARS), which first appeared in southern China in November 2002, caused 8096 cases and 774 deaths in 26 countries before coming to a halt by July 2003 mainly owing to isolation and quarantine (WHO, 2003). In terms of persistence, versatility, potential severity, and speed of spread, however, few viruses rival influenza virus. Endemic in a number of species, including humans, birds, and pigs, influenza virus causes annual outbreaks punctuated by occasional worldwide pandemics, which are characterized by sustained community spread in multiple regions of the world.

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Members of the World Health Organization committee for the review of the 2009 H1N1 pandemic and 2005 International Health Regulations, on whose work this article is largely based, include Preben Aavitsland (Norway), Tjandra Y. Aditama (Indonesia), Silvia Bino (Albania), Eduardo Hage Carmo (Brazil), Martin Cetron (United States), Omar El Menzhi (Morocco), Yuri Fedorov (Russia), Andrew Forsyth (New Zealand), Claudia Gonzalez (Chile), Mohammad Mehdi Gouya (Iran), Amr Mohamed Kandeel (Egypt), Arlene King (Canada), Abdulsalami Nasidi (Nigeria), Paul Odehouri-Koudou (Ivory Coast), Nobuhiko Okabe (Japan), Mahmudur Rahman (Bangladesh), Palliri Ravindran (India), José Ignacio Santos (Mexico), Palanitina Tupuimatagi Toelupe (Samoa), Patricia Ann Troop (United Kingdom), Kumnuan Ungchusak (Thailand), Kuku Voyi (South Africa), Yu Wang (China), and Sam Zaramba (Uganda). The committee secretariat was led by Nick Drager and included Dominique Metais, Faith McLellan, Mary Chamberland, Nadia Day, Alice Ghent, Sue Horsfall, Janet Kincaid, Phillip Lambach, Linda Larsson, Fabienne Maertens, Joan Ntabadde, Les Olson, Magdalena Rabini, Sarah Ramsay, Mick Reid, Chastine Rodriguez, Alexandra Rosado-Miguel, and Natasha Shapovalova.

Beyond spread, the degree to which a pandemic is defined according to the severity of the disease, or whether it may be simply described as often producing many illnesses and deaths, remains ambiguous (Doshi, 2011). At its worst, pandemic influenza can be catastrophic: the great influenza pandemic of 1918–1919 is estimated to have infected 500 million persons worldwide and to have killed 50 to 100 million persons (Taubenberger and Morens, 2006). In a typical year of seasonal outbreaks in the Northern and Southern Hemispheres, influenza virus causes as many as 5 million cases of severe illness in humans and 500,000 deaths (Lozano et al., 2012).

Over the past decade, sporadic cases of severe influenza and deaths in humans have been caused by a number of avian influenza A viruses, including the H5N1 virus, first detected in 1997, and the H7N9 and H10N8 viruses, first reported in 2013. Such sporadic cases may be harbingers of a gathering pandemic, but the likelihood is difficult to judge because it is not known how frequently similar zoonotic episodes occurred silently in the past, when surveillance was more limited, and did not cause pandemics.

The most recent global pandemic was caused by the influenza A (H1N1) strain, which was first detected in North America in 2009 (influenza A[H1N1] pdm09). This event prompted the first activation of provisions under the 2005 International Health Regulations (IHR), which went into effect in 2007 (WHO, 2008). Deliberations that led to the 2005 IHR revisions were shaped by experience in the SARS outbreak of 2003. The regulations delineate the responsibilities of individual countries and the leadership role of the World Health Organization (WHO) in declaring and managing a public health emergency of international concern.

The 2009 H1N1 pandemic presented a public health emergency of uncertain scope, duration, and effect. The experience exposed strengths of the newly implemented IHR as well as a number of deficiencies and defects, including vulnerabilities in global, national, and local public health capacities; limitations of scientific knowledge; difficulties in decision making under conditions of uncertainty; complexities in international cooperation; and challenges in communication among experts, policymakers, and the public.

At the request of the WHO, an international committee, which I chaired, reviewed the experience of the pandemic, with special attention given to the function of the 2005 IHR and the performance of the WHO (WHO, 2011a). Since this was the first time that the 2005 IHR was tested in a real-world situation, it was inevitable that aspects of the response to the series of outbreaks and subsequent pandemic could have been improved. Even though there were areas of outstanding performance, such as the timely identification of the pathogen, the development of sensitive and specific diagnostics, and the creation of highly interactive networks of public health officials, the most fundamental conclusion of the committee, which applies today, is not reassuring: “The world is ill prepared

to respond to a severe influenza pandemic or to any similarly global, sustained and threatening public-health emergency" (WHO, 2011a).

In this article, I focus on lessons from the global response to the 2009 H1N1 pandemic. I identify some of the key successes and shortcomings in the global response, on the basis of the findings and conclusions of the review committee. The article concludes by pointing to steps that can improve global readiness to deal with future pandemics.

Time Course of the 2009 H1N1 Pandemic

The first laboratory-confirmed cases of H1N1 influenza appeared in Mexico in February and March of 2009. Cases that were detected in California in late March were laboratory-confirmed by mid-April. By the end of April, cases had been reported in a number of U.S. states and in countries on various continents, including Canada, Spain, the United Kingdom, New Zealand, Israel, and Germany. On April 25, invoking its authority under the 2005 IHR, the WHO declared a public health emergency of international concern and convened the emergency committee called for in the regulations. The WHO also established a dedicated internal group to coordinate the response to the widening outbreaks. As of June 9, 2009, a total of 73 countries had reported more than 26,000 laboratory-confirmed cases, and the WHO declared on June 11 that the situation met the criteria for phase 6 — that is, a full-fledged pandemic (Table A5-1). By the time the pandemic had waned, in August 2010, virtually all countries had reported laboratory-confirmed cases (Figure A5-1). An interactive graphic showing the timeline of the 2009 H1N1 pandemic is available with the full text of this article at NEJM.org.

Evidence from the first outbreak in Mexico was alarming. An observational study of 899 hospitalized patients showed that 58 (6.5%) became critically ill, and of those, 41% died (Dominguez-Cherit et al., 2009). During the course of the pandemic, mortality among children, young adults, and pregnant women was much higher than in a typical influenza season, and there was substantial variation in severity among different regions of the world (Simonsen et al., 2013). In general, older adults fared relatively well, and the total number of influenza-related deaths worldwide (estimated ranges of 123,000 to 203,000 deaths (Simonsen et al., 2013) and 105,700 to 395,600 deaths (Dawood et al., 2012)) proved similar to the number in a relatively mild year of seasonal influenza. However, because of the proportionately higher mortality among children and young adults, the severity in terms of years of life lost was greater than in a typical year of seasonal influenza (Viboud et al., 2010).

TABLE A5-1 World Health Organization (WHO) Pandemic-Phase Descriptions and Main Actions According to Phase

Phase	Estimated Probability of Pandemic	Description	Main Actions in Affected Countries	Main Actions in Nonaffected Countries
1	Uncertain	No animal influenza virus circulating among animals has been reported to cause infection in humans	Developing and implementing national pandemic-influenza preparedness and response plans and harmonizing them with national emergency preparedness and response plans	Same as in affected countries
2	Uncertain	An animal influenza virus circulating in domesticated or wild animals is known to have caused infection in humans and is therefore considered a specific potential pandemic threat	Same as phase 1	Same as phase 1
3	Uncertain	An animal or human–animal influenza reassortant virus has caused sporadic cases or small clusters of disease in people but has not resulted in a level of human-to- human transmission sufficient to sustain community-level outbreaks	Same as phase 1	Same as phase 1
4	Medium to high	Human-to-human transmission of an animal or human–animal influenza reassortant virus that is able to sustain community-level outbreaks has been verified	Rapid Containment	Readiness for pandemic response

continued

TABLE A5-1 Continued

Phase	Estimated Probability of Pandemic	Description	Main Actions in Affected Countries	Main Actions in Nonaffected Countries
5	High to certain	The same identified virus has caused sustained community-level outbreaks in at least two countries in one WHO region	Pandemic response: each country implements the actions called for in its national plans	Readiness for imminent pandemic response
6	Pandemic in progress	In addition to the criteria for phase 5, the same virus has caused sustained community-level outbreaks in at least one other country in another WHO region	Same as phase 5	Same as phase 5

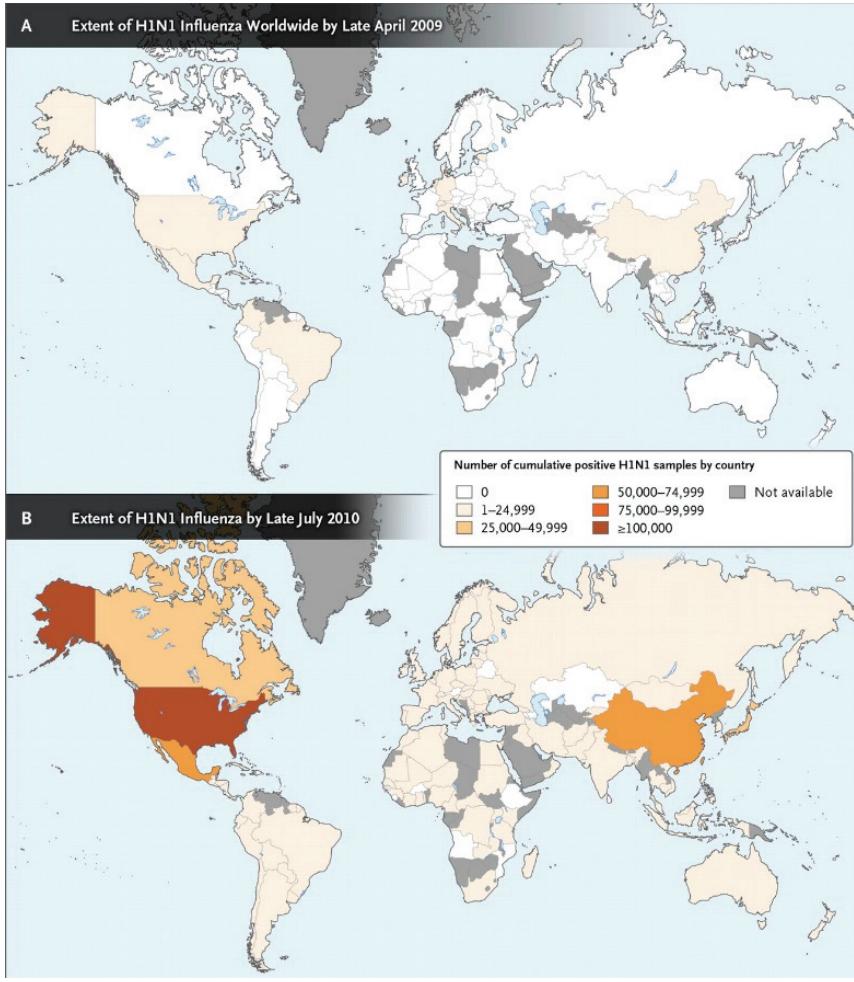

FIGURE A5-1 H1N1 influenza pandemic. Data are from the World Health Organization and http://fluNet.org.

2005 International Health Regulations

A number of provisions of the 2005 IHR proved helpful in dealing with the 2009 H1N1 pandemic. For example, the 2005 IHR established systematic approaches to surveillance, early-warning systems, and response in member states and promoted technical cooperation and sharing of logistic support. Communication among countries and the WHO was strengthened by the establishment

in each member state of National Focal Points—national offices that would be responsible for rapid collection and dissemination of emerging data and guidance.

A static and potentially outdated list of notifiable diseases in previous regulations was replaced by a more flexible flow diagram and decision tool that identified conditions warranting public health action. The 2005 IHR required, for the first time, that member states implementing unilateral measures that interfere with international traffic and trade inform the WHO and that they also provide a public health rationale and scientific justification for those measures. Most important, the 2005 IHR formally assigned to the WHO the authority to declare a public health emergency of international concern and take a leading role in the global response.

Despite these positive features, many member states did not have in place the capacities called for in the IHR, nor were they on a path to meet their obligations by the 2012 deadline specified in the document. Of the 194 eligible states, 128 (66%) responded to a WHO questionnaire on their state of progress in 2011. Only 58% of the responding member states reported having developed national plans to meet their core capacity requirements, and only 10% claimed to have fully established the capacities called for in the IHR (WHO, 2011a).

The IHR fails to specify a basis for virus sharing and vaccine sharing. This has been partially ameliorated in a framework for pandemic-influenza preparedness, adopted in 2011, that calls on member states to encourage vaccine manufacturers to set aside a fraction of their pandemic-vaccine production for donation and for discounted pricing in developing countries (WHO, 2011b). A glaring gap in the IHR, which has not been remedied, is its lack of enforceable sanctions. For example, if a country fails to explain why it restricted trade or travel, no financial penalties or punitive trade sanctions are called for under the 2005 IHR.

World Health Organization

The WHO is an indispensable global resource for leading and coordinating the response to a pandemic. In the 2009 H1N1 pandemic, the WHO had many notable achievements. The organization provided guidance to inform national influenza-preparedness plans, which were in place in 74 countries at the time of the first outbreak in North America, and helped countries monitor their development of IHR core capacities. The WHO Global Influenza Surveillance Network detected, identified, and characterized the virus in a timely manner and monitored the course of the pandemic.

Within 48 hours after the activation of provisions in the 2005 IHR, the WHO convened the first meeting of the emergency committee of experts who would advise the WHO on the status of the pandemic. Within 32 days after the WHO had declared a public health emergency of international concern, the first candidate reassortant vaccine viruses were developed, and vaccine seed strains and control reagents were made available within a few weeks. The Strategic Advisory Group

of Experts on immunization at the WHO provided early recommendations on vaccine target groups and dose. The WHO provided prompt and valuable field assistance to affected countries and efficiently distributed more than 3 million courses of antiviral drugs to 72 countries.

Against this backdrop of accomplishment, the WHO confronted systemic difficulties and made a number of missteps in the course of coping with the unfolding pandemic. Although the WHO is the only global agency with legitimate authority to lead the response to a pandemic, it is burdened by a number of structural impediments. First, the WHO is simultaneously the moral voice for health in the world and the servant of its member states, which authorize the overall program and budget. National interests may conflict with a mandate to equitably protect the health of every person on the planet. Second, the budget of the WHO is incommensurate with the scope of its responsibilities. Only approximately one quarter of the budget comes from member-state assessments, and the rest depends on specific project support from countries and foundations. These budget realities and the personnel-management requirements inherent in being a United Nations agency constrain flexibility.

Third, the WHO is better designed to respond to focal, short-term emergencies, such as investigating an outbreak of hemorrhagic fever in sub-Saharan Africa, or to manage a multiyear, steady-state disease-control program than to mount and sustain the kind of intensive, global response that is required to deal with a rapidly unfolding pandemic. Finally, the regional WHO offices are autonomous, with member states of the region responsible for the election of the regional director, budget, and program. Although this system allows for regional variation to suit local conditions, the arrangement limits the ability of the WHO to direct a globally coherent and coordinated response during a global health emergency.

In anticipation of a possible pandemic before 2009, public health authorities had focused on the threat of avian H5N1 influenza, and a signal feature among recognized cases of H5N1 influenza in humans was mortality exceeding 50% (WHO, 2013). Hence, it was expected that a newly emerging pandemic virus would cause many deaths as well as widespread disease, and the WHO said as much on its website on pandemic preparedness in advance of the 2009 H1N1 pandemic.

The prospects of a pandemic depend on the transmissibility and virulence of the virus and on the susceptibility of the population, which may vary according to age and past exposure to influenza viruses. Although a catastrophic pandemic probably depends on the emergence of a new antigenic type of influenza virus, it does not follow that every newly emerging influenza virus will produce an especially severe burden of influenza. For example, in the 40 years between the mid-1930s and mid-1970s, the 5 years of greatest excess mortality from influenza in the United States were 1937, 1943, 1953, 1957, and 1960, but among these years, only 1957 was marked by a new antigenic type (H2N2), and 1968 (the year

when H3N2 appeared) did not rank in the top five for severity (Dowdle, 1976). The expectation of a very severe pandemic was understandable in the context of H5N1 but not necessarily for every new antigenic type.

Since the formal criteria for advancing from one phase to the next higher phase in an emerging pandemic were based entirely on the extent of spread and not on severity, this led to public confusion about exactly what the WHO meant by a pandemic. The WHO lacked a consistent, measurable, and understandable depiction of the severity of a pandemic. This situation was problematic because, regardless of the definition of a pandemic, the decisions about response logically depend on both spread and severity. In addition, the defining phase structure that was based on spread was needlessly complex in that it defined more stages than there were differentiated responses, and the structure that seemed suitable for planning proved less suited to operational management.

The weekly requests by the WHO for data were overwhelming for some countries, particularly those with limited epidemiologic and laboratory capacity. As the epidemic progressed, it was not always evident to country officials that the data they submitted were being analyzed and used. Rather than focus on laboratory-confirmed cases, a surveillance model that relied on syndromic surveillance and selective, systematic virologic testing might have been more revealing (Lipsitch et al., 2009). Public health officials in some countries, such as the U.K. Health Protection Agency, produced weekly summaries that tracked domestic indicators of influenza spread and severity while noting pertinent global influenza activity, and this approach could hold lessons for other countries as well as for the WHO (HPA, 2014).

When the WHO convened an expert group, typically for a 1- or 2-day consultation, the practice of the organization was not to disclose the identities of the experts until the consultation was concluded. Similarly, the WHO kept confidential the identities of emergency-committee members convened under the provisions of the IHR, who would advise the WHO on the status of the emerging pandemic. Although the intent was to shield the experts from commercial or political influences, the effect was to stoke suspicions about the potential links between individual members of the emergency committee and industry (Flynn, 2010). Although the review committee uncovered no evidence of inappropriate influence on the emergency committee, the decision to keep the members' identities secret fostered suspicions about WHO decision making, which were exacerbated by the failure to apply systematic and open procedures for disclosing, recognizing, and managing conflicts of interest. A practice of confidentiality that was arguably fitting for a 1-day consultation was ill-suited to an advisory function that extended over a period of months.

The failure to acknowledge legitimate criticisms, such as inconsistent descriptions of the meaning of a pandemic and the lack of timely and open disclosure of potential conflicts of interest, undermined the ability of the WHO to respond effectively to unfounded criticisms. For example, the WHO was wrongly

accused of rushing to declare phase 6, or a full-fledged pandemic, because such action would trigger vaccine orders sought by manufacturers. This kind of suspicion proved hard for the WHO to dispel, despite the fact that the declaration of phase 6 was delayed until the sustained community spread in multiple countries in multiple WHO regions was incontrovertible.

The WHO made a number of operational missteps, including conferring with only a subset of the emergency committee, rather than inviting input from the full group, at a crucial point of deciding to declare progression from phase 4 to phase 5. Throughout the pandemic period, the WHO generated an unmanageable number of documents from multiple technical units within the organization and lacked a cohesive, overarching set of procedures and priorities for producing consistent and timely technical guidance. In addition, after the declaration of phase 6, a time when public awareness of the evolving pandemic was especially important, the WHO chose to diminish proactive communication with the media by discontinuing routine press conferences on the pandemic.

The most serious operational shortcoming, however, was the failure to distribute enough influenza vaccine in a timely way. Ultimately, 78 million doses of vaccine were sent to 77 countries, but mainly long after they would have done the most good. At its root, this reflected a shortfall in global vaccine-production capacity and technical delays due to reliance on viral egg cultures for production, as well as distributional problems. Among the latter were variation among wealthier countries and manufacturers in their willingness to donate vaccine, concerns about liability, complex negotiations over legal agreements with both manufacturers and recipient countries, a lack of procedures to bypass national regulatory requirements for imported vaccine, and limited national and local capacities to transport, store, and administer vaccines. Some recipient countries thought that the WHO did not adequately explain that the liability provisions included in their recipient agreements were the same as the provisions accepted by purchasing countries.

Looking Ahead

In light of these structural impediments and operational deficiencies, the world was very fortunate that the 2009 H1N1 influenza pandemic was not more severe. On the basis of its analysis, the review committee offered 15 recommendations to the WHO and the member states (Table A5-2). The report and recommendations were endorsed by the member states at the 64th World Health Assembly in May 2011, and the relevant WHO departments incorporated the recommendations into their biennial work plans (Hardiman et al., 2012). Some recommendations, such as improved protocols for vaccine sharing, have been carried out, some are within the power of the WHO to implement, and others depend on the actions and resources of the member states, which have yet to be committed to this purpose.

TABLE A5-2 Recommendations of the WHO Review Committee on the Functioning of the 2005 International Health Regulations (IHR) in Relation to the 2009 H1N1 Influenza Pandemic

- Accelerate the implementation of the core capacities required by the IHR
- Enhance the WHO Event Information Site*
- Reinforce evidence-based decisions on international travel and trade
- Ensure necessary authority and resources for all National Focal Points†
- Strengthen the internal capacity of the WHO for sustained response
- Improve practices for the appointment of an emergency committee
- Revise pandemic-preparedness guidance
- Develop and apply measures to assess the severity of a pandemic
- Streamline the management of guidance documents
- Develop and implement a strategic, organization-wide communications policy
- Encourage advance agreements for vaccine distribution and delivery
- Establish a more extensive public health reserve workforce globally
- Create a contingency fund for public health emergencies
- Reach an agreement on the sharing of viruses, access to vaccines, and other benefits
- Pursue a comprehensive influenza research and evaluation program

* The Event Information Site is a WHO website that, in the event of a pandemic, would serve as an authoritative resource to disseminate reliable, up-to-date, and readily accessible information related to the pandemic.

† National Focal Points are national offices that are responsible for the rapid collection and dissemination of emerging data and guidance.

Beyond institutional, political, and managerial difficulties, the most fundamental constraints on pandemic preparedness are the limits of scientific understanding and technical capacity. Perhaps because only three or four influenza pandemics tend to occur each century, at least in recent centuries, the annals of influenza are filled with overly confident predictions based on insufficient evidence (Neustadt and Fineberg, 1983). Studies designed to select for avian-origin viruses that can be transmitted more readily than the original virus in mammalian species (gain-of-function studies) may arguably help predict the pandemic potential of naturally occurring viruses but have raised concerns about the possibilities of intentional misuse and unintended consequences (Herfst et al., 2012; Imai et al., 2012). In the current state of scientific knowledge, however, no one can predict with confidence which influenza virus will become dangerous to human health and to what degree. The only way, potentially, to reduce this uncertainty is through a deeper biologic and epidemiologic understanding.

Disease detection, surveillance, and laboratory capacity are improving in many countries. The new techniques of Web-based field reports and analysis of Web-based search patterns can yield valuable intelligence that can give the world a head start on the next emerging pandemic (Brownstein et al., 2009).

In addition to superior surveillance and agreements on virus and vaccine sharing, the world needs better antiviral agents and more effective influenza

vaccines, greater production capacity, and faster throughput. One comprehensive assessment showed that the effectiveness of current influenza vaccines in practice is lower than is typically asserted, especially among elderly persons (Osterholm et al., 2012). The traditional methods of influenza-vaccine production, which rely on egg cultures, are often too slow to keep up with a first wave of pandemic spread, and in total, the annual capacity of influenza-vaccine production covers less than one third of the global population.

In early 2013, the Food and Drug Administration approved the first trivalent influenza vaccine produced with the use of recombinant technology (FDA, 2013), and other production methods are under active research and development. At least four lower-income countries have their own influenza-vaccine manufacturing facilities, and more are on the way. Most important, if research could yield a universal (non–strain-specific), long-lasting, safe, and effective vaccine against influenza, the annual frenzy of action against influenza would be transformed into a proactive, long-term prevention program (Kanekiyo et al., 2013; Treanor, 2004).

In the meantime, influenza outbreaks and pandemics will continue to challenge policy makers and public health leaders to make decisions under conditions of stress and uncertainty. Pandemics will challenge national authorities and the WHO to function more efficiently and effectively with insufficient resources. Preparation beyond planning, with advance protocols and agreements, the commitment of ready reserves of public health experts and a financial line of credit, and the fulfillment of the IHR requirements can all help. Whenever the next influenza pandemic arises, many more lives may be at risk. By heeding the lessons from the 2009 H1N1 pandemic, the international community will be able to cope more successfully the next time.

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A6

STUDYING ZOONOTIC DISEASES IN THE NATURAL HOST

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Introduction

Several factors, including the recent growth and geographic expansion of human populations and the intensification of agriculture combined with habitat disruption caused by climate change and deforestation, has meant that now, more than ever, there is a greater risk of emerging infectious diseases (EIDs) being transmitted to humans from wild and domesticated animals (Jones et al., 2008; Taylor et al., 2001). Moreover, increased global travel means there is a greater likelihood that EIDs will rapidly spread. Over the past three decades the incidence of EIDs has risen in humans, with around 70 percent being zoonotic in nature, and the majority being caused by viruses (Jones et al., 2008; Taylor et al., 2001) (Figure A6-1). Containment of these EID outbreaks has often been difficult owing to their unpredictability and the absence of effective control measures, such as vaccines and antiviral therapeutics. In addition, there is a lack of essential knowledge of the host immune responses induced by zoonotic viruses, particularly those that provide protection.

The Impact of EIDs

The World Health Organization has warned that the source of the next human pandemic is likely to be zoonotic and that wildlife is a prime culprit (see http://www.who.int/zoonoses/diseases/en).

While the current list of known EIDs is a major concern, it is the unknown EIDs out there, with a potential for efficient human-to-human transmission, that may pose the biggest threat. Over the past decade there have been a number of epidemics, raising the concern that they are precursors to a pandemic.

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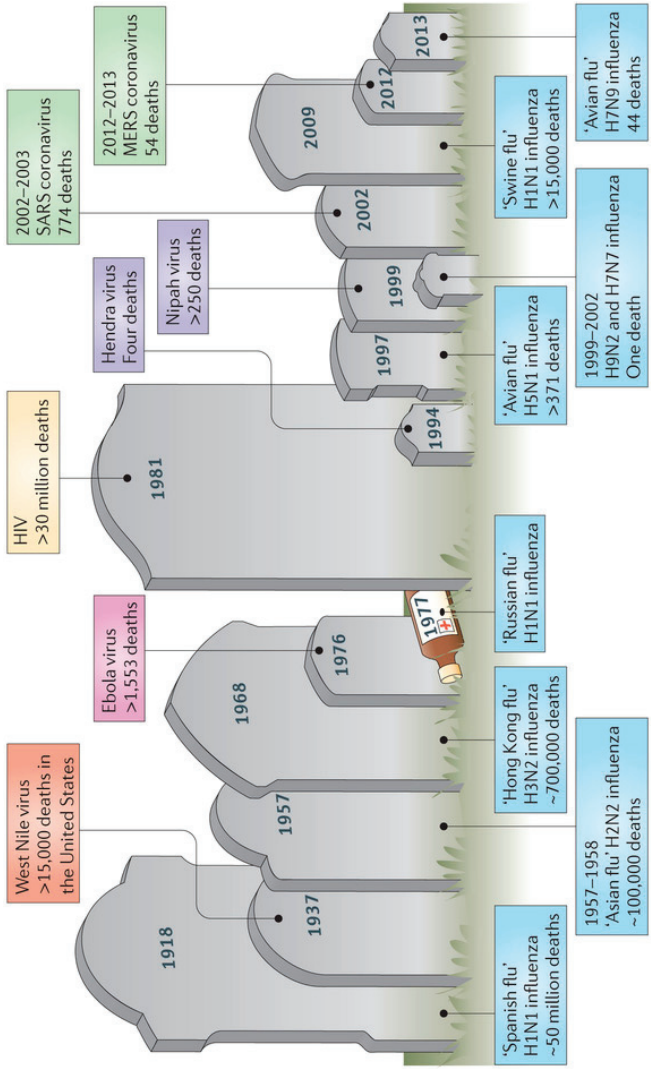

FIGURE A6-1 Emergence of zoonoses. Over the past century, humanity has witnessed the emergence of numerous zoonotic infections that have resulted in varying degrees of human fatalities. Influenza viruses originating from birds account for an important portion of these deaths and recently many new zoonotic viruses originating in bats, such as Hendra virus, Nipah virus, and severe acute respiratory syndrome coronavirus (SARS-CoV), have caused outbreaks with high mortality rates.
NOTE: Human immunodeficiency virus (HIV), West Nile virus (WNV), Novel coronavirus (MERS-CoV). H7N9 avian influenza as of August 11, 2013; MERS-CoV as of September 20, 2013.
SOURCE: Bean et al., 2013.

- The SARS epidemic in 2003–2004 claimed more than 800 lives and cost more than $80 billion to the global economy. It was shown to have involved virus transmission from bats to civet cats to humans.[12]
- In 2012 a novel coronavirus emerged in the Middle East (MERS-CoV) (Zaki et al., 2012) with a 30 percent mortality rate for the more than 500 cases so far confirmed, raising the concerns for a SARS-like pandemic.[13]
- Highly pathogenic H5N1 avian influenza virus has decimated poultry production in Asia and claimed more than 350 lives since 2003 with regular outbreaks continuing.[14]
- A new strain of virus (H7N9), which has never previously been seen in humans, appeared in April 2013. While this current strain of the avian influenza is not in a form that is able to transmit from human to human, there is still the possibility that it could mutate and trigger a serious pandemic (Yu et al., 2013).[15]
- Hendra virus in Australia, Nipah virus in Malaysia and Bangladesh, and hemorrhagic fever viruses (Ebola and Marburg) have over the past two decades emerged from bats via intermediate hosts such as horses and pigs to infect and kill humans.

A One Health Approach

Numerous emerging disease concerns are closely connected to the ever-increasing interactions between humans and wildlife. A number of drivers are associated with the emergence of disease from wildlife and spread to and among humans (Patz et al., 2004):

- The escalated need for food production to meet present and future demand has led to the intrusion of agriculture into previously untouched areas of the native environment (Pulliam et al., 2012).
- The impact of climate change has resulted in disturbances in ecosystems and a redistribution of disease reservoirs and vectors.
- Increased globalization and travel has significantly increased the chance, extent, and spread at which disease transmission occurs.

With this in mind, there has been a growing initiative to more closely address this animal–human–ecosystem interface. The term *One Health* describes a collaborative effort from multiple disciplines to support a holistic approach in the development of health strategies for people, animals, and the environment.

[12] See http://www.who.int/csr/don/archive/disease/severe_acute_respiratory_syndrome/en/index.html.

[13] See http://www.who.int/csr/disease/coronavirus_infections/archive_updates/en/index.html.

[14] See http://www.who.int/influenza/human_animal_interface/H5N1_cumulative_table_archives/en.

[15] See http://www.who.int/influenza/human_animal_interface/influenza_h7n9/en/index.html.

One Health unifies clinical and veterinary health and directly links this with environmental health research. The development of a framework directed at strengthening alliances between these sectors has facilitated the development and application of effective and sustainable community health strategies. There is a growing view that a One Health approach will be critically important for our preparedness for the next zoonotic pandemic.

Coevolution of Hosts and Pathogens

In many cases, natural host reservoirs seem to coexist with human pathogens, including zoonotic viruses, in the absence of disease, demonstrating the importance of this coevolutionary relationship. Bats are one example of a group of mammals that has a long coevolutionary history with the viruses they harbor. Although infection with viruses such as SARS, Hendra, and Ebola appears to result in little pathology in bats, infection of other susceptible hosts often causes severe disease and has fatal consequences. The evolution of unique immune mechanisms for the control of viral replication may be a mechanism for the ability of bats to coexist with viruses. Furthermore, it has been suggested that the severe pathology and disease that often occurs as a result of the spillover of viruses into other vertebrate hosts may also result from the disturbance of this finely tuned interaction of viral proteins with their targets in host cells (Wang et al., 2011). Understanding how bats and other natural reservoirs, such as birds, coexist with human pathogens could potentially lead to the discovery of mechanisms that control viral replication, and these could eventually be applied to help protect other susceptible species.

Animal Models for Zoonotic Pathogens

Traditional Animal Models

Mouse models have been fundamental to our understanding of immune responses to infection and disease outcomes. Indeed, mice have become the traditional "workhorse" because of their ease of handling, fast generation time, and the ready availability of mouse-specific reagents (Legrand et al., 2006). However, for a better understanding of EIDs, the laboratory mouse may not be the most appropriate model. There are often many differences in the symptoms of disease between the natural transmission and human hosts. Frequently, zoonotic infections appear as asymptomatic and nonlethal in the natural reservoir host, yet induce severe and potentially lethal disease in humans or other spillover hosts. Nevertheless, there are numerous factors that are likely to contribute to these differences including anatomical, physiological, metabolic, and behavioral traits as well as how the immune systems of these hosts interact with the same disease agent.

Nontraditional Laboratory Animal Models

There are many examples in the literature where nontraditional animal models have been highly informative for our understanding of host responses to pathogens (Hein and Griebel, 2003). For several decades the chicken has been used to study immunology, sexual development and developmental biology of the limbs, and the nervous system and brain (Le Douarin and Dieterlen-Lievre, 2013). Indeed, with the exception of mice and humans, arguably the most thoroughly characterized immune system is that of the chicken. Other animals have also provided a wealth of knowledge concerning aspects of immunity and human disease. For example, bats are now being used to study several emerging viruses such as Hendra, and ferrets are widely accepted as an excellent model for influenza infection—they are naturally susceptible to infection with human influenza viruses, and the disease pathology they develop resembles that of humans infected with influenza (Belser et al., 2011).

Natural Reservoir Hosts and Spillover Events

Why is it important to use both the natural animal reservoir and spillover host to study the host responses to zoonotic pathogens? In particular, understanding the differences between the immune systems of domesticated and wild animal hosts and comparing them to that of humans is crucial for unravelling the complex disease mechanisms involved in zoonotic infections (Figure A6-2). Furthermore, by studying the pathogen in its natural host we may be able to devise efficient control measures in that host, thereby disrupting their transmission to humans. This has important implications for predicting, preventing, and controlling spillover events and for the development of novel therapeutics and diagnostics. Table A6-1 lists selected zoonotic viruses and their reservoir hosts, susceptible hosts, and transmission hosts.

One example is the case of highly pathogenic avian influenza (HPAI). Waterfowl are natural hosts for avian influenza viruses and after infection with HPAI develop what appears to be a limited inflammatory response of the respiratory system, usually with little or no mortality. By contrast, in chickens and in humans HPAI viruses can induce a rapid and strong inflammatory response and potential hypercytokinemia, often referred to as a cytokine storm (Clark, 2007), and the infection may become systemic and induce severe disease symptoms (Karpala et al., 2011; Lee et al., 2007; Tisoncik et al., 2012). Chickens are acutely susceptible to infection with H5N1 strains of HPAI, which typically cause death within 18–36 hours. Studying waterfowl, such as ducks, and comparing their immune responses to influenza virus with those of the chicken may provide invaluable insight into the "aberrant" immune reactions that occurs in influenza spillover hosts, such as chickens, pigs, and humans (Figure A6-3).

Another interesting example is Hendra virus, which does not cause disease in fruit bats, the natural reservoir for this infection, but induces severe disease

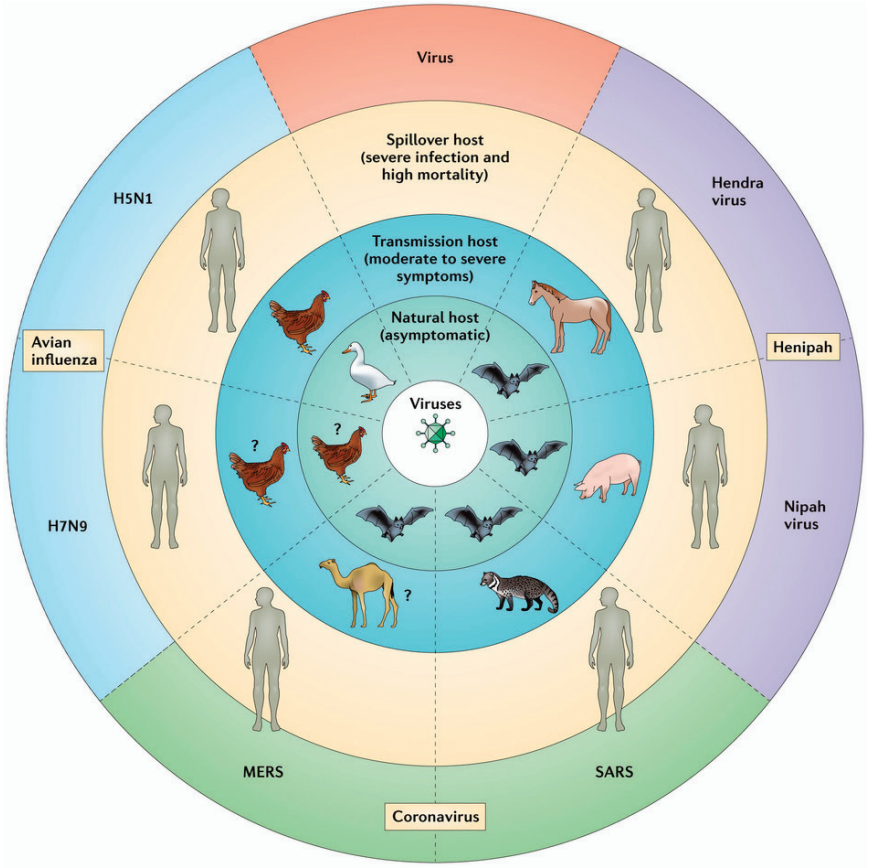

FIGURE A6-2 The outcome of disease severity is influenced by the host–pathogen interaction. Many zoonotic agents cause little or no signs of disease in their natural host such as wild birds and bats, while transmission hosts may present symptoms ranging from moderate (such as pigs for AI) to severe (such as horses for HeV) signs. The terminal or spillover host, such as humans in the case of H5N1 and HeV infections, can present with very severe symptoms and high mortality rates. For some of the most recently emerging EIDs such as H7N9 and MERS-CoV, natural and transmission hosts have not been identified.
SOURCE: Bean et al., 2013.

in horses and humans (reviewed in Mahalingam et al., 2012). The study of disease pathogenesis and immune responses to Hendra virus in horses has led to the development of a horse vaccine that will help reduce the rates of Hendra virus transmission from horses to humans (Mahalingam et al., 2012). Nevertheless, studying zoonotic viruses in wildlife is complex. For example, the fact that

TABLE A6-1 Natural Reservoir Hosts and Susceptible Hosts Involved in Transmission of a Selection of Emerging Zoonotic Viral Diseases, Including Those Deemed to Have Pandemic Potential

Disease (virus)	Known Reservoir Hosts	Other Susceptible Hosts	Transmitted to Humans by
Influenza[a]			
Avian (H5N1, H7N9, H7N7, H9N2, H3N2, and others)	Waterfowl	Bats	Chickens
	Wild birds	Cats	
Swine (H1N1, H3N2)		Dogs	
	Pigs	Ferrets	Pigs
		Foxes	
		Pigs	
		Horses	
		Poultry (chicken, duck, turkey)	
		Marine mammals	
SARS (SARS coronavirus)[b]	Bats	Civet cats	Civet cats
Dengue fever (Dengue virus)[c]	Primates	Unknown	Mosquitoes
Hendra (Hendra virus)[d]	Bats	Horses	Horses
		Ferrets	
Rabies (Rabies virus and other lyssaviruses)[e]	Bats	Cats	Bats
		Cattle	Dogs
		Coyotes	
		Dogs	
		Foxes	
		Horses	
		Mongooses	
		Primates	
		Raccoons	
		Sheep	
		Skunks	
		Wolves	

Ebola viral hemorrhagic fever (Ebola virus)[f]	Bats	Primates	Primates Bats
Japanese encephalitis (Japanese encephalitis virus)[g]	Pigs Wild birds	Horses	Mosquitoes
West Nile virus encephalitis (West Nile virus)[h]	Domestic and wild birds	Bats Camels Horses Marine mammals Reptiles > 30 vertebrate species	Mosquitoes Birds

[a] Centers for Disease Control and Prevention, 2013; World Health Organization, 2013a; World Organisation for Animal Health, 2013; Reperant et al., 2012; Swenson et al., 2010; Tong et al., 2012.

[b] Centers for Disease Control and Prevention, 2013; Shi and Hu, 2008.

[c] Centers for Disease Control and Prevention, 2013; Carver et al., 2009.

[d] Centers for Disease Control and Prevention, 2013; Clayton et al., 2013.

[e] Centers for Disease Control and Prevention, 2013; World Organisation for Animal Health, 2013; Hatz et al., 2012; Rupprecht et al., 2011.

[f] Centers for Disease Control and Prevention, 2013; World Health Organization, 2013a.

[g] Centers for Disease Control and Prevention, 2013.

[h] Centers for Disease Control and Prevention, 2013; World Organisation for Animal Health, 2013.

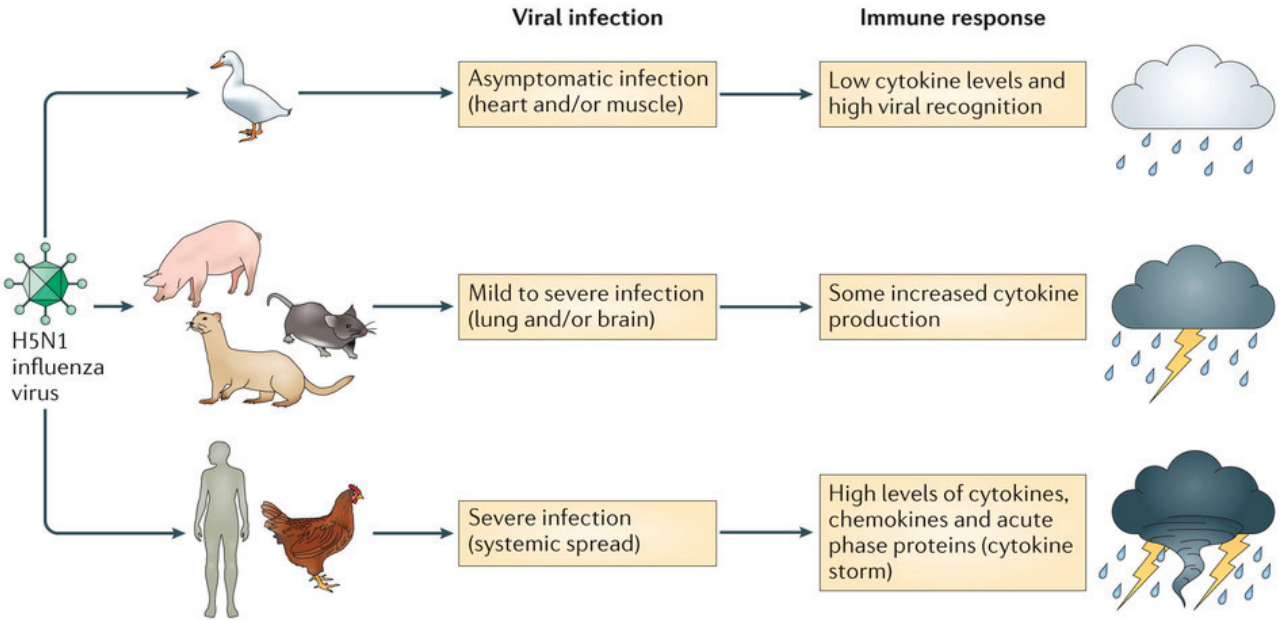

FIGURE A6-3 The host immune response to an infection influences the disease outcome. Infection with H5N1 can cause very different disease outcomes in different reservoir and spillover hosts. Waterfowl such as wild ducks are the natural virus host and develop a limited inflammatory response associated with low levels of cytokine expression. Intermediate hosts such as mice, pigs, and ferrets, often used as laboratory models, display mild to severe symptoms (depending on the H5N1 virus strain used) associated with elevated levels of proinflammatory cytokines. By contrast, spillover hosts such as chickens and humans display a rapid and strong inflammatory response, often referred to as hypercytokinemia (or cytokine storm), and the infection becomes systemic, causing severe disease symptoms and high mortality rates.
SOURCE: Bean et al., 2013.

infectious agents, such as henipaviruses and lyssaviruses, that cause lethal diseases in humans can coexist peacefully with bats raises many questions. It is still unclear how the bat immune system keeps these pathogens in check and enables host survival while allowing enough viral replication to facilitate transmission of the virus to spillover hosts. Other outstanding questions are how host–pathogen interactions are influenced by the genetics of the population, environmental factors, changes in demographics, food supply, co-infections, and interactions with other species as well as particular physiological, anatomical, and metabolic features of the host.

Despite the benefits of using nontraditional animal models, working with these systems presents a number of challenges. These may include a limited access to suitable subjects of the desired species, particularly in the case of fauna that may need to be wild caught and may be members of a species of high conservation value; lack of knowledge or experience in handling and husbandry methods suitable for high levels of biocontainment that will also meet contemporary ethics and welfare requirements; a paucity of reagents such as species-specific antibodies required to conduct traditional immunological experiments; and limited or no genome sequence information for the purpose of developing molecular biology tools. For species not traditionally used in the laboratory, new policies and procedures for housing, husbandry, restraint, and sampling may need to be developed on a case-by-case basis. In such instances, staff within the veterinary departments of zoological parks, zoologists, and veterinarians who specialize in exotic or unusual pets are valuable resources.

Comparative Immunology Studies

A major goal of studying immunology in natural hosts is to explain how infection with the same pathogen can have such vastly different outcomes in different species. Investigation of immunity in natural hosts is therefore an area that may yield significant discoveries and illuminate the nature of successful immune responses to agents that are typically associated with adverse disease outcomes in humans and other species. There is an extensive range of technologies that have been used to understand the pathogenesis and immune responses in reservoir and transmission species. These include molecular genetic tools such as DNA and RNA sequencing, transcriptomics, RNAi, miRNA, and genome-editing meganucleases. In addition, posttranslational analysis tools such proteomics, kinomics, and other protein modifications provide additional information.

Comparative genomics is a powerful approach for identifying genetic determinants underlying phenotypical differences between species. The recent advances in high-throughput sequencing techniques have facilitated whole-genome sequencing of a large number of species, including some that are reservoir hosts of important zoonotic viruses, and others that are susceptible to disease caused by those same viruses. Comparative analysis of these genomes can identify gene

candidates for disease-susceptibility or disease-resistance phenotypes. Quantitative transcriptomics by sequencing, for example RNA-sequencing (RNA-seq) and small RNA-seq, have enormous potential for identifying crucial differences between species in host responses to virus infection. These analyses can provide unprecedented detail and can be easily performed on species for which no species-specific reagents are available, as is the case for the natural hosts of many zoonotic viruses. Another application with strong potential in this field is genome-wide screening using RNAi (reviewed in Meliopoulos et al., 2012). As genome sequencing and RNAi technologies continually develop, it will soon be possible to compare host genes required for virus replication across virus-susceptible and virus-resistant species, such as chickens and ducks in the case of HPAI.

A major goal of sequencing bat genomes was to understand the genetic basis of virus–host interactions in a natural reservoir host (Zhang et al., 2013). More recently, analysis of the duck genome and virus-infected duck transcriptome has continued this trend. It has been observed that avians generally encode fewer cytokines than mammals, and appear to lack α- and θ-defensins. In contrast, ducks featured lineage-specific duplications of β-defensin and butyrophilin-like genes, suggesting a possible connection with the fundamental differences in disease outcome observed between chickens and ducks infected with HPAI (Huang et al., 2013). An area of potential interest will be to revisit genome sequences of animals with the hindsight that they are natural hosts for diseases of relevance to humans, for example the cat, dog, goat, armadillo, and camel genomes.

Clinical Implications of Wild Animal Studies

There are a number of key practical outcomes that research in wild and livestock animal species could achieve. For example, if we better understand influenza infection in pigs and birds, will we be better able to predict where the next pandemic might emerge or be able to develop vaccines or antivirals for the animals that prevent the crossover to humans? For example, if we understand how the bat and duck immune system responds to viruses, will this help us to develop new therapeutics and vaccines for preventing fatal infections caused by these viruses in humans? Can we engineer livestock that will be resilient to EIDs, such as influenza and Nipah viruses, thereby blocking the transmission cycle?

The ability of the segmented influenza genome to continually re-sort within different animal host species has a crucial impact on the epidemiology of influenza outbreaks. For example, the combination of viral gene segments from four virus strains circulating in three different species (human, swine, and poultry) led to the emergence of swine-origin influenza virus (S-OIV) in the human population (Itoh et al., 2009). The relevance of a lack of preexisting immunity became apparent as mortality and morbidity began to rise sharply in the 20- to 40-year-old age group (Chowell et al., 2009), while the older age group was associated with a lower infection risk (Fisman et al., 2009) that was due to a higher prevalence

of preexisting cross-antibodies that cross-reacted with the 2009 H1N1 virus. The impact of a novel influenza virus arising from wildlife species was again felt in February 2013, when an avian-origin influenza virus emerged in Zhejiang, China, causing more than 400 reported human infections and 100 deaths. This virus appears to have emerged from the mixing of influenza viruses from several avian sources, including ducks and wild birds (Chen et al., 2013). It was the first report of an H7N9 influenza virus infecting humans, and was met with little preexisting immunity. Furthermore, vaccines against H7N9 are predicted to be poorly immunogenic due to few T cell epitopes on the H7 molecule compared to other HA subtypes (De Groot et al., 2013). Despite this particular virus being highly pathogenic in humans, natural infections with H7N9 viruses in chickens, ducks, and other birds are asymptomatic and elicit an immune response that can be detected serologically (WHO, 2013b). This is in stark contrast to H5N1 and H7N7 where disease in humans was associated with a highly pathogenic phenotype in poultry (Perdue and Swayne, 2005). The immunologic component to this disease in birds suggests that further study of these emerging viruses in poultry and other bird species are required to understand factors influencing disease susceptibility and transmission.

Identification of key differences in immune pathways between susceptible and nonsusceptible hosts may offer clues to develop disease intervention strategies. As mentioned previously, for HPAI infection, ducks and chickens represent natural and spillover hosts, respectively. Chickens have lost expression of the innate immune sensor retinoic acid-inducible protein I (RIG-I), which may be an important clue in explaining why they suffer close to 100 percent mortality from HPAI. On the other hand, ducks (which have intact RIG-I expression) develop only mild symptoms in response to HPAI infection and usually survive. It has been shown that the transfection of duck RIG-I into chicken cells induced IFN-β promoter activity and limited the replication of low and highly pathogenic avian influenza strains, suggesting a key role of RIG-I in the ability of ducks to be resistant to influenza-mediated disease (Barber et al., 2010). While the consequences of the absence of RIG-I in chickens to other viral infection is not understood, it would nevertheless be of interest to generate transgenic chickens that express duck RIG-I and investigate whether these animals are less susceptible to disease following HPAI infection or indeed infection with other viruses.

Concluding Remarks

What can we learn from nature's experiments? Studying the responses to zoonotic pathogens in the natural reservoir host and comparing it to the responses in spillover hosts will help identify key processes in disease susceptibility and transmission. With the current emphasis on a One Health approach, researchers are turning to an analysis of the immune response of natural host species for a greater understanding of emerging zoonotic diseases. Together with adoption of

new genomic information and genome editing capability we can identify new strategies to prevent and minimize the impact of EIDs and enhance our pandemic preparedness.

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A7

MEDUSA'S UGLY HEAD AGAIN: FROM SARS TO MERS-COV[16]

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Eleven years ago, a novel coronavirus, the severe acute respiratory syndrome coronavirus (SARS-CoV), emerged, causing respiratory illness characterized by relatively high mortality and high rates of transmission in hospitals. The SARS virus taught the scientific community the value of unprecedented collaboration. In February 2013, a similar yet novel coronavirus, the Middle East respiratory syndrome coronavirus (MERS-CoV), was identified. At this writing, approximately 200 cases have been reported and many more are probably undetected (Cauchemez et al., 2014). Like SARS, MERS-CoV infection causes severe respiratory disease for which there is no effective therapy. In this issue of *Annals*, Arabi and colleagues (2014) report a consecutive series of 12 patients with severe respiratory failure, carbon dioxide retention, and extrapulmonary manifestations of sepsis requiring intensive care. One-third of cases were hospital acquired, and 68% of the patients died. Although an intensive search for antivirals continues, a gap remains between this serious disease and effective therapy. Evaluation of the potential effectiveness of convalescent serum therapy and therapeutic drug options is needed to improve our response to emerging diseases.

In Arabi and colleagues' case series, all patients had comorbid illness that may have increased susceptibility to infection. Similar to SARS, MERS-CoV affects middle-aged persons and spares children. However, preexisting chronic illness is more common in patients with severe MERS-CoV–associated pneumonia than in those with SARS: Rates of diabetes, renal disease, and heart disease are 68%, 49%, and 28%, respectively, in patients with MERS versus 24%, 2.6%, and 10%, respectively, among those with SARS (Assiri et al., 2013b). Carefully designed case–control studies are essential to determine the exposures that lead to infection. Such studies could identify potential preventive strategies and, when coupled with translational studies of genetic and other biological factors, could further define the key factors modulating disease severity.

Of note in Arabi and colleagues' report (and similar to SARS) is the nosocomial transmission among close contacts, with 33% of the cases associated with

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health care. Other reports from Jordan (Hijawi et al., 2013), the United Kingdom (HPA, 2013), and the Al-Hasa province of Saudi Arabia (Assiri et al., 2013a) implicated health care transmission in an even greater proportion of cases. In the Al-Hasa report, epidemiologic analysis suggested that 91% of reported cases resulted from transmission in health care facilities. Genomic analysis subsequently identified close phylogenetic clustering of MERS-CoV isolates consistent with human-to-human transmission (Cotton et al., 2013). Although the investigations of Arabi and colleagues and others (Assiri et al., 2013a; Hijawi et al., 2013; HPA, 2013) have found a relatively low risk for MERS-CoV infection and illness in exposed health care personnel, 30 of the first 161 reported MERS-CoV case patients were health care providers and new cases continue to occur in this population (WHO MERS-CoV Research Group, 2013).

Analysis to date suggests that MERS-CoV does not yet have pandemic potential. A model based on published data used the rate of MERS-CoV introduction into the population in the Jordan and Al-Hasa outbreaks to calculate the basic reproductive number (R_0)—that is, the number of secondary cases per index case in a fully susceptible population (Breban et al., 2013). For MERS-CoV, R_0 is estimated to be between 0.60 (95% CI, 0.42 to 0.80) and 0.69 (CI, 0.50 to 0.92). At first blush, this is comforting: Prepandemic SARS virus had an R_0 of 0.8. However, we must keep in mind both the rapid evolution that occurred with SARS and that it emerged in a much more densely populated region. Given the right environment and a crowded part of the world, MERS-CoV might propagate more readily.

As with SARS, we are indebted to international collaboration and a ProMED post that alerted the world to a new virus on 15 September 2012. Early recognition allowed the World Health Organization and other public health authorities to enhance surveillance and develop mitigation strategies. To date, all cases have been directly or indirectly linked to travel to or residence in countries in the Arabian Peninsula. How long will this last, given minimal data on specific exposure risks for infection and persistent health care transmission?

The question remains of whether MERS-CoV infection is occurring due to repeated introductions from an animal reservoir with subsequent limited transmission in humans or from sustained human-to-human transmission, with most cases being subclinical disease in patients without underlying medical conditions. Camels and bats have been implicated as potential reservoirs, but most case patients have not been exposed to these animals and the search for the source of human exposure continues (Perera et al., 2013; Reusken et al., 2013). As reported cases of MERS-CoV increase, we must not lose sight of the most important lesson of SARS: the value of transparency in reporting and of effective international collaboration in public health and research.

Does health care transmission continue because of failure to adhere to infection control practices or despite practices previously believed to be adequate to control the transmission of infection? The concentration of vulnerable patients,

the frequent movement of patients, and the many daily contacts make health care facilities the perfect breeding ground for MERS-CoV transmission. This, in combination with known imperfect adherence to routine infection prevention practices, suggests that early recognition of possible MERS-CoV infection is critical. Intensive surveillance for cases combined with the use of standard, contact, and droplet precautions for persons with suspected or confirmed disease aborted the Al-Hasa outbreak (Assiri et al., 2013a). Because we know little about how the virus is transmitted, it is not surprising that the Centers for Disease Control and Prevention and the World Health Organization disagree on the need for airborne isolation. Data are unavailable to discount either approach.

Arabi and colleagues provide a stark reminder of lessons learned from SARS. Infection with MERS-CoV causes respiratory failure with extrapulmonary organ dysfunction for which there is no effective treatment. Mortality remains high. Health care–associated MERS-CoV transmission to patients, workers, and visitors remains significant but is underplayed. Focus on the health care setting may prevent continued human-to-human transmission among at-risk patients. We applaud these brave authors for providing independent data and enhancing the scientific collaborations that MERS-CoV has created. Globalization and emerging viruses combine to demand new levels of scientific transparency and collaboration to effectively protect populations, a change we must all strive to achieve.

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A8

THE RELATIONSHIP BETWEEN ECO-SOCIAL SYSTEM CHANGES, THE ANIMAL–HUMAN INTERFACE, AND VIRAL DISEASE EMERGENCE

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Introduction

The past 20 years have seen a perceived and probably real increase in the number of viruses emerging that are pathogenic for animals and for both animals and humans. An important question is whether this pattern is indeed real or a consequence of improved diagnostic tools and surveillance. If it is indeed real, as it is generally believed to be, it represents a threat to humanity, and it is therefore important to understand the underlying causal mechanisms in order to be able to effectively predict and control, and ideally prevent, such emergence events. To be able to achieve, it is not only necessary to have good science, but an effective relationship between science and policy is required. To complicate matters further

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both science and policy will need to have a global or regional rather than just a local or national perspective.

Context

Humankind has had a dominating influence on our global ecosystem for some time now, which is expressed in the notion that we are living in the age of the anthropocene (Crutzen, 2002; Ellis et al., 2013). So-called megatrends can be used to express current views of the likely future behavior and trajectory of humanity. The European Environment Agency published a global megatrends study that predicted, among a number of other trends, for the next 30 to 100 years an increasing scarcity of natural resources, increasing economic divergence, increasing urbanisation, and increasing risk of wild animal species extinction and biodiversity loss (Anonymous, 2011). These particular megatrends (as well as others not mentioned here) and their interactions will strongly influence viral emergence and associated policy responses. While a better understanding of the causal mechanisms of emergence is necessary, it has to be recognised that the general public is likely to attribute a higher priority to policy responses to risks other than the ones associated with diseases, which will therefore influence the relative prioritisation of policy makers. Since 2005, the World Economic Forum has commissioned an annual global risk mapping study. The results from 2014 (similar to previous years' reports) demonstrate how much less significance people from around the world attribute to the risk of pandemic disease compared with the five most highly ranked risks: fiscal crises, unemployment, water crises, income disparity, and climate change (Figure A8-1) (Anonymous, 2014a).

Eco-social Changes

Accelerated economic development over the last 20 years has involved a large number of eco-social changes that affect the risk of viral emergence. Increasing urbanization is one of the key features of this development, and it has been predicted that by 2050 6.3 billion of the total human population of 9.3 billion will live in urban areas (Anonymous, 2012). Transport networks have become extremely effective, and as a consequence most locations around the world are within a 6–12-hour travel distance from the nearest city of 50,000 or more people (Anonymous, 2009b). The expansion of human habitat has resulted in a reduction of global forest cover, with 2.3 million sq km being lost against a gain of 0.8 between 2000 and 2012. The tropical climate domain has an increasing trend of 2101 sq km/year in annual forest loss (Hansen et al., 2013). This pattern is influenced by a number of factors, one of them being the increasing demand for food, in particular meat. The graph presented in Figure A8-2 shows a general global trend toward more intensive meat production. While this growth has been close to linear in Europe and North America between 1961 and 2012, China and

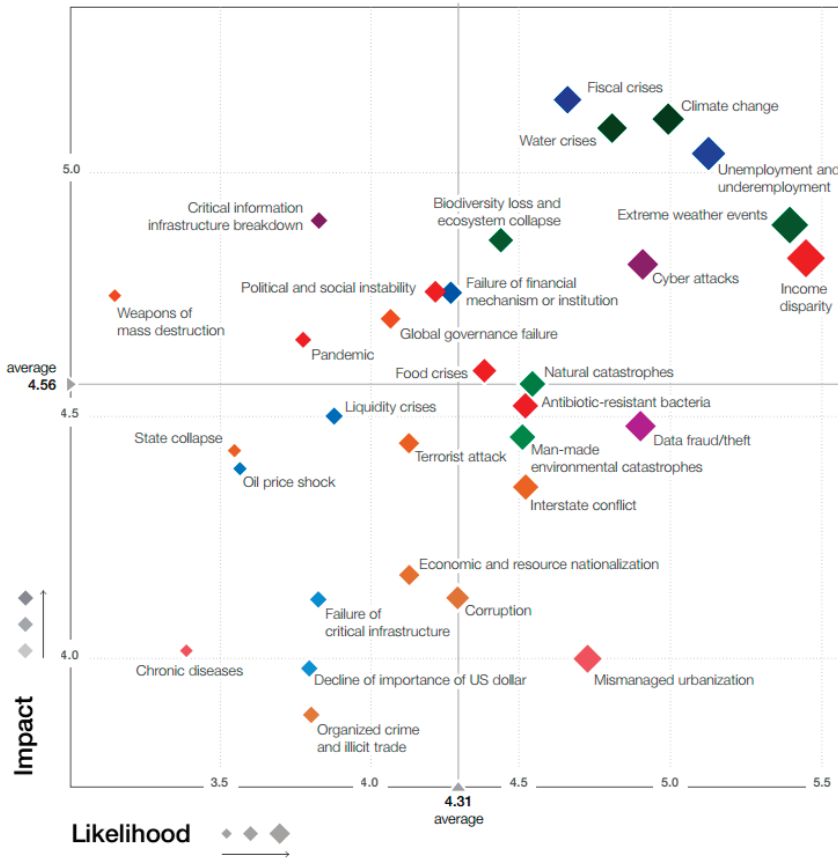

FIGURE A8-1 Perceived impact and likelihood of different risks based on a global risk perception survey, 2013–2014.
SOURCE: Anonymous, 2014a, from "Global Risks 2014," World Economic Forum, Switzerland, 2014.

Southeast Asia have experienced a period of exponential growth resulting in the highest meat production density. This pattern is likely to continue with continuing economic development in Asian countries where human population density is already very high, but also in other regions of the world such as Africa and South America.

The need to increase food production intensity has resulted in initiatives such as the Singapore-Jilin Food Zone project. This includes setting up a 1,450 sq km area in China's Jilin province that is free from foot-and-mouth disease and will be used for food production, including raising pigs, chickens, and cattle at high

density. A key objective of setting up this food production zone is to ensure a high food safety standard by introducing the most up-to-date food production and biosecurity methods. The consumers of the food produced in this zone will be primarily from China and Singapore.

A general global phenomenon has been that the value chains connecting the food producers with consumers, which were mainly local a century ago, in many cases have become globalised. An important driver for this development has been cost-effectiveness together with a demand for increased product variety and quality. An example for this situation is the sevenfold increase in the value of imports of pigs and pig meat products to China from other parts of the world between 2005 and 2011 as a consequence of increased demand for product quality combined with increased purchasing power of Chinese middle class consumers (see Figure A8-3). This indicates the speed at which economic development in one part of the world can lead to major changes in production intensity in other parts and shifts of global patterns of product flow.

Production chains also have become increasingly fragmented and therefore product quality control has become more challenging, as was demonstrated in the European Union during the *E. coli* O104:H4 outbreak in 2011 and the horse meat adulteration scandal in 2013 (Anonymous, 2014b; Appel et al., 2012). Attribution of negative or positive characteristics of food products in such fragmented

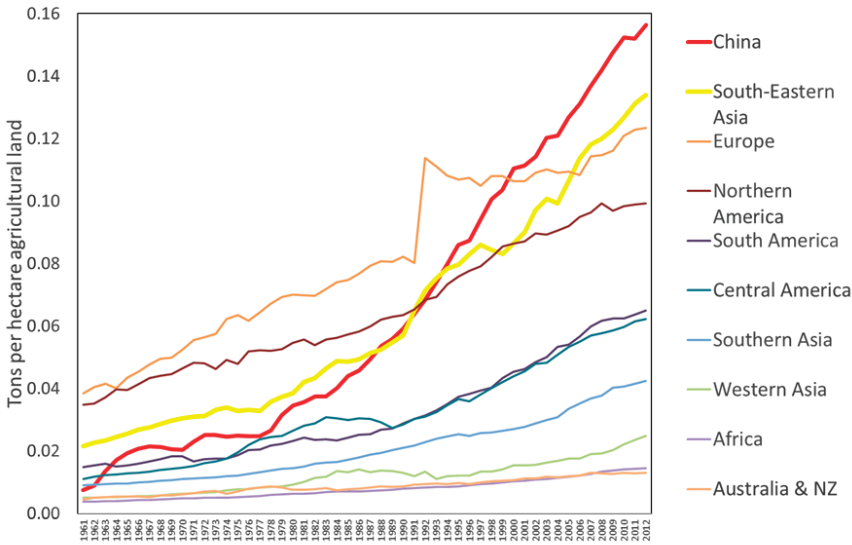

FIGURE A8-2 Temporal pattern of meat production intensity for different parts of the world between 1961 and 2012.
SOURCE: FAOSTAT database.

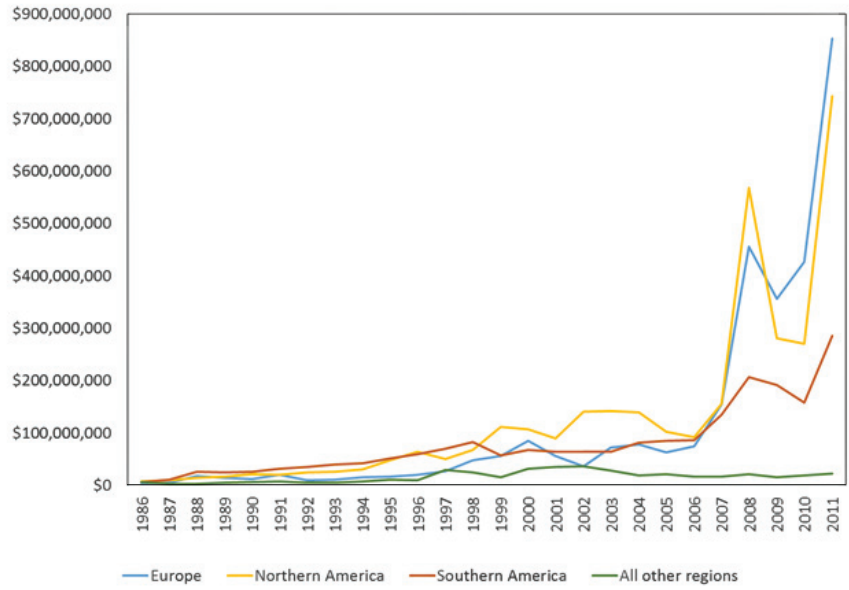

FIGURE A8-3 Temporal pattern of value (in US$) of imports of pigs and pig products to China from various regions of the world between 1986 and 2011.
SOURCE: FAOSTAT database.

production chains is almost impossible, thereby providing producers or other actors involved in the value chain with little incentive to improve product quality. The globalisation of value chains has resulted in trade networks that connect a large number of countries around the world. Ercsey-Ravasz et al. (2012) analysed the complexity of global agri-food trade, which revealed the connectedness between the 44 countries with the highest total food trade activity in 2007. The resulting network also shows the central role of countries such as The Netherlands, Denmark, Ireland, and Belgium in the agri-food trade context. This connectedness in food production chains is associated with benefits in terms of product variety and cost-effectiveness, but it also increases the risk of spread of contaminants or infectious pathogens. The mathematicians Erdõs and Rényi (1960) described the phenomenon, which was later named the connectivity avalanche. It relates to the connectivity between individual entities, called nodes. The underlying process starts with isolated nodes, then clusters consisting of pairs of nodes are formed, followed by clusters involving multiple nodes. The connectivity avalanche occurs at a particular point when adding further connections converts a pattern consisting of multiple clusters into a so-called giant highly connected component with only few isolated nodes remaining, and eventually results in a single connected network (Seeley, 2007). With respect to direct and indirect global connectivity

between humans and animals, we have probably reached a phase that is close to transition toward the connectivity avalanche. If that transition occurs, which is very likely, it will have serious implications for the risk of infectious pathogens.

The extent to which human activity dominates biomass production of the Earth's biosphere has been quantified by Haberl et al. (2007). The results show that in the period 1998–2002 agriculture was responsible for 78 percent of human appropriation of net primary production. It also indicates that there are parts of the world such as in Africa, Russia, and Central Asia where there is potential for an increase in agricultural productivity. Krausmann et al. (2013) took these results further and emphasised the need to improve efficiency of pasture-associated production. The spatial disconnection between consumption and the environmental impact of production of biomass was examined for the year 2000 by Erb et al. (2009). They noted the interdependence and spatial separation of biomass production and consumption which leads to the development of complex socioecological interactions. This work has been taken further by Meyfroidt et al. (2013) who in the context of globalisation emphasized the influence of distant markets on land use changes, particularly through the growing urban consumer class in emerging markets.

There is now increasing recognition that natural ecosystems should be looked at as providing a service to human society, which means that economic values can be attributed to them (Costanza et al., 2011). Ecosystems vary in their characteristics, which includes the diversity of living organisms. They provide human society with various types of natural resources, regulate our environment, and supply us with cultural services (Anonymous, 2003).

Disease Emergence and Spread

The past 30 years have demonstrated the ability in particular of pathogenic viruses to move around the world at increasing speed. This has been facilitated by trade networks of live animals and their products, but probably primarily by human movement. The patterns of spread vary depending on the characteristics of the pathogen, livestock production system characteristics, socioeconomic factors, and the risk management measures put in place (Hui, 2006; Karesh et al., 2012; Plowright et al., 2008). As an example, foot-and-mouth disease virus is a livestock disease that has been present for a long time. It is nowadays only endemic in low- to middle-income countries, whereas most high-income countries are able to maintain freedom from infection. Other infections, such as bluetongue and Schmallenberg virus which involve an insect vector, change their spatial distribution depending on the season of the year. But there has been a trend for them to move further north, probably associated with climate change (Doceul et al., 2013; Koenraadt et al., 2014; Saegerman et al., 2008). Both viruses have recently emerged in northern Europe without it being possible to explain the source of introduction, and in the case of the Schmallenberg virus it was actually

newly detected. African swine fever virus used to be endemic only in Africa, apart from short incursions into the Caribbean, Brazil, and the Iberian Peninsula in the past and current long-term endemicity on the island of Sardinia. In 2007, it appeared in Georgia and has since spread across several Eastern European countries despite extensive control efforts. It is spread through direct or indirect contact between pigs or some tick vector species, and can become endemic in wild pig species (Costard et al., 2009). Highly pathogenic avian influenza virus (HPAIV) H5N1 emerged in China in 1996 and has since spread around Southeast Asia and parts of South Asia where it has become endemic in several countries. Incursions occurred into Africa and Europe, but resulted in endemicity only in Egypt. Wild waterfowl and poultry trade are the main mechanisms for long-distance spread, whereas local spread is influenced by the characteristics of the local poultry production system, in particular the density of ducks (Pfeiffer et al., 2011). Other examples of viral emergence are porcine reproductive and respiratory syndrome and porcine circoviral disease, which were first described in 1987 and 1997, respectively (Baekbo et al., 2012; Lunney et al., 2010). Both diseases are now present in the pig production systems of most countries around the world, causing significant losses particularly in intensive pig production (Rose et al., 2012; Rowland and Morrison, 2012). There are a variety of other zoonotic virus diseases that have emerged in the past 15 years from animal populations and caused disease in humans, including HPAIV H5N1, HPAIV H1N1, HPAIV H7N9, SARS, West Nile virus, and Nipah virus (Jones et al., 2013). Most of these events have been associated with intensification of production resulting in amplification of transmission within the relevant domestic animal species followed by spillover into humans (HPAIV H5N1, HPAIV H1N1, HPAIV H7N9). In some instances, the emergence has been due to changes of the interface between wild and domestic animals and humans (Nipah virus, West Nile virus, SARS). The pathogen's ability to transmit among humans then determined its further spread, and here SARS and in particular HPAIV H1N1 were very effective.

Systems Perspective to Risk Assessment

The eco-social changes that have occurred particularly in the past 20 years have generated an environment that significantly enhances the ability for viral pathogens to change and spread very quickly around the world. Any attempt to assess the risks associated with such events needs to acknowledge the complexity of this global system. This requires an interdisciplinary approach, which is the background to the development of the One Health and EcoHealth approaches (Zinsstag, 2012). An example of the challenge represented by these complex systems is the emergence and continuing presence of HPAIV H5N1 in large parts of Asia. A key driver of the spread of the virus is human behaviour influenced by cultural and economic factors. Figure A8-4 shows the complexity of the resulting eco-social system, which is described in more detail in Pfeiffer et al. (2013). It

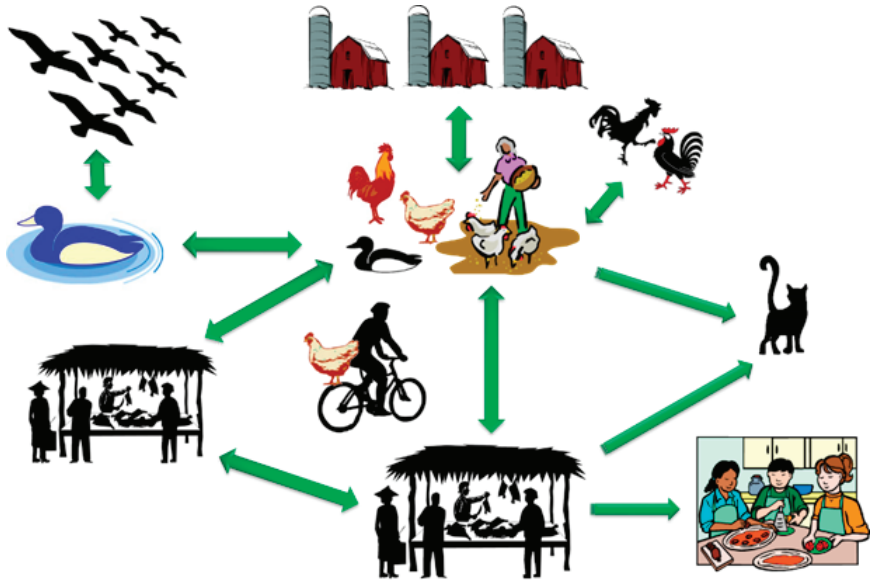

FIGURE A8-4 Eco-social system components influencing spread of HPAIV H5N1 in parts of Asia.

centres around small- to medium-size poultry producers keeping chickens as well as domestic waterfowl. They are connected with each other and with consumers through live bird trade networks that often also cross national boundaries. The density of birds in these populations and intensity of the trade varies in space and time, influenced by the variation in demand for and therefore the price of poultry meat. There are links between this production system and wild waterfowl populations, fighting cock activity, and large-scale industrial poultry production that will also influence spread. The risk management of HPAIV H5N1 in these populations is based on disease outbreak detection, followed by extensive poultry culling and potentially large-scale vaccination. These control methods are often not entirely supported by the stakeholders in the system, which is probably one reason for the reoccurrence of infection. It therefore needs to be accepted that with these control tools it is currently impossible to eliminate infection from these systems. Only Thailand has managed to achieve apparent freedom from infection in domestic poultry since 2008, without ever using vaccination. Most countries surrounding Thailand, and also Bangladesh, Indonesia, and China, continue to report outbreaks, with several of them applying large-scale poultry vaccination campaigns. It is notable that while HPAIV H5N1 occasionally spreads from its epicentre in Southeast Asia and East Asia to Europe and Africa, of those long-distance moves the virus only managed to become endemic in Egypt. It was never reported from the Philippines, Australia/New Zealand, or the Americas. This local

and global pattern of HPAIV H5N1 demonstrates the importance of the features of the local eco-social system in relation to the ability of the virus to become endemic. The particular practice of small- to medium-scale poultry producers engaging in live poultry trade together with presence of ducks appears to play a key role in maintenance of HPAIV H5N1. Increased demand from urban consumers has resulted in intensification of this type of trade activity, and thereby is likely to have provided an environment that supports virus maintenance as well as evolution, as reflected in the continuing emergence of new HPAIV H5N1 clades and variants of avian influenza.

From Risk Assessment to Management

Risk assessment of disease threats provides a synthesis of scientific evidence that is then used to develop appropriate risk management. The emphasis within that context has been on biomedical science, and socioeconomic drivers of disease risk most of the time have been ignored. This means policy makers and scientists tend to focus their efforts on coming up with a technical "tool" that allows mitigating the risk, ideally through intervention using a treatment or vaccine. Within human health the importance of behaviour change as a social tool has been recognised since the emergence of HIV/AIDS. It is now clear that effective management of the disease threats associated with eco-social changes needs to be based on a systems perspective. Coker et al. (2011) presented a conceptual framework for such an approach within a One Health context. Figure A8-5 shows

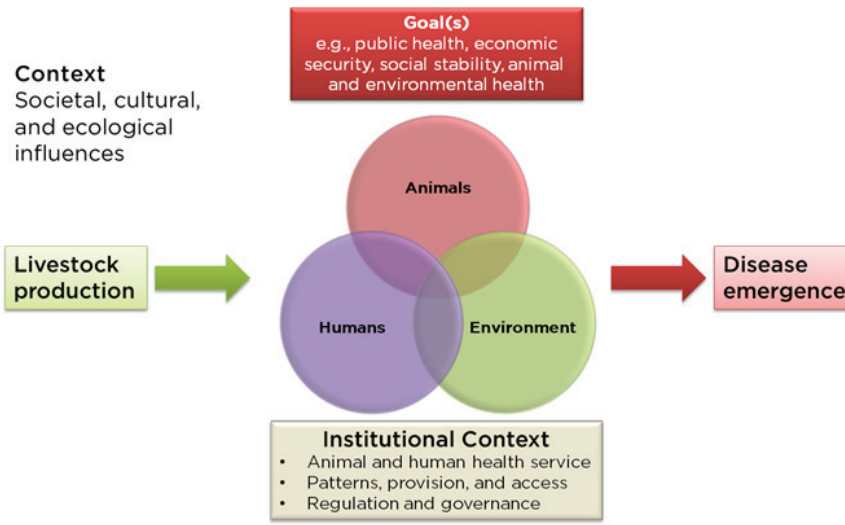

FIGURE A8-5 A systems perspective on disease emergence.

its key elements for the example of livestock production where it emphasizes the importance of the institutional and the wider societal context. These two factors will influence the goals that different stakeholders associate with the risk of disease emergence, and these goals are likely to vary among stakeholders and may even contradict between some of them.

Taking the risk assessment to the logical next step, the management of the risk, Millstone et al. (2004) characterised three different models linking science with risk management: the technocratic, the decisionistic, and the transparent models for policy development. The key difference is that the technocratic approach focuses primarily on science as the primary and theoretically only source of information for policy development. This method has been used widely, or at least was considered to be the basis of so-called science-based policy development. With the decisionistic approach, social and economic considerations are taken into account when policy makers decide on the risk management policy, but neither prior to nor during the risk assessment. This is the currently most widely used approach, and it could be called science-led (as distinct from science-based) decision making. Both approaches do not adequately take account of the wider socioeconomic context and the goals and therefore the motivations of key stakeholders that are all recognised as having significant influence on the effectiveness of any risk management policy. The transparent approach to policy development described by Millstone et al. (2004) addresses this shortcoming. Here, prior to starting the risk assessment, a risk assessment policy is developed that takes the institutional and wider societal context into account. The weaknesses of the currently used technocratic and decisionistic approaches to decision making about health risks have also been pointed out in a report produced by the Committee on Improving Risk Analysis Approaches Used by the U.S. EPA (Anonymous, 2009a).

Pfeiffer (2014) reviewed the role of science in decision making for animal and zoonotic diseases in the context of disease emergence and eco-social changes that have occurred in the past 30 years. He suggests the need to adopt an interdisciplinary approach to the science and to embed the risk assessment within a risk governance framework, such as developed by the International Risk Governance Council (Anonymous, 2008). Figure A8-6 shows the components of this framework, which add a framing and evaluation component to the risk analysis frameworks, defined by the World Organisation for Animal Health (Anonymous, 2013b) and *Codex Alimentarius* (Anonymous, 2013a). Both these components formalise the need to reflect the wider system context, particularly in relation to social and economic factors, and therefore the engagement with relevant stakeholders. Such an approach to policy development will foster an interdisciplinary approach to the science as well as the resulting decision making.

FIGURE A8-6 Components of the risk governance framework.
SOURCE: Adapted from Anonymous, 2008.

Conclusions

Eco-social changes that have occurred particularly rapidly and on a global scale in parallel with global economic development in the past 20 years have included significant changes to the animal–human interface. This has had the important side effect of providing a highly efficient environment for emergence of viral pathogens. Human interventions used to have a local, national, and sometimes regional effect, whereas now, this effect is often global. Complex and dynamic relationships, including interactions and feedback effects between natural and man-made phenomena, drive the behaviour of the global ecosystem. Scientific approaches need to be adapted to this situation, in particular by embracing systems thinking and interdisciplinary research approaches. While this includes linking animal with human health, it is as important to include the environmental and social sciences. But it is important that the required science is purpose driven, ideally informed by risk assessment, and, for it to be useful, it needs to be embedded in risk governance frameworks that explicitly take account of the societal context within which risks occur and are to be managed.

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A9

FROM RISK ANALYSIS TO RISK GOVERNANCE—ADAPTING TO AN EVER MORE COMPLEX FUTURE[21]

Dirk U. ***Pfeiffer***[22]

Summary

Risk analysis is now widely accepted amongst veterinary authorities and other stakeholders around the world as a conceptual framework for integrating scientific evidence into animal health decision making. The resulting risk management for most diseases primarily involves linking epidemiological understanding with diagnostics and/or vaccines. Recent disease outbreaks such as Nipah virus, SARS, avian influenza H5N1, bluetongue serotype 8 and Schmallenberg virus have led to realising that we need to explicitly take into account the underlying complex interactions between environmental, epidemiological and social factors which are often also spatially and temporally heterogeneous as well as interconnected across affected regions and beyond. A particular challenge is to obtain adequate understanding of the influence of human behaviour and to translate this into effective mechanisms leading to appropriate behaviour change where necessary. Both, the One Health and the ecohealth approaches reflect the need for such a holistic systems perspective, however the current implementation of risk analysis frameworks for animal health and food safety is still dominated by a natural or biomedical perspective of science as is the implementation of control and prevention policies. This article proposes to integrate the risk analysis approach with a risk governance framework which explicitly adds the socio-economic context to policy development and emphasizes the need for organisational change and stakeholder engagement.

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Keywords: Animal health, Disease control, Interdisciplinarity, Policy, Systems perspective

Introduction

Risk analysis frameworks for animal health and food safety, as defined in the OIE Animal Health Code (Anonymous, 2013a) and the Codex Alimentarius (Anonymous, 2013b) have had major influence on the adoption of science-led decision making in animal health around the world (Anonymous, 2010). Veterinary authorities in most countries have used it to inform the development of disease control and prevention policies. The emphasis of these frameworks has been on risk pathways defined by epidemiological system characteristics taking account of scientific knowledge in relation to the relevant infectious pathogen, its host's characteristics and the associated diagnostic methods. This has resulted in an improved transparency of the policies for disease control and international negotiations. At the same time, however, the risk of emergence and spread of existing and new pathogens has increased as a consequence of global changes in food production, animal-human interfaces and human movement networks, as well as many other factors that characterise the age of the anthropocene (Crutzen, 2002; McMichael, 2014). Examples for such events in relation to animal health have been the emergence of bluetongue virus serotype 8 and Schmallenberg virus in Northern Europe, the Nipah virus outbreak in Malaysia and the highly pathogenic avian influenza virus (HPAIV) H5N1 epidemics in South-East and East Asia. This increased disease threat has led to the realisation that effective control and prevention of animal and human diseases require the development of new approaches to risk management that integrate knowledge about epidemiological risk factors with environmental and social risk factors. The One Health and ecohealth approaches are a result of this vision; but while the risk analysis framework provides sufficient flexibility to accommodate the holistic principles of a One Health or ecohealth approach, established practice around the world currently focuses primarily on biomedical and epidemiological system aspects. The following is a brief review of the scientific principles underlying risk analysis and its role in policy development. The article concludes stressing the need to embed risk analysis in animal health within risk governance frameworks so as to allow the development of more effective risk management policies, particularly when dealing with significant uncertainty in relation to the likelihood of disease occurrence and its consequences.

Science and Knowledge

As has been remarked by Hansson and Aven, it is essential to reflect on the role of science in the context of decision making when examining the use of risk analysis in policy development (Hansson and Aven, 2014). An important purpose of science is to generate the knowledge that allows us to understand cause-effect relationships within the world we live in (Van den Hove, 2007). Until the end of the 19th century, it was believed that these relationships were of a deterministic nature, in that with complete knowledge it will be possible to precisely predict

the behaviour of natural systems. The fact that uncertainty is an inherent feature of natural systems has only been recognised since the beginning of the 20th century (Sarewitz and Pielke, 1999). General public thinking is still dominated though by a conscious or subconscious preference for deterministic interpretation of cause-effect relationships. It is the aim of scientific research to reduce and where possible remove the uncertainty about cause-effect relationships, thereby improving the ability to effectively prevent or control diseases both in animal and human health. In this respect, the traditional perspective has been to emphasize the importance of the biomedical sciences, and the general view was that only reductionist science would lead to meaningful advances in scientific knowledge. This resulted in a specific research focus at the organism and the molecular level. As a consequence, the importance of the effects generated by the interactions between entities within complex systems was not recognised or at least underestimated (Parkes et al., 2005). The emphasis on reductionism also resulted in the development of rigid boundaries separating different scientific disciplines, hence compromising the effectiveness of interdisciplinary approaches (Gieryn, 1983). While research projects involving multiple disciplines have been encouraged by funding agencies for some time, such activities typically lead to working in parallel (i.e. multidisciplinary projects) rather than in an integrated fashion (i.e. interdisciplinary projects). As a result the outputs of this type of research may well be of high scientific quality from a single discipline perspective but typically are unlikely to generate integrated knowledge. It is now recognised that to be able to deal with disease threats more effectively, it is essential to appreciate the complexity of the underlying system, including its biological, environmental and social dimensions (Fish et al., 2011; Leach and Scoones, 2013). High quality reductionist and disciplinary science is necessary, but its outputs need to be integrated using interand transdisciplinary approaches (Lowe et al., 2013; Stokols et al., 2008; Wilkinson et al., 2011). In order to generate knowledge suitable for designing effective risk management policies, scientific researchers also need to recognise the potential importance of integrating a wide variety of knowledge perspectives in addition to scientific ones (Parkes et al., 2005). It is also important for policymakers and society in general to accept that certainty about cause-effect relationships in complex systems is never completely attainable (Jasanoff, 2007).

Interdisciplinary and Transdisciplinary Research

The effective development of inter- and transdisciplinary research is compromised by a disciplinary and epistemological silo mentality amongst scientists which is still promoted by research and academic institutions as well as funding agencies (Syme, 2008). The most difficult barrier to overcome is the one between the 2 disciplinary blocks comprising the natural and social sciences (Lele and Norgaard, 2005). An element of such a process will have to be that scientists become more comfortable with epistemological pluralism (Miller et al., 2008).

Lyall et al. (2011) provide a practical introduction to the implementation of interdisciplinary research projects. An integrated perspective towards the research question can be facilitated by developing an agreed conceptual framework outlining the relevant elements in the underlying eco-social system, such as the one described by Coker et al. (2011). The definition of transdisciplinary research varies in that some researchers view it as several disciplines working together for extended periods of time and developing novel conceptual and methodological frameworks, whereas others define it as adding a participatory dimension to interdisciplinary research (Klein, 2008). The terms team science and action research have also been used to emphasise the translational aspect of transdisciplinary research (Stokols, 2006; Stokols et al., 2008).

A particular challenge in inter- and transdisciplinary research is the need to use and integrate qualitative and quantitative data analysis approaches. Social scientists are usually very comfortable with this, whereas natural scientists tend to believe that qualitative data lacks scientific rigour and are therefore not suitable for generating knowledge that enhances our understanding of cause-effect relationships (Lele and Norgaard, 2005). Quantitative approaches emphasise the importance of measurement precision and representativeness in relation to a larger or other population to which inferences from the research are to be applied. A recognised strength of qualitative data is the accuracy of the data collected concerning individuals in the sample. However, such data are less, if not completely, unsuitable for inferences beyond the sampled individuals. Mixed methods analysis techniques have been used in social sciences for some time to integrate qualitative and quantitative data analysis, which are for this reason able to benefit from the strengths of both approaches in data collection and analysis (Creswell, 2014).

Systems Perspective (Ecohealth/One Health)

Since the emergence of HPAIV H5N1, there has been increasing recognition that the complexity of ecosocial systems needs to be better understood to be able to deal effectively with current and future endemic, emerging and new infectious disease threats (Leach and Scoones, 2013; Pfeiffer, 2013; Pfeiffer et al., 2013; van Helden et al., 2013). The One Health and ecohealth approaches are a result of this development; while these approaches vary somewhat in the underlying concepts, they are now likely to converge towards a single approach which should reduce confusion and therefore increase acceptance amongst stakeholders (Zinsstag, 2012). The animal health scientists and policymakers found it relatively easy to accept the relevance of these concepts, while it appears to have been more difficult in human health. For risk questions suitable in the context of a One Health approach, the active engagement of ecological and environmental sciences and associated policy development is still quite poor, the situation is even worse with respect to the social sciences. But it is inevitable that as a result

of the need for more effective risk management, policymakers will increasingly demand use of integrative approaches, and therefore the research communities will have to accept their relevance and integrated research will eventually also become part of mainstream academic education. One example of a major challenge that humanity will have to urgently deal with is the emergence and spread of antimicrobial resistance (Laxminarayan et al., 2013). Antimicrobials have become an essential risk management tool for protecting animal and human health from infectious disease threats as well as for achieving food security and safety. As a result, enormous quantities of antimicrobials are used in humans and animals for curative and preventive purposes, which in turn have become a major driver of emergence of resistance. There are also still some antimicrobial compounds that are used both in humans and animals, whereas many are now restricted to only human use. Attempts to regulate usage need to adopt a systems perspective able to take into account the variety of economic and social drivers that influence antimicrobial usage in humans as well as animals.

Risk Analysis and Risk Governance

A more effective link between scientific knowledge and policy development/implementation has been achieved by the widespread adoption of risk analysis frameworks concerning animal health, food safety and many other areas (Anonymous, 2009; Anonymous, 2010; Anonymous, 2011; Vose, 2008). A key component of this framework is communication amongst the stakeholders involved or affected by the particular risk that is to be mitigated. Where risk management policies have been ineffective, poor communication between risk managers and risk assessors has often been mentioned as one of the reasons. A particular challenge is the communication of uncertainty by scientists to both decision makers and stakeholders affected by the decisions. It is widely recognised that quantitative information in relation to risk and uncertainty is difficult to communicate, as a result of differences in education and/or variation in risk perception amongst recipients of the relevant information (Hermans et al., 2012). Nonetheless, this admittedly very important issue has also detracted attention from the fact that the emphasis of risk assessment and management on biomedical drivers of the disease process often misses some of the key ecosocial factors influencing disease risk, and that these may well be a more important reason for ineffective risk management. For example, human behaviour has significant influence on animal disease emergence and the impact of any intervention (Aven and Renn, 2010). Kasperson et al. (1988) developed a conceptual framework describing the influence of psychological, social, institutional and cultural processes on risk (i.e. the social amplification of risk). Slovic et al. (2004) emphasized the various dimensions of the concept of risk by referring to ‘risk as analysis,’ ‘risk as feelings,’ and ‘risk as politics’. Given the extensively developed scientific theory and practical knowledge in relation to human behavioural drivers of risk, it is surprising that

animal health risk assessment and management rarely take these factors explicitly into account (Brown, 2008). Furthermore, the emphasis on independence between risk assessment and management has had a detrimental effect on the utility of the generated outputs, in that risk assessors and risk managers often find it difficult to work together (Anonymous, 2009; Anonymous, 2011; Ely et al., 2009a). While it is essential to maintain a conceptual separation between risk assessment and management, and thereby prevent risk managers from introducing undue bias into the risk assessment process, it is important to consider risk management options in the process of assessing the risk. Indeed, this also more appropriately reflects the difference between what Jasanoff (1995) defined as 'research science' and 'regulatory science', in that risk assessment as a scientific approach is usually conducted in response to a specific policy need and may inform actual regulatory actions, as distinct from scientific endeavours primarily aimed at improving knowledge. The influence of institutional and organisational factors also needs to be considered in the process of risk-based policy development. Rothstein and Downer (2012) and Huber and Rothstein (2013) found that various aspects of organisational culture can adversely affect the impact of adopting a risk analysis approach in a government department. It was suggested that risk-based approaches were used to 'cloak' entrenched behaviours and perceptions as 'rational' and transparent policy. In another study, Rothstein et al. (2013) concluded that the adoption of risk-based policymaking (i.e. risk analysis) varies significantly between 3 European countries as a result of differences in societal, organisational and/or political norms and accountability in relation to risk governance. Stakeholders usually interpret animal health and food safety risk analysis frameworks as technical tools to support decision making, without realising or wanting to realise that they usually also require changes in institutional and organisational structures as well as behaviours, if they are to be effective. As part of a comprehensive review of risk analysis, the International Risk Governance Council (IRGC) identified 25 different deficits in risk governance structures and processes (Aven, 2011). Apart from technical deficiencies, such as incomplete understanding of underlying biological processes, these included, for example, incomplete stakeholder consultation, inability to acknowledge incompleteness of knowledge and failure to take account of important factors, such as risk perception and risk acceptance.

Many of the aspects discussed above can also be examined in the context of the direction of the flow of information and the sequence of actions involved in risk analysis, and how all this influences the effectiveness of the resulting policies for risk management. Usually, a linear information flow underpins the development of risk management policies, in that following a risk problem identification (i.e. hazard identification) a risk assessment is conducted, which tends to be dominated by a biomedical science perspective. The output from the risk assessment informs the policy development which is then communicated to relevant stakeholders. A commonly used variation on this approach is that

the interpretation or evaluation of the outcomes of the risk assessment and the development of the risk management strategy are shaped by other information, such as the one concerning social and economic factors. Millstone et al. (2004) named the first option the technocratic and the second the decisionistic model. Given their linear nature and the biomedical science focus, both approaches do not adequately acknowledge the influence of system complexity including feedback loops on risk, stakeholder perceptions in response to risk and/or risk mitigation, and the potential for endorsing different mitigation options. Millstone et al. (2004) therefore proposed the need to adopt a transparent model based on a process that starts with development of a risk assessment policy grounded on socioeconomic and political considerations involving a wide group of stakeholders rather than starting with risk problem identification performed by a narrow group of stakeholders, which often ends up being just the policymakers. This approach places major emphasis on communication and stakeholder participation during risk analysis which, while being more demanding on resources, should enhance the likelihood of policy acceptance by key stakeholders.

Recognising the limitations of the risk analysis framework, some scholars (Renn, 2005; Aven and Renn, 2010) have proposed the IRGC risk governance framework that explicitly integrates the factual dimension of risk with its sociocultural context. The term 'risk governance' reflects the wider societal context of policy making. It can be defined as "the totality of actors, rules, conventions, processes, and mechanisms concerned with how relevant risk information is collected, analysed and communicated and management decisions are taken" (Aven and Renn, 2010; Hermans et al., 2012). The components of the IRGC risk governance framework are pre-assessment, risk appraisal, tolerability & acceptability judgement and risk management (Renn, 2005). Pre-assessment, tolerability and acceptability components have a particularly strong stakeholder engagement emphasis, whereas risk appraisal and risk management are broadly similar to the risk assessment and risk management components in the OIE's risk analysis framework for animal health. Roodenrijs et al. (2014) evaluated the feasibility of applying the IRGC framework for recent Q-fever and Schmallenberg virus outbreaks in the Netherlands. They found it to be broadly applicable but noted that one of the challenges will be to decide on the breadth of stakeholder input that will be required, particularly during the early phases of a disease outbreak when the situation is dominated by uncertainty. Through its extensive stakeholder engagement, the IRGC framework performs particularly well for risks associated with significant ambiguity, for example when there is wide variation in societal values and risk perception and therefore disagreement with respect to the appropriateness of different policy options. The IRGC risk governance framework has recently been adapted for application in food safety governance (Dreyer and Renn, 2009). The resulting general framework consists of the 4 sequential components of risk framing, risk assessment, risk evaluation and risk management (Ely et al., 2009b). Both, risk framing and evaluation involve integrating

socio-political considerations into the risk governance process, and thereby expand the very broad and somewhat vaguely defined risk communication component in the risk analysis framework.

Policy Development and Implementation

Decision-making in relation to risk has become more challenging not only because of the physical and biological aspects of ecological and environmental changes together with vastly increased global connectedness, but also due to the increasing heterogeneity in social values and individual preference associated with educational and economic development. Rittel and Webber (1973) already recognised this trend as one of several factors contributing to the difficulty of policymakers being able to deal effectively in particular with so-called 'wicked problems'. There are various examples of this type of decision-making challenge, including global issues such as climate change or locally relevant ones such as tuberculosis control in cattle in Great Britain.

Policy development is ultimately about making a judgment leading to a decision for a particular risk mitigation strategy, which will then either be effective (and potentially also accepted by stakeholders) or not. This decision will be informed by several factors, such as risk estimates, resource availability, stakeholder values and legislation. It therefore integrates facts with values (Hansson and Aven, 2014). The knowledge about the likelihood of event occurrence and the significance of its consequences together are widely interpreted as the 'risk'. Traditional risk assessment will aim to quantify this risk. Nonetheless, it is important to recognise that risk is a complex multidimensional concept (Kasperson et al., 1988; Slovic et al., 2004) and therefore primarily focusing on scientific knowledge as the basis for a risk mitigation strategy is unlikely to achieve the desired outcomes (Hermans et al., 2012). To more adequately reflect this complexity, Stirling (2010) developed an uncertainty matrix which uses the knowledge in relation to the probability of the event and its consequences (including risk management options) as its 2 dimensions. He thereby defines the 4 knowledge states of 'risk', 'uncertainty', 'ambiguity' and 'ignorance'. Using this approach, the detection of bovine spongiform encephalopathy (BSE) during the first couple of years after detection represents an example of the knowledge state of 'ignorance' where there is major uncertainty with respect to probability of occurrence and lack of knowledge about the consequences of occurrence. The situation with bovine tuberculosis in Great Britain offers an instance for the 'ambiguity' knowledge state, in that there is relatively good knowledge about the probability of infection in cattle but significant variation in knowledge and opinion about the consequences of occurrence and any interventions. An example for the knowledge state of 'risk' is the occurrence of bovine virus diarrhoea (BVD) in intensive livestock production systems where the probability of BVD occurrence is relatively well understood and the consequences are known and there is little disagreement

about the management options. It may indeed be more appropriate to refer to this particular knowledge state as 'simple risk' (Renn et al., 2011). The knowledge state of 'uncertainty' applies to exotic diseases such as foot-and-mouth disease, where the introduction of the causative virus is subject to uncertainty but the consequences are well understood and the management tools established. The risk analysis framework for animal health performs best for the knowledge state of 'simple risk', less so for that of 'uncertainty', but it is of limited utility when confronted with 'ambiguity' or 'ignorance'. Policy makers should use these 4 broad categories to inform their choice of tools for integrating different types of knowledge such that it optimises their chances of being able to make good decisions. It is very understandable that policy makers are most comfortable in the knowledge state of 'simple risk', since they have to deal with very limited uncertainty in relation to event occurrence and its consequences. At the same time, it is surprising that both the science-policy interface and government decision making processes are usually 'optimised' for the 'simple risk' states and to a lesser extent for 'uncertainty' knowledge states, despite of both these representing less difficult challenges for decision making compared with the knowledge states of 'ambiguity' and 'ignorance'. Indeed, there have been many challenges to animal health in the past 20 years that have been in the 3 knowledge state categories of 'uncertainty', 'ambiguity' or 'ignorance'. In these situations, targeted public engagement strategies become particularly important and knowledge generated using qualitative analytical methods is likely to be as useful or even more useful than quantitative analysis (Stirling, 2012). These cases unveil the limitations of risk analysis frameworks for animal health and food safety which have a primary biomedical focus (Ely et al., 2009b). The risk framing phase of the IRGC risk governance framework will allow policy makers to clarify which knowledge state applies to a particular hazard, and inform decision making in relation to the most appropriate risk assessment methods. It involves explicit interaction between risk assessors and managers as well as any other important stakeholders. The evaluation of the findings from the risk assessment is aimed at assessing the tolerability or acceptability of the risk and, therefore, determines whether nothing will have to be done, further risk assessment or a risk mitigation policy will be required. This is also the stage where a decision to invoke the precautionary principle can be made (Renn, 2008; Stirling and Gee, 2002). Public engagement is a key aspect of the IRGC risk governance framework, and it needs to be based on a detailed stakeholder analysis to be conducted during the risk framing phase. Mills et al. (2011) present an example of this process for identifying stakeholder groups with 'interest' and 'influence' in plant health issues, and they emphasize that appropriate stakeholder choice for involvement in a risk assessment will strongly benefit the acceptance of any risk management policies. Overall, the IRGC risk governance framework should be used as a model for an evolutionary adaptation of the current risk analysis frameworks for animal health and food safety that will take advantage of the experience with their use in the last 20 years and our

improved understanding of decision making processes, particularly in terms of the role of a wider range of sciences.

Conclusions

As a result of technological development, globalisation, environmental change and modern society's expectations, policy development in animal health has become an ever more challenging process. The still widely used linear technocratic models for policy development have limited effectiveness when dealing with risks occurring within complex eco-social systems. The utility of the established risk analysis frameworks for animal health and food safety could be enhanced if they were subsumed into a risk governance framework that better recognises the wider meaning of the term 'risk'. Specifically, the addition of risk framing and risk evaluation to the current animal health risk analysis components of hazard identification, risk assessment, management and communication places a more explicit emphasis on the socio-economic and participatory dimensions of policy responses to risk. Furthermore, the risk assessment process itself has to take account of the breadth of factors influencing pathogen transmission from the molecular to the population/landscape/regional level, including socio-economic factors, and interactions between factors as well as emergent properties at system level. This requires an inter- or transdisciplinary research approach which is comfortable with bringing together knowledge from different scientific disciplines including that generated by quantitative and qualitative approaches, rather than being dominated by the natural and biomedical sciences and quantitative methods, as is currently the case. It is also important to consider the impact of organisational culture on risk management. Indeed, organisational behaviour varies within and between countries and regions, such that it may be possible to implement effective science-led decision making in some countries with relative ease but only with major difficulty or not at all in others. Finally, and may be most importantly, a risk governance approach will have to optimise its public engagement component based on the socioeconomic risk characteristics of the hazard, since this will positively influence appropriateness and acceptance, and therefore impact of policies.

Conflict of Interest

The author has no conflicts of interest to declare.

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A10

A ONE HEALTH PERSPECTIVE ON HPAI H5N1 IN THE GREATER MEKONG SUB-REGION[23]

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Abstract

Highly pathogenic avian influenza H5N1 has been a global concern for almost 10 years since its epidemic emergence in South-east Asia in 2003/2004. Despite large investment of resources into the region, the infection has not been eradicated and continues to result in outbreaks in poultry and a small number of human fatalities. This review synthesizes the knowledge base generated by a vast number of research activities conducted in the region and beyond, and adopts an interdisciplinary perspective consistent with the one health paradigm towards analysing the problem and formulating possible policy solutions. A key outcome of the work has been the need to integrate socio-economic and anthropological dimensions with any disease control and prevention activities traditionally informed by primarily epidemiological, virological and pathological attributes of the infection in poultry and wild waterbirds. Recommendations at a broad conceptual level are presented that acknowledge the diversity in the region with respect to livestock production, as well as the changing nature of the risk landscape as a consequence of the rapid economic development which some of the countries in the Greater Mekong sub-region are currently undergoing, as well as their strong trade links with China as the major economic power in East Asia.

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Introduction

Since its emergence, highly pathogenic avian influenza (HPAI) subtype H5N1 has attracted considerable public and media attention because the virus involved has been shown to be capable of producing fatal disease in humans. While there is fear that the virus may mutate into a strain capable of sustained human-to-human transmission, the greatest impact to date has been on the highly diverse poultry food systems in some affected countries. In response to this, HPAI H5N1 control measures have focused on implementing prevention and eradication measures in poultry populations, with more than 175 million birds culled in South-east Asia alone. The control methods used were based on classical approaches designed from a single discipline, i.e. a veterinary, perspective. They primarily involved culling, movement control and vaccination, which have proven to be effective for dealing with small to medium-size outbreaks of a relatively short duration. In the case of HPAI H5N1, it quickly became apparent that the infection cannot be eradicated from South-East Asia and China, and therefore classical control approaches will neither be effective nor sustainable. In order to improve local and global capacity for evidence-based decision making in the control of HPAI H5N1, inter- and intra-disciplinary approaches need to be adopted to develop cost-effective and efficient approaches for disease risk reduction. The current review examines the HPAI H5N1 epidemiology in the Greater Mekong sub-region (GMS), specifically the region represented by Cambodia, Lao People's Democratic Republic (PDR), Thailand and Viet Nam, and explores cross-disciplinary approaches to its control. A significant part of the evidence base considered here are the findings from an interdisciplinary project conducted by authors of this review.

Background on HPAI H5N1 in the Greater Mekong Sub-region

Ecology/biology of avian influenza viruses

Avian influenza viruses (AIVs) have high mutation rates typical of RNA viruses (faulty transcription) resulting in relatively high rates of antigenic drift. In addition, due to their segmented genome (8 segments), genetic reassortment can occur in hosts that are infected by more than one AIV strain, facilitating host adaptation and resulting in high rates of genetic shift. AIVs therefore have a comparatively high evolutionary capacity to adapt to new hosts and changing environments (Holmes, 2010; Lee and Saif, 2009).

AIVs representing nearly all 146 combinations of haemagglutinin (HA) (H1–H16) and neuraminidase (NA) (N1–N9) have been isolated from wild waterfowl where they cause asymptomatic infection and are considered to be endemic (Dugan et al., 2008; Webby and Webster, 2001; Webster et al., 1980). Generally, AIVs exhibit host specificity and are easily transmitted within the aquatic environment from one waterfowl species to another through the faecal–oral route.

AIVs circulating in wild birds can spill over to domestic poultry, in which, initially, they are of low pathogenicity, causing mild respiratory disease. Non-waterfowl wild bird species appear to play a less important role for virus circulation, but can still fulfil a function as so-called bridge species that expose domestic poultry to infection (Cardona et al., 2009; Martin et al., 2006; Vandegrift et al., 2010; Yee et al., 2009). In the 1990s, low pathogenicity AIVs (LPAIVs) have dramatically spread globally in domestic poultry, establishing chicken-adapted lineages. Several major outbreaks of avian influenza in domestic poultry due to H9N2 subtype occurred in the late 1990s in Germany, Italy, Ireland, South Africa, the USA, Korea, and China. While only few reports of HPAI in poultry are available for the 40-year period 1950–1990, 16 incidents of distinct HPAIV emergence have been recorded in the Americas, Australia, Europe, South Asia and South-east Asia since 1990. Severe epidemics have been associated with subtypes H5N2 in Mexico, H7N3 in Pakistan, H5N1 in China and beyond, H7N1 in Italy, H7N7 in Holland, and H7N3 in Canada, heavily burdening national animal health systems and causing massive losses to poultry industries (Capua and Alexander, 2007; Sims et al., 2005; Yee et al., 2009). HPAIV H5N1 emerged in South China in 1996, caused a major health scare in Hong Kong when the first human cases of infection and death were reported in 1998, continued to circulate and evolve in southern China for another 5 years, and expanded to other countries in South-east Asia in late 2003. In a second wave of expansion in 2005/2006, HPAIV H5N1 reached Central Asia, the Middle East, Europe, and Africa. Despite major efforts to control HPAIV H5N1, it is now firmly established in parts of China, Viet Nam, Cambodia, Indonesia, Bangladesh, India and Egypt (Fournié et al., 2012). Forty-four distinct HPAIV H5N1 genotypes have been identified between 1996 and 2006, with changes in dominant genotypes reflecting major reassortment events and establishment of distinct lineages in poultry in different geographical regions indicating separate foci of endemicity (Pfeiffer et al., 2011).

Poultry sector dynamics and consumer preferences

Poultry production in the GMS is heterogeneous in all its aspects, with the use of different species, different production and marketing systems, and supports a very diverse range of products and services. Typically, poultry are an integral feature of smallholder agriculture, where the majority of households keep a small (tens of birds) flock of 'indigenous', dual-purpose (meat and eggs) birds to meet household consumption needs, social obligations and minor cash expenses, the latter by sales through informal, live bird marketing channels (Burgos et al., 2008a; Burgos et al., 2008b; Desvaux et al., 2008; Rushton et al., 2005; Safman, 2009). This traditional, extensive poultry production system is virtually ubiquitous throughout the GMS. Comparisons produced by Rushton et al. (2005) and by Otte et al. (2008) based on various heterogeneous data sources in 2004–2005 suggest that extensive poultry producers (backyard subsistence and small commercial

farmers) represent over 90% of farmers and poultry in Cambodia and Lao PDR, about 70–80% for both in Viet Nam. In Thailand, this group also represents the vast majority of producers, but only 10% of poultry. While the data sources for these figures vary in quality and are based on data from several years ago, the basic patterns are likely to be accurate. Simultaneously, intensive industrial poultry production systems, which follow the production model developed in industrialized countries, have been established particularly in Thailand where they produce 90% of poultry, but are still uncommon in the other GMS countries (Otte et al., 2008; Rushton et al., 2005). These two poultry production systems are extremes, between which 'hybrid' and/or intermediate, semi-intensive systems exist, including partial scavenging with feed supplementation, indigenous birds crossed with industrial poultry lines, partial reliance on 'formal' input supply systems, but operating at intermediate scales (hundreds of birds) and relying primarily on 'traditional', informal live bird marketing networks. Each production model has adaptive advantages and disadvantages and none is likely to disappear completely. The marketing channels for small scale producers are varied. Small scale producers sell birds through five different channels: aggregators, market vendors, households and other farmers, and restaurants. Aggregators are currently the most common buyers (Behnke et al., 2012; Desvaux et al., 2008; Heft-Neal et al., 2012a; Heft-Neal et al., 2012b; Métras et al., 2011; Otte et al., 2008; Soares Magalhães et al., 2010a).

Free-grazing duck systems are a prominent feature in rice paddy areas in the GMS. Primarily intended for egg production, their farmers transport them intermittently or continuously to graze in rice fields. In southern Viet Nam, particularly in the Mekong river delta, this itinerant livestock practice is widespread (Men, 2010). Free-grazing duck flocks (up to several thousand ducks) can travel 10–20 km per day, moving across commune, provincial, and even national borders. For the owners of rice fields, ducks offer pest control and fertilization services, while for duck farmers, free-range grazing reduces the cost of feed by up to 50% (Edan, 2006). Consequently, free-range grazing is an essential component of farmer livelihoods. These interactions are a highly productive utilization of resources for owners of both rice fields and ducks, but introduce serious animal and public health risks from an AIV perspective (Desvaux et al., 2008; Heft-Neal et al., 2012a; Henning et al., 2012; Minh et al., 2010).

In Thailand, large-scale industrial poultry production is one of the economy's most important sources of animal-derived food, employment, and income. This intensive, industrial system is characterized by (a) being organized by stages of production with separate primary breeders, multipliers, and finishing producers (often contract farmers), (b) a small number of breeding companies dominating the global supply of genetic material, (c) specialization in meat or eggs and use of specific birds for each product, (d) use of high density feeds tailored to specific stages and lines of production, (e) increasing scales of production (thousands of birds) and (f) growing interconnectedness with the processing and agrifood

marketing industries (Heft-Neal et al., 2012b). In Cambodia and Lao PDR, the 'formal', industrial poultry sector occupies a minor share in national poultry production (about 10% of poultry meat), while the situation in Viet Nam is intermediate between that of Thailand and Cambodia/Lao PDR (about a quarter of poultry meat) (Behnke et al., 2012; Ear, 2009; Heft-Neal et al., 2012a; Rushton et al., 2005; Vu, 2009). In each of these emerging economies, poultry production generally has grown faster than real incomes because the diet is shifting towards meat, but industrial production has been growing faster than other categories, driven by high levels of investment and restructuring of urban food supply chains. Although the market share of smallholder poultry production is shrinking, market-oriented smallholder producers still outnumber large-scale industrial production units (Otte et al., 2008).

Most grocery shopping occurs at traditional wet markets, although that is changing in urban centres, particularly in Thailand where supermarkets are taking on a major role (Anonymous, 2009, 2010, 2011b, 2011c; Reardon et al., 2012). Wet markets sell live and slaughtered whole fresh local chickens, while supermarkets sell frozen birds and fresh cuts of industrial chickens (Anonymous, 2011a). Live birds are cheaper than slaughtered ones and live chickens are preferred because customers can determine their quality and health. Across the region, consumers in markets with comparable access to local and industrial birds placed a premium of 30–100% on the former (per kilo of rendered meat) (Heft-Neal et al., 2012a; Heft-Neal et al., 2012b; Ifft et al., 2011).

Consumers in different regions consistently rate safety as the most important attribute of poultry meat. However, while consumers are concerned about safety, they are limited in their ability to accurately evaluate the safety levels of the meat they purchase. Consumers that purchase live birds base safety considerations on the birds' movement and appearance while people that purchase slaughtered birds evaluate the meat colour and texture. It was very rare that anyone ranked price or taste higher than the safety of the product they buy (Heft-Neal et al., 2012b). Overall, the lack of knowledge of the farm source was the greatest reason for concern about safety, followed closely by disease risk and freshness considerations. Although many consumers prefer the taste of traditional poultry varieties, most urban Thai households primarily consume industrial breeds of chicken in part because they place a high premium on safety (Heft-Neal et al., 2012b; Ifft et al., 2011).

Household poultry keeping and marketing

Nearly all rural households in the GMS keep poultry for both sustenance and income, specializing in traditional bird varieties raised in low-input systems. Smallholders invest little to no resources in poultry production and sales of poultry account for only a small percentage of household cash incomes (less than 5%). Nonetheless, the minimal investment in production means that the percentage

returns are extremely high and marketing poultry provides supplemental cash income to some of the poorest households in the region (Heft-Neal et al., 2012b; Otte et al., 2008). Because they are a millennial fixture of rural life in the GMS, poultry are deeply embedded in society and customs. Small flocks in and around households reduce pest damage, provide highly concentrated manure for direct application and composting, and offer surveillance against predatory animals and strangers. On a more personal level, poultry are popular as individual and family pets, and throughout this region they support an extensive, culturally important, and very lucrative cock fighting industry. The importance of this activity is reflected in the value of the most successful fighting cocks, which can sell for multiples of average annual household income. Finally, poultry are also integrated in many spiritual practices and festival events (Lockerbie, 2008; Lockerbie and Herring, 2009; Thu Hang, 2010).

Market-oriented smallholder farmers source their inputs (eggs, day old chicks, some feed and supplements) from small commercial counterparts, and they are linked to downstream markets by individual aggregators and small poultry product vendors in local live bird markets (LBM) (Heft-Neal et al., 2012b; Métras et al., 2011). Aggregators reduce transactions and search cost for farmers, but act as monopsonists, reducing farmer bargaining power and their incentives to invest in product quality. Aggregators also blend bird stocks and obscure the origin of individual birds. The former activity can sharply increase infection risk, while the latter creates moral hazard and adverse selection that further undermine the incentive for farmers to invest in larger scale and product quality. For their part, LBM offer a variety of direct benefits to merchants and consumers, including freshness, discernable product variety and quality, and traditional food values that continue to elicit price premia in many GMS markets. Whatever the share of income from poultry, smallholder independent farmers exhibit negligible autonomous biosecurity adoption behaviour. They will often perceive the occurrence of disease in their animals as a periodic and natural event (Fielding et al., 2009; Seng et al., 2008). By contrast, most contract and large scale household producers have adopted some form of biosecurity measures in order to conform to contracts and/or protect investments undertaken. However, large(r)-scale producers could still benefit from increased access to technical knowledge and inputs. Both anecdotal evidence and direct observation around the GMS reveal extensive, diverse, and continuous transboundary trade in poultry products, despite the fact that such trade is either forbidden or much more strictly circumscribed. These flows, especially of live birds and eggs, through both kinship and commercial networks can extend from sources to destinations hundreds of kilometres from border crossings (Fournié et al., 2012; Van Kerkhove, 2012).

Epidemiology of HPAI in the Greater Mekong Sub-region

Spatial and temporal patterns of HPAI H5N1 occurrence

In the initial epidemic waves, HPAI H5N1 risk in Thailand and Viet Nam was statistically associated with duck abundance, human population and rice cropping intensity but less strongly with chicken numbers (Gilbert et al., 2008). In Viet Nam, the two main HPAI H5N1 risk clusters (Red and Mekong river deltas) not only coincide with irrigated rice areas in the lowlands, but also with areas of good market access and high poultry transaction frequency (Pfeiffer et al., 2007). The latter suggests that the trade network, in which LBMs fulfil a key role, facilitates spread of the virus. A striking feature of the different epidemic waves in Thailand and Viet Nam is that they did not appear to be synchronous, which raises questions about the underlying factors that may define 'hot' periods during which increased virus circulation can be expected. In Viet Nam, the initial epidemics occurred before and during the Tét holiday period when demand for poultry and pork meat is particularly high, suggestive of poultry movements as important determinants of local epidemics (Pfeiffer et al., 2007). In Cambodia and Lao PDR, HPAI H5N1 outbreaks occurred sporadically, and are probably associated with cross-border poultry trade: in the case of south-eastern Cambodia as spillover from southern Viet Nam and in Lao PDR as a result of poultry trade with southern China and northern Viet Nam. The small extent of the commercial poultry sectors in Cambodia and Lao PDR is a possible reason for the small size of the epidemics in these countries and endemicity is unlikely to develop due to the comparatively low density of poultry. Thailand experienced only a very small number of outbreaks between the major outbreak waves in 2004 and 2008. These outbreaks, caused by descendants of the original HPAIV H5N1 clades, suggested the existence of a local virus reservoir and are believed to have been associated with live poultry trade and cock fighting activities of farmers. In Viet Nam, since introduction of interventions (including large-scale vaccination campaigns in late 2005) outbreak incidence has been reduced significantly. There are still small-scale epidemics around the Tét holiday period, but also at other times of the year. The main foci of infection remain in the two large river deltas, particularly in the Mekong river delta (Pfeiffer et al., 2007; Pfeiffer et al., 2011). Since 2008, HPAI H5N1 incidence in Viet Nam has been about 30–70 outbreaks per year involving single to multiple poultry flocks, up to 10 per year in Lao PDR and Cambodia, and none have been reported from Thailand (*data source*: FAO EMPRES-i). This represents a major achievement considering that in 2004 Thailand and Viet Nam had reported almost 2000 and 3000 outbreaks, respectively, which in 2005 dropped to about 200 and 2000, respectively. Myanmar reported 4 outbreak waves between 2006 and 2010 affecting different parts of the country, which based on clade types appeared to be epidemiologically connected with events in neighbouring GMS countries (Mon et al., 2012; Pfeiffer et al., 2011).

In the Red river delta, the predominant virus clades have changed over time while the original clade still dominates in the Mekong river delta (Pfeiffer et al., 2011). This suggests different mechanisms of introduction and maintenance between the Red river delta and Mekong river delta. Northern Viet Nam seems to be subject to more frequent introductions of virus from southern China, whereas the Mekong river delta may have a local reservoir of circulating virus. Mechanisms for local maintenance of virus presence are unclear, but are particularly important in southern Viet Nam (and bordering areas of Cambodia) since introductions from outside the region seem to be less common (Pfeiffer et al., 2011). Unvaccinated ducks have been implicated on various occasions as the cause of outbreaks in that region (*source*: HPAI H5N1 timeline document on www.who.int). The area within the Mekong river delta where the outbreaks occurred is known for a high duck density and large numbers of free-grazing ducks (Pfeiffer et al., 2011).

Risk of between flock transmission of HPAIV H5N1 and of transmission from poultry to humans

The likelihood of exposure of domestic poultry flocks to HPAIV H5N1 is influenced by production system characteristics and associated husbandry measures. The published data describing differences in infection risk between poultry production types needs to be interpreted cautiously, since it is likely to be affected by reporting bias and other factors compromising surveillance system sensitivity (Desvaux et al., 2006; Pfeiffer et al., 2007; Tiensin et al., 2005; Trevennec et al., 2011). Still, it is possible to identify general epidemiological patterns on the basis of an assessment of the published information, as, for example, presented in some detail in Fournie et al. (2012). The systems within which poultry are kept in the GMS are complex. Most farming households will keep chickens, for subsistence and many for cock fighting, together with other agricultural production activities, such as rice production or aquaculture (Paul et al., 2011). The chickens may be scavenging freely or be kept in small cage enclosures, hence very limited if any bio-exclusion or -containment measures are likely to be in place (Fournié et al., 2012). Transmission of HPAIV H5N1 can occur directly through contact between chickens from the same as well as neighbouring flocks, and given the large quantities of viruses excreted by clinically diseased chickens also indirectly by contamination of clothing or equipment (Songserm et al., 2006). Since there is a high likelihood of HPAIV H5N1 infected chickens developing obvious clinical signs and mortality, outbreaks will have significant adverse effects on farmers' livelihoods, and in the absence of vaccination are highly likely to be reported. The percentage of farmers keeping waterfowl will be high in areas with significant surface water area, such in river deltas or around lake areas, and can then be linked to aquaculture. These systems may be able to maintain HPAIV H5N1 without it being recognized, given that waterfowl are able to carry the virus without developing clinical disease (Desvaux et al., 2011;

Gilbert et al., 2006; Gilbert and Pfeiffer, 2012; Henning et al., 2011; Kim et al., 2009; Sturm-Ramirez et al., 2005). Subsequent to the 2003–2005 outbreak waves in the GMS, industrialized poultry farms, primarily chicken farms in Thailand, have established bio-exclusion measures which have been effective at preventing introduction of infection, although it needs to be acknowledged that levels of infection in Thailand have been very low for several years and apparently zero since 2008. Considering these system features, it would seem that production systems involving waterfowl, such as in rice producing river delta areas, have the highest potential to maintain the virus locally, whereas the systems dominated by chickens produced for subsistence or small to medium scale commercial production are likely to require introduction from elsewhere, either through wild birds or through live poultry trade (Tiensin et al., 2009).

While wild birds in some instances might have been associated with the introduction of infection into the domestic poultry population, this source has several orders of magnitude lower importance for the spread and maintenance of HPAIV H5N1 infection, compared with human activities associated with domestic poultry. This conclusion is supported by the relatively clear trade association of the early outbreak waves in Viet Nam through their occurrence around the Tét holiday periods, and outbreak occurrence in northern Viet Nam along recognized trade routes (e.g. Dien Bien Phu and several other locations along the border between Viet Nam and China) (Pfeiffer et al., 2007). Also, the risk pathway from release of live HPAIV H5N1 by wild birds through to exposure of domestic poultry that then has infection as a consequence is likely to be less effective, than any risk pathways associated with the poultry value chain.

The poultry trading network has an important role in the spatial spread of infection. The network involves farmers, poultry traders and consumers, with the traders linking between different farms when collecting birds as well as through unsold birds going back from an LBM to the home of the trader (Soares Magalhães et al., 2010a; Van Kerkhove et al., 2009). Data from Viet Nam indicate that LBMs host a highly dynamic population consisting of a mixture of domestic and occasionally wild bird species, representing a potentially large geographic area from which birds were sourced. Infected poultry will shed large amounts of virus, resulting in significant environmental contamination. It is therefore likely that within villages, through poultry traders collecting birds and at live bird markets there is a high risk of indirect transmission through contaminated humans or fomites. As mentioned above, infected waterfowl species can shed virus without necessarily progressing to a clinical disease stage, and therefore are likely to have a key role in the spread and maintenance of infection (Boyce et al., 2009; Keawcharoen et al., 2011; Sakoda et al., 2012).

Live bird markets are a key feature of the epidemiology of HPAIV H5N1 in that they allow the mixing of birds from a large number of sources and of different species, including chickens and waterfowl (Amonsin et al., 2008; Kung et al., 2007; Lee et al., 2010; Martin et al., 2011). Given the likely absence of

hygiene at most LBMs, they thereby can be seen as large flocks that have a high turnover (daily) linked to a multitude of source and target populations, and may be able to maintain silent infection, without necessary occurrence of noticeable outbreaks (Fournié et al., 2011). This also increases the potential for antigenic drift as well as reassortment (Pfeiffer et al., 2011). Fighting cocks are ubiquitous amongst the backyard and small scale commercial poultry producers in Thailand and other GMS countries, and result in additional mechanisms of potential spread of infection through movements to and from cock fighting events (Tiensin et al., 2009).

The intensity of transmission of HPAIV H5N1 during the 2004 epidemic in Thailand was quantified using a basic reproduction number R_0 between 2 and 5 (Tiensin et al., 2007). A transmission model for the North of Viet Nam confirmed the Red river delta as a hotspot for sustained onward transmission (Walker et al., 2010). This finding is consistent with spatial cluster analyses conducted for Viet Nam which identified clusters in the Red and Mekong river deltas (Pfeiffer et al., 2007).

The risk of HPAIV H5N1 transmission from poultry to humans is very low, as evidenced in the low morbidity, but case fatality rates are very high. Wang et al. (2012) suggest that non-fatal human cases are likely to be severely underreported, and that therefore current case fatality estimates of over 50% are too high. Exposure risk is highest amongst producers as well as in LBMs (Van Kerkhove et al., 2011; Wan et al., 2011). Viet Nam has had the highest reported human cases and fatalities in the GMS with 59 deaths and 119 cases between 2003 and 2011. Second is Thailand with 25 cases and 17 fatalities, followed by 18 cases and 16 fatalities in Cambodia and 2 cases and 2 fatalities in Lao PDR (*source*: WHO, January 2012). It needs to be emphasized that in particular the case numbers are likely to be an underestimate due to underdiagnosis and underreporting.

Epidemiologic investigations of human HPAI H5N1 cases have shown that transmission of HPAIV H5N1 from poultry to humans is currently limited to individuals who may have been in contact with the highest potential concentrations of virus shed by poultry (Van Kerkhove et al., 2011). This suggests that there may be a minimum level of virus concentration needed for effective transmission to occur and that circulating HPAIV H5N1 strains have not yet mutated to transmit easily from poultry to human, and clearly not from human to human. The mode of transmission varies within and between countries ranging from exposure to poultry or poultry products during a visit to a LBM to preparing infected poultry or swimming or bathing in ponds, which are frequented by poultry (Shinde et al., 2011; Van Kerkhove et al., 2011; Wan et al., 2011).

It has to be concluded that infection of humans with HPAIV H5N1 currently is fairly unlikely, even in the absence of specific hygienic prevention measures. Nevertheless, any human case of infection apart from the high case fatality rate, represents potential for virus reassortment that could produce a virus variant that

is transmissible between humans (Amendola et al., 2011; Van Kerkhove et al., 2011).

HPAI H5N1 Risk Management and Its Impact

Driving forces of national HPAI H5N1 risk management policy

Thailand is one of the world's largest poultry meat exporters (*source*: FAOSTAT). Therefore, the risk management response of the Thai government to the emergence of HPAI H5N1 in 2003/2004, and in particular the major epidemic in 2004 was very much influenced by the highly integrated intensive poultry producer stakeholder group as well as by the extensive publicity around the relatively small number of human fatalities (Safman, 2009). It was considered crucial to achieve status of disease freedom as soon as possible, and therefore during the 2004 epidemic a control policy of large-scale culling without vaccination was adopted (Safman, 2009). The risk management since then has been aimed at minimizing the likelihood of reoccurrence, and key components have been the introduction of intensive nationwide surveillance and of a compartmentalisation scheme for commercial poultry farms. The influence of backyard and small-scale chicken as well as duck farmers appears to have been much less significant, as has been that of cockfighting enthusiasts which represent a large part of rural communities (Safman, 2009).

In Viet Nam, policy development at national level is driven by state actors, i.e. the Vietnamese Communist party with a weak link to other sections of society, particularly with farmers who represent 70% of the population (Vu, 2009). Furthermore, the effectiveness of policy implementation at central government level is compromised by the relative independence of local authorities (Vu, 2009). This situation results in different control policies between provinces or districts, such as for example different levels of compensation between provinces (Vu, 2011). Significant introductions of foreign aid also had a strong influence on policy development (Vu, 2009). While the occurrence of HPAI H5N1 had not been acknowledged by the Vietnamese authorities until the beginning of 2004, from then on its control was given high priority, such that between 2005 and 2006 the Vietnamese government spent US $266 million on avian influenza control (Safman, 2009). The occurrence of the epidemic with at the time the highest number of reported human fatalities and the associated media reaction also resulted in rural and primitive farming practices being blamed for it (Lockerbie, 2008; Vu, 2011). The key difference in the control strategy compared with Thailand was the use of large-scale vaccination. In Vietnam, 65% of poultry producers were smallholder free-range systems which contributed 60–70% of all chickens sold per year. Industrial farming systems produced 18–20% of chickens, but only represented 0.1% of all poultry farms (Desvaux et al., 2008). Vietnam does not have significant live poultry and associated products exports. As a consequence of

this poultry production system structure, the industry stakeholders had relatively little influence on the policy response (Herington, 2010; Vu, 2009).

Both, Lao PDR and Cambodia only reported a very small number of outbreaks during the major epidemics in 2004/2005 within the GMS. They have low poultry density, and their policy response was strongly influenced by foreign aid and influence, largely due to poor animal and human health infrastructures (Burgos et al., 2008b; Ear, 2009).

National control measures and their efficacy

All GMS countries considered in this review engaged in promoting improved biosecurity at farm level as a method for preventing introduction of infection to poultry flocks. The specific approaches were broadly consistent with recommendations made by international organizations (Anonymous, 2008). But as discussed by Cristalli and Capua (2007), the incentives for promotion or adoption varied significantly between countries, with Thailand having achieved the highest level of awareness, and Cambodia and Lao PDR the lowest.

In Thailand, measures adopted for disease containment adhered closely to provisions laid out by FAO, WHO and OIE. These included a comprehensive cull of all susceptible poultry from farms located within a 5-km radius. Compensation was among the highest paid in South-east Asia. Movement restrictions were imposed within a 50-km radius of outbreak locations. A 90-day ban imposed on export of poultry from affected areas, redundant to prohibitions from other countries (Tiensin et al., 2005). From mid-2004, due to the reduction in outbreaks achieved by the disease containment policy, it was possible to focus on large-scale active surveillance involving diagnostic assessment of very large numbers of samples collected from farms, as well as in relation to movements and slaughter. Any outbreaks were controlled using culling within zones of only 1-km radius. Information campaigns were implemented in relation to human health protection and poultry biosecurity (Meyer and Preechajarn, 2006; Safman, 2009). To specifically protect industrial poultry farms from infection through exposure to potential presence of infection in backyard and small-scale commercial production systems, a government-funded scheme was implemented that involved establishment of disease-free compartments surrounding some industrial poultry farms. The biosecurity protocol involves intensive surveillance for infection in a 2-km buffer zone around the compartmentalized farms, as well as other measures (Meyer and Preechajarn, 2006; Ratananakorn and Wilson, 2011).

Viet Nam implemented a wide range of control measures, including large-scale culling, movement controls and closure of live poultry markets, banning poultry keeping in some major cities, and campaigns to educate the public about preventive measures. The culling policy was revised after the first epidemic wave (44 million birds culled) as it became clear that extensive culling based on pre-established geographic criteria (i.e. 1-km radius ring culling) was too expensive

and hard to perform given that farmers were not willing to give up apparently healthy birds (Vu, 2009). In addition to the direct cost of culling, farmers demanded compensation, which represented a major fiscal burden. In subsequent waves, targeted culling of high-risk bird populations immediately adjacent to infected farms was employed, dramatically reducing the number of birds culled. From 2005 onwards, Viet Nam launched comprehensive, nationwide vaccination campaigns for all birds, to a large extent funded by donors (Vu, 2009). Vaccination coverage achieved by the mass vaccination campaigns was at best moderate (Walker et al., 2010). Although the within-flock basic reproduction number of infection (R_0) has been significantly reduced in the fourth epidemic wave (vaccination-based control policy) when compared to the second epidemic wave (depopulation-based control policy), the mean within-flock R_0 of the fourth epidemic wave was still not significantly below unity, suggesting problems with obtaining the required vaccination coverage within some flocks (FAO, 2011; Soares Magalhães et al., 2010b).

Cambodia's control policy involves poultry movement restrictions and permitted culling of infected flocks without compensation. Also, 3-km protection zones and 10-km surveillance zones were established around outbreaks (Burgos et al., 2008a; Ear, 2009). Temporary suspension of sales and purchases of birds was mandated. However, law enforcement is weak and compliance is low (Burgos et al., 2008a; Ear, 2009).

Experience from Viet Nam (and also China) has shown that large-scale vaccination does not eliminate infection (FAO, 2011; Hinrichs and Otte, 2012; Peyre et al., 2009; Pfeiffer et al., 2011). Overall, control measures in place during the 2007 wave of outbreaks in Viet Nam reduced the number of communes capable of spreading infection by an estimated 11%. This was achieved at a far lower social and economic cost than during previous waves. However these gains have to be balanced against the cost of maintaining levels of effective vaccination protection in an endemic situation (Hinrichs and Otte, 2012). As estimates suggest that the infectious period at population level has increased following vaccination, the impact of waning levels of immunity as the initial impetus to vaccinate is lost, coupled with the effects these changes may have upon the ability to detect outbreaks, remains an issue which needs to be addressed (Walker et al., 2010). On the other hand, a control strategy without vaccination involving a combination of activities including intensive surveillance such as practiced in Thailand around compartmentalized poultry production units appears to be able to eliminate infection, and apparently prevent outbreaks of disease (Pfeiffer et al., 2011).

An important aspect of effective prevention of spread in the event of outbreaks is their early detection, as has been demonstrated by mathematical models (Walker et al., 2010). The most cost-effective mechanism for achieving this goal will be to incentivise farmers to report any suspect cases and for the animal health authorities to be able to react quickly. A generic set of guidelines for on-farm biosecurity has been published by the Food and Agriculture Organization

of the United Nations (FAO), and local stakeholders will implement adaptations of these which are relevant in their specific context (Anonymous, 2008, 2011a). It is important to recognize that biosecurity does not come in 'black or white' but in shades of grey. It is incremental, i.e. one measure can be put on top of another, and sensibly should address the biggest risk(s) first. This, however, means that biosecurity is to a large extent context-specific and, although in qualitative terms it is known how HPAIV H5N1 may spread, there is only limited quantitative data on the relative importance of different pathways of infection in different production systems. As all investments, investing in biosecurity is subject to the law of diminishing returns and it is neither economically efficient, nor biologically feasible, to reach 100% biosecurity. For privately funded investment in biosecurity the benefit to the individual needs to at least cover the cost over the lifetime of the investment. Given that investing in biosecurity has a fixed cost component, cost per bird protected will be lower for larger production units than for smaller production units, hence economic incentives differ by scale of production (in addition to the fact that larger flocks may have more transactions and therefore often more risky contacts than small flocks). Consequently, smallholder behaviour of limited investment into biosecurity is economically rational. Therefore, approaches to disease control need to be congruent with local social, cultural, economic and political realities (Fielding et al., 2009; Seng et al., 2008). Policies aimed at behaviour change which should be to HPAI H5N1 control, need to build on an understanding of existing behaviour, as the latter is likely to have very solid foundations, otherwise they are likely to fail. Biosecurity 'kills several birds with one stone' and returns at the beginning of the 'biosecurity function' are high. If context-specific (i.e. proven to work and not requiring radical changes in a given environment and production system), the introduction/improvement of biosecurity is potentially pro-poor rather than anti-poor, provided producers have access to the required capital and knowledge, and are given sufficient time and support to adapt.

Livelihoods and economic impacts of HPAI H5N1 disease and control

HPAI H5N1 affects animal production via three main pathways. Firstly, it causes direct losses to producers and other actors connected to the production and marketing of poultry through morbidity and mortality and the private costs associated with *ex ante* risk mitigation or *ex post* coping measures and the need to reinvest in replacement birds. Second, HPAI H5N1 has severe impacts through government intervention, which carries a cost borne by the public at large and affects producers and associated up- and downstream actors. Thirdly, HPAI H5N1 impacts arise through demand shocks created by consumer fears of contracting the disease. In concert, these impacts can lead to irreversible industry readjustments.

On a national scale, direct poultry losses from HPAI H5N1 disease and related culling were minor in Cambodia and Lao PDR, while both in Thailand and Viet Nam some 60 million birds were culled during the initial waves in 2004, which at the time represented between 20 and 30% of the standing poultry population (McLeod et al., 2005; Otte et al., 2008). Compensation payments and other public mitigation measures implemented by the respective governments transferred some of the financial burden from the private to the public sector (World Bank, 2006). Apart from direct losses, movement restrictions, marketing bans and consumer reluctance to purchase poultry and poultry products led to a severe drop in activity throughout the entire market-oriented sector of the poultry industry in the GMS, affecting feed producers, traders, processors and retailers (not eligible for compensation). The economic downturn of the poultry sector was partially compensated by increasing activity and prices in sectors producing substitute food products (Ifft et al., 2011; Otte et al., 2008).

The industrial/corporate poultry sector has adapted to HPAI H5N1 by exerting increasing control over every stage of production and raising sanitary standards (Behnke et al., 2012; McLeod, 2010; Otte et al., 2008; Walker et al., 2012). The high costs required to build the necessary infrastructure and difficulty of securing loans without collateral, make it unlikely that low-income households would be able to enter into any stage of industrial poultry production. Even farmers that presently have contracts may have difficulty adapting to the highly competitive conditions if they are required to make expensive upgrades to farm infrastructure. The high fixed costs of processing, controlled primarily by the integrators, pose another barrier prohibiting entry of independent farms into the system. Additionally, in Thailand, because of export orientation, processing plays an increasingly important role in the organization of poultry production (Heft-Neal et al., 2012b; Otte et al., 2008). Collectively, small-scale subsistence-oriented poultry keepers suffered the largest cumulative economic losses from HPAI H5N1 disease and control in the GMS while the disease posed the highest livelihoods threat to market-oriented poultry producers and market agents (in their majority usually relatively small-scale enterprises) specialized in poultry. The reason for this discrepancy is that the latter only represent a minority of producers, but a minority whose livelihoods are most affected by longer lasting HPAI H5N1 outbreaks and/or protracted control measures due to their relatively high investments and specialization in poultry (Otte et al., 2008).

Alternative approaches to HPAI H5N1 control

Animal diseases are part and parcel of farmers' everyday experience and local responses are determined at least as much by local cultural as by imposed technical rationales. There is a direct link between the perceived value of poultry and the optimum disease management approach from an individual farmer's perspective. Higher valuation of live poultry will increase the care taken, possibly

enhancing monitoring efforts and thereby reducing the culling radius. Enhancing the value of poultry, via improved marketing and safety, would ultimately result in less drastic HPAI H5N1 control policies. Numerically, small farmers and enterprises dominate the market populations across GMS agrifood systems. These networks confer livelihoods on such low income agents only because the costs of participation are very low. If control measures impose significant additional costs on the operations of any category of participation in these markets, they will be forced out quickly (Ifft et al., 2011). Moreover, because of low savings and the need to re-commit to some other livelihood activity, displacement like this can be irreversible. By promoting risk sharing supply chain relationships, such as contracting, certification, and traceability, individual agents can contribute to a local commons of lower disease risk, more credible product quality, and higher value added across low income networks extending from farmers to consumer households. In these circumstances, every value chain participant has a shared interest in more diligent safety production, distribution, and marketing practices. Such virtuous cycles of value creation/sharing can overcome endemic problems of moral hazard and adverse selection (Heft-Neal et al., 2012a; Heft-Neal et al., 2012b). Based on a simple statistical value of life calculation, the gain from reduced pandemic risk is in the billions of dollars, annually (Sproul et al., 2012). The private sector is unlikely to invest optimally in development of improved surveillance and risk reduction measures. Therefore, development of disease surveillance technologies has a global public good element, and their development should be supported by public sources. To deal with distributional issues within and across countries and regions, a regime of penalties should be accompanied by fixed transfers, including from third countries which benefit from reduced disease risk.

Conclusions

The HPAI H5N1 situation in the GMS countries illustrates the importance for adopting an interdisciplinary (or one health) approach towards risk assessment and management when dealing with disease problems. The countries are diverse across all aspects relevant to animal disease control, including the role of agriculture in the overall economy, livestock sector and market structure, individual and societal risk perceptions related to livelihoods and public health, national and local governance systems. This diversity limits the generality of national solutions and poses a challenge to multilateral coordination. Standard disease response prescriptions that populate international guidelines and are replicated in country plans assume well-functioning human and animal health systems, rapid and efficient response capacity, and up-to-date epidemiological information and technical expertise, none of which prevail in most GMS countries. Technocratic, expert-driven, top-down solutions falter in the face of bureaucratic and political complexity, institutional weakness, and local market imperfections.

Diseases can be controlled and even eradicated without having to reduce transmission risk to ZERO. To be cost effective, control measures should first be applied to the highest risk groups/areas/activities and proceed down the risk hierarchy as resources allow and aggregate risk necessitates. Disease control authorities need to recognize that the risk of livestock disease is a combined result of biological processes and economic as well as social behaviour extending across the entire agrifood sector, including livestock keepers, their input suppliers, their downstream market partners, and of agents within the animal and public health system itself. 'Conventional' disease control strategies, emphasizing public surveillance and economic sanctions, present significant long-term fiscal obligations and adverse incentive problems.

In the short term it will be impossible to eradicate HPAI H5N1 infection from the region. It is entirely feasible, however, to reduce rates of transmission to a degree that forestalls development of local reservoirs of infection and detects incursions before they have spread 'out of control'. Targeted control measures, such as reducing infection risks at LBMs, as well as prevention measures aimed at domestic duck production, would make important contributions to this 'second-best' objective. Transboundary HPAI H5N1 transmission risk within the GMS appears to be high and Thailand, Lao PDR, and Viet Nam are exposed to HPAIV introductions from southern China. In this setting, national and international resources for domestic eradication will not achieve their objectives, suggesting an urgent need for more determined multilateral policy coordination (Pfeiffer et al., 2011).

Poultry are rarely the primary source of income for rural households, and within the household level or small scale poultry 'enterprise', HPAI H5N1 is not normally the disease of primary concern. If this disease is seen as exceptional by other stakeholders, emergency responses need to communicate this with meaningful development responses that reward smallholders for internalizing national or global health risks. Unfortunately, these two 'response modalities' are decoupled both at international and national levels.

In the context of emergency response, risk management of HPAI H5N1 has not been integrated with other poultry or livestock disease issues, even though these may matter more to the smallholders. Support for producer 'diversification' and quality improvements appear a more promising tool for HPAI H5N1 risk reduction than targeted compensation for stock losses. The same reasoning applies to production and trade bans, which cannot be enforced and may make matters worse.

HPAIV H5N1 now appears to be endemic in parts of the GMS and domestic and (especially) external public resources for control measures will be difficult to sustain at previous levels (Pfeiffer et al., 2011). Attempting to improve the biosecurity of millions of backyard producers is an ineffective use of scarce resources, especially through public funds in countries with many high development priorities. Publicly funded, routine large-scale vaccination campaigns are

costly and appear to be inefficient (Swayne et al., 2011). Targeted vaccination of specific high-risk groups can achieve comparable risk reduction at a fraction of the cost (Hinrichs and Otte, 2012). For within-country areas with apparent endemic infection (e.g. Mekong delta in Viet Nam), eradication programmes should be considered, but carefully targeted at the mechanisms responsible for maintenance of infection. Hygiene and diagnostic effectiveness needs to be improved in LBMs and associated value chains. These include poultry trade networks (e.g. allow movement in one direction—downstream; limit distance travelled), live bird markets (rest days, species segregation) and targeted duck surveillance, including accreditation of infection-free duck farms. Establishment of infection-free zones or compartments is possible, as has been demonstrated by Thailand, and can be used as 'success stories' and technology incubators. Economic outcomes for these groups may also induce emulation/adoption elsewhere. Cross-border trade, particularly with southern China, is an important mechanism for recurrent introduction of infection to the GMS region. This risk needs to be managed, or national eradication programmes will be futile. Simple prohibitions of cross-border trade are ineffective and create informal flows that make infection processes unobservable. The only practical solution is multilateral coordination to effectively monitor flows of animals, products, and infrastructure. Reducing virus prevalence in poultry will significantly reduce the risk of humans to become infected, and this can be further reduced by public education campaigns limiting high risk behaviour.

Conflicts of Interest

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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A11

ZOONOSIS EMERGENCE LINKED TO AGRICULTURAL INTENSIFICATION AND ENVIRONMENTAL CHANGE[27]

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Abstract

A systematic review was conducted by a multidisciplinary team to analyze qualitatively best available scientific evidence on the effect of agricultural intensification and environmental changes on the risk of zoonoses for which there are epidemiological interactions between wildlife and livestock. The study found several examples in which agricultural intensification and/or environmental change were associated with an increased risk of zoonotic disease emergence, driven by the impact of an expanding human population and changing human behavior on the environment. We conclude that the rate of future zoonotic disease emergence or reemergence will be closely linked to the evolution of the agriculture–environment nexus. However, available research inadequately addresses the complexity and interrelatedness of environmental, biological, economic, and social dimensions of zoonotic pathogen emergence, which significantly limits our ability to predict, prevent, and respond to zoonotic disease emergence.

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Since prehistoric time, major changes in human disease burden, spatial distribution, and pathogen types have arisen largely owing to human activity. The change from small hunter-gatherer to large agricultural communities was associated with the emergence of human contagious diseases, many of which are of animal origin. Travel and colonization facilitated the introduction of disease to naïve populations. In the last century, improved nutrition and hygiene and the use of vaccines and antimicrobials reduced the infectious disease burden. However, in recent decades, increasing global travel and trade, expanding human and livestock populations, and changing behavior have been linked to a rise in disease emergence risk and the potential for pandemics (Harper and Armelagos, 2010; McMichael, 2004; Morse, 1995).

An analysis of human pathogens revealed that 58% of species were zoonotic, and 13% were emerging, of which 73% were zoonotic (Woolhouse and Gowtage-Sequeria, 2005). A similar study found that 26% of human pathogens also infected both domestic and wild animals (Cleaveland et al., 2001). Emerging pathogens are more likely to be viruses than other pathogen types and more likely to have a broad host range (Cleaveland et al., 2001; Woolhouse and Gowtage-Sequeria, 2005). Many recently emerged zoonoses originated in wildlife, and the risk of emerging zoonotic disease events of wildlife origin is higher nearer to the equator (Jones et al., 2008). The human health burden and livelihood impact of zoonotic disease in developing countries are greater than in the developed world, but lack of diagnosis and underreporting mean that the contribution of zoonotic disease to total human disease burden is not sufficiently understood (Maudlin et al., 2009).

The interaction of humans or livestock with wildlife exposes them to sylvatic disease cycles and the risk of spillover of potential pathogens (Figure A11-1). Livestock may become intermediate or amplifier hosts in which pathogens can evolve and spill over into humans, or humans can be infected directly from wildlife or vectors (Childs et al., 2007). Human behavioral changes, driven by increasing population, economic and technological development, and the associated spatial expansion of agriculture, are creating novel as well as more intensive interactions between humans, livestock, and wildlife. These changes have been implicated as drivers of some recent emerging disease events (McMichael, 2004; Morse, 1995; Woolhouse and Gowtage-Sequeria, 2005) that had important impacts on human livelihoods and health. Sustainable agricultural food systems that minimize the risk of emerging disease will therefore be needed to meet the food requirements of the rising global population, while protecting human health and conserving biodiversity and the environment. These will require a better understanding of the drivers of disease emergence.

To inform the research policy of the United Kingdom's Department of International Development, a systematic review was conducted to analyze qualitatively scientific knowledge in relation to the effect of agricultural intensification and environmental changes on risk of zoonoses at the wildlife–livestock–human interface.

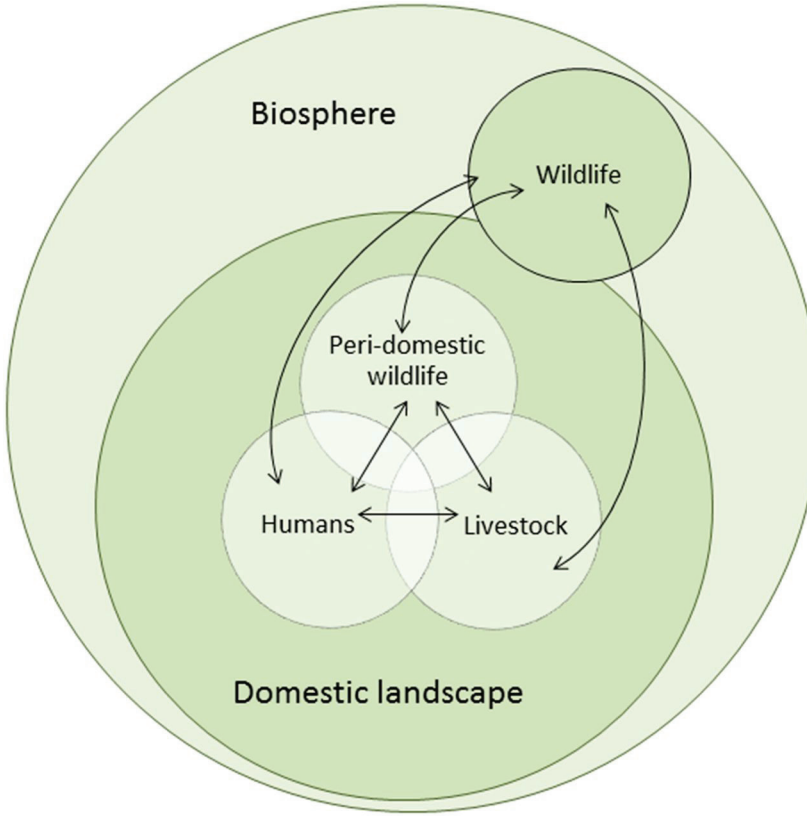

FIGURE A11-1 Pathogen flow at the wildlife–livestock–human interface. Arrows indicate direct, indirect, or vector-borne candidate pathogen flow. In each host species there is a vast array of constantly evolving microorganisms, some of which are pathogenic in the host. These are a source of new organisms for other host species, some of which may be pathogenic in the new host or may evolve in the new host to become pathogenic. If the pathogen is also transmissible in the new host species then a new transmission cycle may be established. The rate and direction of candidate pathogen flow will depend on the nature and intensity of interaction between wildlife, livestock, and human compartments and the characteristics of the compartments (Table A11-1).

Results

In summary, the review found strong evidence that modern farming practices and intensified systems can be linked to disease emergence and amplification (Brown, 2004; Cutler et al., 2010; Daszak et al., 2000; Dorny et al., 2009; Epstein et al., 2006; Gould and Higgs, 2009; Gummow, 2010; McMichael, 2004; Newell

et al., 2010). However, the evidence is not sufficient to judge whether the net effect of intensified agricultural production is more or less propitious to disease emergence and amplification than if it was not used. Expansion of agriculture promotes encroachment into wildlife habitats, leading to ecosystem changes and bringing humans and livestock into closer proximity to wildlife and vectors, and the sylvatic cycles of potential zoonotic pathogens. This greater intensity of interaction creates opportunities for spillover of previously unknown pathogens into livestock or humans and establishment of new transmission cycles. Anthropogenic environmental changes arising from settlement and agriculture include habitat fragmentation, deforestation, and replacement of natural vegetation by crops. These modify wildlife population structure and migration and reduce biodiversity by creating environments that favor particular hosts, vectors, and/or pathogens.

Direct Pathogen Spillover from Wildlife to Humans

Examples of direct pathogen spillover from wildlife to humans are many. The emergence of HIV is believed to have arisen from hunting of nonhuman primates for food in central African forests, and outbreaks of Ebola hemorrhagic fever have been associated with hunting in Gabon and the Republic of Congo (Daszak et al., 2000; Gummow, 2010; Leroy et al., 2004). Transmission of rabies by vampire bats to cattle and humans was associated with forest activities in South America (Belotto et al., 2005), and Kyasanur Forest disease outbreaks followed encroachment of agriculture and cattle into Indian forests (Chomel et al., 2007; Varna, 2001). The early human cases of severe acute respiratory syndrome (SARS) were associated with captive wildlife contact. It is likely that SARS corona virus-like virus of bats was transmitted, in the wild or in live animal markets, to various species of wild animal, such as masked palm civets (*Parguma larvata*), and spilled over into humans through contact with these intermediate hosts or their tissues, before establishing human–human transmission (Li et al., 2005).

Anthropogenic Environmental Change

Encroachment of human settlements and agriculture on natural ecosystems results in expansion of ecotones (transition zones between adjacent ecological systems), where species assemblages from different habitats mix. This provides new opportunities for pathogen spillover, genetic diversification, and adaptation. Associations between disease emergence and ecotones have been suggested for several diseases, including yellow fever, Lyme disease, hantavirus pulmonary syndrome, Nipah virus encephalitis, influenza, rabies, cholera, leptospirosis, malaria, and human African trypanosomiasis (Despommier et al., 2006). Most of these are zoonoses, and several involve both wildlife and livestock in their epidemiology.

Geographical expansion of Japanese encephalitis virus (JEV) in Southeast Asia has been associated with increasing irrigated rice production and pig farming due to an expanding human population (Pfeffer and Dobler, 2010; van den Hurk et al., 2009; Vora, 2008). The primary mosquito vector of JEV, *Culex tritaeniorhynchus*, breeds in irrigated areas, feeding primarily on herons and egrets but also on domestic and wild mammals. Although humans are dead-end hosts, pigs develop viremia and are amplifiers for human infection (Pfeffer and Dobler, 2010; van den Hurk et al., 2009). The combination of irrigated fields, which increase the density of vectors and water birds, and pig farming increases the risk of virus spillover into humans. Relocation of pigs away from households, in combination with human vaccination and vector control, has helped to decrease the incidence of human JEV in Japan, Taiwan, and Korea (van den Hurk et al., 2009).

A study of tsetse fly density and natural habitat fragmentation in eastern Zambia found that density was lowest in areas of greatest fragmentation, and intense human settlement and habitat clearance for agriculture has resulted in the disappearance of tsetse flies, which transmit human and animal trypanosomiasis (Ducheyne et al., 2009).

A study of the gut bacterium *Escherichia coli* in humans, livestock, and wildlife around Kibale National Park in Uganda found that isolates from humans and livestock living near forest fragments were genetically more similar to those from nonhuman primates in the forest fragments than to bacteria carried by nonhuman primates living in nearby undisturbed forest. The degree of similarity increased with the level of anthropogenic disturbance in the forest fragment (Goldberg et al., 2008). A second study in Bwindi Impenetrable National Park in Uganda found that the genetic similarity between *E. coli* isolated from humans and livestock and that of mountain gorillas increased with greater habitat overlap (Rwego et al., 2008). Higher interspecies transmission, which may be in either direction, is therefore likely to arise from greater ecological overlap.

The recent emergence of bat-associated viruses in Australia—Hendra virus, Australian bat lyssavirus, and Menangle virus—is associated with loss of bat habitat due to deforestation and agricultural expansion. Changes in the location, size, and structure of bat colonies, and foraging in periurban fruit trees have led to greater contact with livestock and humans, increasing the probability of pathogen spillover (Daszak et al., 2006; Field, 2009).

Loss of biodiversity can exacerbate the risk of pathogen spillover. In low diversity communities, vectors attain higher pathogen prevalences because they feed more frequently on primary reservoirs (Ostfeld, 2009; Vora, 2008). Conversely, vectors in high biodiversity communities feed on a wider range of hosts, some of which are poor pathogen reservoirs, often resulting in lower pathogen prevalence at ecological community level, as evidenced by the negative correlation between bird diversity and human West Nile virus incidence in the United States (Ostfeld, 2009). Forest fragmentation in North America has led to an increased risk of Lyme disease in humans as a result of reduced biodiversity and

the associated increase in the density of the white-footed mouse (*Peromyscus leucopus*), an efficient host for the causative agent, *Borrelia burgdorferi*, and its tick vector (Mathews, 2009; Pongsiri et al., 2009). Ticks occurring in forests with high vertebrate diversity have lower *B. burgdorferi* infection prevalence than ticks in low vertebrate diversity habitats, and there is a greater abundance of ticks in low diversity habitats (Ostfeld, 2009). The reemergence in Brazil of Chagas disease, caused by *Trypanosoma cruzi*, has been attributed to anthropogenic environmental change leading to low mammal diversity and abundance of the common opossum, *Didelphis aurita* (Vaz et al., 2007). *T. cruzi* sero-prevalence in small wild mammals in fragmented habitats was found to be higher than in continuous forest habitat owing to low small mammal diversity and increased marsupial abundance. Similar effects have been observed for leishmaniasis, Rocky Mountain spotted fever, and schistosomiasis (Vora, 2008).

Water management activities may result in increased density of breeding sites for mosquitoes. Rift Valley fever epidemics have occurred after the construction of dams and irrigation canals (Pepin et al., 2010). Liver fluke and its intermediate snail host have adapted to the irrigation systems of the Nile Delta in Egypt and in Peru, leading to increasing incidence of human fascioliasis (Mas-Coma et al., 2005). The effect of fertilizer use on disease dynamics varies depending on the pathogen, the host, the ecosystem, and the level of environmental nutrient enrichment (ENE), but parasites with complex life cycles, especially trematodes, increase in abundance under nutrient-rich conditions because their intermediate hosts—snails, worms, crustaceans—have increased population density and survival of infection. Increases in ENE in tropical and subtropical regions as agriculture develops may have an important impact because of the diversity of infectious pathogens in these areas (Johnson et al., 2010). The use of manure as a fertilizer may increase transmission of food-borne pathogens such as verotoxigenic *E. coli* and *Salmonella* (Newell et al., 2010).

Intensification of Livestock Farming

Intensification of livestock production, especially pigs and poultry, facilitates disease transmission by increasing population size and density (Cutler et al., 2010; Drew, 2011; Graham et al., 2008), although effective management and biosecurity measures will mitigate the between-herd spread of zoonotic diseases, such as brucellosis and tuberculosis (Perry et al., 2013). As an alternative to investing in improved husbandry or in situations of poor animal health service provision, antimicrobials are often used for growth promotion, disease prevention, or therapeutically, which in turn promotes the evolution of antimicrobial resistance in zoonotic pathogens (Gilchrist et al., 2007). Intensification also requires greater frequency of movement of people and vehicles on and off farms, which further increases the risk of pathogen transmission (Leibler et al., 2010).

Intensive livestock farming can promote disease transmission through environmental pathways (Graham et al., 2008). Ventilation systems expel material, including pathogens such as *Campylobacter* and avian influenza virus, into the environment, increasing risk of transmission to wild and domestic animals. Large quantities of waste are produced that contain a variety of pathogens capable of survival for several months if left untreated. Much of the waste is spread on land, where it can come into contact with wild animals and contaminate water. Similarly, use of animal waste in aquaculture leads to potential contact with wild birds (Graham et al., 2008).

Intensive farms use fewer workers per animal, thereby reducing the number of people exposed to zoonoses compared with extensive systems. However, several cross-sectional studies report higher sero-prevalence in farm workers of pandemic H1N1/09 influenza, hepatitis E, and highly pathogenic avian influenza H5 and H7 (Gilchrist et al., 2007; Graham et al., 2008) compared with the general community.

Intensive livestock systems generally have high density populations of low genetic diversity, which may favor increased transmission and adaptation (Drew, 2011). Epidemiological modeling experiments indicate that lower genetic diversity was associated with an increased probability of a major epidemic or no epidemic at all, whereas a more diverse population had a higher probability of a minor epidemic (Springbett et al., 2003).

Food-borne bacterial pathogens evolve in response to environmental changes, developing new virulence properties and occupying new niches, including antimicrobial resistance (Newell et al., 2010). Such evolution can be facilitated by intensified livestock systems. Increases in human salmonellosis have been due to the adaptation of *Salmonella enteritidis* phage type 4 to the poultry reproductive tract, and the emergence of vero cytotoxin-producing *E. coli* O157 to infect humans via contaminated beef and by environmental transmission (Newell et al., 2010).

Nipah Virus Emergence Linked to Livestock Intensification and Environmental Change

The first known outbreak of Nipah virus occurred in Malaysia during 1998–1999, causing respiratory disease in pigs and high case fatality in humans. Epidemiological outbreak investigation showed that pig and human cases had occurred in 1997 on a large intensive pig farm in northern Malaysia (Epstein et al., 2006), where Nipah virus-infected fruit bats were attracted to fruit trees planted around the farm. This provided the opportunity for virus spillover to susceptible pigs via consumption of fruit contaminated with bat saliva or urine. Respiratory spread of infection between pigs was facilitated by high pig and farm density and transport of pigs between farms to the main outbreak area in south Malaysia (Daszak et al., 2006; Field, 2009). Pigs then acted as amplifier hosts for human infection (Field, 2009). Almost all human cases had contact with pigs; there was no evidence of

direct spillover from bats to humans or of human-to-human transmission (Epstein et al., 2006). The outbreak was controlled by mass culling of pigs, and there have been no further outbreaks of Nipah virus in Malaysia (Epstein et al., 2006). Nipah virus was found to be closely related to Hendra virus, for which the reservoir hosts are *Pteropus sp.* fruit bats. A high sero-prevalence was found in several species of Malaysian bats, suggesting that they are reservoirs and that the virus is endemic (Chua et al., 2002; Epstein et al., 2006; Yob et al., 2001). Epstein et al. (2006) and Daszak et al. (2006) propose that Malaysian bats have historically been infected with Nipah virus and that there has probably been sporadic bat-to-pig and pig-to-human transmission. They hypothesize that the initial 1997 outbreak on the index pig farm died out quickly, causing only a few human cases, but the reintroduction of virus into a partially immune population in 1998 resulted in prolonged circulation on the farm, increasing the risk of spread to other farms and to humans. When infected pigs were sold from the affected farm to the south, where there was a high density of smaller intensive pig farms and a high human density, a large outbreak occurred in humans, stimulating an investigation and the discovery of Nipah virus as the causative agent (Daszak et al., 2006; Epstein et al., 2006). They conclude that the emergence of Nipah virus was primarily driven by intensification of the pig industry combined with fruit production in an area already populated by Nipah virus-infected fruit bats.

In contrast, seasonal clusters of human Nipah encephalitis cases occurred in Bangladesh and India between 2001 and 2005, with no apparent intermediate host (Field, 2009). Serological surveys found Nipah virus antibodies in *Pteropus giganteus* fruit bats but no evidence of infection in pigs or other animals (Daszak et al., 2006; Hsu et al., 2004). It is believed that humans in these outbreaks acquired infection initially from bats via contaminated date palm sap and that the outbreaks spread through human-to-human transmission (Daszak et al., 2006; Epstein et al., 2006). There is serological evidence that henipaviruses occur throughout the range of pteropid bat species, which occur from Madagascar to South and Southeast Asia, Australasia, and Pacific Islands (Epstein et al., 2006). Surveys in bats in India, Indonesia, Cambodia, Thailand, and Madagascar have found Nipah virus RNA or virus-neutralizing antibodies (Epstein et al., 2008; Iehle et al., 2007; Reynes et al., 2005; Sendow et al., 2006; Wacharapluesadee et al., 2010). Nipah and Hendra virus-neutralizing antibodies and henipavirus RNA were also found in *Eidolon helvum* fruit bats sampled in Ghana in West Africa, demonstrating that henipaviruses are not restricted to the range of pteropid bats (Drexler et al., 2009; Hayman et al., 2008).

Influenza A Virus Emergence Linked to Poultry Farming Practices

Influenza A viruses are segmented RNA viruses that evolve constantly by reassortment and mutation to create new strains of varying pathogenicity and host range (Landolt and Olsen, 2007; Pekosz and Glass, 2008). They are found

in birds, humans, pigs, horses, cats, dogs, and other animals (Landolt and Olsen, 2007; Riedel, 2006). Aquatic birds are considered to be the natural reservoir hosts (Irvine and Brown, 2009; Landolt and Olsen, 2007) and seem to host a variety of ephemeral variants rather than a single discrete strain (Dugan et al., 2008). Avian influenza is usually subclinical or of low pathogenicity in wild birds (Artois et al., 2009), but some strains may be highly pathogenic when introduced to domestic poultry (Landolt and Olsen, 2007). Swine influenza occurs in several subtypes in pigs worldwide, and infection may be transmitted between pigs, birds, and humans (Irvine and Brown, 2009; Landolt and Olsen, 2007).

Both extensive and intensive farming practices can influence the likelihood of influenza virus spillover from wild birds to domestic birds and pigs and the subsequent evolution and amplification in domestic animals and transmission to humans. Rice paddies combined with free-grazing duck farming in wetland areas bring wild water birds into close proximity with domestic water birds (Artois et al., 2009; Gilbert et al., 2007). The latter are susceptible to infection but less likely to develop disease than chickens and are infectious to other domestic poultry by direct contact or environmental contamination (Sims et al., 2005). Other low biosecurity rearing systems, such as scavenging poultry, household poultry, and small-scale commercial poultry, also allow direct or indirect contact between wild and domestic birds (Artois et al., 2009; Sims et al., 2005).

Although high biodiversity of the wild bird population can increase the risk of pathogen spillover, low genetic diversity in the domestic population encourages rapid dissemination of infection if the latter are susceptible (Drew, 2011; Keesing et al., 2010). The expansion of intensive livestock production in the last few decades, particularly for short generation interval species such as poultry and pigs, creates large high density populations in which there is an increased probability of adaptation of an introduced influenza virus and amplification for transmission between farms, to humans, and to wild animals (Gilbert et al., 2007; Graham et al., 2008; Kapan et al., 2006). The increased trade in poultry and poultry products can rapidly spread infection to new farms, areas, or countries, whether by small-scale informal or formal trade or large-scale commercial trade. Live bird markets in particular play an important role in disseminating infection and provide opportunities for cross-species transmission between domestic and wild birds (Fevre et al., 2006; Sims et al., 2005).

The human disease impact of recently emerged human pathogenic influenza viruses has been lower than was observed during the pandemics of the last century, but the potential remains for the evolution of a variant that is both highly transmissible to humans and of high pathogenicity (Landolt and Olsen, 2007). Farming systems that allow contact between wild and domestic birds and pigs and have large high density populations that facilitate transmission, adaption, and amplification are increasing the risk that such a pandemic variant will emerge.

Discussion

The results from this work will inform the research policy of the United Kingdom Department of International Development. Given the broad nature of the study question and the potential for significant biases, we decided that a systematic review approach was required, so that the scientific knowledge base could be examined in a structured and transparent manner. A key objective was to obtain as complete a literature database as possible. Most of the publications that were included did not present data and results suitable for quantitative analysis, such as metaanalysis, and therefore the interpretation needed to be based on qualitative methods.

Some of the limitations of our approach included the following: few papers described primary research; different review papers tended to be based on the same small number of primary research papers; the diversity of studies prevented metaanalysis; and non-English language papers were excluded from the initial database search.

This systematic review found several examples of zoonotic disease emergence at the wildlife–livestock–human interface that were associated with varying combinations of agricultural intensification and environmental change, such as habitat fragmentation and ecotones, reduced biodiversity, agricultural changes, and increasing human density in ecosystems. Expansion of livestock production, especially in proximity to wildlife habitats, has facilitated pathogen spillover from wildlife to livestock and vice versa and increased the likelihood that livestock become amplifying hosts in which pathogens can evolve and become transmissible to humans. Some wildlife species have adapted to and thrived in the ecological landscape created by human settlement and agriculture and have become reservoirs for disease in livestock and humans. Table A11-1 provides a conceptual framework of the characteristics of the types of wildlife–livestock–human interface where zoonotic disease has emerged or reemerged.

Human population growth and associated changes and increases in demand for food and other commodities are drivers of environmental change, such as urbanization, agricultural expansion and intensification, and habitat alteration. These play an important role in the emergence and reemergence of infectious diseases by affecting ecological systems at landscape and community levels, as well as host and pathogen population dynamics. Climate variability interacts with these environmental changes to contribute to disease emergence (Wilcox and Colwell, 2005). Changes in the ecosystem can lead to increased pathogen transmission between hosts or greater contact with new host populations or host species. This occurs against a background of pathogen evolution and selection pressure, leading to emergence of pathogen strains that are adapted to the new conditions (Daszak et al., 2001). The intensity of the interface between wildlife, humans, and domestic animal species has never been static, and all biological systems have an inherent capacity for both resilience and adaptation (Redman and

TABLE A11-1 Conceptual Framework of Types of Wildlife–Livestock–Human Interfaces and Their Characteristics

Type of wildlife–livestock–human interface	Level of biodiversity	Characteristics of livestock population	Connectedness between populations	Examples of zoonotic disease with altered dynamics
"Pristine" ecosystem with human incursion to harvest wildlife and other resources	High	No livestock	Very low, small populations and limited contact	Ebola, HIV, SARS, Nipah virus in Bangladesh and India
Ecotones and fragmentation of natural ecosystems: farming edges, human incursion to harvest natural resources	High but decreasing	Few livestock, multiple species, mostly extensive systems	Increasing contact between people, livestock, and wild animals	Kyasanur Forest disease, Bat rabies, *E. coli* interspecies transmission in Uganda, Nipah virus in Malaysia
Evolving landscape: rapid intensification of agriculture and livestock, alongside extensive and backyard farming	Low, but increasing peridomestic wildlife	Many livestock, both intensive and genetically homogenous, as well as extensive and genetically diverse	High contacts between intensive and extensive livestock, people, and peridomestic wildlife. Less with endangered wildlife.	Avian influenza, Japanese encephalitis virus in Asia
Managed landscape: islands of intensive farming, highly regulated. Farm land converted to recreational and conservancy	Low, but increased number of certain peridomestic wildlife species	Many livestock, mainly intensive, genetically homogeneous, biosecure	Fewer contacts between livestock, and people; increasing contacts with wildlife.	Bat-associated viruses in Australia, West Nile virus in United States, Lyme disease in United States

Kinzig, 2003), but the current pace of anthropogenic change could be too fast to allow system adaptation and overwhelm resilience.

In pristine or natural ecosystems, coevolution of host and pathogens tends to favor low pathogenicity microorganisms. In intensive systems, genetic selection and management of livestock creates frequent contact opportunities, high animal numbers, and low genetic diversity, providing opportunities for "wild" microorganisms to invade and amplify or for existing pathogens to evolve to new and more pathogenic forms. Human influence on the ecosystem through farming practices, extensive transportation networks, sale of live animals, and juxtaposition of agriculture or recreation with wildlife all contribute to emergence and shifting virulence of pathogens.

Key features of the systems within which these processes occur are their complexity, connectedness, feedback loops, and emerging properties. These cannot be captured by the single- or multidisciplinary approaches that the majority of published research is still based on, and simple globally generalizable explanations for zoonoses emergence are not possible. Instead the geographical diversity and complexity of systems requires local interdisciplinary studies to be conducted to generate locally relevant solutions. A priority for research therefore should be a holistic perspective on pathogen dynamics at the wildlife–livestock–human interface, based on an interdisciplinary approach to the examination of biological, ecological, economic, and social drivers of pathogen emergence. Investigations are required on the frequency and risks of pathogen flow between species, the mechanisms of amplification and persistence, the influence of different livestock production systems, and the socioeconomic context, to identify possible interventions to reduce pathogen emergence, as well as more effective strategies for responding to such events.

In conclusion, we find that available research clearly indicates the significance of the zoonotic disease threat associated with the wildlife–livestock interface. However, it inadequately addresses the complexity, context specificity, and interrelatedness of the environmental, biological, and social dimensions of zoonotic pathogen emergence and has therefore failed to generate scientific evidence to underpin effective management of zoonotic disease risk at the wildlife–livestock interface.

Methods

A qualitative systematic review was carried out during late 2010 to early 2011 by a multidisciplinary team with expertise in epidemiology, socioeconomics, and ecology. A systematic review is an analytical research study design that follows a structured approach toward selecting, analyzing, and interpreting available empirical evidence in an integrated way to answer a specific research question while explicitly taking potential bias into account (Tricco et al., 2011). The full protocol for conducting the review is provided as supplementary material

(*SI Methods*, Fig. S1, and Tables S1–S3) and is summarized here. The overall objective of the study was to analyze scientific knowledge in relation to zoonotic disease transmission by direct or indirect livestock–wildlife interaction, with emphasis on risk factors, drivers, and trajectories of transmission. This article focuses on those study findings that provide evidence of the effect of agricultural intensification and environmental change on zoonosis at the wildlife–livestock–human interface.

The overall objective was broken down into seven themes, for which literature database search terms and algorithms were defined. More than 280 unique algorithms were used and more than 100 keywords. Several databases were explored to assess the number and quality of papers identified, and PubMed (www.ncbi.nlm.nih.gov/pubmed) and CAB Direct (www.cabdirect.org) were selected. The initial search criteria were English language papers published from 2006 to 2010 describing primary research and reviews. A total of 1,022 relevant published papers were identified by searching their titles and abstracts for specified key words. The abstracts were independently reviewed by at least two reviewers to identify those that contained relevant information, and 261 papers were selected to be assessed for eligibility using forms for data extraction and assessment of study quality (*SI Methods*). One hundred forty-five papers were eligible for inclusion. A further 133 papers were identified by screening the reference lists of the eligible papers and inclusion of relevant papers already known to the team. This resulted in a total of 278 eligible papers, 57 of which were relevant to the topic of this article. Because of the wide variation in type of study, geographical location, pathogens, and host species it was not possible to conduct quantitative metaanalysis, so information was extracted, summarized, and organized by emerging themes; these are the headings used in *Results* in this article.

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A12

GLOBAL TRENDS IN EMERGING VIRAL DISEASES OF WILDLIFE ORIGIN

*Jonathan **Sleeman***[31] *and Hon Ip*[31]

Introduction

Fifty years ago, infectious diseases were rarely considered threats to wildlife populations, and the study of wildlife diseases was largely a neglected endeavor. Furthermore, public health leaders at that time had declared that "it is time to close the book on infectious diseases and the war against pestilence won," a quote attributed to Dr. William H. Stewart in 1967. There is some debate whether he actually said these words; however, they reflect the widespread belief at that time (Spellberg, 2008). Leap forward to today, and the book on infectious diseases has been dusted off. There is general consensus that the global environment favors the emergence of infectious diseases, and in particular, diseases of wildlife origin (Taylor et al., 2001). Examples of drivers of these infectious diseases include climate and landscape changes, human demographic and behavior changes, global travel and trade, microbial adaptation, and lack of appropriate infrastructure for wildlife disease control and prevention (Daszak et al., 2001). The consequences of these emerging diseases are global and profound with increased burden on the public health system, negative impacts on the global economy and food security, declines and extinctions of wildlife species, and subsequent loss of ecosystem integrity. For example, 35 million people are currently living with HIV infection globally (http://www.who.int/gho/hiv/en); 400 million poultry have been culled since 2003 as a result of efforts to control highly pathogenic H5N1 avian influenza (http://www.fao.org/avianflu/en/index.html), and there are increasing biological and ecological consequences.

Examples of health threats to biodiversity include the "spillover" of human diseases to great ape populations (Köndgen et al., 2008), the near-extirpation of

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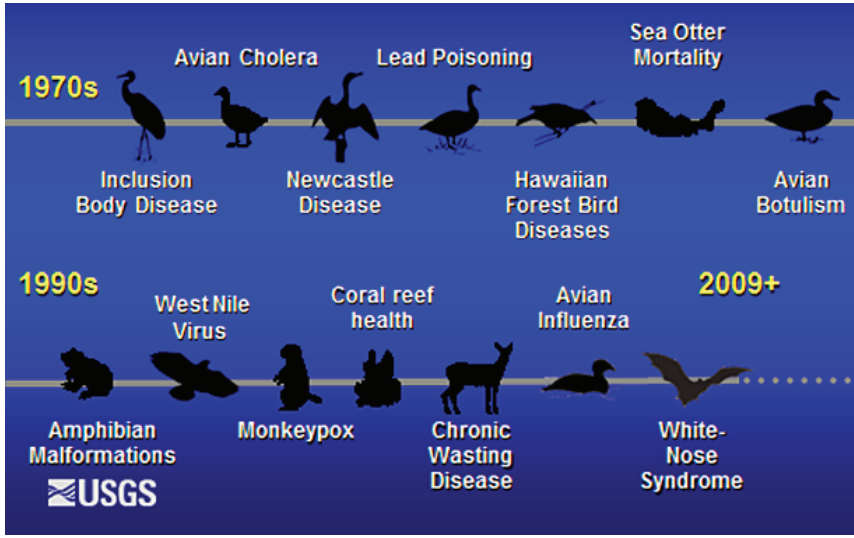

FIGURE A12-1 Emerging diseases investigated by the U.S. Geological Survey. SOURCE: U.S. Geological Survey.

the black-footed ferret from canine distemper and sylvatic plague (for a review see Abbott et al., 2012), and threats to Hawaiian forest birds from introduced pathogens such as avian malaria and avian pox (van Riper et al., 1986, 2002). There are also newly discovered pathogens or diseases that have resulted in population declines, and global extinctions of several species. Examples include *Batrachochytrium dendrobatidis*, which causes a cutaneous fungal infection of amphibians and is linked to declines of amphibians globally (Kriger and Hero, 2009); and recently discovered *Pseudogymnoascus (Geomyces) destructans*, the etiologic agent of white-nose syndrome (WNS), which has caused precipitous declines of North American bat species (Blehert et al., 2009). Furthermore, there is increasing evidence of the subsequent impacts on human and ecosystem health; for example, increasing risk of exposure to Lyme disease as a consequence of decreased biodiversity (LoGiudice et al., 2003) as well as the economic cost of the loss of bats due to decreased insect control services (Boyles et al., 2011). Figure A12-1 is a timeline of important diseases investigated by the U.S. Geological Survey since the 1970s, which illustrates three factors:

1. The unprecedented emergence of new pathogens and geographic spread of known pathogens since the 1990s;

2. Diseases are increasingly causing large-scale, negative impacts on wildlife populations and spreading over larger geographic areas rather than remaining localized; and
3. Diseases are increasingly of concern for multiple sectors, including public health, agriculture and wildlife management agencies.

Of increasing concern are these novel diseases such as WNS as they are hard to anticipate, particularly devastating to human health or wildlife populations, challenging to manage, spread over large geographic areas in short time periods, and may result in ecological ripple effects that are difficult to predict.

The following article provides examples of recently emerged viral diseases of wildlife origin. The examples have been selected to illustrate the drivers of emerging viral diseases, both novel pathogens and previously known diseases, the impacts of these diseases, as well as the role of wildlife both as "villains" or reservoirs as well as "victims" of these viral diseases. The article also discusses potential management strategies for emerging viral diseases in wildlife populations and future science directions in wildlife health to prevent, prepare, respond to, and recover from these disease events. Finally, the concept of One Health and its potential role in developing solutions to these issues of mutual concern is discussed.

Avian Influenza

Wild Birds as Victims?

Extensive phylogenetic analysis of avian influenza viruses has shown that wild birds do not normally harbor highly pathogenic lineages; instead waterfowl and shorebirds in particular are the reservoirs of a vast diversity of low pathogenic avian influenza (LPAI) viruses (Rohm et al., 1995; Sakoda et al., 2010). These LPAI viruses, following their introduction into domestic poultry, then evolve into highly pathogenic strains (Monne et al., 2014). The adaptation to respiratory transmission and ability to replicate in extra-intestinal organs in poultry can take as little as weeks to months following lapses in biosecurity in facilities (Berhane et al., 2009).

Highly pathogenic avian influenza (HPAI) outbreaks are rare in wild birds. Sparrows, starlings, and other peridomestic free-living species have occasionally died in association with HPAI outbreaks in poultry, but these are usually few in number and were exposed to the same virus as in nearby outbreaks in poultry (Alexander, 2007). Of the 31 HPAI outbreaks that have occurred since the discovery in 1955 that fowl plague, a devastating disease in poultry, was caused by avian influenza viruses, only two have involved significant number of wild birds (Table A12-1). The first large-scale wild bird HPAI outbreak killed more than 1,300 common terns (*Sterna hirundo*) in 1961 in South Africa (Becker, 1966).

TABLE A12-1 Highly Pathogenic Avian Influenza Outbreaks in Poultry and Wild Birds Since 1959

Year	Strain	Location	Species	Number of Animals
1959	H5N1	Scotland, UK	Chicken	Unknown
1961	H5N3	South Africa	Gulls	1,300
1963	H7N3	England, UK	Turkey	29,000
1966	H5N9	Ontario, Canada	Turkey	8,000
1975	H7N7	Victoria, Australia	Chicken	58,000
1979	H7N7	Germany	Chicken	600,000
1979	H7N7	England, UK	Turkey	9,000
1983	H5N2	Pennsylvania, US	Chicken	>17 million
1983	H5N8	Ireland	Ducks	307,000
1985	H7N7	Victoria, Australia	Chicken	240,000
1991	H5N1	England, UK	Turkey	8,000
1992	H7N3	Victoria, Australia	Chicken	18,000
1994	H7N3	Queensland, Australia	Chicken	22,000
1994	H5N2	Mexico	Chicken	Unknown
1995	H7N3	Pakistan	Chicken	>6 million
1996	H5N1	Hong Kong	Chicken	3 million
1997	H7N4	New South Wales, Australia	Chicken	160,000
1997	H5N2	Italy	Chicken	8,000
1999	H7N1	Italy	Chicken	14 million
2003	H5N1	Hong Kong, China	Chicken	1.5 million
2002	H7N3	Chile	Chicken	700,000
2003	H7N7	Netherlands	Chicken	>25 million
2004	H7N3	British Columbia, Canada	Chicken	17 million
2004	H5N2	Texas, US	Chicken	6,600
2004	H5N2	South Africa	Ostrich	30,000
2004-Present	H5N1	Asia, Europe, Africa	Chicken	>400 million
2005	H5N2	British Columbia, Canada	Chicken	16 million
2007	H7N3	Saskatchewan, Canada	Chicken	53,000
2008	H7N7	England, UK	Chicken	15,000
2011	H5N2	South Africa	Ostrich	26,000
2014	H5N8	South Korea	Chicken	14 million

It was speculated at the time that unspecified stress in the colony might have "converted a latent into an overt infection" (Becker, 1966). Most of the HPAI outbreaks listed in Table A12-1 were limited in geographical location and were eliminated through prompt management actions and resulted in limited wild bird

exposure. However, the HPAI H5N1 outbreak is exceptional as it had gone largely unchecked from its emergence in 1996 until the events of 2005 (the extraordinary efforts by the government of Hong Kong in 2001 and 2003 are obvious exceptions), resulting in significant spread by human action throughout Southeast Asia (FAO, 2011a). As might be expected from such a large-scale infection in poultry, spill back into wild birds was likely. The HPAI H5N1 outbreaks in wild birds that occurred in 2005–2006, particularly the event in Qinghai, China, where more than 6,000 birds including 3,282 bar-headed geese perished, was the second HPAI outbreak with significant wild bird involvement (Chen et al., 2005; Liu et al., 2005).

Wild Birds as Vectors?

The spectacular feats of long-distance migration that some species of birds undertake makes the concept of long distance disease spread easy to assume. For example, because the strain of West Nile virus (WNV) isolated in New York in 1999 is most closely related to WNV that caused an outbreak in geese and storks in Israel 1 year earlier, Rappole et al. (2000) proposed 31 species of birds that might act as vectors between the Old and New World (Rappole et al., 2000). Other mechanisms of introduction such as the importation of a WNV-infected bird, mosquitoes, or even an infected person are also possible and the exact mechanism of how WNV arrived in North America remains a subject of debate (Roehrig, 2013).

The spread of WNV following its introduction into the Americas in 1999 may serve as an informative example of the spread of a newly introduced disease agent across the landscape. Since WNV has a wide host range (infection has been documented in more than 300 species of birds) and it is a mosquito-transmitted arbovirus infecting at least 10 genera of mosquitoes, the virus was predicted to spread rapidly from its initial introduced location in New York. However, the virus was restricted to the Northeast United States and the Atlantic Flyway for 3 years despite multiple cycles of annual migration, expanding only to the Gulf states in 2001. That year, the first evidence of WNV infection in birds of the Mississippi Flyway was reported, and in the following year, the virus was reported in the rest of the continental United States. However, it was not until 2005, 6 years after its introduction, that WNV was detected in all 48 contiguous states (Gubler, 2007).

Large-scale surveillance programs have detected infections of HPAI H5N1 in very few healthy wild birds, but wild birds are likely to have introduced Clade 2.3.2 HPAI H5N1 viruses into Russia and Mongolia in 2009 and Romania in 2010 (FAO, 2011b). The outbreaks of HPAI H5N1 in Japan in 2010/2011 were preceded by the detection of the virus in wild bird fecal samples collected from Lake Onuma, Hokkaido, in October 2010 (Kajihara et al., 2011; Sakoda et al., 2012). A similar situation occurred in South Korea over the same period, with initial detection in healthy wild birds, followed by outbreaks in poultry farms (Kim et al., 2012). In both Korea and Japan, the virus was assumed to have been

introduced by migratory birds, and following introduction into poultry, the subsequent spread from farm to farm was due to agricultural practices. Together, Japan and South Korea culled 7.3 million birds during eradication efforts (Korea, 2011; Tsukamoto, 2012). Conversely, the persistence of viruses belonging to Clade 1 within Vietnam while having been extirpated elsewhere, and the continued circulation of Clade 2.1 viruses in Indonesia since 2004 and Clade 2.2.1 virus in Egypt since 2006 without any evidence of long-distance export of these clades to adjacent or distant countries, suggests that long-distance transportation of HPAI H5N1 by migratory birds is not usually a significant mechanism of transmission (Scotch et al., 2013; WHO, 2011).

Phylogenetics

The genetic sequence of a virus can be used in phylogenetic studies and when appropriately applied can be used to infer the evolutionary relationship and possible routes of introduction and transmission. For example, HPAI H5N1 outbreaks have occurred in South Korea and Japan in 2004, 2007, and 2010. During each outbreak, genetic information of the viruses provided information independent of the traditional epidemiological investigations to support determinations of the mechanism and pattern of spread. Moreover, the comparison of nucleotide sequence differences showed that the viruses belonged to different clades in different years (2004, Clade 2.5; 2007, Clade 2.2; 2010, Clade 2.3.2.1) and supported the countries' assertion that the stamping-out policies were effective in the eradication of the virus each time (Kim et al., 2012; Sakoda et al., 2012).

Genetic information can also be used to infer the possible origin of an outbreak strain. The persistence of HPAI H5N1 in China since 1996 has allowed it to re-assort with other avian influenza viruses circulating in poultry (Chen et al., 2004). The HPAI H5N8 virus that was first detected in South Korea in January 2014 is characterized by a hemagglutinin from the HPAI H5N1 Clade 2.3.4.6 lineage, but the other genes are from a variety of viruses including other H5N8, H5N2, and H11N9 viruses (Lee et al., 2014). These types of re-assortments might provide the progeny virus with improved fitness such as increased ability to replicate in domestic poultry or amantadine resistance. Similarly, the nucleotide sequence of the strains of Bluetongue virus found in the European Union (EU) has been used in phylogenetic comparisons with those found elsewhere to infer possible sources of introductions (Purse et al., 2005).

A Direct Pipeline?

Do existing viruses always have to mutate or undergo adaptation before they are able to infect new hosts? Recent examples of avian influenza A viruses suggest that some contemporary viruses already possess the ability to infect mammals efficiently. An avian influenza H3N8 virus was shown to be the causative

agent in a large-scale mortality event of harbor seals (*Phoca vitulina*) in the northeastern United States in 2011 (Anthony et al., 2012). The virus was most closely related to contemporary wild bird H3N8 viruses in North America and did not contain RNA segments from mammalian influenza viruses, including canine and equine H3N8 lineages. In a second example, an H7N9 virus was found in an 87-year-old man from Shanghai, China, who had died in March 2013 of pneumonia. Since then, more than 450 cases of additional H7N9 infection have been reported with an estimated 32 percent fatality rate. In spite of the serious disease in people, these H7N9 viruses do not cause mortality in experimentally infected chickens and have been found in apparently healthy poultry in live bird markets (Morens et al., 2013). In both the seal H3N8 and poultry H7N9 viruses, mutations that confer increased ability to replicate in mammalian hosts were already present in the avian viruses, suggesting these viruses have a preexisting ability to cross species barriers.

In summary, the literature reveals a complex picture of the role of migratory birds in avian influenza epidemiology, and the view that migratory birds are primarily responsible for highly pathogenic avian influenza outbreaks or drive viral diversity is too simplistic. However, knowing that influenza viruses will move from wild to domestic birds and to people, focusing on interventions such as farm biosecurity will remain key to reducing risks.

There is also a need for ongoing surveillance of genetic diversity. A recent paper showed that gene segments from the 1918 Spanish flu virus circulate today in wild birds and that an artificial construct that brings together modern descendants of the 1918 virus is capable of elevated pathogenicity in mice as well as being able to be spread by respiratory droplets (Watanabe et al., 2014). As the authors of the paper note, a better understanding of the genetic diversity and the molecular mechanisms of pathogenicity will aid in improved risk assessment and outbreak preparedness.

Global Trade: Schmallenberg Virus

Schmallenberg virus (SBV) is a bunyavirus and a member of the Simbu serogroup (OIE, 2013). Its introduction in Europe serves as an example of the expansion of a virus into an area where susceptible hosts and suitable vectors already exist. Schmallenberg virus was first detected in Europe in November 2011 in Schmallenberg, Germany, and 1 month later in The Netherlands (Tarlinton et al., 2012). Since then, 16 countries in Europe have been affected. Infection with SBV is associated with deformities in lambs and calves, abortions, and decreased milk production in cattle, goats, and sheep. The virus is suspected to have been first introduced into Europe in early 2011 because there is no evidence of SBV in archived samples prior to 2011 and its effects only became apparent during the 2011 fall lambing season (Beer et al., 2013). While the actual route of introduction has not been established, the virus may have been introduced by midges that

arrived in shipments of cut flowers, or produce arriving daily from Africa into the clusters of international airports including Amsterdam, Brussels, and Cologne as well as seaports such as Rotterdam (Beer et al., 2013). These are also areas with a high density of susceptible hosts including cattle and sheep as well as established populations of *Culicoides* species that are competent vectors of SBV.

Climate Change: Bluetongue Virus

Bluetongue virus (BTV) is an orbivirus belonging to the family Reoviridae and is transmitted by *Culicoides* spp. midges. Bluetongue virus had historically been restricted to a zone between approximately 40°N and 35°S, and its expansion into Europe is an example of spread as a result of climate change leading to conditions permissive to sustained transmission. Since 1998, the geographical range of BTV has expanded, sometimes as far as 700 km northward. Between 1998 and 2005, BTV belonging to five serogroups (BTV-1, BTV-2, BTV-4, BTV-9, and BTV-16) was present continuously in the Mediterranean basin (Saegerman et al., 2008). In 2006, BTV-8 was detected for the first time in Europe, initially in The Netherlands and by 2007, more than 60,000 farms in six countries were affected (Wilson and Mellor, 2009). At least six countries (Bulgaria, France, Italy, Macedonia, Tunisia, and Yugoslavia) had never had BTV previously. Prior to 2004, BTV was associated with periodic expansion and contraction from endemic into adjacent areas as conditions favorable for transmission such as vector availability alternated (Walton, 2004), but the widespread outbreak of BTV-8 in 2006 suggests that factors, including climate change, allowing for sustained transmission, including successful overwintering, may now be present in the EU (Purse et al., 2005; Saegerman et al., 2008).

Diseases at the Human–Primate Interface

The hunting, butchering, and consumption of primates is recognized as a major source of viral disease emergence. This has resulted in cross-species transmission of several retroviruses to humans including simian immunodeficiency virus (SIV), simian T-lymphotropic virus (STLV), and simian foamy virus, the former of which resulted in a human disease of pandemic proportions in the form of human immunodeficiency virus and AIDS (Gao et al., 1999). The extent of the wildlife trade is difficult to measure due to the clandestine nature of the business; however, bushmeat continues to present threats to public health. In a recent study, samples were collected at several international airports from illegally imported nonhuman primate and rodent species, including baboon, chimpanzee, mangabey, guenon, green monkey, cane rat, and rat (Smith et al., 2012). Pathogen screening identified retroviruses (simian foamy virus) and/or herpes viruses (cytomegalovirus and lymphocryptovirus) in the primate samples. These results demonstrated that illegal bushmeat importation into the United States could act as a conduit

for pathogen spread, and the authors suggested that implementation of disease surveillance of the wildlife trade would help facilitate prevention of disease emergence. The uncontrolled extraction and trade of wildlife is also a threat to the persistence of fish and wildlife species, and changing attitudes toward this trade would not only benefit public health, but would assist in the conservation of threatened and endangered species.

Recent outbreaks of zoonotic diseases in African great apes also illustrate the potential role of infectious diseases in jeopardizing the persistence of great ape populations. Controlled contact as well as unavoidable contact between humans and great apes is increasing due to ecotourism, expanding human populations, as well as other ecologic factors such as deforestation and the bushmeat trade (Figure A12-2) (Adams et al., 2001; Guerrera et al., 2003; Walsh et al., 2003). Great apes are especially vulnerable to human diseases due to the close taxonomic relationship, and there are increasing reports of human-associated diseases in great ape populations including outbreaks of sarcoptic mange in mountain gorillas (*Gorilla beringei beringei*) (Graczyk et al., 2001; Kalema-Zikusoka et al., 2002) suspected to be of human origin. More recently, Köndgen et al. (2008) presented evidence of human paramyxovirus transmission from humans to wild chimpanzees (*Pan troglodytes*) that resulted in respiratory disease, mortality, and decline of the chimpanzee population. In these areas where humans and primates coexist, improvements in public health infrastructure and measures to reduce disease transmission (for example, the creation of open defecation free zones) would benefit the human populations that have high burdens of disease and unmet health needs as well as these endangered species. This, again, illustrates

FIGURE A12-2 Contact between humans and great apes is increasing due to ecotourism, and without preventive measures could result in cross-species pathogen exchange.

the connectivity between human and wildlife health, and provides an additional conservation-related argument for the improvement of public health in these developing countries.

Bats and Emerging Viral Diseases

Bats are being increasingly recognized as an important reservoir of zoonotic viruses of different families, including SARS coronavirus, Nipah virus, Hendra virus, and Ebola virus (Smith and Wang, 2013). The question of whether bats have unique biological features making them ideal reservoir hosts has been the subject of recent discussion (Kupferschmidt, 2013). However, these unique features may also increase their susceptibility to infectious diseases. *Pseudogymnoascus (Geomyces) destructans*, the etiologic agent of white-nose syndrome (WNS), which has caused precipitous declines of North American bat species, infects bats during hibernation. It has been hypothesized that bats are particularly vulnerable to infection during that period of their life cycle due to natural immunosuppression (Blehert et al., 2009).

The triptych of bats as reservoirs of zoonotic viruses, their ecological and economic importance (Boyles et al., 2009), and threats to their persistence creates the question on how humans and bats can coexist. A recent study of 2007–2008 outbreaks of Marburg virus associated with caves in Uganda may provide some answers (Amman et al., 2012). These caves were used by local population for mining, were also tourist attractions, and contained large population of *Rousettus aegyptiacus* fruit bats, which were implicated as the reservoir of Marburg virus. Between August 2008 and November 2009, 1,622 bats were captured and tested for Marburg virus, and the Q-RT-PCR data showed distinct pulses of virus infection in older juvenile bats that temporarily coincided with the peak twice yearly birthing seasons. Retrospective analysis of historical human infections suspected to have been the result of discrete spillover events directly from nature found 83 percent (54/65) of events occurred during these seasonal pulses in virus circulation, perhaps demonstrating periods of increased risk for human infection. These results provide a basis for risk-reduction strategies through temporal separation of human caving activities and bats during the high-risk birthing seasons.

Future Directions in Wildlife Health

Early detection of emerging viral diseases in wildlife is an important component of an overall strategy to prevent, prepare for, and respond to emerging infectious diseases. A primary component is the field epidemiological capacity and network to detect and respond to unusual wildlife mortality events. This includes natural resource agency field biologists and wildlife health professionals as well as epidemiologists trained in wildlife disease outbreak investigation and surveillance. State-of-the-art wildlife diagnostic laboratory capacity, including

virology, microbiology, chemistry, and pathology to detect and identify novel emerging pathogens is essential, including a network of laboratories specializing in wildlife pathogen detection and characterization. The application of new molecular diagnostic technologies such as next-generation sequencing has opened up previously unknown avenues for pathogen discovery (Relman, 1998; Wang et al., 2003) and should become mainstream in this context. Such systems will also contribute to upstream surveillance for hazards, and strengthen the capacity of nations to detect infectious diseases that may represent potential public health emergencies (Baker et al., 2010).

We have established Earth (Landsat: landsat.usgs.gov) and climate monitoring systems (NOAA: www.nesdis.noaa.gov) that provide continuous imagery, atmospheric measurements, and climatic data, and we have global public health surveillance systems for human diseases (WHO: http://www.who.int/research/en), yet we lack the same ongoing, systematic collection of data for fish and wildlife health. Collection and integration of data from such a long-term data system with data from a variety of sources, including human and animal health data, climatic, ecologic, hydrologic, geologic, and socioeconomic data, among other sources, will allow a deeper understanding of the environmental drivers and the generation of predictive models of "hot spots" of disease emergence (Jones et al., 2008). This will ultimately allow for the targeting of resources to geographic areas and populations at greater risk and the prevention of disease emergence and spread. The development of new analytical models will also provide us with the mathematical tools to identify and anticipate threats to wildlife, understand the distribution, dynamics, and impacts of disease, and ultimately provide better information for guiding management decisions. Recognizing that not all diseases will be predicted and prevented, the biggest deficiency is a suite of tools that can be mobilized to manage diseases in wildlife populations. The current methods such as culling are crude, unpopular, and generally ineffective. Vaccines are probably the primary, cost-effective public health and veterinary intervention available and have been used widely to save millions of lives and reduce economic losses. Very few vaccines are available for use in free-ranging wildlife populations due to the challenges of delivery; however, the oral rabies vaccine has reduced the prevalence of rabies infection in wildlife, and was used to successfully eradicate fox rabies from Western Europe (Brochier et al., 1991). Further research in the development of safe and effective vaccines that can be mass delivered to wildlife populations, as is being done to develop a sylvatic plague vaccine for prairie dogs (Abbott et al., 2012), would allow for this technique to be increasingly applied to vaccinate upstream and prevent pathogen spillover. Increased focus on other disease management tools should include biocontrol strategies and research on social attitudes and behaviors related to natural resources and disease management.

Finally, we need robust partnerships to address these pressing issues of mutual concern. While the One Health concept recognizes the interconnectedness of human, animal, wildlife, and ecosystem health, the infrastructure to respond

TABLE A12-2 Factors That Contributed to the Success of One Health Projects

- Sense of urgency and common purpose
- Delegated authority or mandate to conduct the work
- Good governance: An interagency steering committee or working group is formed to oversee the work
- Foundation of trust exists among key individuals in different agencies, built on a willingness to acknowledge the other agencies' concerns
- Mutually agreed-upon outcomes are science based
- Clearly defined roles and responsibilities
- Leadership rotates rather than being monopolized by one sector

SOURCE: Rubin et al., 2014.

to wildlife emerging diseases and wildlife health emergencies is lacking. Until we have the operational framework (the network of partners, with appropriate governance, policies, procedures, etc.) by which agencies and institutions with a stake in wildlife diseases cooperate and collaborate to achieve optimal outcomes for human, animal, and ecosystem health, the third leg of the One Health stool (the three legs being human health, domestic animal health, and wildlife health) will always be missing. In one sense this is a leadership challenge. Interdisciplinary teams are more likely to be successful when there is a unified task and shared goals and values, and when personal relationships are developed from a foundation of trust and respect (Anholt et al., 2012). A recent review of successful One Health projects revealed common factors that contributed to their success (Rubin et al. 2014; Table A12-2). Consequently, what are the common core values of One Health, and do we have the individual leadership skills, such as an ability to think beyond the boundaries of one's own agency or institution to make One Health successful? Addressing emerging viral diseases is a shared leadership responsibility we all must willingly accept, and doing so will help us make significant progress.

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A13

ROLE OF POULTRY IN SPREAD OF NOVEL H7N9 INFLUENZA VIRUS IN CHINA[32]

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Abstract

The recent outbreak of H7N9 influenza in China has resulted in many human cases with a high fatality rate. Poultry are the likely source of infection for humans based on sequence analysis and virus isolations from live bird markets, but it's not clear which species of birds are most likely to be infected and shedding sufficient levels of virus to infect humans. Intranasal inoculation of chickens, Japanese quail, pigeons, Pekin ducks, Mallard ducks, Muscovy ducks, and Embden geese with 10^6 EID_{50} of the A/Anhui/1/2013 virus resulted in infection but no clinical disease signs. Virus shedding in quail and chickens was much higher and prolonged than in the other species. Quail effectively transmitted the virus to direct contacts but pigeons and Pekin ducks did not. In all species, virus was detected at much higher titers from oropharyngeal swabs than cloacal swabs. The HA gene from samples collected from selected experimentally infected birds were sequenced and three amino acid differences were commonly observed when compared to A/Anhui/1/2013: N123D, N149D, and L217Q. Leucine at position 217 is highly conserved for avian isolates and is associated with α2,6 sialic acid binding. Different amino acid combinations were observed suggesting that the inoculum had viral subpopulations that were selected after passage in birds. These experimental studies corroborate that certain poultry species are reservoirs of the H7N9 influenza virus, and that the virus is highly upper respiratorytropic so testing of bird species should preferentially be conducted with oropharyngeal swabs for best sensitivity.

IMPORTANCE: The recent outbreak of H7N9 in China has resulted in a number of human infections with a high case fatality rate. The source of the viral outbreak is suspected to be from poultry, but definitive data for

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the source of the infection is not known. This study provides experimental data to show that quail and chickens are susceptible to infection and shed large amounts of virus and are likely important in the spread of the virus to humans. Other poultry species, including Muscovy ducks, can be infected and shed virus, but are less likely to play a role of transmitting the virus to humans. Pigeons were previously suggested as a possible source of virus because of isolation of virus from several pigeons in poultry markets in China, but experimental studies show they are generally resistant to infection and are unlikely to play a role in spread of the virus.

On 1 April 2013, the People's Republic of China reported the first 3 human cases of a novel H7N9 subtype of influenza A virus. Over the subsequent days, the number of confirmed cases ballooned to over 82, with over 17 fatalities occurring in 6 different provinces (Li et al., 2014). Sequence analysis of the virus showed the H7 and N9 genes to be those of avian influenza viruses (AIVs) of Eurasian lineage, but at only 95% similarity to other isolates in the public sequence databases, the viruses were uniquely different from what had previously been described (Gao et al., 2013). However, the internal genes were all closely related to the well-established H9N2 lineage of avian influenza virus circulating in poultry in the region since at least 1997 (Gao et al., 2013; Guan et al., 1999). This H9N2 lineage virus has also been associated with human disease (Butt et al., 2005; Peiris et al., 1999).

The epidemiology of H7N9 virus showed that human cases were widely distributed in the affected provinces and there was no strong evidence of human-to-human transmission. Because the genome sequences of the isolates showed that they were genetically related to AIVs, Chinese veterinary officials quickly started testing poultry associated with live bird markets and commercial poultry operations and wild birds in the regions where human infections were being reported. The H7N9 viruses were detected at relatively low rates in avian species in the live bird markets, including chickens, pigeons, and ducks, and the environment (Lam et al., 2013; Shi et al., 2013a). Additional evidence of an epidemiological link of exposure to birds in markets has been found in some human cases (Chen et al., 2013b; Shi et al., 2013b). Therefore, live bird markets were suspected of being a source of human infections, and Chinese officials required temporary closure of live poultry markets in the affected provinces, resulting in an immediate reduction of human cases, providing further evidence of a role of live poultry markets in the spread of the virus and that closure of the markets is an effective control strategy (Yu et al., 2014). However, it is not clear which species of birds are most likely to be infected and are shedding levels of virus sufficient to infect humans. The lack of understanding of the virus ecology in birds has recently resulted in an additional number of human cases, demonstrating that the virus still circulates in China (Chen et al., 2013a). Based on the initial reports of this virus and previous experience with avian influenza, we evaluated the potential role of different poultry species in the epidemiology of H7N9 influenza.

Materials and Methods

Virus

The virus used in this study was A/Anhui/1/2013 (H7N9), which was kindly provided by the Centers for Disease Control and Prevention, Atlanta, GA. The virus was propagated in specific-pathogen-free (SPF) embryonating chicken eggs (ECEs) according to standard procedures (Senne, 1998). Allantoic fluid was diluted in brain heart infusion (BHI) medium (BD Bioscience, Sparks, MD) in order to prepare an inoculum with 10^2, 10^4, or 10^6 50% egg infective doses (EID_{50}) per 0.1 ml per bird. All challenge doses were confirmed by back-titration in ECEs. All experiments using the H7N9 influenza virus, including work with animals, were reviewed by the institutional biosecurity committee and were performed in biosecurity level 3 enhanced (BSL-3E) and animal biosecurity level 3 enhanced (ABSL-3E) facilities at the Southeast Poultry Research Laboratory (SEPRL), Agricultural Research Service, United States Department of Agriculture (USDA), and all personnel were required to wear a powered air-purifying respirator with high-efficiency particulate air (HEPA) filtration (3M, St. Paul, MN).

Birds

Fifty-nine-week-old SPF White Leghorn chickens (*Gallus gallus domesticus*) (egg layer type) were obtained from SEPRL in-house flocks. Four-week-old Japanese quail (*Coturnix japonica*), 6- to 12-month-old rock pigeons (*Columbia livia domestica*), 2-week-old Pekin ducks (*Anas platyrhynchos* var. *domestica*), 2-week-old Mallard ducks (*Anas platyrhynchos*), 2-week-old Muscovy ducks (*Cairina moschata*), and 2-week-old Embden geese (*Anser anser domesticus*) were obtained from commercial farms. Birds were housed in self-contained isolation units that were ventilated under negative pressure with HEPA-filtered air and maintained under continuous lighting. Serum samples were collected from all birds immediately prior to challenge and found to be negative for antibodies to the H7 subtype of influenza virus by hemagglutination inhibition assay, as described below. Feed and water were provided with *ad libitum* access. All bird experiments were approved by and performed under the regulations of the SEPRL Institutional Animal Care and Use Committee.

Pathogenicity and Virus Transmission Studies

Eleven chickens, 11 Japanese quail, 11 pigeons, 11 Pekin ducks, 11 Mallard ducks, 7 Muscovy ducks, and 9 Embden geese were intranasally (i.n.) inoculated through the choanal cleft with an inoculum containing $10^{6.0}$ EID_{50} of A/Anhui/1/2013 (H7N9) in 0.1 ml. At 3 days postinoculation (dpi), 2 to 3 birds from each group were euthanized and gross lesions were recorded. The following tissues were collected in 10% neutral buffered formalin solution to determine microscopic lesions and the extent of virus replication in tissues: nasal turbinates,

trachea, lung, air sac, comb, eyelid, heart, brain, esophagus, proventriculus, ventriculus, duodenum, jejunum, cecal tonsils, pancreas, liver, spleen, bursa, thymus, Harderian gland, kidney, gonads, adrenal gland, and muscle from the breast and left thigh. Lung, spleen, intestine, kidney, and thigh and breast muscle tissues, as well as allantoic fluid from eggs laid by inoculated chickens, were collected separately in BHI and kept frozen at −70°C for subsequent virus detection. The remaining birds were observed for clinical signs over an 11-day period, during which time any clinical signs were recorded.

To evaluate the susceptibility of quail, pigeons, and Pekin ducks, three doses of virus (10^2, 10^4, or 10^6 EID_{50}) were administered i.n. to groups of five birds. At 2 dpi, three uninfected birds were placed in the same cage with the directly inoculated birds in each dose group to determine the transmission potential of the virus by contact exposure.

Oropharyngeal (OP) and cloacal (C) swab specimens were collected at 2, 4, 6, 8, and 11 dpi from directly inoculated birds and at 2, 4, 6, and 9 days after birds infected through contact exposure to determine virus shedding. Swab specimens were collected in 2 ml of BHI medium with 1× antibiotic-antimycotic. All birds remaining at the end of the experiment were euthanized by the intravenous (i.v.) administration of sodium pentobarbital (100 mg/kg of body weight).

RNA Extraction and Quantitative rRT-PCR

RNA was extracted from swab and tissue specimens using a MagMAX 96 AI/ND viral RNA isolation kit (Ambion, Inc. Austin, TX) with a KingFisher magnetic particle processor (Thermo Scientific, Waltham, MA). Quantitative real-time reverse transcription-PCR (rRT-PCR) was performed using a SmartCycler (version 2.0) apparatus and an AgPath-ID OneStep RT-PCR kit (Ambion, Inc.). The processing of the samples from the chicken, quail, and pigeon experiment was performed at the same time, and an H7 assay was used for quantitation (Slomka et al., 2009). For the duck and goose samples, the matrix gene assay was used for quantitation (Spackman et al., 2002). A standard curve for virus quantification was established with RNA extracted from dilutions of the same titrated stock of virus used to inoculate the birds. Viral titers were extrapolated from the standard curve. Data were analyzed using Prism (v.5.01) software (GraphPad Software Inc.). Two-way analysis of variance with Tukey's posttest was used to the compare virus titers in oropharyngeal swab specimens. For statistical purposes, all oropharyngeal swab specimens from which viruses were not detected were given a numeric value of $10^{1.0}$ EID_{50}/ml. Statistical significance was set at a P value of <0.05.

Virus Replication in Tissues

Virus replication in lung, spleen, intestine, kidney, and muscle tissues from 2 to 3 birds was examined at 3 days following intranasal infection with the H7N9 virus. Titers of infectious virus were determined in ECEs or by rRT-PCR, as described above. Allantoic fluid collected from virus-inoculated laying chickens was also examined by rRT-PCR for the presence of virus.

HI Assays

Hemagglutination inhibition (HI) assays were used to evaluate antibody to H7 influenza virus prior to challenge and to confirm exposure and infection with serum collected at 11 dpi and preadsorbed with 10% chicken red blood cells (Pedersen, 2008). The HI assay was conducted in accordance with standard procedures. Briefly, 2-fold serial dilutions of 25µl of serum were made in 25µl of phosphate-buffered saline (PBS). Diluted sera were incubated for 30 min at 4°C with 4 hemagglutination units (HAU)/25µl of A/Anhui/1/2013 (H7N9) virus which had been treated with 0.1% beta-propiolactone (the pH was adjusted to 7.0 with sodium bicarbonate), and then 50µl of 0.1% chicken red blood cells was added. The test result was evaluated after 30 min of incubation at room temperature. Titers were calculated as the reciprocal of the last HI-positive serum dilution, and samples with HI titers of 8 or below were considered negative.

Histopathology and IHC

Tissues were prepared for histopathology and immunohistochemistry (IHC) as previously described (Pantin-Jackwood and Swayne, 2007). Briefly, collected tissues were fixed by submersion in 10% neutral buffered formalin and embedded in paraffin. In addition, the nasal cavity was decalcified for 2 days. Sections were made at 5µm and were stained with hematoxylin-eosin (HE). A duplicate section was immunohistochemically stained by first microwaving the sections in Citra antigen retrieval solution (BioGenex, San Ramon, CA) for antigen exposure. A 1:2,000 dilution of a mouse-derived monoclonal antibody (P13C11) (Perdue et al., 1994) specific for type A influenza virus nucleoprotein (NP) was applied, and the mixture was allowed to incubate overnight at 4°C (Perkins and Swayne, 2001). The primary antibody was then detected by the application of biotinylated goat anti-mouse IgG secondary antibody using a biotin-streptavidin detection system (supersensitive multilink immunodetection system; BioGenex). Fast Red TR (BioGenex) served as the substrate chromogen, and hematoxylin was used as a counterstain. All tissues were systematically screened for microscopic lesions and virus antigen staining.

Sequencing

Viral RNA from selected OP and cloacal swab samples collected at 2 to 11 dpi from birds of each species directly inoculated with virus and from contact-exposed birds was directly amplified by one-step reverse transcription-PCR (RT-PCR) for the HA1 gene and the region of the PB2 protein around amino acid position 627. Selected viruses isolated from the experimentally inoculated birds and propagated in ECEs were also sequenced for comparison. A commercial one-step RT-PCR kit (Qiagen Inc., Valencia, CA) and primers which matched the sequence of A/Anhui/1/2013 (H7N9) virus and which were directed to the conserved sequences at the ends of the gene segments were used. Primer sequences are available upon request. Templates were then purified by agarose gel extraction with a QIAquick gel extraction kit (Qiagen Inc., Valencia, CA) and quantified by UV spectroscopy. A BigDye Terminator kit (Applied Biosystems, Foster City, CA) was used for cycle sequencing, and the samples were subsequently run on an ABI 3730 DNA analyzer (Applied Biosystems, Foster City, CA). Bioinformatics analysis looking at the amino acid differences of the hemagglutinin (HA) gene was performed using the Influenza Research Database, which was used to look for single nucleotide polymorphisms (Squires et al., 2012).

TABLE A13-1 Susceptibility of Poultry to Influenza A (H7N9) Virus

	No. of birds virus positive/ total no. of birds sampled at the following times[a]:			
	2 dpi		4 dpi	
Group	OP swabs	C swabs	OP swabs	C swabs
Chicken layers	10/11 (6.0)^{A}	4/11 (2.1)	8/8 (6.4)^{AC}	4/8 (2.5)
Quail	11/11 (7.6)^{B}	10/11 (2.9)	8/8 (7.5)^{A}	8/8 (3.2)
Pigeons	7/11 (2.5)^{C}	5/11 (1.9)	1/8 (1.7)^{B}	0/8
Pekin ducks	9/11 (5.1)^{C}	0/11	3/8 (4.5)^{BD}	0/8
Mallard ducks	10/11 (4.0)^{C}	0/11	2/8 (4.2)^{BD}	0/8
Muscovy ducks	7/7 (5.3)^{A}	2/7 (3.2)	5/5 (5.9)^{CE}	3/5 (4.9)
Embden geese	7/9 (3.4)^{C}	0/9	5/6 (4.4)^{DE}	0/6

[a] Results of testing for influenza A (H7N9) virus in oropharyngeal (OP) and cloacal (C) swab specimens from birds inoculated intranasally with 10^6 EID_{50} of the virus. The results are presented as the average viral shedding for each day, and samples negative by rRT-PCR were given a value 1 log unit lower than the limit of detection. Values in parentheses are the mean virus titer for positive samples determined by quantitative rRT-PCR and are reported as the $\log_{10}$ number of EID_{50}/ml. The

Results

Pathogenicity of H7N9 Influenza Virus in Different Poultry Species

No clinical disease signs were observed in any of the directly inoculated or contact-exposed birds during the 11-day observation period. Results for virus shedding are presented in Table A13-1. Virus was detected in OP swabs taken at 2 dpi from all the quail and Muscovy ducks and from 10 of 11 chickens, 7 of 11 pigeons, 9 of 11 Pekin ducks, 10 of 11 Mallard ducks, and 7 of 9 Embden geese inoculated with $10^{6.0}$ EID_{50} of the virus. At this time point, virus was detected in cloacal swabs from 4 of 11 chickens, 10 of 11 quail, 5 of 11 pigeons, 2 of 7 Muscovy ducks, and in none of the Pekin ducks, Mallard ducks, and geese. At 4 dpi, virus was detected in OP swabs of all chickens, quail, and Muscovy ducks but in smaller numbers of pigeons, Pekin ducks, Mallard ducks, and geese. Cloacal swab specimens from all quail, 3 of 5 Muscovy ducks, and 4 of 8 the chickens were virus positive, but swab specimens from the rest of the birds were negative. By 6 dpi, pigeons, Pekin ducks, and Mallard ducks had stop shedding by both the OP and C routes. Two of 6 geese had positive OP swabs at this time but were negative for both OP and C swabs on the rest of the days. Limited numbers of

6 dpi		8 dpi		11 dpi		
OP swabs	C swabs	OP swabs	C swabs	OP swabs	C swabs	Serology[b]
7/7 (5.5)[A]	3/8 (2.1)	6/8 (3.3)[A]	0/8	1/8 (1.8)[A]	0/8	5/6 (64–128)
7/8 (2.6)[B]	7/8 (2.6)	6/8 (2.9)[A]	4/8 (2.2)	1/8 (2.0)[A]	1/8 (1.7)	8/8 (32–128)
0/8	0/8	0/8	0/8	0/8	0/8	0/7 (5)
0/8	0/8	0/8	0/8	0/8	0/8	3/8 (16)
0/8	0/8	0/8	0/8	0/8	0/8	1/8 (16)
5/5 (4.0)[BC]	2/5 (4.1)	3/5 (3.1)[A]	3/5 (3.4)	0/5	0/5	4/5 (16–32)
2/6 (3.4)[C]	0/6	0/6	0/6	0/6	0/6	0/6

estimated lower limit of sensitivity of the rRT-PCR test was 10^2, as determined by the limit of detection on the standard curve. For oropharyngeal virus shedding, the results for groups with different uppercase letters are significantly different ($P < 0.05$). dpi, days postinoculation.

[b] Data represent the number of positive birds/total number of birds tested (range of titers or titer by hemagglutination inhibition assay). Titers of 8 or below were considered negative.

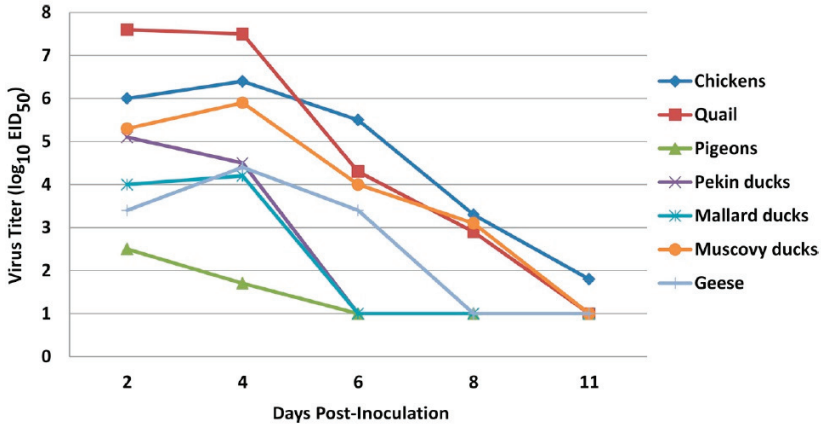


FIGURE A13-1 Comparison of oropharyngeal virus shedding after experimental challenge. Oropharyngeal shedding was detected at 2, 4, 6, 8, and 11 dpi with 10^6 EID_{50} of A/Anhui/1/2013 (H7N9) virus. The rRT-PCR values were interpolated by quantitative real-time RT-PCR using a standard curve generated with the challenge isolate. The estimated lower limit of sensitivity of the rRT-PCR test was 10^2, as determined by the limit of detection on the standard curve. The results are presented as the average viral shedding for each day, and samples negative by rRT-PCR were given a value 1 log unit lower than the limit of detection.

chicken and quail continued shedding virus until 11 dpi, and some Muscovy ducks were still shedding virus at 8 dpi.

High virus titers were present in OP swabs, with quail shedding an average of $10^{7.5}$ EID_{50} on days 2 and 4 dpi and chickens shedding $10^{6.2}$ EID_{50}, on average, on the same days. Most chickens and quail continued shedding virus at 6 and 8 dpi, although the titers had decreased (Figure A13-1). The titers in cloacal swabs averaged 3 to 4 log units lower than those in OP swabs. Only 7 of 11 directly exposed pigeons in the pathogenesis experimental group shed detectable virus at 2 dpi, with most shedding virus at low titers close to the assay limit of detection. One pigeon with the highest titer at day 2 continued to shed virus on day 4, but no other pigeons had detectable viral shedding for the remainder of the study. In the waterfowl study, Muscovy ducks had the highest numbers of birds shedding virus and the highest titers, with all birds shedding virus by the oropharyngeal route on days 2, 4, and 6 and some shedding virus by the cloaca until day 8. The Pekin ducks, Mallard ducks, and Embden geese had similar patterns of infection, with most directly inoculated birds shedding virus by the oropharyngeal route on day 2 and with decreasing numbers of positive birds being detected on days 4 and 6 and no virus being detected from the cloacal swabs (Table A13-1).

Susceptibility and Transmission of H7N9 Influenza Virus in Quail, Pigeons, and Pekin Ducks

Quail, pigeons, and Pekin ducks were chosen on the basis of the differences in pathogenicity observed as described above. Results are presented in Table A13-2. All directly inoculated quail in all three dose groups eventually became infected, and virus was transmitted to all contacts. Only a single quail receiving 10^2 EID_{50} was infected at 2 dpi, but it shed enough virus to infect its cage mates. In contrast, although some pigeons in the groups inoculated with 10^4 and 10^6 EID_{50} were shedding virus at 2 dpi, none of the contact-exposed pigeons became infected. No virus was detected from the pigeons that received 10^2 EID_{50} of the virus. Similarly, 2 of 5 and 4 of 5 Pekin ducks in the groups receiving 10^4 and 10^6 EID_{50}, respectively, became infected, but the virus was transmitted to only 2 contact ducks, which shed virus for only a short period of time.

Gross and Microscopic Lesions and Virus Antigen Staining in Tissues

Very mild gross lesions were observed at necropsy and mainly consisted of mild sinusitis. Microscopic lesions were consistent with low-pathogenic AIV (LPAIV) infection. Most of the lesions were confined to the upper respiratory tract. In the chickens and quail, the virus caused moderate to severe catarrhal and/or lymphocytic rhinitis and sinusitis, with mucocellular exudates containing sloughed epithelial cells, submucosal edema, and glandular hyperplasia (Figure A13-2). The trachea presented mild degenerative changes of the overlying epithelium, mild lymphocytic infiltration in the submucosa, and mild edema (Figure A13-2). In the lung, mild congestion, mild interstitial inflammation with mixed mononuclear cells, and mild catarrhal bronchitis were observed. Lesions in the gastrointestinal tract consisted of mild proliferation of gut-associated lymphoid tissues (GALTs). The remaining organs lacked significant histopathologic lesions. In ducks and geese, similar to the chickens and quail, most of the microscopic lesions were found in the upper respiratory tract (nasal turbinates, trachea); however, no lesions were observed in any other tissues, including the enteric tract. Mild to severe catarrhal rhinitis with congestion and loss of epithelial cells lining the nasal cavity was present in some ducks. In others, lymphoplasmacytic inflammation of the nasal submucosa was observed. Tracheitis with exudates and epithelial loss was common. In geese, mild to moderate lymphocytic rhinitis was the only lesion observed. No significant lesions were found in noninoculated birds.

In order to determine sites of virus replication, immunohistochemical staining for AIV antigen with an antibody to NP was conducted. Common viral staining was present in the epithelial cells and macrophages of the nasal cavity and adjacent glands in all of the quail and chickens examined (Figure A13-2). Similar viral antigen staining was present in the nasal epithelium of 2 of 3 Pekin ducks, 3 of 3 Mallards, and 1 of 2 Muscovy ducks but not in the geese or pigeons. Viral staining was also present in epithelial cells, macro phages, and desquamated

TABLE A13-2 Transmission of Influenza A (H7N9) Virus in Quail, Pigeons, and Ducks

	No. of birds virus positive/ total no. of birds sampled at the following times[a]:			
	2 dpi		4 dpi	
Group/virus dose	OP swabs	C swabs	OP swabs	C swabs
Quail/10^2 EID_{50}	1/5 (2.4)	0/5	2/5 (3.0)	2/5 (1.9)
Quail/10^2 EID_{50} contacts			1/3 (1.7)	0/3
Quail/10^4 EID_{50}	5/5 (7.5)	5/5 (3.0)	5/5 (7.4)	4/5 (2.7)
Quail/10^4 EID_{50} contacts			3/3 (7.6)	2/3 (2.3)
Quail/10^6 EID_{50}	5/5 (7.6)	5/5 (2.9)	5/5 (7.5)	5/5 (3.2)
Quail/10^6 EID_{50} contacts			3/3 (7.7)	3/3 (3.0)
Pigeon/10^2 EID_{50}	0/5	0/5	0/5	0/5
Pigeon/10^2 EID_{50} contacts			0/3	0/3
Pigeon/10^4 EID_{50}	2/5 (2.2)	1/5	0/5	0/5
Pigeon/10^4 EID_{50} contacts			0/3	0/3
Pigeon/10^6 EID_{50}	2/5 (2.5)	2/5 (1.9)	0/5	0/5
Pigeon/10^6 EID_{50} contacts			0/3	0/3
Pekin duck/10^2 EID_{50}	0/5	0/5	0/5	0/5
Pekin duck/10^2 EID_{50} contacts			0/3	0/3
Pekin duck/10^4 EID_{50}	2/5 (3.7)	0/5	1/5 (4.4)	0/5
Pekin duck/10^4 EID_{50} contacts			0/3	0/3
Pekin duck/10^6 EID_{50}	4/5 (4.0)	0/5	1/5 (2.7)	0/5
Pekin duck/10^6 EID_{50} contacts			0/3	0/3

[a] Results of testing for influenza A (H7N9) virus in oropharyngeal (OP) and cloacal (C) swabs specimens from inoculated birds and birds exposed through contact. Values in parentheses are the mean virus titer for positive samples determined by quantitative rRT-PCR and are reported as the $\log_{10}$ number of EID_{50}/ml. The estimated lower limit of sensitivity of the rRT-PCR test was 10^2, as

cells of the trachea of one quail and one Muscovy duck and in enterocytes and submucosal macrophages in the intestine of one quail and two Muscovy ducks.

Virus Detection in Tissue Samples

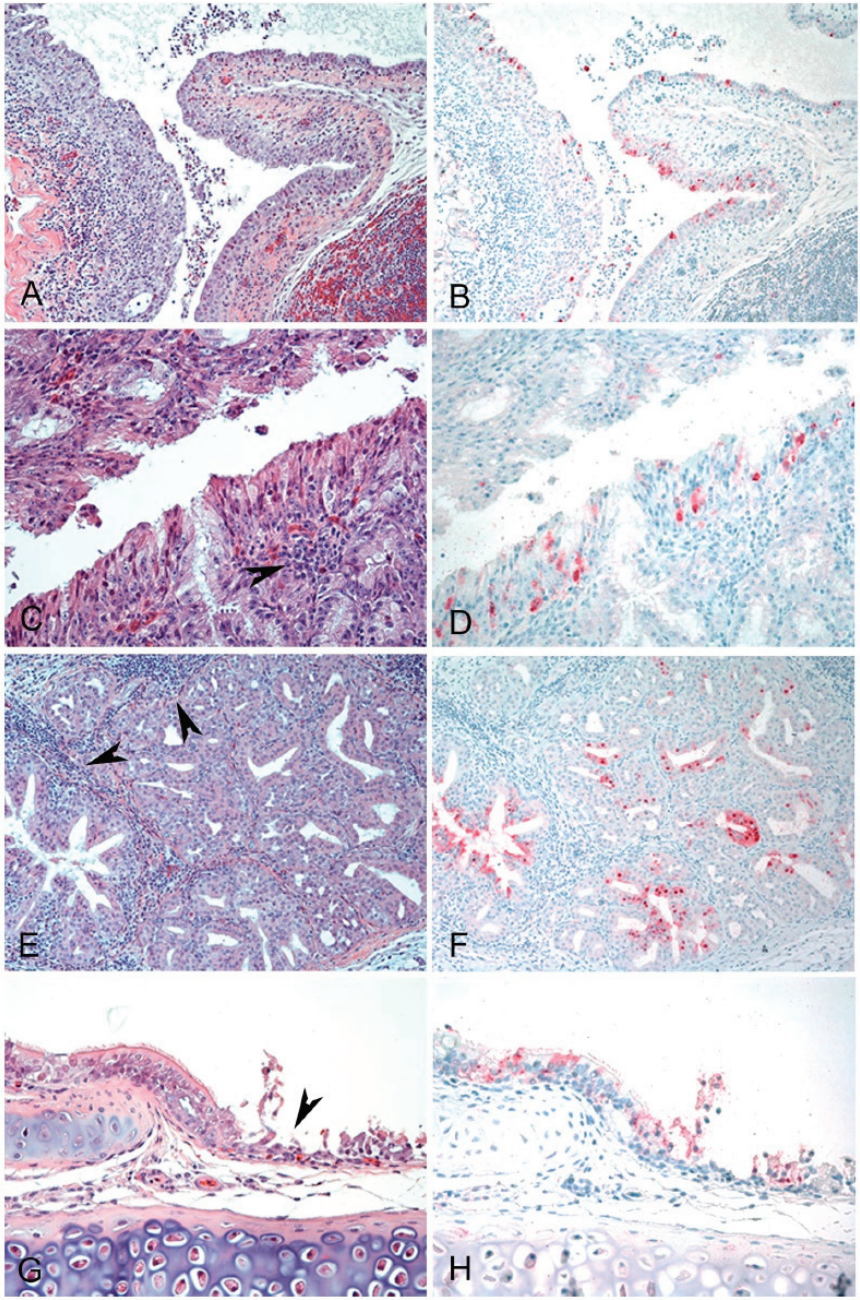
Virus isolation and/or virus detection by rRT-PCR from tissues collected at 3 dpi from birds infected with 10^6 EID_{50} of the H7N9 virus was attempted. Low

6 dpi		8 dpi		11 dpi		
OP swabs	C swabs	OP swabs	C swabs	OP swabs	C swabs	Serology[b]
5/5 (5.8)	3/5 (2.1)	5/5 (6.1)	4/5 (4.8)	5/5	4/5	3/5 (8–64)
3/3 (4.0)	0/3	3/3 (6.1)	0/3	3/3 (7.0)	2/3 (2.1)	1/3 (8)
5/5 (5.8)	4/5 (2.7)	4/5 (2.8)	4/5 (2.7)	0/5	0/5	5/5 (16–256)
3/3 (6.1)	2/3 (2.6)	3/3 (4.5)	1/3 (2.2)	1/3 (2.3)	2/3 (2.3)	3/3 (32)
5/5 (4.3)	3/5 (2.6)	3/5 (2.9)	3/5 (2.2)	2/5	1/5	5/5 (74)
3/3 (7.9)	2/3 (2.7)	3/3 (4.9)	3/3 (3.2)	3/3 (2.2)	3/3 (2.2)	3/3 (8–128)
0/5	0/5	0/5	0/5	0/5	0/5	1/5 (16)
0/3	0/3	0/3	0/3	0/3	0/3	0/3
0/5	0/5	0/5	0/5	0/5	0/5	0/5
0/3	0/3	0/3	0/3	0/3	0/3	0/3
0/5	0/5	0/5	0/5	0/5	0/5	0/5
0/3	0/3	0/3	0/3	0/3	0/3	0/3
0/5	1/5	0/5	0/5	0/5	0/5	0/5
0/3	0/3	0/3	0/3	0/3	0/3	0/3
0/5	0/5	0/5	0/5	0/5	0/5	1/5 (16)
1/3 (3.8)	0/3	2/3 (4.5)	0/3	0/3	0/3	0/3
0/5	0/5	0/5	0/5	0/5	0/5	1/5 (32)
0/3	0/3	0/3	0/3	0/3	0/3	0/3

determined by the limit of detection on the standard curve. The results are presented as the average viral shedding for each day, and samples negative by rRT-PCR were given a cycle threshold value 1 log unit lower than the limit of detection. dpi, days postinoculation.

[b] Data represent the number of positive birds/total number of birds tested (range of titers or titer by hemagglutination inhibition assay). Titers of 8 or below were considered negative.

virus titers ($10^{0.97}$ to $10^{1.23}$ EID_{50}) were detected in the intestine of two Muscovy ducks, one quail, and one chicken; in the spleen of three quail, one chicken, and one Mallard duck; and in the kidney of one Pekin duck, two Mallard ducks, one Muscovy duck, and two geese. However, the lungs and muscle tissues and the contents of eggs laid by infected chickens were virus negative.



Serology

When examined at 11 dpi, all quail, most chickens and Muscovy ducks, and some Pekin and Mallard ducks infected with 10^6 EID_{50} of the H7N9 virus had detectable titers of antibodies against the virus, but no pigeons or geese seroconverted (Table A13-1). All quail given 10^4 to 10^6 EID_{50} of the virus and 3 of 5 quail given 10^2 EID_{50} also seroconverted (Table A13-2), and antibodies were also detected in contact-exposed quail. However, most pigeons and Pekin ducks had undetectable antibody titers regardless of the virus dose given.

Sequencing

The HA1 gene and part of the PB2 gene were RT-PCR amplified and sequenced from selected virus-positive OP swabs collected at 2 to 11 dpi from inoculated chickens ($n = 3$), quail ($n = 2$), a pigeon ($n = 1$), a Pekin duck ($n = 1$), and a Muscovy duck ($n = 1$) and from contact control birds chickens ($n = 3$) and quail ($n = 6$). Similar numbers of sequences were obtained from virus isolated in embryonating chicken eggs from OP swabs. All samples had lysine (K) at position 627 of the PB2 gene, which is the same as the sequence of the parent human isolate. Lysine at this position is associated with virulence in mammals (Hatta, 2001). However, three amino acid differences were commonly observed in the HA1 gene sequence compared to the A/Anhui/1/ 2013 sequence: N123D, N149D, and L217Q (H7 numbering) (Table A13-3). Both the virus that was originally received from China and the virus that was used as the inoculum for these experiments and that had been passaged once in embryonating chicken eggs were sequenced. While the virus received from China had asparagine (N) residues at both positions 123 and 149, the egg-passaged inoculum virus had aspartic acid

FIGURE A13-2 Histopathology and immunohistochemical staining for avian influenza virus antigen in tissues of quail intranasally infected with the A/Anhui/1/2013 (H7N9) virus 3 dpi. Virus is stained in red. (A and B) Nasal epithelium. Severe necrotizing rhinitis with submucosal congestion and edema, glandular hyperplasia, and lymphoplasmacytic infiltration (A) and viral antigen in epithelial cells (B) are shown (magnification, ×200). (C and D) Nasal epithelium. Necrosis of epithelial cells and lymphocytic infiltration (arrowhead) (C) and viral antigen in epithelial cells (D) are shown (magnification, ×400). (E and F) Nasal gland. Lymphocytic infiltration in submucosa (arrowheads) (E) and viral antigen in epithelial cells (F) are shown (magnification, ×200). (G and H) Trachea. Necrosis of epithelial cells (arrowhead) (G) and viral antigen staining in epithelial cells (H) are shown (magnification, ×400).

TABLE A13-3 Comparison of Common Sequence Polymorphisms in H7 HA1 and PB2 Proteins Between Parental Strain A/Anhui/1/2013 (H7N9) and Derivative Viruses Recovered from Birds Inoculated with A/Anhui/1/2013[a]

Virus or virus source	No. of viruses with identical sequences	Source	Sequence in the following protein[b]:			
			H7 position 123 (132)	H7 Position 149 (158)	H7 position 217 (226)	PB2 position 627
A/Anhui/1/2013 (egg passage 2)	1	Original	N	N	L	K
A/Anhui/1/2013 (egg passage 3)	1	Inoculum	D	D	L	K
Chicken	1	Challenged	D	D	Q	K
Chicken	2	Challenged	N	N	Q	K
Chicken, egg isolation	1		D	D	Q	K
Chicken, egg isolation	2		N	N	Q	K
Quail	1	Challenged	D	D	Q	K
Quail	1	Challenged	D	D	L	K
Quail	5	Contact	D	D	Q	K
Quail	1	Contact	D	D	L	K
Quail, egg isolation	8		D	D	Q	K
Pigeon, egg isolation	1		D	D	Q	K
Pekin duck	1	Challenged	D	D	Q	K
Mallard duck	1	Challenged	N	N	L	K
Muscovy duck	1	Challenged	N	N	L	K

[a] Viruses were obtained directly from swabs or after egg passage.
[b] Numbers in parentheses are the analogous positions in H3.

(D) at both positions. Both isolates maintained the leucine (L) at position 217. Four different combinations of amino acids were observed in the infected birds. Most birds in this study continued to have aspartic acid at positions 123 and 149, but some isolates from chickens and Mallard and Pekin ducks had asparagine at both positions. The most consistent difference observed was the change at position 217 from leucine (L) to glutamine (Q). Position 217 correlates to position 226 in H3 human influenza viruses, and position 226 is critical for determining specificity to either α2,6 human-like sialic acid receptors or α2,3 avian-like sialic acid receptors (Connor et al., 1994). Most of the Chinese H7N9 sequences have leucine at this position, but passage in poultry seems to provide selection pressure for glutamine. Leucine was still found in a minority of isolates from both challenged and contact-infected birds. All the viruses that were passaged in eggs had glutamine at position 217. Examination of GenBank for sequence variation of Eurasian H7 influenza viruses at position 217 showed that over 99% of AIVs have glutamine at this position, and none are reported to have leucine. At position 123, asparagine is found in over 97% of viruses and aspartic acid is found in less than 3% of viruses, and at position 149, asparagine is found in 99% of isolates, with no viruses having aspartic acid.

Discussion

Quail are experimentally susceptible to many subtypes of both mammalian and avian influenza viruses (Bonfante et al., 2013; Cilloni et al., 2010; Makarova et al., 2003; Perez et al., 2003) and have been proposed to be a bridging species or disease amplifiers between wild waterfowl and domestic gallinaceous poultry (Cilloni et al., 2010; Hossain et al., 2008; Sorrell and Perez, 2007; Thontiravong et al., 2012; Yamada et al., 2012). In this study, we demonstrate that quail are susceptible to even a low-dose challenge of the Chinese H7N9 virus. The virus replicated to high titers in the upper respiratory tract for at least a week, and the virus transmitted easily by direct contact to cage mates. Although quail are proportionally a minor poultry species, they have the potential to be a major reservoir of the H7N9 virus for transmission to other poultry and to humans. The adult chickens in this study also shed high levels of virus, indicating that chickens are also an important source of virus which could be infecting humans either through direct contact or by aerosolization of virus, which occurs in particular during the slaughter process in live bird markets (Belser, 2010).

Our data support the suggestion that pigeons are generally resistant to AIV infection, with only 1 of 26 pigeons shedding moderate titers of virus. Historically, isolation of AIV from pigeons is rare, with less than 40 sequences appearing in GenBank, including the sequences of 8 H9N2 viruses and no H7 viruses (Squires et al., 2012). Experimentally, pigeons have generally been resistant to H5N1 highly pathogenic AIV (HPAIV) infection (Boon et al., 2007; Liu et al., 2007; Perkins and Swayne, 2001; Yamamoto et al., 2012); however, inoculation

of pigeons with high virus doses or with specific strains resulted in infrequent morbidity and mortality (Jia et al., 2008; Klopfleisch et al., 2006; Werner et al., 2007). Experimental data suggest that pigeons are unlikely to play a major role in the maintenance and transmission of the Chinese H7N9 virus. Exposure to high levels of virus from chickens or other species in live bird markets could explain the reported H7N9 isolations from pigeons (Shi et al., 2013a).

In this study, we also examined the pathogenesis of the H7N9 influenza virus in three different types of ducks and one type of goose. Pekin and Muscovy ducks and Embden geese are domestic waterfowl frequently present in live bird markets in China. Although closely related to Pekin ducks, we chose to also include Mallard ducks to address the possibility that wild birds could become infected with this virus by contact with domestic poultry and possibly spread the virus to other areas. Wild Mallard ducks also have one of the highest isolation rates for AIV and are a primary reservoir in the wild (Stallknecht et al., 2008). All four species could be infected by high-dose challenge with the virus, but the birds did not show any clinically observable disease and most species shed relatively small amounts of virus for shorter periods of time than quail and chickens. There was some evidence of contact transmission in Pekin ducks, but the ducks infected through contact shed little virus and shed virus for only short periods of time. Of the four species, Muscovy ducks shed the most virus. This is not surprising, since Muscovy ducks have been shown to be more susceptible to infection with highly pathogenic H5N1 AIV strains, show more severe disease, and shed larger amounts of virus than other domestic duck species (Cagle et al., 2011, 2012; Pantin-Jackwood et al., 2013). Muscovy ducks, it must be remembered, are a different species (*Cairina moschata*) than Pekin and Mallard ducks (*Anas platyrhynchos*) and should not be expected to have a similar response to infection (Brown et al., 2006). These differences in response to AIV infection in different waterfowl species should be taken into account when determining which species are involved in the transmission of emerging viruses. In this study, Muscovy ducks appeared to play a more important role as a possible biological vector of H7N9 AIV than Pekin ducks, Mallard ducks, and Embden geese.

AIV is maintained in wild birds, but occasionally, the virus can spread from its natural reservoir to poultry. Wild-bird AIVs are generally poorly adapted to domestic galliformes (chickens, quail, partridge), but as conditions permit, the virus can be transmitted and adapt to the new host. Wild aquatic birds do not typically show clinical signs of infection with AIVs, and although AIVs can replicate in cells of both the respiratory and intestinal tracts, in ducks they are reported to favor the intestinal tract (Swayne and Slemons, 2008; Webster et al., 1978). The results of these studies are consistent with those of previous studies indicating that chicken-adapted AIVs replicate better in chickens than in ducks (Pillai et al., 2010; Spackman et al., 2010). The underlining mechanism is not clear, but a shorter neuraminidase protein due to a deletion in the stalk region may be linked to this feature (Banks et al., 2001; Matrosovich et al., 1999; Mundt et al., 2009).

Control of H7N9 influenza is complicated by the lack of disease signs in poultry. Detection of LPAIVs is more difficult than detection of HPAIVs, like H5N1, because testing cannot target sick or dead birds like syndromic surveillance can. Critically, the data from both quail and chickens show high levels of viral replication in the upper respiratory tract and the shedding of much less virus in cloacal swabs, findings which are not unexpected, because poultry-adapted AIVs are typically shed at much higher levels in the respiratory tract in gallinaceous poultry (Claes et al., 2013; Gonzales et al., 2012; Marché et al., 2012; Pillai et al., 2010; Spackman et al., 2010). The disease pathogenesis of the H7N9 virus was unusual, in that virus replication was primarily restricted to the upper respiratory tract for all the species examined and the virus did not replicate well in lungs. Testing of gallinaceous and waterfowl bird species should preferentially be conducted with OP swabs for the best sensitivity.

One of the unusual features of this H7N9 virus was the presence of leucine at position 217 in the HA1 protein, a sequence which many have speculated increased the ability of the virus to infect humans. This position, analogous to position 226 of H3 viruses, forms part of the receptor binding site. The presence of leucine at this position is associated with binding to α2,6-sialic acid, which is the primary type of sialic acid found in the human upper respiratory tract. The presence of glutamine at this position is associated with binding to α2,3-sialic acid, the most common type of sialic acid found in avian species (Naeve et al., 1984; Vines et al., 1998). Receptor binding studies have confirmed that the human H7N9 virus with leucine at position 226 binds more strongly to α2,6-sialic acid than an avian H7 strain, but the human virus also had strong binding to α2,3-sialic acid (Xiong et al., 2013). Examining published Eurasian H7 sequences, glutamine is almost exclusively found at position 217 in avian influenza viruses, so the presence of leucine in the human isolate appears to support a change for adaptation to humans. The change of leucine back to glutamine in most of the challenged birds shows positive selection for glutamine in poultry and because of the mixed results suggests that the challenge virus was a mixed population. The change of L217Q in egg-passaged virus is also not surprising, as egg adaptation of human viruses is a common occurrence (Rocha et al., 1993). Experimental studies examining a European H7 virus showed that when the double mutation of Q226L and G228S was introduced into the hemagglutinin protein, the tropism of the hemagglutinin changed from α2,3-sialic acid to α2,6-sialic acid (Spackman et al., 2002), further supporting our observations. The single mutation Q226L was not examined, but because most Chinese H7N9 viruses already have leucine, it suggests that a single additional change could result in a major shift in viral tropism. Two other amino acid changes at positions 123 and 149 were also commonly observed. The amino acid at position 123 is part of the 120 loop that is projected to form part of the receptor binding site, but it is not clear how this amino acid might affect viral binding (Yang et al., 2012). The role of aspartic acid at position 149 also remains unclear. The data from this study and the sequence data for H7 viruses

in general suggest a conundrum about the H7N9 reservoir, because although the H7N9 virus with leucine at position 226 was transmitted in poultry, the majority of transmitted virus in this study had glutamine. Are there other animal species in the Chinese wet markets that may be a bridge species that supports leucine at position 226, or does the Q226L mutation occur after the virus jumps the species barrier to humans?

The epidemiology of the H7N9 virus outbreak clearly has an important poultry component, on the basis of the sequence analysis and the epidemiology of the virus, and these experimental studies have shown that chickens and quail are likely important reservoirs of the virus and pigeons are not. The role of domestic waterfowl is less clear, with relatively high levels of virus being shed by Muscovy ducks and smaller amounts being shed by Pekin ducks and Embden geese. Efforts in China have targeted live bird markets in an attempt to control the virus, and understanding the contribution of poultry farms, wholesale markets, and the markets themselves in the maintenance of the virus is critical. In the live bird market system in the United States, poultry farms are generally free of infected birds, but with H7N2 LPAIV, wholesale markets seemed to be the major sources of the virus (Bulaga et al., 2003).

The H7 virus has infected humans on numerous occasions, although the clinical disease has usually been mild. The Chinese H7N9 virus is unusual in the manifestation of severe disease in humans with a high case fatality rate. The viral sequence suggests that the virus is poised to mutate to a form that has α2,6-sialic acid receptor specificity, which is likely a prerequisite for human-to-human transmission, which could lead to a highly virulent pandemic. An understanding of viral pathogenesis and key reservoir species can potentially allow interventions in animals to stop a future human pandemic.

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Appendix B

Agenda

Emerging Viral Diseases—The One Health Connection

March 18–19, 2014
500 Fifth Street, NW
Washington DC

DAY ONE: TUESDAY, MARCH 18, 2014

8:00–8:30: Registration and continental breakfast

8:30–8:45: Welcoming remarks and overview: Drs. David A. Relman, James M. Hughes, Lonnie King

8:45–9:30: **KEYNOTE:** Challenges and trends in emerging viral diseases: A global perspective
Keiji Fukuda, The World Health Organization

9:30–10:00: **DISCUSSION**

10:00–10:30: BREAK

SESSION I: OVERVIEW OF EMERGING VIRAL DISEASES
Moderator: Peter Daszak

10:30–11:00: Global trends in emerging viral diseases of wildlife origin
Jonathan Sleeman, USGS National Wildlife Health Center

11:00–11:30: The relationship between eco-social system changes, the animal–human interface and viral disease emergence
Dirk Pfeiffer, Royal Veterinary College

11:30–12:00: Studying immunity to zoonotic diseases in the natural host—Keeping it real
John Lowenthal, CSIRO

12:00–12:30: Emerging and reemerging viral diseases: A view from NIAID
Anthony Fauci, NIAID

12:30–1:00: **DISCUSSION**

1:00–1:45: LUNCH

SESSION II: THE EMERGENCE OF A NOVEL BETACORONAVIRUS IN THE MIDDLE EAST—LOCAL, REGIONAL, AND GLOBAL IMPACTS
Moderator: Lonnie King

1:45–2:15: Human coronavirus emergence and cross-species adaptation
Ralph Baric, **University of North Carolina**

2:15–2:45: Animal coronaviruses: Lessons for MERS and SARS human coronaviruses
Linda Saif, Ohio State University

2:45–3:15: BREAK

3:15–3:45: Investigating the ecology and animal origins of MERS-CoV
Jonathan Epstein and **Kevin Olival, EcoHealth Alliance**

3:45–4:15: MERS-CoV: Its epidemiology, transmissibility, pandemic potential, and prevention
Trish M. Perl, **Johns Hopkins School of Medicine**

4:15–4:45: The potential for the international spread of Middle East respiratory syndrome in association with mass gathering events in the Kingdom of Saudi Arabia
Kamran Khan, St. Michael's Hospital, Toronto, Canada

4:45–5:30: **DISCUSSION**

5:30–5:45: **SUMMARY AND CONCLUDING REMARKS**

5:50: ADJOURNMENT

DAY TWO: WEDNESDAY, MARCH 19, 2014

8:30–9:00: Registration and continental breakfast

9:00–9:15: Welcome and summary of day one—Dr. David Relman

9:15–10:00: **KEYNOTE:** Lessons learned from IHR implementation and WHO performance in the 2009 (H1N1) influenza pandemic
Harvey V. Fineberg, President, Institute of Medicine

10:00–10:30: **DISCUSSION**

10:30–10:45: BREAK

SESSION III: DISCUSSION OF THE EMERGENCE OF THE INFLUENZA A VIRUSES IN ASIA—H5N1, H1N1, H7N9—OTHERS
Moderator: James M. Hughes

10:45–11:15: Similarities and differences between the novel H7N9 and H5N1 influenza A viruses
Ruben Donis, CDC

11:15–11:45: Studies on H7N9 virus infectivity and transmission in poultry and field assessment of epidemiology and control
David Swayne, USDA

11:45–12:15: Epidemiology and characteristics of influenza A H7N9 infections
Daniel Jernigan, CDC

12:15–12:45: **DISCUSSION**

12:45–1:30: LUNCH

SESSION IV: HOW IS THE DOMESTIC AND INTERNATIONAL COMMUNITY RESPONDING TO THESE VIRAL DISEASES?
Moderator: Jeffrey Duchin

1:30–2:00: Responses to the emergence of H7N9: The OIE perspective
Alex Thiermann, OIE

2:00–2:30: Coordinated responses to the emergence of the H7N9 avian influenza A virus in the Asian and Pacific regions—The USAID perspective
Dennis Carroll, USAID

2:30–3:00: Challenges in assessing and preventing transmission of β-coronaviruses in hospital/health care facilities
Allison McGeer, Mt. Sinai Hospital, Toronto, Canada

3:00–3:30: BREAK

3:30–4:00: Using what we know from science to contribute to predicting the pandemic potential of zoonotic influenza viruses
Derek Smith, Cambridge (UK)

4:00–4:30: A pandemic risk assessment framework to triage animal influenza viruses and minimize pathotyping studies
Ruben Donis, CDC

4:30–5:00: **DISCUSSION**

5:00–5:15: **CONCLUDING REMARKS**

5:15: ADJOURNMENT

Appendix C

Acronyms

AAHL	Australian Animal Health Laboratory
ABSL-3E	animal biosecurity level 3 enhanced
ACE2	angiotensin I converting enzyme 2
AI	avian influenza
AIDS	acquired immune deficiency syndrome
ASF	African swine fever
BHI	brain heart infusion
BSL	biosafety level
BT	Bluetongue
C	cloacal
CCD	colony collapse disorder
CDC	Centers for Disease Control and Prevention
CDV	canine distemper virus
CNISN	Chinese National Influenza-Like Illness Surveillance Network
CoV	coronavirus
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DPP4	dipeptidyl peptidase-4
DURC	dual-use research of concern
ECE	embryonating chicken egg
EHD	epizootic hemorrhagic disease
EID	emerging infectious disease

EPT	emerging pandemic threat
FAO	Food and Agriculture Organization of the United Nations
GALT	gut-associated lymphoid tissue
GISN	Global Influenza Surveillance Network
GISRS	Global Influenza Surveillance and Response System
HA	hemagglutinin
HAART	highly active antiretroviral therapy
HEPA	high-efficiency particulate air
HIV	human immunodeficiency virus
HPAI	highly pathogenic avian influenza
HPAIV	highly pathogenic avian influenza virus
IHR	International Health Regulations
ILI	influenza-like illness
IOM	Institute of Medicine
IRAT	Influenza Risk Assessment Tool
ISG	interferon-stimulated gene
iv	intravenous
LBM	live bird market
LPAI	low pathogenic avian influenza
MDG	Millennium Development Goal
MERS	Middle East respiratory syndrome
NA	neuraminidase
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NRC	National Research Council
OECD	Organisation for Economic Co-operation and Development
OIE	World Organization for Animal Health (formally known as Office International des Epizooties)
OP	oropharyngeal
PCR	polymerase chain reaction
PEDV	porcine epidemic diarrhea virus
PEPFAR	President's Emergency Plan for AIDS Relief
PHEIC	public health emergency of international concern
PIP	Pandemic Influenza Preparedness

PRCV	porcine respiratory coronavirus
ProMED	Program for Monitoring Emerging Diseases
PUE	Pneumonia of Unknown Etiology
PVS	Tool for the Evaluation of Performance of Veterinary Services
RIG-I	retinoic acid-inducible protein I
RNA	ribonucleic acid
SARI	Severe Acute Respiratory Infection
SARS	Severe Acute Respiratory Syndrome
SBV	Schmallenberg virus
SDCV	swine delta-coronavirus
SEPRL	Southeast Poultry Research Laboratory
SIV	Simian Immunodeficiency Virus
SPF	specific-pathogen-free
STLV	Simian T-lymphotropic Virus
TGEV	transmissible gastroenteritis virus
USAID	U.S. Agency for International Development
USDA	U.S. Department of Agriculture
USGS	U.S. Geological Survey
WHO	World Health Organization
WNS	white-nose syndrome
WNV	West Nile virus

Appendix D

Glossary

Abiotic: Nonliving chemical and physical factors in an environment.

Aerosolize: To disperse (as a medicine, bactericide, or insecticide) as an aerosol.

African swine fever: A highly contagious tick-borne hemorrhagic disease of pigs, warthogs, European wild boar, and American wild pigs. With high virulence forms of the virus, it is characterized by high fever, loss of appetite, hemorrhages in the skin and internal organs, and death in 2–10 days on average. Mortality rates may be as high as 100%. It is caused by a DNA virus of the *Asfarviridae* family.

Agent (of disease): Factor such as a microorganism whose presence is essential for the occurrence of a disease.

Alveolitis: An inflammation of the alveoli of the lungs caused by the inhalation of an allergen.

Anophelines: A genus of mosquitoes that includes all mosquitoes that transmit malaria to humans.

Anthropogenic: Caused or produced by humans.

Anthroponotic: Transmission from human to human and potentially from human to animal.

Antibiotic: Class of substances that can kill or inhibit the growth of some groups of microorganisms. Used in this report to refer to chemicals active against bacteria. Originally antibiotics were derived from natural sources (e.g., penicillin from molds), but many currently used antibiotics are semisynthetic and modified with additions of man-made chemical components. See *antimicrobials.*

Antibiotic resistance: Property of bacteria that confers the capacity to inactivate or exclude antibiotics or a mechanism that blocks the inhibitory or killing effects of antibiotics.

Antibody: A protein produced by the immune system in response to the introduction of a substance (an antigen) recognized as foreign by the body's immune system. Antibody interacts with the other components of the immune system and can render the antigen harmless, although for various reasons this may not always occur.

Antimicrobials: Class of substances that can destroy or inhibit the growth of pathogenic groups of microorganisms, including bacteria, viruses, parasites, and fungi.

Antiretroviral: A substance that stops or suppresses the activity of a retrovirus such as HIV.

Arboviral diseases: Shortened form of *arthropod-borne virus*. Any of a group of viruses that are transmitted to man and animals by mosquitoes, ticks, and sand flies; they include such agents as yellow fever and eastern, western, and Venezuelan equine encephalitis viruses.

Arthropod: As used in this report, refers to insects and ticks, many of which are medically important as vectors of infectious diseases.

Arthropod-borne: Capable of being transmitted by insect and tick (arthropod) vectors.

Asymptomatic: Presenting no symptoms of disease.

Avian influenza: Any of several highly variable diseases of domestic and wild birds that are caused by orthomyxoviruses and characterized usually by respiratory symptoms but sometimes by gastrointestinal, integumentary, and urogenital symptoms.

Bacteria: Microscopic, single-celled organisms that have some biochemical and structural features different from those of animal and plant cells.

Biosafety: Safety with respect to the effects of biological research on humans and the environment.

Biosafety Level 3 (BSL-3): Is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents, and must be supervised by scientists competent in handling infectious agents and associated procedures. All procedures involving the manipulation of infectious materials must be conducted within biological safety cabinets or other physical containment devices. A BSL-3 laboratory has special engineering and design features.

Biosafety Level 4 (BSL-4): Is required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that is frequently fatal, for which there are no vaccines or treatments, or a related agent with unknown risk of transmission. Agents with a close or identical antigenic relationship to agents requiring BSL-4 containment must be handled at this level until sufficient data are obtained either to confirm continued work at this level, or redesignate the level. Laboratory staff must have specific and thorough training in handling extremely hazardous infectious agents. Laboratory staff must understand the primary and secondary containment functions of standard and special practices, containment equipment, and laboratory design characteristics. All laboratory staff and supervisors must be competent in handling agents and procedures requiring BSL-4 containment. The laboratory supervisor in accordance with institutional policies controls access to the laboratory.

Biota: The animal and plant life of a given region.

Bluetongue disease: Bluetongue disease or catarrhal fever is a noncontagious, insect-borne, viral disease of ruminants, mainly sheep and less frequently cattle, goats, buffalo, deer, dromedaries, and antelope. It is caused by the Bluetongue virus.

Bronchiolitis: An acute viral infection of the small air passages of the lungs called the bronchioles.

Bushmeat: Wildlife species that are hunted in the "bush" or forests.

Canine distemper virus: A highly contagious, systemic, viral disease of dogs seen worldwide. Clinically, it is characterized by a diphasic fever, leukopenia, gastrointestinal and respiratory catarrh, and frequently pneumonic and neurologic

complications. Its epidemiology is complicated by the large number of species susceptible to infection. The disease is seen in Canidae (dog, fox, wolf, raccoon dog), Mustelidae (ferret, mink, skunk, wolverine, marten, badger, otter), most Procyonidae (raccoon, coatimundi), some Viveridae (binturong, palm civet), Ailuridae (red panda), Ursidae (bear), Elephantidae (Asian elephant), primates (Japanese monkey), and large Felidae. Domestic dogs (including feral populations) are considered to be the reservoir species in most, if not all, locations.

Chemoprophylaxis: The use of drugs or biologics taken by asymptomatic persons to reduce the risk of developing a disease.

Chikungunya: A febrile disease that resembles dengue, occurs especially in parts of Africa, India, and southeastern Asia, and is caused by a togavirus of the genus *Alphavirus* (species *Chikungunya virus*) transmitted by mosquitoes especially of the genus *Aedes*— also called *chikungunya fever.*

Cholera: Any of several diseases of humans and domestic animals usually marked by severe gastrointestinal symptoms; an acute diarrheal disease caused by an enterotoxin produced by a comma-shaped Gram-negative bacillus of the genus *Vibrio* (*V. cholerae* syn. *V. comma*) when it is present in large numbers in the proximal part of the human small intestine.

Climate: Average meteorological conditions over a specified time period, usually at least a month, resulting from interactions among the atmosphere, oceans, and land surface. Climate variations occur over a wide range of spatial and temporal scales.

Climate change: A change of climate that is attributed directly or indirectly to human activity that alters the composition of the global atmosphere and which is in addition to natural climate variability observed over comparable time periods.

Cloaca: The common chamber into which the intestinal, urinary, and generative canals discharge especially in monotreme mammals, birds, reptiles, amphibians, and elasmobranch fishes; the terminal part of the embryonic hindgut of a mammal before it divides into rectum, bladder, and genital precursors; a passage in a bone leading to a cavity containing a sequestrum.

Colony collapse disorder: A pathological condition affecting a large number of honeybee colonies, in which various stresses may lead to the abrupt disappearance of worker bees from the hive, leaving only the queen and newly hatched bees behind and thus causing the colony to stop functioning.

Communicable disease: An infectious disease transmissible (as from person to person) by direct contact with an infected individual or the individual's discharges or by indirect means (as by a vector).

Coronavirus: Any of a family (Coronaviridae) of single-stranded RNA viruses that have a lipid envelope with club-shaped projections and include some causing respiratory symptoms in humans.

Cytokine: Any of a class of immunoregulatory proteins (as interleukin, tumor necrosis factor, and interferon) that are secreted by cells, especially of the immune system.

Dengue fever: An acute infectious disease that is characterized by headache, severe joint pain, and a rash and that is caused by a single-stranded RNA virus of the genus *Flavivirus* (species *Dengue virus*) transmitted by mosquitoes of the genus *Aedes*— also called *breakbone fever, dandy fever, dengue fever*.

Disease: As used in this report, refers to a situation in which infection has elicited signs and symptoms in the infected individual; the infection has become clinically apparent.

Dual-use research of concern: In the life sciences, research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, materiel, or national security.

***E. coli*:** A straight rod-shaped Gram-negative bacterium (*Escherichia coli* of the family Enterobacteriaceae) that is used in public health as an indicator of fecal pollution (as of water or food) and in medicine and genetics as a research organism and that occurs in various strains that may live as harmless inhabitants of the human lower intestine or may produce a toxin causing intestinal illness.

Ebola: A hemorrhagic fever caused by the Ebola virus.

Ecosystem: Mutually interrelated communities of species and abiotic components, existing as a system with specific interactions and exchange of matter, energy, and information.

El Niño: A warming of the surface waters of the tropical Pacific that occurs every 3 to 5 years, temporarily affecting weather worldwide.

Emerging infection: Either a newly recognized, clinically distinct infectious disease or a known infectious disease whose reported incidence is increasing in a given place or among a specific population.

Emerging infections: Any infectious disease that has come to medical attention within the last two decades or for which there is a threat that its prevalence will increase in the near future. Many times, such diseases exist in nature as zoonoses and emerge as human pathogens only when humans come into contact with a formerly isolated animal population, such as monkeys in a rain forest that are no longer isolated because of deforestation. Drug-resistant organisms could also be included as the cause of emerging infections since they exist because of human influence. Some recent examples of agents responsible for emerging infections include human immunodeficiency virus, Ebola virus, multi-drug resistant *Mycobacterium tuberculosis,* and influenza A (H1N1).

Emerging infectious diseases: Infections that are rapidly increasing in incidence or geographic range.

Endemic: Present in a community or common among a group of people; said of a disease prevailing continually in a region.

Enteric: Of, relating to, or affecting the intestines.

Enterovirus: Any of a genus (*Enterovirus*) of picornaviruses (as the causative agent of poliomyelitis) that typically occur in the gastrointestinal tract but may be involved in respiratory ailments, meningitis, and neurological disorders.

Enzootic: A disease of low morbidity that is constantly present in an animal community.

Epidemic: The condition in which a disease spreads rapidly through a community in which that disease is normally not present or is present at a low level.

Epidemiology: Study of the distribution and determinants of health-related states or events in specified populations. Epidemiology is the basic quantitative science of public health.

Epizootic: A disease of high morbidity that is only occasionally present in an animal community.

Eradication: Reduction of the worldwide incidence of a disease to zero as a result of deliberate efforts.

Etiologic agent: The organism that causes a disease.

Etiological: Of or pertaining to causes or origins.

Etiology: Science and study of the causes of diseases and their mode of operation.

Extrinsic incubation period: Time required for the development of a disease agent in a vector from the time of uptake of the agent to the time the vector is infective.

Gallinaceous birds: Also called galliforms, belong to an order (*Galliformes*) of heavy-bodied ground-feeding birds that includes the turkey, grouse, chicken, New and Old World quail, ptarmigan, partridge, and pheasant.

Genomics: The study of all the genes in a person, as well as interactions of those genes with each other and with that person's environment. (http://www.cdc.gov/genomics/faq.htm)

Global warming: The gradual increase, observed or projected, in global surface temperature, as one of the consequences of radiative forcing caused by anthropogenic emissions.

Globalization: The increased interconnectedness and interdependence of peoples and countries, is generally understood to include two interrelated elements: the opening of borders to increasingly fast flows of goods, services, finance, people, and ideas across international borders; and the changes in institutional and policy regimes at the international and national levels that facilitate or promote such flows. (http://www.who.int/trade/glossary/story043/en/index.html)

Hantavirus: Any of a genus (*Hantavirus*) of bunyaviruses (as the Hantaan virus) that are transmitted by rodent feces and urine and cause hantavirus pulmonary syndrome and hemorrhagic fevers marked by renal necrosis.

Hemagglutinin protein: Species-specific binding protein that allows for the virus to bind to the cell membrane of host respiratory cells and propagate through cellular processes.

Herd immunity: A reduction in the probability of infection that is held to apply to susceptible members of a population in which a significant proportion of the individuals are immune because the chance of coming in contact with an infected individual is less.

Host (disease): Person or other living animal that affords subsistence or lodgment to an infectious agent under natural conditions.

Immune competence: The ability of the immune system to respond appropriately to an antigenic stimulation.

Immunoassay: A technique or test (as the enzyme-linked immunosorbent assay) used to detect the presence or quantity of a substance (as a protein) based on its capacity to act as an antigen or antibody.

Immunocompromised: A condition (caused, for example, by the administration of immunosuppressive drugs or irradiation, malnutrition, aging, or a condition such as cancer or HIV disease) in which an individual's immune system is unable to respond adequately to a foreign substance.

Incidence: Number of cases of a disease commencing, or of persons falling ill, during a given period of time in a specified population. Incidence rate is the number of new cases of a specific disease diagnosed or reported during a defined interval of time divided by the number of all persons in a defined population during the same time.

Index case: An instance of a disease or a genetically determined condition that is discovered first and leads to the discovery of others in a family or population.

Infection: The invasion of the body or a part of the body by a pathogenic agent, such as a microorganism or virus. Under favorable conditions the agent develops or multiplies, the results of which may produce injurious effects. Infection should not be confused with disease.

Influenza: An acute highly contagious virus disease that is caused by various strains of orthomyxoviruses belonging to three major types now considered as three separate genera and that is characterized by sudden onset, fever, prostration, severe aches and pains, and progressive inflammation of the respiratory mucous membrane—often used with the letter *A*, *B*, or *C* to denote disease caused by a virus of a specific one of the three genera; any human respiratory infection of undetermined cause—not used technically; any of numerous febrile usually virus diseases of domestic animals (as shipping fever of horses and swine influenza) marked by respiratory symptoms, inflammation of mucous membranes, and often systemic involvement.

Intermediate host: A host that is normally used by a parasite in the course of its life cycle and in which it may multiply asexually but not sexually.

International Health Regulations (IHR): An international legal instrument that is binding on 194 countries across the globe, including all the member states of WHO. Their aim is to help the international community prevent and respond to acute public health risks that have the potential to cross borders and threaten people worldwide. The IHR, which entered into force on June 15, 2007, requires countries to report certain disease outbreaks and public health events to WHO. Building on the unique experience of WHO in global disease surveillance, alert, and response, the IHR defines the rights and obligations of countries to report public health events, and establishes a number of procedures that WHO must follow in its work to uphold global public health security.

Interstitial pneumonia: Any of several chronic lung diseases of unknown etiology that affect interstitial tissues of the lung without filling of the alveolae and that may follow damage to the alveolar walls or involve interstitial histological changes.

Lassa: A disease especially of Africa that is caused by the Lassa virus and is characterized by a high fever, headaches, mouth ulcers, muscle aches, small hemorrhages under the skin, heart and kidney failure, and a high mortality rate.

Microbe: A microorganism or biologic agent that can replicate in humans (including bacteria, viruses, protozoa, fungi, and prions).

Microbial threat: Microbes that lead to disease in humans.

Microbiology: A branch of biology dealing especially with microscopic forms of life.

Migration: The regular, usually seasonal, movement of all or part of an animal population to and from a given area.

Millennium Development Goals: Eight international development goals that were established following the Millennium Summit of the United Nations in 2000, following the adoption of the United Nations Millennium Declaration. These goals—which range from halving extreme poverty rates to halting the spread of HIV/AIDS and providing universal primary education, all by the target date of 2015—form a blueprint agreed to by all the world's countries and all the world's leading development institutions. They have galvanized unprecedented efforts to meet the needs of the world's poorest.

Mitigation: Initiatives that reduce the risk from natural and man-made hazards. With respect to climate change, mitigation usually refers to actions taken to reduce the emissions or enhance the sinks of greenhouse gases.

Morbidity: Diseased condition or state.

Mortality: The number of deaths in a given time or place; the proportion of deaths to population.

Mutation: Genetic change that can occur either randomly or at an accelerated rate through exposure to radiation or certain chemicals (mutagens) and may lead to change in structure of the protein coded by the mutated gene.

Neuraminidase: A substance used (as in detecting or measuring a component, in preparing a product, or in developing photographs) because of its chemical or biological activity.

Nucleoprotein: Any of a group of substances found in the nuclei of all living cells and in viruses and composed of a protein and a nucleic acid.

One Health: The collaborative effort of multiple disciplines working locally, nationally, and globally to attain optimal health for people, animals, and our environment.

Outbreak: Localized occurrence as opposed to a generalized epidemic.

Pandemic: Epidemic occurring over a wide geographic area and affecting an exceptionally high proportion of the population.

Parainfluenza: Any of several paramyxoviruses (genus *Paramyxovirus*) that are associated with or responsible for some respiratory infections especially in children— also called *parainfluenza.*

Pathogen: Organism capable of causing disease.

Pathogenic: Capable of causing disease.

Pathology: The branch of medicine concerned with disease, especially its structure and its functional effects on the body.

Phylogeny: The connections between all groups of organisms as understood by ancestor/descendant relationships.

Physiochemical: Of or relating to physiological chemistry.

Prevalence: Proportion of persons in a population currently affected by a particular disease. Prevalence rate is the number of cases of a specific disease at a particular time divided by the population at that time living in the same region.

ProMED: The Program for Monitoring Emerging Diseases. An Internet-based reporting system dedicated to rapid global dissemination of information on outbreaks of infectious diseases and acute exposures to toxins that affect human health, including those in animals and in plants grown for food or animal feed.

Prophylaxis: Measures designed to preserve health (as of an individual or of society) and prevent the spread of disease.

Public health: The art and science of dealing with the protection and improvement of community health by organized community effort and including preventive medicine and sanitary and social health.

Public health emergency of international concern: An extraordinary event that is determined (1) to constitute a public health risk to other states through the international spread of disease; and (2) to potentially require a coordinated international response. This definition implies a situation that is serious, unusual, or unexpected; carries implications for public health beyond the affected state's national border; and may require immediate international action.

Quarantine: The enforced isolation or restriction of free movement imposed to prevent the spread of a contagious disease.

***Ranavirus*:** A genus in the family Iridoviridae that causes disease in amphibians

Resistance: See *antibiotic resistance.*

Retrovirus: Any of large family of RNA viruses that includes lentiviruses and oncoviruses, so called because they carry reverse transcriptase.

Risk: Probability that an event will occur; a measure of the degree of loss expected by the occurrence of a loss.

Schmallenberg virus: A virus first identified in Schmallenberg, Germany, in 2011, which causes brain and limb malformations in cattle and lambs. It is thought to be a negative-sense, single-stranded RNA virus of the Bunyaviridae family, genus *Orthobunyavirus*.

Shoe-leather epidemiology: Often synonymous with *field epidemiology* or *intervention epidemiology*. All three terms imply investigations initiated in response

to urgent public health problems and for which the investigative team does much of its work in the field (i.e., outside the office or laboratory).

Species barrier: Difficulty or impossibility for an infectious agent to pass from one species to another (due to differences between species).

Surveillance: Used in this workshop summary to refer to data collection and record keeping to track the emergence and spread of disease-causing organisms such as antibiotic-resistant bacteria.

Syndrome: A group or recognizable pattern of symptoms or abnormalities that indicate a particular trait or disease. (http://www.genome.gov/glossary.cfm?key=syndrome)

Transmission: Process by which a pathogen passes from a source of infection to a new host.

Vaccine: A preparation of living, attenuated, or killed bacteria or viruses, fractions thereof, or synthesized or recombinant antigens identical or similar to those found in the disease-causing organism that is administered to raise immunity to a particular microorganism.

Vector: An organism, such as an insect, that transmits a pathogen from one host to another.

Vector-borne: Transmitted from one host to another by a vector.

Vector-borne disease: (1) *Mechanical*: This includes simple mechanical carriage by a crawling or flying insect through soiling of its feet or proboscis or by passage of organisms through its gastrointestinal tract. This does not require multiplication or development of the organism. (2) *Biological*: Propagation (multiplication), cyclic development, or a combination of these (cyclopropagative) is required before the arthropod can transmit the infective form of the agent to humans. An incubation period (extrinsic) is required following infection before the arthropod becomes infective. The infectious agent may be passed vertically to succeeding generations (transovarian transmission); transstadial transmission indicates its passage from one stage of the life cycle to another, as nymph to adult. Transmission may be by injection of salivary gland fluid during biting, or by regurgitation or deposition on the skin of feces or other material capable of penetrating the bite wound or an area of trauma from scratching or rubbing. This transmission is by an infected nonvertebrate host and not simple mechanical carriage by a vector or vehicle. However, an arthropod in either role is termed a vector.

Viremia: The presence of virus in the blood of a host.

Virulence: The ability of any infectious agent to produce disease. The virulence of a microorganism (such as a bacterium or virus) is a measure of the severity of the disease it is capable of causing.

West Nile virus: A flavivirus (genus *Flavivirus*) that causes an illness marked by fever, headache, muscle ache, skin rash, and sometimes encephalitis or meningitis, that is spread chiefly by mosquitoes and that is closely related to the viruses causing Japanese B encephalitis and Saint Louis encephalitis.

White-nose syndrome: An emergent disease caused by the fungus *Geomyces destructans*. The fungus invades bats' skin where it is not covered by fur, such as the muzzle, wings, and ears, forming white patches on these areas, giving rise to the name. The fungus attacks bats while they are hibernating, disrupting their hibernation and potentially causing starvation or dehydration.

Zoonotic infection: Infection that causes disease in human populations but can be perpetuated solely in nonhuman host animals (e.g., bubonic plague); may be enzootic.

Appendix E

Speaker Biographies

Ralph Baric, Ph.D., is a professor in the Department of Epidemiology at the University of North Carolina, a World Technology Award Finalist, a member of the Biological Experts Science Group, a fellow of the American Association for Microbiology, a senior editor of *PLoS Pathogens*, and a member of the editorial board of other specialty journals. His group has published more than 200 papers, many in highly visible journals like *PNAS*, *Nature Medicine*, *Science*, *PLoS Medicine*, and *PLoS Pathogens*. The Baric laboratory uses genetic, immunologic, molecular, and biochemical approaches to study the molecular mechanisms regulating virus replication, pathogenesis, molecular evolution, and cross-species transmission using emerging coronaviruses (SARS-CoV, MERS-CoV), flaviviruses (Dengue), and noroviruses as model systems. The SARS-CoV and MERS-CoV are emerging respiratory coronaviruses that most likely originated in bats and circumvented the globe in 2003–2004, and in 2012–2013, causing 10 to 44 percent mortality rates, respectively. His group has pioneered new strategies for developing reverse genetic approaches for manipulating coronavirus genomes, and developed synthetic genome approaches to reconstruct emerging viruses from in silico sequence information and/or live attenuated virus vaccine design. His group also uses systems biology and systems genetic approaches to identify host susceptibility loci and signaling pathways that regulate severe end-stage lung disease following respiratory virus infection in young and aged animals and human populations.

Dennis Carroll, Ph.D., currently serves as the Director of the U.S. Agency for International Development's (USAID's) Pandemic Influenza and other Emerging Threats Unit. In this position Dr. Carroll is responsible for providing strategic and

operational leadership for the agency's programs addressing new and emerging disease threats, which has included leading the agency's response to the H5N1 avian influenza and H1N1 pandemic viral threats. He is presently coordinating the rollout of USAID's new Emerging Pandemic Threats program—a global effort to combat new disease threats before they can become significant threats to human health.

Dr. Carroll was initially detailed to USAID from the U.S. Centers for Disease Control and Prevention as a senior public health advisor in 1991. In 1995 he was named the agency's Senior Infectious Diseases advisor, responsible for overseeing the agency's programs in malaria, tuberculosis, antimicrobial resistance, disease surveillance, as well as neglected and emerging infectious diseases. In this capacity Dr. Carroll was directly involved in the development and introduction of a range of new technologies for disease prevention and control, including community-based delivery of treatment of onchocerciasis, rapid diagnostics for malaria, new treatment therapies for drug-resistant malaria, intermittent therapy for pregnant women and "long-lasting" insecticide-treated bed nets for prevention of malaria. He was responsible for the initial design and development of the President's Malaria Initiative. Dr. Carroll officially left the CDC and joined USAID in 2005 when he assumed responsibility for leading the USAID response to the spread of avian influenza.

Dr. Carroll has a doctorate in biomedical research with a special focus in tropical infectious diseases from the University of Massachusetts Amherst. He was a Research Scientist at Cold Spring Harbor Laboratory where he studied the molecular mechanics of viral infection. Dr. Carroll has received awards from both the CDC and USAID, including the 2006 USAID Science and Technology Award for his work on malaria and avian influenza, and the 2008 Administrator's Management Innovation Award for his management of the Agency's Avian and Pandemic Influenza program.

Ruben Donis, Ph.D., serves as the Associate Director for Policy, Evaluation, and Preparedness for the CDC's Influenza Division. Before this position, Dr. Donis was chief of the former Molecular Virology and Vaccines Branch in the Division.

Dr. Donis earned his Veterinary Medicine diploma from the University of Buenos Aires and his Ph.D. in Virology from Cornell University. He completed his postdoctoral work at St. Jude Children's Research Hospital, where he specialized in influenza molecular virology. Prior to joining the CDC in 2003, Dr. Donis served on the faculty of the University of Nebraska-Lincoln (UNL), where he participated in the leadership of the UNL Center for Biotechnology, and conducted research on influenza and flavivirus molecular biology.

At the CDC, Dr. Donis oversees risk assessment studies that analyze structural and functional properties of emerging influenza viruses, including genome reassortment and virus-receptor interactions. The division monitors the evolution and pandemic potential of animal influenza viruses to inform development of

prepandemic candidate viruses for vaccine production, with a view to mitigating the public health impact of future pandemics. Dr. Donis has more than 25 years of research experience with influenza virus molecular biology and virus–host interactions. He currently serves as an adjunct professor of microbiology at Emory University.

Jon Epstein, M.D., is a veterinary epidemiologist and Associate Vice President of EcoHealth Alliance. He also serves as the Executive Director of the Consortium for Conservation Medicine, a multidisciplinary partnership of five U.S.-based research institutions dedicated to training the next generation of One Health scientists. He is the Asia Regional Coordinator for the USAID Emerging Pandemic Threats PREDICT program and also serves on the Steering Committee of the One Health Alliance of South Asia (OHASA), a multidisciplinary network linking health scientists and ministry officials in India, Pakistan, Bangladesh, and Nepal. His current research interests include bat-borne emerging zoonotic viruses such as Nipah virus, Ebola virus, and coronaviruses including SARS CoV and the recently discovered novel CoV from the Arabian peninsula. In 2004, he was part of the team that identified bats as the natural wildlife reservoir for SARS coronavirus (SARS-CoV) in China. Dr. Epstein is currently investigating Nipah virus in Bangladesh, where outbreaks occur in people almost every year with mortality rates reaching more than 90 percent. The aim of this research is to better understand the factors that cause Nipah virus to spill over from bats, and to develop models that will predict and help prevent future outbreaks.

Dr. Epstein holds adjunct faculty positions at Columbia University's Mailman School of Public Health and the Department of Ecology, Evolution, and Environmental Biology; Tufts Cummings School of Veterinary Medicine and Tufts School of Medicine; and Mount Sinai School of Medicine. His work has been published in several leading scientific journals, including *Emerging Infectious Diseases*, *PLoS Pathogens*, *Proceedings of the National Academy of Sciences of the United States of America*, the *Journal of Applied Ecology*, and *Science*. He has been an invited speaker at meetings held by the Institute of Medicine and the World Health Organization. He holds advisory positions on two committees in the International Union for the Conservation of Nature (IUCN): the Wildlife Health Specialist Group and the Bat Specialist Group.

Anthony S. Fauci, M.D., is director of the National Institute of Allergy and Infectious Diseases (NIAID) at the National Institutes of Health. Since his appointment as NIAID director in 1984, Dr. Fauci has overseen an extensive research portfolio devoted to preventing, diagnosing, and treating infectious and immune-mediated diseases. Dr. Fauci also is chief of the NIAID Laboratory of Immunoregulation, where he has made numerous important discoveries related to HIV/AIDS and is one of the most-cited scientists in the field. Dr. Fauci serves as one of the key advisors to the White House and Department of Health and Human

Services on global AIDS issues, and on initiatives to bolster medical and public health preparedness against emerging infectious disease threats such as pandemic influenza. He was one of the principal architects of the President's Emergency Plan for AIDS Relief (PEPFAR), which has already been responsible for saving millions of lives throughout the developing world.

Dr. Fauci is a member of the U.S. National Academy of Sciences and is the recipient of numerous prestigious awards for his scientific and global health accomplishments, including the National Medal of Science, the Mary Woodard Lasker Award for Public Service, and the Presidential Medal of Freedom. He has been awarded 38 honorary doctoral degrees and is the author, co-author, or editor of more than 1,200 scientific publications, including several major textbooks.

Harvey V. Fineberg, M.D., Ph.D., is former President of the Institute of Medicine, serving from 2003 to 2014. He served as Provost of Harvard University from 1997 to 2001, following 13 years as Dean of the Harvard School of Public Health. He has devoted most of his academic career to the fields of health policy and medical decision making, including assessment of medical technology, evaluation and use of vaccines, and dissemination of medical innovations. Dr. Fineberg helped found and served as president of the Society for Medical Decision Making and has been a consultant to the World Health Organization. He serves on the boards of the William and Flora Hewlett Foundation, the Carnegie Endowment for International Peace, the Josiah Macy Jr. Foundation, The China Medical Board, and the Association François-Xavier Bagnoud (USA).

Dr. Fineberg is co-author of the books *Clinical Decision Analysis*, *Innovators in Physician Education*, and *The Epidemic That Never Was*, an analysis of the controversial federal immunization program against swine flu in 1976. He has co-edited books on such diverse topics as AIDS prevention, vaccine safety, and understanding risk in society. He has also authored numerous articles published in professional journals. Dr. Fineberg received the Stephen Smith Medal for Distinguished Contributions in Public Health from the New York Academy of Medicine, the Frank A. Calderone Prize in Public Health, awarded by the Mailman School of Public Health at Columbia University, the Henry G. Friesen International Prize in Health Research, awarded by Friends of Canadian Institutes of Health Research, the Harvard Medal from the Harvard Alumni Association, and a number of honorary degrees. He earned his bachelor's and doctoral degrees from Harvard University.

Keiji Fukuda, M.D., M.P.H., has been Assistant Director-General (ADG) for Health Security, World Health Organization (WHO) since September 1, 2010. Before this, he was Special Adviser on Pandemic Influenza to the Director-General, ADG for Health Security and Environment ad interim, Director of the Global Influenza Programme (GIP), and Coordinator of GIP and Scientist in GIP.

Dr. Fukuda has extensive global and national public health, field, and research experience related to emerging diseases (including chronic fatigue syndrome, SARS, avian influenza H5N1 and H7N9, MERS, and pandemic influenza H1N1), regional and global emergencies due to other causes, and international negotiations and activities. His current responsibilities are focused on global health security including food safety, pandemic and epidemic infectious diseases, including antimicrobial drug resistance, global alert and monitoring for health security-related events, and implementation of the International Health Regulations as well as the Pandemic Influenza Preparedness Framework and the Codex Alimentarius. Before coming to WHO, Dr. Fukuda was Chief of the Epidemiology Unit, Influenza Branch at the U.S. CDC. He is a physician and received his B.A. from Oberlin College, M.D. from the University of Vermont, and M.P.H. from the University of California, Berkeley.

Daniel B. Jernigan, M.D., M.P.H., is the Deputy Director of the Influenza Division in the National Center for Immunization and Respiratory Diseases at the CDC. The Influenza Division is responsible for national surveillance of influenza and serves as a WHO Collaborating Center for the Surveillance, Epidemiology, and Control of Influenza. The division provides epidemiologic and laboratory leadership in various research, investigative, and preparedness activities for seasonal, avian, and pandemic influenza.

Dr. Jernigan received his bachelor of science from Duke University, his doctor of medicine from Baylor College of Medicine, and his master of public health from the University of Texas. He is board certified in internal medicine and has completed an additional residency in preventive medicine. Dr. Jernigan joined the CDC's Epidemic Intelligence Service in 1994 working in the Respiratory Diseases Branch, and has remained at the CDC since that time.

Dr. Jernigan is active in the field of infectious diseases epidemiology and response. He has published peer-reviewed articles and book chapters on various emerging infectious diseases topics and has supervised outbreak investigations of viral, bacterial, and fungal infections associated with emerging and antibiotic-resistant pathogens. These findings led to improvements in disease detection and infection control. He has led epidemiology and surveillance teams for national and international responses, including bioterrorism-related anthrax, West Nile virus, SARS in Asia, and public health management following natural disasters. Most recently, he served as the Senior Science Officer and Lead for the Epidemiology and Laboratory Task Force responding to the 2009 H1N1 influenza pandemic.

In his current role as Deputy Director of the Influenza Division, Dr. Jernigan serves as Senior Medical Officer, and Senior Public Health Service Officer for the Influenza Division. He is responsible for oversight and direction of 241 staff members with primary supervision of budget, communications, policy, preparedness, and program support. He is responsible for implementation of a broad influenza diagnostic strategy, including research and development of new influenza

diagnostic tests and manufacturing, distribution, and compliance with quality and regulatory requirements. Dr. Jernigan also serves as a principle investigator for influenza research and public health evaluation activities.

Kamran Khan, M.D., M.P.H., is an infectious disease physician and scientist at St. Michael's Hospital in Toronto, an associate professor of medicine at the University of Toronto, and the Founder of BioDiaspora (www.biodiaspora.com). Dr. Khan's research interests focus on emerging infectious disease threats and their potential spread and impacts in an increasingly interconnected and interdependent world. To support time-sensitive decision making during public health emergencies, Dr. Khan developed BioDiaspora, a Web-based GIS application capable of generating predictive analytics in near real time. He is currently partnering with the Division of Global Migration and Quarantine at the CDC under a project named BioMosaic to strengthen preparedness and response to public health emergencies from infectious diseases.

John Lowenthal, Ph.D., obtained his Ph.D. in immunology from the Walter and Eliza Hall Institute for Medical Research, University of Melbourne in 1983. He completed postdoctoral research at the Ludwig Institute for Cancer Research in Lausanne, Switzerland, and at the Howard Hughes Medical Institute, Duke University in North Carolina. He joined Commonwealth Scientific and Industrial Research Organisation (CSIRO) in 1990 to establish an Avian Immunology group and is a Senior Principal Research Scientist based at CSIRO's Australian Animal Health Laboratory in Geelong, Victoria.

Dr. Lowenthal's research group takes a One Health approach to fighting emerging infectious diseases. This work involves veterinary health and immunology, including studying the innate immune responses to viral diseases under high biocontainment; assessing the ability of immune modulators to improve vaccine efficacy; developing novel therapeutics for zoonotic viruses such as H5N1 avian influenza and Hendra virus; and developing disease-resilient animals. He is an adjunct professor at the Deakin University School of Medicine and has published more than 150 journal articles (6,000 citations), produced more than 140 conference presentations, and is an inventor on 15 patents.

Allison McGeer, M.D., MsC., trained in internal medicine and infectious diseases at the University of Toronto and then completed a fellowship in hospital epidemiology at Yale New Haven Hospital in 1989–1990. She has served on the Canadian National Advisory Committee on Immunization, and is currently a member of the infection control subcommittee of the Ontario Provincial Infectious Diseases Advisory Committee. Her areas of research interest are the epidemiology of influenza infection, the prevention of health care–associated infection, and adult immunization.

Kevin Olival, Ph.D., is a Senior Research Scientist at EcoHealth Alliance. He has investigated the ecology, evolution, diversity, and dynamics of bats and their viruses for more than a decade. This includes extensive field studies of Nipah virus in Malaysia and Bangladesh; using phylogeography and population genetics to understand the dynamics of Nipah; discovering several novel bat pathogens; and building models to predict pathogen diversity and spillover potential in mammals. Olival is a senior scientist on the USAID PREDICT project as part of the modeling team and surveillance coordinator in Thailand and Indonesia. Over the past 4 years, he has led field expeditions and bat surveillance workshops in Bangladesh, Cambodia, India, Indonesia, Kingdom of Saudi Arabia (KSA), Malaysia, the Philippines, and Thailand; and has coordinated global research activities under a NIAID R01 grant on the "Risk of viral emergence from bats." He has led two field expeditions to KSA (Oct 2012, April 2013) working with the KSA Ministry of Health and Columbia University, where he is adjunct faculty. This led to the first discovery of MERS-CoV in Arabian bats and additional data to better understand the ecology of bats in KSA and the diversity of the coronaviruses they harbor.

Trish Perl, M.D., M.S., is a professor in the Departments of Medicine (Infectious Diseases) and Pathology at Johns Hopkins University School of Medicine in Baltimore, Maryland, and in the Department of Epidemiology at the Johns Hopkins Bloomberg School of Public Health. She is Senior Epidemiologist for The Johns Hopkins Health System. Dr. Perl received her bachelor of arts and medical degree from the University of North Carolina at Chapel Hill and a master of science degree from McGill University in Montreal, Canada. She completed a residency in internal medicine and a fellowship in infectious diseases and clinical epidemiology at the University of Iowa in Iowa City, Iowa.

She has extensive practical and research experience in the field of health care-associated infections and resistant and epidemiologically significant organisms and is world renowned for her innovation and research in the field and the use of research knowledge in the health care setting. Dr. Perl is the former President of the Society of Hospital Epidemiologists of America (SHEA) and has served on advisory panels for the IOM, the CDC, and WHO, and been a consultant to the NIH and ARHQ. She was the Courage Fund Visiting Professor in 2008–2010. An active researcher, Dr. Perl has been a principal and co-principal investigator for multiple studies funded by the CDC and the U.S. Department of Veterans Affairs over the years. She has authored or co-authored more than 200 peer-reviewed articles. In addition, she has written multiple chapters and contributed to guidelines and policies relevant to health care–associated infections at the institutional, state, and federal level.

Dirk Pfeiffer, DrMedVet, MACVSc, Ph.D., DipECVPH, graduated in veterinary medicine in Germany in 1984. He obtained his Ph.D. in veterinary epidemiology from Massey University, Palmerston North, New Zealand, in 1994, and

worked as an academic in New Zealand for 9 years. He has been holding the Chair in Veterinary Epidemiology at the Royal Veterinary College (RVC) since 1999. Dr. Pfeiffer has been involved in epidemiological research since 1985 and worked on animal health issues in developing as well as developed countries. He has published 191 peer-reviewed publications. He is the Head of the Veterinary Epidemiology, Economics & Public Health Group within RVC comprising 11 academic staff and about 30 Ph.D. students and researchers. Dr. Pfeiffer is head of the RVC's FAO Reference Centre for Veterinary Epidemiology. He is the lead author of a textbook on spatial epidemiology, author of the chapter on spatial analysis in the key veterinary epidemiology textbook *Veterinary Epidemiologic Research* as well as the author of the textbook *Introduction to Veterinary Epidemiology*. He teaches epidemiology at undergraduate and postgraduate levels and has designed and taught international training courses in veterinary epidemiology, risk analysis, and spatial analysis in Europe, North America, Australasia, and Africa. Dr. Pfeiffer provides scientific expertise to various national and international organizations.

Linda Saif, M.S., Ph.D., is a Distinguished University Professor at Ohio State University (OSU) in the Food Animal Health Research Program (OARDC) and the Veterinary Preventive Medicine Department (CVM, OSU). She is a virologist and immunologist, whose research focuses on comparative aspects of enteric and respiratory viral infections (coronaviruses, rotaviruses, and caliciviruses) of food animals and humans. Her lab studies mucosal immunity and vaccine development and is currently focusing on the impact of malnutrition and micronutrient deficiencies on vaccines and interactions of probiotics and the gut microbiota with the neonatal immune system and vaccines. Her team's discovery of the gut-mammary secretory IgA axis (initial description of the common mucosal immune system) in swine was a breakthrough for development of maternal coronavirus vaccines to passively protect neonatal animals. Her lab identified new enteric viruses (group C rotavirus, caliciviruses), characterized their pathogenesis and developed novel cultivation methods, diagnostic assays and vaccines for them. Her current research emphasizes novel bioengineered virus-like particle (VLP) vaccines and adjuvants (vitamin A, probiotics) to prevent viral diarrheas in humans and animals and their evaluation in germfree animal disease models. Her lab also investigates the interrelationships among animal viruses and their human counterparts to assess their zoonotic potential and mechanisms of interspecies transmission.

Dr. Saif is a member of the U.S. National Academy of Sciences and the Argentine Academia Nacional de Agronomía y Veterinaria. She is an elected Fellow of the American College of Veterinary Microbiologists, the AAAS, and the American Academy of Microbiology. She has served as a member of advisory teams for various organizations (USAID, CDC, WHO, etc.), she was a Fulbright Scholar (Argentina) and she serves on several journal editorial boards (including

Proceedings of the National Academy of Sciences of the United States of America). Her laboratory serves as a WHO International Reference Lab for Animal Coronaviruses within the SARS Coronavirus Network and as an International Reference Lab for TGEV porcine coronavirus for the Office International des Epizooties, Paris, France. Dr. Saif has authored or co-authored more than 300 journal publications and 57 book chapters pertaining to her research.

Jonathan Sleeman, VetMB, ACZM, is currently the Center Director for the U.S. Geological Survey's National Wildlife Health Center where he leads a team that provides national leadership to safeguard wildlife and ecosystem health through multidisciplinary research and technical assistance to federal, state, and tribal agencies as well as internationally as an OIE Collaborating Centre. He is also an adjunct professor at the University of Wisconsin School of Veterinary Medicine. He has authored more than 50 peer-reviewed publications and several book chapters all on the topics of wildlife and ecosystem health. He is active in various scientific organizations, and serves on several committees for the U.S. Animal Health Association, Association of Fish and Wildlife Agencies, U.S. Fish and Wildlife Service, and the CDC. He is board certified by the American College of Zoological Medicine, and received his veterinary degree and master's degree in zoology from the University of Cambridge, England. Previous positions include Director of the Mountain Gorilla Veterinary Center in Rwanda and Wildlife Veterinarian for the Virginia Department of Game and Inland Fisheries.

Derek Smith, M.S., Ph.D., is Professor of Infectious Disease Informatics at Cambridge University in the United Kingdom and is also Director of the Centre for Pathogen Evolution, and Director of WHO Collaborating Centre for Modelling, Evolution, and Control of Emerging Infectious Diseases, both also at Cambridge University.

He is a member of the Viroscience Department at Erasmus Medical Center in The Netherlands. He is also a member of the WHO Influenza Vaccine Strain Selection Committee, and is involved in vaccine strain selection for other human and nonhuman pathogens for the FAO and OIE. His research is focused on how pathogens evolve, to what extent this evolution is predictable, and determining public and animal health measures against such ever-changing pathogens. He received a U.S. National Institutes of Health Director's Pioneer Award in 2005 for his work on antigenic cartography, a method that enables detailed study of pathogen evolution.

David Swayne, D.V.M., M.Sc., Ph.D., is a research veterinarian in avian influenza. Since 1987, his personal research has focused on pathobiology and control of high pathogenicity avian influenza with more than 262 peer-reviewed publications and more than 237 invited presentations. He is a former faculty member at Ohio State University and for the past 20 years has been the Director

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Alejandro Thiermann, D.V.M., Ph.D., is the Senior International Organization's Coordinator for the Animal and Plant Health Inspection Services, USDA-APHIS. He has been seconded full-time by USDA to the OIE to serve as senior advisor to the Director General of the OIE. He is President of the Terrestrial Animal Health Standards Commission, the OIE's international standard-setting body. He has served in this commission since 1994. Prior to coming to Paris, from October 1996 and until September 2001, he served as Senior Trade Coordinator and Regional Director for USDA-APHIS in Brussels, with responsibility over Europe, Africa, Middle East, Russia, and the former Soviet Republics. During 1997 to 1999 he was twice elected Chairman of the World Trade Organisation, Sanitary and Phytosanitary (WTO-SPS) Committee. Dr. Thiermann joined USDA-APHIS in 1989 as the Deputy Administrator for International Services. Before joining APHIS, he was the National Program Leader for animal health research under the USDA Agriculture Research Service (ARS). A native of Chile, Dr. Thiermann received his doctorate of veterinary medicine degree from the University of Chile at Santiago, and a Ph.D. degree in microbiology and immunology from the School of Medicine at Wayne State University in Michigan.