Perspectives on Research with H5N1 Avian Influenza: Scientific Inquiry, Communication, Controversy: Summary of a Workshop

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Perspectives on Research with H5N1 Avian Influenza

Scientific Inquiry, Communication, Controversy

SUMMARY OF A WORKSHOP

Karin Matchett, Anne-Marie Mazza, and Steven Kendall, *Rapporteurs*

Committee on Science, Technology, and Law
Policy and Global Affairs

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Forum on Microbial Threats
Board on Global Health

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This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the National Academies' 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 report as sound as possible and to ensure that the report meets institutional standards for quality and objectivity. The review comments and draft manuscript remain confidential to protect the integrity of the process.

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Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the content of the report, nor did they see the final draft before its release. The review of this report was overseen by Huda Akil, University of Michigan. Appointed by the National Academies, she was responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the rapporteurs and the institution.

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Introduction

THE H5N1 CONTROVERSY

The submission for publication of a scientific manuscript is typically an unremarkable event. When, however, it became public knowledge in December 2011 that two research groups, working independently, had recently submitted manuscripts for publication in *Nature* and *Science* that reported on their work on mammalian transmissibility of an H5N1 avian influenza strain—a lethal strain with suspected pandemic potential whose worldwide behavior had been monitored closely in recent years—the information caused a vigorous and far-reaching international debate about the appropriateness of and communication of the researchers' work, the risks associated with the work, partial or complete censorship of scientific publications, and dual-use research of concern in general.

Using well-known techniques, the groups had selected for influenza strains highly transmissible between ferrets, identified and sequenced the strains' genetic mutations, inserted the mutated genes into a new virus, and, by observing the behavior of the newly constructed viruses, demonstrated a causal link between the mutated genes and degree of transmissibility between mammals.

The research projects were led by Ron Fouchier at Erasmus Medical Center in Rotterdam, the Netherlands, and Yoshihiro Kawaoka at the University of Wisconsin, Madison. The work identified mutations in the H5N1 HA gene. When the mutated gene was inserted into another influenza strain (H1N1), the resulting strain was more transmissible between ferrets, mammals whose response to influenza infection is thought to be predictive of the human response.

Concerns were raised both about the accidental release of the newly constructed strains and about the possibility that the research results could be used by people seeking to do harm. While some argued that the research should not be published (or that it should not have been undertaken to begin with), others argued that openness is essential to the success of the scientific enterprise and that the insights gained as a result of the research might yield public health benefits that outweighed any risks associated with the research.

The two research papers were brought to the attention of the U.S. National Science Advisory Board for Biosecurity (NSABB),[1] which weighed the potential benefits and risks of full publication of the research. In December 2011, the NSABB recommended that the papers be published absent certain details of the experimental design. In January 2012, the two research groups and other influenza researchers called for a temporary moratorium on research involving H5N1 influenza viruses that might lead to the creation of highly pathogenic, highly transmissible strains. In February 2012, the World Health Organization (WHO) convened a meeting of public health and influenza experts to discuss the manuscripts. Following that meeting, the WHO recommended that the manuscripts be published in full, but only after biosecurity and communication issues had been addressed. On March 29, 2012, the U.S. Department of Health and Human Services' Office of Biotechnology Activities, which convenes and manages the NSABB, released a new policy for the oversight of life sciences dual-use research of concern.[2] On March 29-30, 2012, the NSABB reconsidered the manuscripts, and in light of additional risk/benefit information, voted, although not unanimously, in favor of the publication of both revised papers in full.

The response of both the scientific community and the public to Fouchier and Kawaoka's research illuminates the unsettled landscape of the current national and international governance and regulation of scientific research that could, either advertently or inadvertently, result in great harm.[3] Traditionally, the scientific community has had a strong culture of openness, and when high risk research has warranted restrictions, those restrictions have taken the form of security classification mechanisms or the regulation of certain toxic or pathogenic microorganisms and substances.

[1] The NSABB is a federal advisory committee established in 2004 to provide "advice, guidance, and leadership" regarding federally-funded dual use research. See the website of the Office of Biotechnology Activities, "About NSABB," http://oba.od.nih.gov/biosecurity/about_nsabb.html.

[2] It is important to note that the new policy applies to *all* federally funded research, not just research funded by the Department of Health and Human Services.

[3] In the context of this report, regulation is broadly defined to include rules, principles, statutes, or other forms of guidance.

The debate over the Fouchier and Kawaoka manuscripts indicates that there are significant issues related to high risk life sciences research that must be addressed, including the need to further clarify how both information and materials are handled in a world where sensitive information and materials are generated at an accelerating pace.

Recognizing that the H5N1 research is only the most recent scientific activity subject to widespread attention due to safety and security concerns, on May 1, 2012, the National Academy of Sciences' Committee on Science, Technology, and Law, in conjunction with the National Academy of Sciences' Board on Life Sciences and the Institute of Medicine's Forum on Microbial Threats, convened a one-day public workshop. The workshop was organized by an ad hoc committee "for the purposes of (1) discussing the H5N1 controversy; (2) considering responses by the National Institute of Allergy and Infectious Diseases (NIAID), which had funded this research, the WHO, the U.S. National Science Advisory Board for Biosecurity (NSABB), scientific publishers, and members of the international research community; and (3) providing a forum wherein the concerns and interests of the broader community of stakeholders, including policy makers, biosafety and biosecurity experts, non-governmental organizations, international organizations, and the general public may be articulated. The workshop was to "examine possible points of intervention from research conceptualization through publication" and to "discuss the current regulation and oversight of research whose results may raise bio-security concerns in the U.S. and abroad and . . . consider alternative mechanisms for the oversight and governance of such research."

Workshop participants were asked to look toward the future and consider new paradigms for the evaluation, oversight, and communication of research identified as warranting special consideration; to evaluate the potential need for enhanced biosafety and biosecurity oversight; and to reflect on how a new mechanism might be structured and implemented.

This workshop summary has been prepared by the workshop rapporteurs as a factual summary of what occurred at the workshop. The statements made are those of the rapporteurs or individual workshop participants and do not necessarily represent the views of all workshop participants, the planners of the workshop, or the National Academies.

DUAL-USE RESEARCH

In general, dual-use research is defined as research undertaken to generate information that may be used to protect national security or public health but which, if misused, may cause harm. Dual-use research concerns effectively emerged in the mid-20th century, particularly in the context of wartime nuclear research and concurrent advances in biological knowledge

and laboratory technologies. Dual-use research and the question of how or whether to regulate it raises difficult questions about scientific freedom, communication of scientific knowledge, and access to that knowledge. Proposals to limit access to scientific research forcefully encounter deeply held values in scientific communities around norms of openness, access, and transparency. As scientific knowledge and technologies move forward and as fears have grown that these technologies could be used for harm, society must continually assess both the benefits of research of concern and how best to regulate it. Regulatory precedents for governing such research were established in a fundamentally different era, an era when sensitive information was available to a small, select group, the primary areas of the research were physical and chemical (rather than biological), and the principal aggressors were nation states. The flurry of activity in the winter and spring of 2011/2012 ignited an important debate about research undertaken in a very different world; a world where extraordinary advances in the biological sciences and biotechnology are common, a wired, technically sophisticated world, and a world where scientifically savvy individuals work to protect public health and safety and, in some cases, to cause harm.

2

The Quickening Pace of Biological Research and Current Challenges in Biosecurity

DEVELOPMENTS IN MICROBIOLOGY

In the workshop's first session, **Roger Brent, Member of the Division of Basic Sciences and Adjunct Member of the Division of Public Health Sciences, The Fred Hutchinson Cancer Research Center and Affiliate Professor, Department of Genome Sciences, University of Washington**, provided an overview of the major developments in molecular biology over the past 40 years. Brent highlighted key developments in scientists' ability to deconstruct and recombine DNA and RNA, beginning with their capacity to remove and make copies of bacterial DNA and reinsert it into organisms of a different species, and extending to scientists' current capacity to engineer viral genomes. (For a timeline of events and related publications, see Appendix A.)

The development of recombinant DNA technology in the early 1970s marked the beginning of technical capabilities that would, within the next three decades, enable the scientific community to move genetic material between species, induce bacteria to synthesize new proteins using foreign genes inserted for that purpose, and build new genomes that reveal genes of great interest to those charged with protecting the public health. Between 1973 and 1978, scientific advances led to the ability to compel *E. coli* bacteria to produce complex recombinant proteins. In 1978, researchers engineered a bacterial species to produce human insulin, and in 1982, researchers successfully transferred bacterial genes into plants, thereby conferring new traits to agriculturally important species.

The 1980s brought further developments in genetic manipulation and gene replacement, most importantly, the ability to reverse engineer a virus. The first complete clone of an animal virus genome was for a plus strand virus, which was synthesized and expressed, in 1981 (bacterial viruses had been genetically manipulated previously). In 1993, scientists synthesized and expressed a negative strand virus (influenza is also a negative strand virus) and had, by 2007, synthesized and expressed a double stranded virus.

By the 2000s, it was possible to add or remove biological functions genetically to examine the effect on a pathogen's virulence or transmissibility. This capability allowed laboratory scientists to investigate evolutionary questions in a manner that had never before been possible. A common experimental design involved creating an environment hospitable only to organisms possessing a specific trait—for example, virulence or transmissibility. Genetic material from surviving organisms would be sequenced in order to identify the mutation(s) responsible for specifically selected traits. Genetic material associated with the mutations would be extracted and inserted into new viruses to determine whether they caused the appearance of the trait.

In parallel with these developments, there was ever increasing access by ever larger numbers of people to the tools and information needed to manipulate potentially lethal viruses. The equipment necessary to rapidly sequence and reconstruct genomes, for instance, has become affordable and knowledgeable, both of genomics and of how to use the relevant equipment, has become readily available. As a result, access to potentially dangerous information has expanded well beyond the boundaries of what has traditionally been considered the scientific community, both in the United States and internationally.

With particular regard to influenza viruses, Brent noted that researchers have publicly stated, since at least 2004, the goal of constructing human-transmissible H5N1. The rationale behind the goal is that the relative ease or difficulty of the task will provide an indicator of the relative risk posed by the H5N1 virus to public health.

Regulatory Developments Prompted by These Advances

In 1974, in light of the development of recombinant DNA technology and the uncertainties surrounding its safety, the scientific community imposed a moratorium on further research, and in 1975, convened a conference at the Asilomar Conference Center in California for the purposes of defining a framework for governing recombinant DNA technology and its products. The Asilomar Conference (see Box 2-1) was followed in 1976 by NIH's regulatory framework for recombinant DNA, which included local control (in the form of Institutional Biohazard Committees) and national

BOX 2-1
Asilomar Conference on Recombinant DNA

As recombinant DNA techniques became more widespread in the 1970s, concern grew in the scientific community and among the public that microbes manipulated through recombinant DNA techniques could endanger the health of humans and the environment.

In 1971, researchers inserted the genome of a tumor-causing virus, SV40, into a bacterial plasmid that could reproduce in *E. coli*. As the research proceeded, concerns arose that if this engineered strain of *E. coli* were accidentally released into the human population, it could cause a cancer epidemic. Scientists voluntarily halted the experiments until a determination could be made regarding the risk of the experimental plasmid spreading to strains of *E. coli* that exist naturally in the human body.

A group of leading scientists asked the National Academy of Sciences (NAS) to assess the concerns and provide recommendations on how to proceed. The resulting NAS Committee on Recombinant DNA Molecules issued a letter endorsing a voluntary moratorium on specific types of recombinant DNA research "until the potential hazards of such recombinant DNA molecules have been better evaluated or until adequate methods are developed for preventing their spread." In the letter, the committee acknowledged that it was difficult to estimate risk and recommended that an international conference of involved scientists be held to examine the question more closely.[a]

The resulting Asilomar Conference on Recombinant DNA took place in February 1975 in Pacific Grove, California. Its purpose was to make recommendations on whether to end the moratorium, and if so, under what circumstances. One-hundred fifty participants gathered, including biologists, lawyers, physicians, and journalists. The discussions were vigorous and contentious, and encompassed views ranging from the insistence that no limits be placed on scientists' freedom to the view that limits should be entertained. Participants from the scientific community felt strongly that if they did not arrive at a path forward, that path would likely be determined by others.

The outcome of the conference was a nearly unanimous agreement to lift the moratorium and to require that recombinant DNA research be carried out according to yet-to-be-determined guidelines that would define levels of physical and biological containment based upon the potential risk posed by a given research project.[b]

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[a] Committee on Recombinant DNA Molecules, "Potential Biohazards of Recombinant DNA Molecules," *Proceedings of the National Academy of Sciences* 71, no. 7 (July 1974): 2593-2594.

[b] Paul Berg, et al., "Summary Statement of the Asilomar Conference on Recombinant DNA Molecules," *Proceedings of the National Academy of Sciences* 72, no. 6 (June 1975): 1981-1984; Susan Wright, *Molecular Politics: Developing American and British Regulatory Policy for Genetic Engineering,* 1972-1982 (Chicago: University of Chicago Press, 1994).

oversight (in the form of the Recombinant DNA Advisory Committee[1]). In 1977, public representatives were given seats on the Recombinant Advisory Committee. According to Brent, between 1977 and 1982 the combination of directed experimentation, a lack of evidence of harm, and a deeper understanding of the pertinent scientific questions to ask or address resulted in more flexible oversight and an exemption of most experiments from NIH guidelines.

Policy Considerations

Dr. Brent concluded his remarks by offering observations about the wisdom of attempting to regulate research. He stated that any regulation places burdens on researchers. Great care, he argued, should therefore be taken before attempting to hinder the unfettered pursuit of research. Brent then observed that, if research with potential benefits as well as dangers to public health were performed, there is no system in place to release the experimental details selectively. He stated that he believes that it would be very difficult to devise such a system. Brent further remarked upon the difficulty of formulating workable policies regarding research funding or the publication of research when no scientific consensus exists about what right behavior is. He observed that weighing foreseeable benefits versus risks to public health requires an omniscience that humans do not possess. Brent also observed that knowledge, once obtained, cannot be undone. For Brent, the fact that knowledge about the virulence and transmissibility of, for example, lethal human-transmissible influenza strains, would be available from the point of production onward, is an important consideration. Experts, he remarked, are individually less likely to predict a distinct benefit *or* risk from their research. With regard to the H5N1 controversy itself, Brent viewed the incident as an ethical failure and indicative of a far too fragmented scientific community.

[1] The Recombinant DNA Advisory Committee (RAC) is a federal advisory committee that "issues recommendations to the NIH Director that are conveyed through the NIH Office of Biotechnology Activities (OBA), which is responsible for the NIH system of oversight of recombinant DNA research." The RAC developed and suggests changes to a set of NIH guidelines (now known as the NIH Guidelines for Research Involving Recombinant DNA Molecules) to "govern the safe conduct of recombinant DNA research by outlining appropriate biosafety practices and containment measures." It is important to note that compliance with these guidelines "is *mandatory* for investigators at institutions receiving NIH funds for research involving recombinant DNA,"[emphasis added] but *voluntary* for institutions, companies, or individuals not subject to NIH requirements. See the website of the Office of Biotechnology Activities, "About Recombinant DNA Advisory Committee (RAC)," http://oba.od.nih.gov/rdna_rac/rac_about.html.

Discussion

The moderator of the workshop's opening session, **David Baltimore, Robert Andrews Millikan Professor of Biology and President Emeritus, California Institute of Technology**, stated that his view about the Asilomar Conference diverged in one respect from Brent's, namely, that, while Brent considered the conference and period immediately afterwards to be a lamentably short period of self-governance, Baltimore considered the period to be the first step in a process of continued self-governance. He also expressed the opinion that the Asilomar model is an appropriate one for the situation at hand.

Brent clarified his larger point, stating that he sees the Asilomar/governance process as a manifestation of what works well in our national culture, which is that an external entity is given regulatory power and the people whose activities it regulates lobby that entity; in other words, "a representative democracy is easier on us all."

DEVELOPMENTS AT THE INTERSECTION OF BIOSECURITY AND SCIENCE

David Relman, Thomas C. and Joan M. Merigan Professor, Departments of Medicine, and of Microbiology and Immunology, Stanford University and Chief, Infectious Disease Section, VA Palo Alto Health Care System, discussed developments in national security and responses to dual-use research, highlighting the tension between the long-established value of openness in science (particularly strong in the life sciences) and the ever-changing needs of national security. Relman reiterated that concerns about dual-use research rose to the fore with weapons research in the Cold-War era and with questions about what should be done with the information and materials generated.

Cold War Deliberations

In 1982, the National Research Council (NRC) released the report *Scientific Communication and National Security* (known as the Corson Report after the authoring panel's chair Dale Corson of Cornell University). The Corson panel defined three categories of university research. "The first, and by far the largest share," the panel observed, "are those activities in which the benefits of total openness overshadow their possible near-term military benefits to the Soviet Union. There are also those areas of research for which classification is clearly indicated [and] between the two lies a

small 'gray area' of research activities for which limited restrictions short of classification are appropriate."[2]

Following release of the Corson Report and intergovernmental discussions, in 1985 the Reagan administration issued National Security Decision Directive 189 (NSDD-189), which declared that, "to the maximum extent possible," it was the policy of the administration that "the communication of the products of [federally-funded] fundamental research [should] remain unrestricted," but "where the national security requires control, the mechanism for control of information generated . . . is classification."[3]

The Dawn of the 21st Century: New Scientific and Political Developments

During the late 1990s and early 2000s, attention focused on the potential for using life sciences research to deliberately cause large-scale harm. This was due, in part, to advances in life sciences and growing concern over increases in the numbers of people seeking to do harm (as exemplified, for instance, by the anthrax mailings in the fall of 2001). In 2004, the NRC published *Biotechnology Research in an Age of Terrorism* (known as the Fink Report after the authoring committee's chair Gerald Fink of the Massachusetts Institute of Technology). This report recognized the potential for misuse of knowledge in the biological sciences, described seven classes of experiments of concern, and recognized the need for oversight throughout the life cycle of research to protect against misuse. The report looked to journal editors rather than the government as gatekeepers for decisions about publication and recommended creating a national science advisory board for biodefense to provide advice, guidance, and leadership for the review and oversight of research of concern. While the report emphasized the importance of self-governance by the scientific community, the report's authors recognized a need for the development of federal guidelines through a process similar to that adopted with recombinant DNA.

In 2004, in response to the Fink Report, the National Science Advisory Board for Biosecurity (NSABB) was created. The NSABB was to be an advisory board to the U.S. Department of Health and Human Services and all other federal agencies that support life sciences research. Its purpose "is to provide . . . advice, guidance, and leadership regarding biosecurity oversight of dual use research" and to "provide advice on and recommend specific strategies for the efficient and effective oversight of federally conducted or supported dual use biological research." The NSABB was tasked to advise

[2] Panel on Scientific Communication and National Security, *Scientific Communication and National Security* (Washington, DC: National Academy Press, 1982).

[3] National Security Decision Directive 189 (NSDD.189): National Policy on the Transfer of Scientific, Technical, and Engineering Information, September 21, 1985.

on (1) "policies governing publication, public communication, and dissemination of dual use research methodologies and results," (2) "programs for outreach, education and training in dual use research issues for scientists, laboratory workers, students, and trainees in relevant disciplines," and (3) "the development, utilization, and promotion of codes of conduct." It was also to "recommend strategies for fostering international engagement on dual use biological research issues."[4]

Following the creation of the NSABB, both the NRC and the NSABB released additional biosecurity reports. The NRC, for instance, published *Globalization, Biosecurity, and the Future of the Life Sciences* (2006), *Science and Security in a Post 9/11 World* (2007), and *Review of the Scientific Approaches Used During the FBI's Investigation of the 2001 Anthrax Letters* (2011). In 2011, the NSABB released its *Recommendations on Communication of Experimental Adaptation of Avian Influenza A/H5N1*.

Dual-use Research in The Life Sciences

Dr. Relman cited the NSABB criterion for identifying dual-use research of concern. That criterion is "research that, based on current understanding, can be reasonably anticipated to provide knowledge, products, or technologies that could be *directly misapplied* by others to pose a threat to public health and safety, agricultural crops and other plants, animals, the environment or materiel"[5] [emphasis added by the speaker]. He observed that two principles—the scope (magnitude) and the immediacy of the impact of the research—were an intrinsic part of this definition and emphasized that the evaluation of dual-use potential is seen to be based upon: (1) a current understanding regarding the implications of the research results, and (2) a reasonable anticipation that research results could be misapplied.

Relman discussed how the concept of dual-use research of concern originally pertained to science and technology that could be applied to both civilian and military purposes (helicopters and satellite technology, for example). In recent years, Relman noted that the distinction has broadened and shifted and now signals research intended for beneficial purposes that also has the potential to be misused for harm. Regarding research on infectious agents specifically, Relman referred to research that the NSABB specifically identified as worthwhile but which may also need special review.

[4] U.S. Department of Health and Human Services, "Charter, National Science Advisory Board for Biosecurity," as renewed April 4, 2012.

[5] National Science Advisory Board for Biosecurity, "Proposed Framework for the Oversight of Dual Use Life Sciences Research: Strategies for Minimizing the Potential Misuse of Research," (June 2007):17.

Of greatest concern are those experiments that have the potential to produce information, products, or technologies that could:

- Enhance the harmful consequences of a biological agent or toxin;
- Disrupt immunity or the effectiveness of an immunization without a clinical and/or agricultural justification;
- Confer to a biological agent or toxin, resistance to clinically and/or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin, or facilitate their ability to evade detection methodologies;
- Increase the stability, transmissibility, or the ability to disseminate a biological agent or toxin;
- Alter the host range or tropism of a biological agent or toxin;
- Enhance the susceptibility of a host population; or
- Generate a novel pathogenic agent or toxin, or reconstitute an eradicated or extinct biological agent.[6]

Continuing the discussion on biosecurity, **Lawrence Kerr, Deputy Director for Global Biological Threats, Office of the Director of National Intelligence,** spoke about government regulations and recommendations, with a focus on defining threat, risk, vulnerability, and consequence. Kerr began his remarks with a historical overview, citing concern within the U.S. national security community during World War II that the country could lose its military superiority. At that time, Kerr stated, the President's Scientific Research Board strongly advised that "security regulations . . . should not attempt to cover basic principles of fundamental knowledge."[7] In 1949, Kerr continued, President Truman told a panel convened by the American Association for the Advancement of Science that "[c]ontinuous research by our best scientists is the key to American leadership and true national security."

Kerr noted that a later Executive Order stated that "basic science research information not clearly related to the national security may not be classified." He elaborated on the Corson Report's argument against "security by secrecy" and observed that, at the time of the report's 1982 publication, there was no practical way to restrict international scientific exchange without also hindering communication within U.S. borders. Following the Corson Report, Kerr said, those in governmental, nongovernmental, national, and international circles attempted unsuccessfully to find frameworks for handling research in a "gray zone"—research not immediately

[6] Ibid., 18-21.

[7] The President's Scientific Research Board was established by President Truman in October 1946.

related to national security (and therefore not classifiable) but with possible national security implications. In 1985, Kerr noted, NSDD-189 declared that "to the maximum extent possible, the [communication of] products of fundamental research [should] remain unrestricted." Kerr stated that federal agencies were responsible for reviewing research projects at the time of a funding decision and for periodically reviewing research findings. Shortly after the events of September 11, 2001 and the subsequent anthrax mailings, Kerr said, National Security Advisor Condoleezza Rice reaffirmed that NSDD-189 would remain in effect.

Kerr proceeded to discuss "risk" as a function of the threat, vulnerability, and consequence of or to an action. Kerr noted that vulnerability and consequences (or impact) can be discussed and sometimes measured. Regarding infectious diseases, vulnerability can be measured on the basis of a population's past experience (or lack of experience) with a particular pathogen. Vulnerability, he said, can be mitigated by the public health community's possession of countermeasures and its ability to deliver them effectively. Consequences, he continued, are the magnitude of the damage, given a specific attack type at a specific time that damages a specific target. Threat, Kerr observed, is more difficult to forecast. Threat is the probability that a specific target will be attacked in a certain way during a specific time period, and includes a consideration of the intent of a person seeking to do harm and that person's capability to do such harm. Threat, he argued, is therefore extremely difficult to predict because intent is an emotional state, and thus, the assessment of "threat" is beyond the purview of most scientists.

Kerr discussed levels of risk associated with three situations and suggested how risk might be mitigated in each case: (1) unintentional risk associated with the research and technologies themselves (e.g., accidental exposure, contamination, accidental release) and intentional risk from outside of the laboratory (e.g., people gaining access to the pathogens) and inside the laboratory (e.g., lab workers being bribed); (2) risk associated with the information obtained from research (e.g., ill-willed people using that information for nefarious purposes or loss of public trust in the government and scientific establishment); and (3) risk associated with the withholding of dual-use research and information (that might, for example, hinder planning and implementation of preparedness and response plans, impede surveillance activities and the development of countermeasures, or harm international relationships of the United States).

Discussion

How will federal agencies and departments acquire the expertise necessary to make the regulatory decisions with which they will be tasked?

Individual panel members pointed to the expertise of individual scientists working outside of government and expressed hope that these scientists will take up the task. They also suggested that effective practices across institutions should be assimilated into a "toolkit" created for the purpose of assisting all institutions with this regulatory task.

Should current policy discussions be organized around the life sciences overall, or are the regulatory questions best applied on a case-by-case basis? Dr. Baltimore expressed the opinion that in many cases, for example, in the case of H5N1 research, policy decisions must be based on an assessment of the specific hazards associated with a specific pathogen and that it would be difficult to arrive at general guiding principles.

To what degree should those in other disciplines be paying attention to these deliberations? How cross-disciplinary are these issues? Many panelists suggested that people in numerous related fields pay close attention to the H5N1 controversy and associated discussions, because similar dual-use questions are pertinent to synthetic biology, systems biology, biological engineering, chemistry, physics, and many types of engineering.

3

Influenza

VIRULENCE, TRANSMISSIBILITY, AND THE IMPORTANCE OF MUTATIONS FOR INFLUENZA A VIRUSES

In the workshop's second session, **Dr. Jeffery Taubenberger, Chief of the Viral Pathogenesis and Evolution Section, National Institute for Allergy and Infectious Diseases, National Institutes of Health,** and **Robert G. Webster, Rose Marie Thomas Chair in Virology, St. Jude Children's Hospital and Director, World Health Organization Collaborating Laboratory on Animal Influenza**, discussed the structure and function of influenza viruses.

Viruses are small, infectious agents consisting of genetic material encapsulated within a protein coat. To reproduce, viruses require host cells to which they attach and into which they inject their genetic material. Viruses overtake and use the host's cellular machinery to replicate themselves. This eventually causes the host cell to burst and viral particles are released back into the environment.

Viral surface proteins largely define a virus's identity and behavior. In influenza, surface HA (hemagglutinin) and NA (neuraminidase) proteins have opposite roles. Hemagglutinin enables the virus to bind to the host cell, and neuraminidase enables the virus to release itself from the host cell's surface to seek a new host cell.

Differences in Virulence and Transmissibility

Influenza strains differ in their ability to cause disease (pathogenicity) and to travel between hosts (transmissibility). The influenza virus, in gen-

eral, when well-adapted to a host, is among the most highly transmissible viruses known. If a highly transmissible strain is also highly virulent (i.e., highly likely to cause serious disease), the mortality rate for those infected can be significant. Most influenza strains are transmissible only between members of the same species. Other strains may pass from one species to another. For a pandemic to occur in humans, a human-transmissible strain is required. It is only when a strain becomes transmissible from an animal species to humans and then gains the ability to travel easily among humans that a human pandemic may result.

Differences in transmissibility are due to a strain's genetic make-up. The influenza viruses that most concern scientists are strains of influenza A. The genetic material of influenza A viruses is a single strand of RNA with eight gene segments. (See Figure 3-1.) The genes encoding hemagglutinin and neuraminidase are carried on different segments; therefore, the particular combination of HA and NA type is easily changed, both in nature and in the laboratory. If a single host is infected with two different strains of influenza, a new viral strain may emerge, in a process known as antigenic shift, through a process of gene segment reassortment that results in an influenza virus with a new combination of the hemagglutinin and neuraminidase proteins. Variation is also often introduced during viral reproduction. The replication process in viruses is imprecise, and errors are frequently introduced into viral RNA sequences. This imprecision often benefits the virus, since different variations may confer survival advantages in different environments. New genetic signatures can result in altered traits which may improve the strain's ability to evade the host's immunological defenses. While, in many cases, the new traits do not adversely affect the human host, they may, in some cases, cause serious harm. Regardless, the influenza virus's rapid evolution means that the influenza vaccine must be reformulated each year.

Influenza A Viruses and Their Many Hosts

Influenza A viruses infect a wide variety of animals that include—in addition to humans—whales, seals, pigs, horses, domesticated poultry, and wild birds. (See Figure 3-2.) Wild birds are the principal hosts, and throughout the world there is a large reservoir of influenza viruses in hundreds of avian species. In general, influenza-infected birds are asymptomatic. When an infected bird is symptomatic, mild gastrointestinal symptoms are most common.

An influenza strain that crosses a species barrier is cause for greater concern. The pathogenicity of such a strain is often much higher on the other side of the species barrier, as has been the case with H5 and H7 influ-

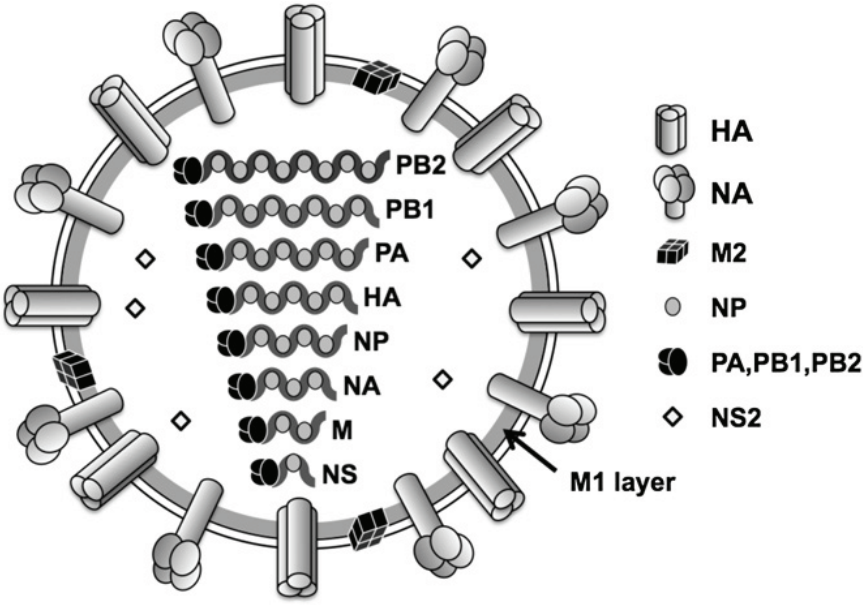

FIGURE 3-1 Diagrammatic Representation of an Influenza A Virus. The two major surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA), along with small numbers of the matrix 2 (M2) ion channel protein, are embedded in a lipid bilayer. The matrix 1 (M1) protein underlies the envelope and interacts with the surface proteins and also with the ribonucleoproteins (RNPs). RNPs consist of the eight negative-stranded RNA segments and nucleoprotein (NP) and the polymerase complex heterotrimer (PB2, PB1, and PA). The nuclear export protein (NEP, or nonstructural protein 2, NS2) is contained within the virion, but the nonstructural protein 1 (NS1) is not.
SOURCE: Courtesy of Jeffery K. Taubenberger, NIH. This image is a work of the National Institutes of Health, taken or made during the course of an employee's official duties. As a work of the U.S. federal government, the image is in the public domain.

enza sub-types that have been transmitted from wild birds to domesticated poultry.

Pigs are potential sources of new strains capable of infecting humans, as pigs are subject to infection by avian influenza, swine influenza, and human influenza. If a pig is infected simultaneously with influenza strains adapted to different species, it may thus serve as a mixing vessel wherein the interaction of the assortment of viruses may more likely give rise to a strain that is highly pathogenic for humans.

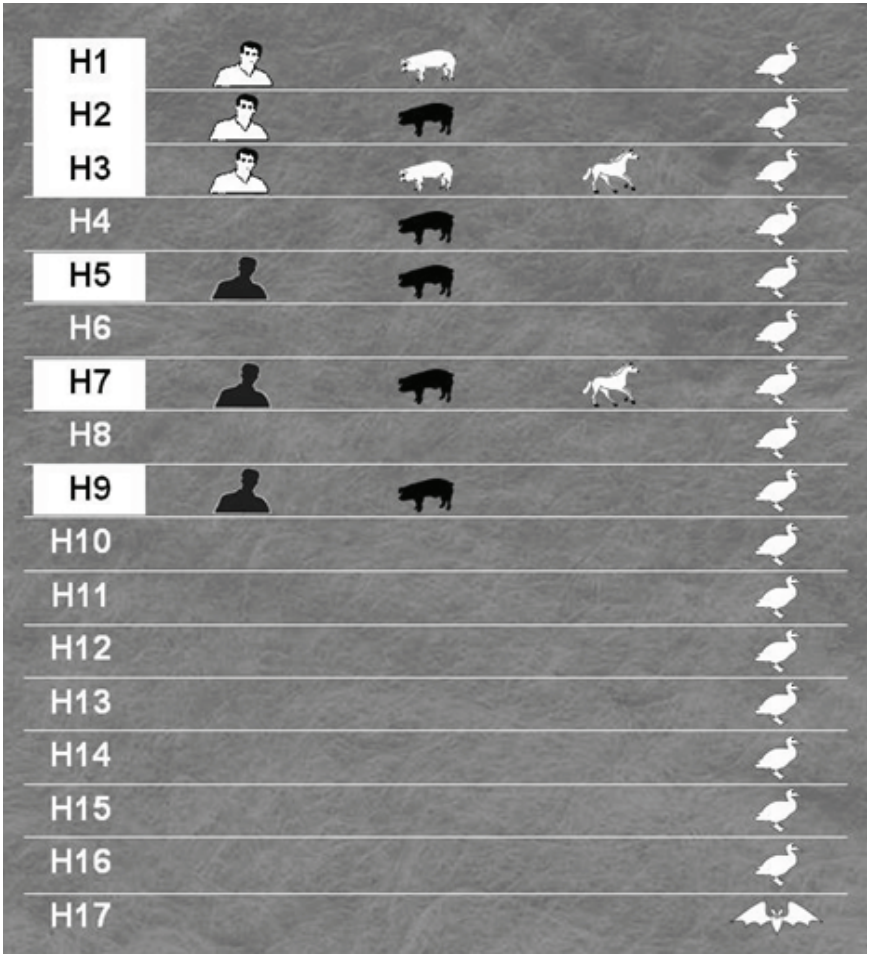

FIGURE 3-2 Influenza A Virus Host Range. Of the 17 subtypes of influenza A viruses isolated to date, the majority have come from aquatic birds. In the figure above, white figures indicate instances where the viruses have established permanent lineages, and black figures indicate where sporadic viral infection has occurred. Of the 17 subtypes of the influenza A virus, only the H1, H2, and H3 subtypes have caused pandemics in humans. Subtypes H5, H7, and H9 transmit sporadically to humans but to date have not established permanent lineages or been transmitted between humans. Subtypes of the influenza A viruses have also caused infections in pigs. The H7 subtype has caused infection and established a permanent lineage in horses. This indicates that avian H7 viruses have successfully established lineages in mammals. Recently, the H17 subtype has been described in bats.
SOURCE: Courtesy of Robert G. Webster, St. Jude Children's Research Hospital.

Human Influenza: Seasonal Outbreaks and Unpredictable Pandemics

During the winter season, influenza strains are transmitted rapidly among human beings and cause major illness and death. It is estimated that between 3,000 and 49,000 people in the U.S. die from seasonal influenza every year.[1] The influenza strains that cause seasonal outbreaks continually evolve as they circulate among human populations and can acquire decreased or increased virulence. Pandemic outbreaks, in contrast, originate with an animal-adapted strain. They occur when a human-adapted strain receives a new "HA" gene from an animal-adapted strain or when an entire animal-adapted strain becomes adapted to humans.

Four influenza pandemics have been observed in the past 100 years: "Spanish" flu (H1N1) in 1918, "Hong Kong" flu (H3N2) in 1968, "Asian" flu (H2N2) in 1957, and "swine" flu (H1N1) in 2009. In the intervening years, these four strains have circulated as seasonal flu and "mixed" with other animal-adapted strains to form new variants.

CASE STUDIES: THE 1918 SPANISH INFLUENZA PANDEMIC AND H5N1

Dr. Taubenberger discussed his research on the Spanish influenza outbreak of 1918, and Dr. Webster discussed past and recent developments in H5N1 research. Drs. Taubenberger and Webster addressed a number of questions, including: What was the scientific rationale for undertaking the research? What security and safety risks were considered prior to beginning the research? How, and by which individuals or entities other than the researchers themselves, were these risks weighed, and what kinds of precautionary or counter-measures were mandated or considered? Was the process for the risk-benefit assessment prior to the research optimal, and what might have been done differently? Why has the reaction to the H5N1 research played out differently from other dual-use research? What is different or distinct about the science, design and conduct of research, policy implications, or governance of the H5N1 research as opposed to earlier work such as the reconstruction of the 1918 Spanish Flu virus? What lessons can we learn regarding the design, conduct, communication, and oversight of future life sciences research of concern? Is there scientific research where the risks of undertaking the research outweigh the benefits of undertaking the research, and who or what institutional or societal processes are or should be in place to make such determinations?

[1] Centers for Disease Control and Prevention, "Estimating Seasonal Influenza-Associated Deaths in the United States: CDC Study Confirms Variability of Flu," June 24, 2011, http://www.cdc.gov/flu/about/disease/us_flu-related_deaths.htm.

The 1918 Spanish Influenza Pandemic

Dr. Taubenberger stated that the Spanish flu, an H1N1 strain, killed 40-50 million people worldwide during 1918-1919, including almost 700,000 people in the United States (1-2 percent of those infected died). At the conclusion of the pandemic, this strain lost much of its virulence but persisted globally, circulating and causing typical seasonal flu. (See Figure 3-3.)

In the mid-1990s, Taubenberger's research group and others investigated the Spanish flu using genetic and virologic techniques to explore the virus's origin, pathogenicity, and transmissibility in experimental models. The research was motivated by questions such as: How did the strain evolve and adapt to humans? Could the mutations be used to improve current surveillance strategies? Why was the strain pathogenic? Could data generated as the result of the research be used to develop new therapies and vaccines?

Taubenberger's team recovered viral RNA fragments from two formalin-fixed samples from autopsy collections in Washington, DC and London and one frozen, unfixed sample from Alaska and used RT-PCR to sequence the fragments. Taubenberger observed that both the public and the scientific community were aware of this research as it proceeded and noted that, in comparison with the reception of the research of Drs. Fouchier and Kawaoka, the research caused little alarm.[2] Researchers rebuilt cDNAs, cloned the cDNAs into bacterial plasmids, and, in high containment facilities, reconstructed the virus's complete genome by reverse genetics.[3] Taubenberger's team also constructed novel viruses that contained one or more of the genes from the original 1918 strain. In 1997, the first genetic sequences were published, and in 2005, the entire genome was published with the details about the methods used to reconstruct the virus.

Taubenberger stated that the research was subject to several forms of regulatory oversight. The research was funded by the Armed Forces Institute of Pathology, the Departments of Defense and Veterans Affairs, the American Registry of Pathology, and the National Institutes of Health,

[2] While not discussed explicitly at the workshop, there has been significant discussion about the way that certain H5N1 research results were conveyed and subsequently interpreted by the press. See, for example, Katherine Harmon, "What Will the Next Influenza Pandemic Look Like?," *Scientific American*, September 19, 2011, http://www.scientificamerican.com/article.cfm?id=next-influenza-pandemic.; Deborah MacKenzie, "Five Easy Mutations to Make Bird Flu a Lethal Pandemic," *New Scientist* 2831 (September 26, 2011): 3.; Editorial, "An Engineered Doomsday," *New York Times*, January 7, 2012, http://www.nytimes.com/2012/01/08/opinion/sunday/an-engineered-doomsday.html?_r=0.; Peter M. Sandman, "Science versus Spin: How Ron Fouchier and Other Scientists Miscommunicated about the Bioengineered Bird Flu Controversy," June 7, 2012, http://www.psandman.com/articles/Fouchier.htm.

[3] The genome of the 1918 influenza virus was assembled at Mount Sinai School of Medicine (laboratories of Drs. Basler, García-Sastre, and Palese) and the infectious virus was rescued at the Centers for Disease Control and Prevention (laboratory of Dr. Tumpey).

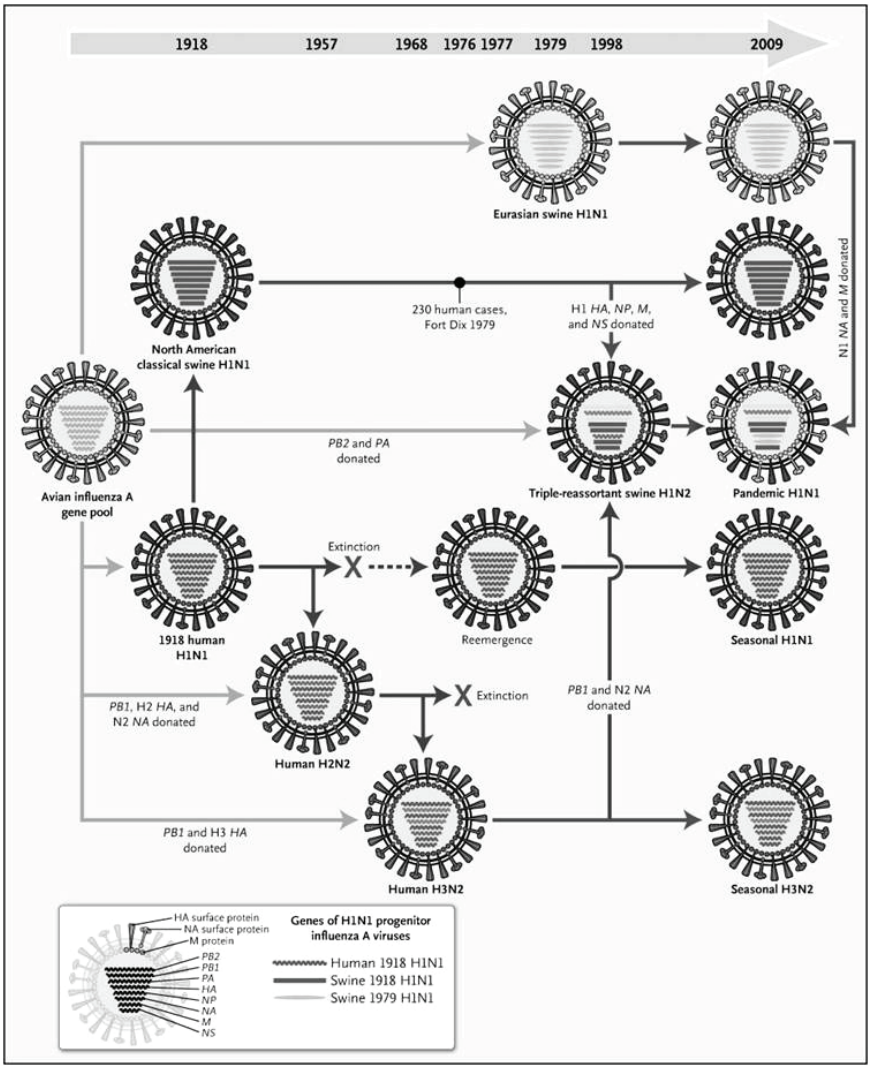

FIGURE 3-3 Descendants of the 1918 H1N1 "Spanish" Influenza Virus. All subsequent pandemic and seasonal influenza viruses are descended in part from the 1918 virus.

SOURCE: Courtesy of Jeffery K. Taubenberger, NIH. This image is a work of the National Institutes of Health, taken or made during the course of an employee's official duties. As a work of the U.S. federal government, the image is in the public domain.

and was carried out in U.S. government and academic research laboratories (see Box 3-1). In 2005, the NSABB reviewed the research and supported publication of the research results. Since 2005, the reconstructed virus has been regulated by the Centers for Disease Control and Prevention (CDC) as a select agent requiring Biosafety Level 3 enhanced containment.

What knowledge was gained from the reconstruction of the 1918 Spanish influenza virus? Taubenberger discussed how his group identified a number of factors that influenced the virulence and host adaptability of the Spanish influenza virus. Specifically, they discovered the importance of the host's inflammatory response on the pathogen's virulence and identified mutations in the virus's hemagglutinin that facilitated the virus's binding to upper airway cells in humans.

Taubenberger also noted that this research led to the discovery of a new protein common to all influenza viruses. Phylogenetic analyses have shown that all subsequent human seasonal and pandemic viruses are descendants of the 1918 pandemic virus. Data also suggest that evolutionary

BOX 3-1
Biosafety Levels for Infectious Disease Research

Federally funded research on infectious agents must be carried out in laboratories where appropriate protection and containment measures are in place. The measures employed are based on the pathogen. Guidelines are outlined and regularly updated in *Biosafety in Microbial and Biomedical Laboratories*, a publication of the CDC and the NIH. These guidelines describe the safeguards that must be in place in a facility, the safety equipment that must be used in the laboratory, and the safety practices that must be used by laboratory personnel.

The current biosafety levels—BSL-1 through BSL-4—are based on variables specific to the pathogen, e.g., its health effects and the existence of vaccines and effective treatments for those infected. The appropriate biosafety level for a specific research program depends on the infectivity and transmissibility of the pathogen, the severity of the disease that it is capable of causing, and the type of work being performed. For pathogens that can cause moderate or severe disease, an additional consideration is whether the pathogen is "indigenous or exotic."

Influenza research is carried out in facilities classified as BSL-2, BSL-3, or enhanced BSL-3. BSL-2 facilities are generally used for research on currently circulating human strains of influenza and on avian strains with low pathogenicity. BSL-3 facilities are employed for research on human-adapted strains that are not currently circulating and against which human populations may lack immunity and for research on highly pathogenic avian influenza. Enhanced BSL-3 facilities include additional precautions which are customized to the estimated risk; these precautions may include enhanced respiratory protection, HEPA filtration of exhaust air from the laboratory, and personal body showers and additional changes of lab clothing.

pressures exerted on influenza A viruses infecting wild birds are distinct from those exerted on influenza A viruses adapted to non-native hosts, such as humans and other mammals. In wild birds, influenza A viruses exist as a set of transient, unstable genomes with frequent reassortment that produces a great variety of HA and NA subtype combinations. There is evolutionary pressure toward diversity of surface proteins and toward stabilization of the virus's internal genes. When a virus leaves the large, diverse gene pool of its avian host and encounters a non-avian host, its evolution is more linear, with the genetic composition of the original infecting virus forming the basis for subsequent generations of the virus.

Taubenberger's team also learned that their original hypothesis—that if they elucidated how the 1918 virus adapted to humans and what the mutations were, they could understand all future pandemics—was incorrect. Instead, they discovered that some of the evolutionary adaptations of the 1918 strain were not present in the 2009 pandemic. The influenza virus, it appears, creates alternate solutions to the same problems.

H5N1

Dr. Webster presented an overview of the host population for influenza viruses. There are 16 HA subtypes in influenza viruses found in acquatic birds, but only three subtypes—those expressing H1, H2, and H3 hemagglutinins—have successfully established themselves in human populations. H5, H7, and H9 viruses have infected humans but failed to establish permanent presence. Birds are the ultimate source of all influenza A viruses that infect humans. In birds, influenza A viruses are transmitted by the fecal-oral route via water and infect a bird's intestinal tract. Only strains with low pathogenicity are present in bird populations, and thus wild birds exhibit few symptoms. These strains form the basis of the gene pool from which highly pathogenic strains emerge and spread to other hosts.

Dr. Webster focused his discussion on H5 and H7 viruses and the process by which they are transformed from low to high pathogenicity. In wild birds, H5 and H7 viruses persist in the low pathogenicity form (although there is a question as to whether this is changing for H5). After H5 and H7 viruses are transmitted to domesticated birds, they sometimes become highly pathogenic. Specifically, the hemagglutinin sometimes acquires an insertional mutation of basic amino acids at the cleavage site, which confer greater virulence. Once an H5 virus becomes highly pathogenic in a domestic poultry population, 100 percent of the infected birds die. Webster observed that this knowledge has been used for many years in agricultural surveillance and control programs—the presence of these amino acids signals that the virus must be eradicated.

Control and Preparedness

Webster discussed how experience has shown that culling infected flocks, and compensating bird owners—as is the practice in Japan, South Korea, and European countries—is very successful in containing avian influenza outbreaks. Countries that opted for vaccination, for example China, Egypt, and Indonesia, have been less successful in their containment efforts and have even witnessed a more rapid evolution of the virus.

In 2004, pandemic preparedness programs were undertaken in response to an endemic presence of H5N1 in Eurasia due to the fear that the virus might spread to Australia and the Americas. In actuality, the viral strain that has since spread most rapidly throughout the world is H1N1—a low pathogenicity but highly transmissible form. At least 6000 human deaths resulted from H1N1 infections between April and November 2009.

Research Environment into which the Fouchier and Kawaoka Papers Appeared

Webster proceeded to discuss research questions that are being pursued.

In 2006, the National Institute of Allergy and Infectious Diseases called for researchers to study how influenza viruses move between different animal populations as well as the evolutionary pressures that lead to the emergence of new sub-types, with an emphasis on learning what factors allow the transmission of a subtype to humans.[4] In 2009, the WHO recommended that researchers investigate the pathogenicity, infectivity, and transmissibility of influenza viruses.[5] Webster noted that various approvals are required before such research may be conducted. These include peer review of the research grant proposal, approval (for some studies) by the NIH Office of Biotechnology Activities, and approvals at the research institution consisting of an Institutional Biosafety Committee and Institutional Animal Care and Use Committee. Webster noted that facilities-related procedures include registration of select agents, influenza-specific enhancements to standard BSL-3 facilities, registration with either the U.S. Department of Agriculture (USDA) or Centers for Disease Control and Prevention (CDC), and repeated inspections of the facilities security and inventory. In addition, material-transfer documentation includes importation permits (USDA and CDC) and export permits (U.S. Department of Commerce). Webster also referred to laboratory security and biosafety measures designed to restrict

[4] National Institute for Allergy and Infectious Diseases, "Report on the Blue Ribbon Panel for Influenza Research," June 2007.

[5] World Health Organization, "WHO Public Health Research Agenda for Influenza, Version 1, 2009," (2010): 9.

access to authorized personnel, protect the health of such personnel, and for tracking the movement of viral inventories.

The H5N1 Papers, The Approval Process, and Public Reaction to Fouchier and Kawaoka's Papers

Webster noted that Drs. Kawaoka and Fouchier's research demonstrated that H5N1 strains may potentially mutate to become transmissible between ferrets.[6] In the transmissible form produced in the laboratory, researchers identified specific attributes of the hemagglutinin protein and its stability. Furthermore, they recognized the importance of glycosylation and observed that there are multiple routes whereby a strain can become transmissible between ferrets.

In the fall of 2011, the NSABB reviewed the two papers and, in December, recommended that the papers should not be published in full. This decision was based on information that indicated that the resultant strains were not only highly transmissible but also highly pathogenic (the latter in the case of the Fouchier study). However, the characterization of the strains' virulence was subsequently revised when it was acknowledged that many strains of influenza are highly virulent when inoculated directly into the trachea, the pathway used in these experiments. This additional context was provided at a meeting of international influenza experts convened by the WHO in February 2012.[7] On March 29 and 30, 2012, this new information was reviewed at a second meeting of the NSABB, along with national security information. At this meeting, the NSABB reconsidered its earlier recommendation regarding the publication of a redacted version of the manuscripts. As a result, in part, of this new information, the NSABB reversed its original recommendation and recommended that both papers be published in full. However, while the NSABB was unanimous in its opinion that the Kawaoka paper be published in full, it was not unanimous in its decision to recommend publication of the Fouchier paper.[8]

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[6] It should be noted that the mutant viruses that became transmissible between ferrets lost virulence in the ferret system.

[7] The experts at the WHO meeting called for publication of both papers in full but also called for a continuation of the existing moratorium on such research until there could be more discussion about biosecurity and biosafety issues. See Jon Cohen, "WHO Meeting of Flu Experts Calls for Full Publication of Controversial H5N1 Papers," *ScienceInsider*, February 17, 2012, http://news.sciencemag.org/scienceinsider/2012/02/who-meeting-of-flu-experts-calls.html.

[8] Although the deliberative activity of the NSABB was not discussed in detail at the workshop, it is important to place the NSABB deliberations in context. Scientific manuscripts often do not provide sufficient information about research projects' aims or the precautions that were taken, obligating boards such as the NSABB to act without complete information about the experimental design and results, and thus without full information

The question of full or partial publication resonates beyond the scientific community and into the realm of international health policy and relations. Both research groups had utilized viral strains provided by countries in Asia: Fouchier's from Indonesia and Kawaoka's from Vietnam. Cooperative agreements between the WHO and public health leaders in these countries made these transfers possible. To stimulate such exchanges, the WHO provides vaccines and antiviral drugs to contributing nations. The redaction of key experimental information might complicate relationships among international parties engaged in, and benefiting from, influenza research.

Benefits and Risks

Webster discussed the benefits versus the risks of undertaking research on H5N1. He believes that the Fouchier and Kawaoka research has produced benefits that include knowledge that H5N1 may acquire mutations that permit transmission between mammals. Webster noted that the risks of conducting such research include high lethality to humans, the intentional or unintentional escape of the virus from the laboratory, and the possibility that the knowledge gained from such research could be used to develop biological weapons. He noted, however, that nature is perfectly capable of creating the same mutations that were generated in the laboratory and that the threat of a pandemic is a real one. He observed that the global community's task is to manage and not avoid risks.

Webster made several observations about the pandemic potential of H5N1 viruses. He observed that the research community is getting closer to knowing which subset of H5N1 possesses the necessary mutations to cause a pandemic and therefore, potentially, the strains on which efforts at control or eradication might be focused. He suggested that further research might focus on such questions as: Does the virulence of H5N1 in mam-

about the risk involved. Further, the NSABB review process does not dictate that it acquire knowledge of key details about risks and benefits before proceeding to a decision.

A letter sent by a member of the NSABB to the NIH highlights particular concerns about the circumstances of the meeting wherein the NSABB reconvened to reconsider its earlier recommendations. The author notes that no disinterested subject matter experts were asked to speak to the current state of the art in the use of reverse genetics technology or to discuss the implications of such work. Likewise, the author observed that no input was invited from people working on H5N1 surveillance and control as to the benefits for surveillance/control of the publication of the mutational data. According to the letter, a security briefing given at the meeting lacked a basis in knowledge of the threat, both historical and present-day, specific to influenza. Further, the letter states that, while investigative research using interviews with prominent professionals in public health was published in *Science* and *Nature*, the work was not incorporated into NSABB's process. See Michael T. Osterholm, "Letter to Amy P. Patterson, M.D., Associate Director for Science Policy, National Institutes of Health," April 12, 2012, http://news.sciencemag.org/scienceinsider/NSABB%20letter%20final%2041212_3.pdf.

mals increase or decrease with transmission? Are the transmissible strains of H5N1 more likely to reassort with the pandemic-causing H1N1 strains?

Asked whether important questions can be addressed using less pathogenic strains, Webster replied that some of them can. He observed, however, that many of the key questions can only be answered by using highly pathogenic strains; for example, questions concerning the host inflammatory response and its regulation.

Discussion

What tools do we currently have for use in the face of a pandemic? The session moderator, **Dr. Alice Huang, Senior Faculty Associate in Biology, California Institute of Technology,** asked what tools are available for responding to pandemics. Taubenberger and Webster both spoke about the effectiveness of quarantine, vaccination, and antiviral medications. Taubenberger noted that the effectiveness of each strategy depends on the pathogen. With an illness in which infected people shed the virus only *after* they become symptomatic, controlling an outbreak is easier than with illnesses where those infected begin to shed the virus before they become symptomatic. Those who contracted SARS (severe acute respiratory syndrome) in 2003, for example, exhibited symptoms before shedding the SARS virus,[9] and it was possible to recognize and quarantine the affected persons. In the case of influenza, a host sheds the virus before exhibiting any symptoms. Therefore, quarantine as a strategy is not as effective with influenza. Likewise, existing vaccines may not be effective countermeasures against future influenza strains. Influenza strains evolve quickly, and as a result, vaccines must be strain-specific. The formulation of strain-specific vaccines takes months: antiviral medications are, therefore, the best countermeasure. Webster noted, however, that only two families of antiviral medications are available: neuraminidase inhibitors and ion channel blockers. He observed that there is a great need to develop new families of antiviral drugs as well as a universal influenza vaccine.

To the question of whether there is an important distinction to be made between doing research on a naturally occurng pathogen versus a pathogen that has been generated by researchers and may never appear in nature, both Webster and Taubenberger indicated that there is not. Webster believes that all viruses that could be created in a laboratory already exist in nature. Taubenberger agreed, noting that chance determines whether a variant reaches a new host that it is well adapted to.

Taubenberger added that at the beginning of his research on the 1918 strain, it was best to understand a virus that had evolved in nature. Thus,

[9] The SARS virus is a member of the coronavirus family not previously observed in humans.

even though there were significant risks, given the knowledge that the strain in question could produce a pandemic, the potential health benefits of reconstructing the virus outweighed those risks. Further, information gained from the research might be particularly useful in informing preparations for a future pandemic. Regarding H5N1, Taubenberger sees value in using reverse genetics to construct influenza strains with specific characteristics in order to study the biology of the pathogen. He believes that valuable information is gained from manipulating the virus's genetic code and studying the effect on the pathogen's phenotype.

4

Scientists and the Social Contract

In the workshop's third session, five panelists discussed the notion of a social contract between scientists and society. The panel considered: (1) how the rapid pace of advances in the life sciences changes how we weigh risk versus benefit, (2) how research that possesses some element of risk is regulated, (3) how mechanisms currently in place can guide how scientists weigh risks/benefits and whether these mechanisms are appropriate, (4) whether there are types of research that should not be pursued or supported by the federal government or research whose findings should be restricted, (5) whether there is a place for classification in life sciences research, and (6) the role of the public in scientific decision-making. Each panelist was given an opportunity to offer opening remarks on the topic.

Robert Cook-Deegan, Director for Genome Ethics, Law, and Policy, Institute for Genome Sciences and Policy, Duke University, reflected on the distinction between biosafety and biosecurity and, with regards to the latter, on efforts to thwart the deliberate misuse of biological knowledge. Cook-Deegan observed that a major difference between biosafety and biosecurity is the degree to which politics and policy making will be influenced by external events. A more readily apparent difference between biosafety and biosecurity is the addition, on institutional biosafety committees, of the expertise necessary to assess the level of threat—by anticipating and preventing deliberate misuse of research materials or information. Cook-Deegan acknowledged, however, that the influence of external events cannot be avoided, particularly events that are unpredictable, unprecedented, or violent.

Cook-Deegan observed that many past events were not preventable. He used the examples of Sverdlovsk[1] and Amerithrax[2] as examples and argued that neither situation could have been prevented by the measures under discussion at the workshop. A workshop participant pointed out that the Sverdlovsk incident was partly accidental and complicated by the failure to admit what had happened and a government cover-up. Cook-Deegan concurred and observed that personnel decisions and laboratory oversight is critical. In Cook-Deegan's view, complete risk characterization will never be possible, regardless of the level of risk-assessment expertise. Nevertheless, Cook-Deegan observed that the twin questions of whether to "perform" or to "publish" research will continue to arise in the foreseeable future and that we must have solid procedures in place for making determinations about how to address this. Cook-Deegan noted that one of the recommendations of the Fink Report was that such a mechanism should be developed for this purpose and observed that the Fink committee had recommended that the purview of existing institutional biosafety committees be expanded to include determinations about the threat of a research project's potential misuse.

Cook-Deegan observed that emotions run so high in the current debate because there are deeply held opinions about scientific freedom. Given the strong culture of openness in the biological sciences, Cook-Deegan believes that the current deliberations should be based on a presumption that the strong tendency toward openness in life sciences research will remain. He added that this tendency will serve the U.S. well in its international relationships, as access to information will assist other countries in protecting themselves.

Ruth Berkelman, Rollins Professor and Director, Center for Public Health Preparedness and Research, Emory University and Director, Emory Preparedness and Emergency Response Research Center, defined the social contract as the giving up of certain rights in exchange for the benefit of the protection of society or the community. She discussed the example of policies surrounding clinical research studies on human subjects, policies that, while imposing a "burden" on the research enterprise, have ensured that such research proceeds in an atmosphere of public support and cooperation. In contrast, regulatory systems—national or international—to protect populations of people (and ecosystems overall) have not developed

[1] In 1979, an anthrax outbreak occurred in the city of Sverdlovsk (now named Ekaterinburg) in the Soviet Union. Anthrax infections occurred in livestock and humans who had eaten contaminated meat. The source of the pathogen was determined to be an accidental release of *Bacillus anthracis* (anthrax) spores from a military-run microbiology laboratory.

[2] In September and October 2001, letters containing anthrax spores were sent through the U.S. postal system. As a result, twenty-two people are known to have been infected with anthrax. Five died. The FBI investigation of the mailings is known as Amerithrax.

in tandem. Berkelman emphasized the need for local, national, and international mechanisms which are developed transparently, critiqued regularly, and include public representation. She concluded her remarks by noting that scientific freedom and the public interest rarely come into conflict, but when they do, the public interest must come first. When scientists put the public's interest first, she observed, the public will be in the best position to continue offering its trust and support.

Greg Kaebnick, Research Scholar, The Hastings Center, stated, "I don't think there is any such thing" as a contract between society and scientists. He noted, however, that there is a "nested" relationship in which science occurs within a "social milieu" and is subject to the social values and norms in the way that all of us are. A social contract that is seen as a contract between co-equals, between a group and society, he observed, tends to be framed in terms of the group securing social privileges—in this case, the privilege of self-governance. Kaebnick encouraged the audience to think of the relationship in nested terms wherein the group is part of society and has certain obligations.

Kaebnick discussed how, for many people within the scientific community, science is considered technical and value-free. When this perspective is in play, he noted, the concept of a social contract between science and society becomes an effort by scientists either to keep broader social values at bay or to protect scientific values from the social values that surround them.

Given this, Kaebnick saw a system of pure self-governance as cause for concern. He recommended that any such system have a broad range of social inputs. Kaebnick acknowledged that a system may need to rely somewhat on self-governance simply because the field is highly technical and changing rapidly. Kaebnick also acknowledged that it is quite difficult to decide who among the public would be involved and how. He urged that such a system ensure that the participants well understand the science. He envisioned, rather than an open consultation with the public, a mechanism whereby existing scientific bodies (such as institutional biosafety committees or the National Science Advisory Board for Biosecurity) are enriched by the presence of non-scientists; people who are committed to the process and committed to learning the science.

Daniel Kevles, Stanley Woodward Professor of History and Professor of History of Medicine, American Studies, and Law (adjunct), Yale University, discussed the H5N1 controversy in the context of two historical examples—nuclear weapons in the early 1940s and recombinant DNA in the 1970s. He noted that, in both cases, scientists acted responsibly. Nuclear physicists halted the publication of research on nuclear fission in the early stages of the nuclear era and, in the latter case, scientists imposed a moratorium on recombinant DNA research while the biosafety questions could begin to be addressed. Kevles also addressed the role of the public.

He noted ways in which public involvement in both situations was critical. In the case of recombinant DNA specifically, he noted the importance of the public role in both the deliberations about and establishment of regulations regarding recombinant DNA. The public, he observed, was part of state and local governmental processes, advisory committees, and institutional review boards, for example.

In the case of both recombinant DNA and nuclear fission, consideration was taken both of the organisms/technologies themselves and information about them. In the case of recombinant DNA, Kevles noted that the danger was primarily associated with the organisms themselves and not their possible misuse as weapons. Containment measures therefore had to do with securing laboratory facilities to prevent the organisms' escape. In contrast, with nuclear fission, the threat was not in the experimental material itself (though fissionable material was a critical ingredient), but rather in the technological knowledge that enabled its manipulation.

Following World War II, scientists considered how nuclear science could be advanced while protecting national security. Upon the release of the Smyth Report,[3] they drew a "bright line" between research that would be available publicly and research that would be classified. A group of scientists also led an effort to establish international control of nuclear research.

Regarding the question of whether public policy should be formulated in a general or abstract way or formulated specifically to a given research area or project, Kevles was in favor of specific oversight of particular knowledge or technology.

Journalist **Carl Zimmer** has written about the H5N1 controversy for the *New York Times* and other publications. He spoke about the role of journalism in scientific debates and deliberations, affirmed the importance of journalism in bringing scientific debates to the public's attention, and discussed journalism's function in distinguishing real risk from baseless fear. In the case of the H5N1 papers, Zimmer noted, journalists found themselves writing about research that they did not have access to: the relevant research papers had not been released, and although many scientists were quite open with journalists, a number were unwilling to talk with the media.

Zimmer reflected on a commonly heard accusation that, in the uproar about the Fouchier and Kawaoka papers, journalists exaggerated the risks of conducting the research. While acknowledging that some irresponsible coverage of the controversy took place, Zimmer pointed out that in most cases journalists were basing their analyses on information provided

[3] Henry DeWolf Smyth, "Nuclear Energy: A General Account of Methods of Using Atomic Energy for Military Purposes Under the Auspices of the United States Government 1940-1945."

directly from scientists. He admonished scientists who unjustly use journalists as scapegoats.

In future instances involving scientific controversy, Zimmer called for more transparency on the part of the scientific community. Zimmer concluded his remarks by observing that there are cases where scientists have done an exceptionally good job of communicating with the public on, for example, public health topics. He urged the broader scientific community to learn from such examples.

Discussion

Session moderator **Harold T. Shapiro, President Emeritus and Professor of Economics and Public Affairs, Princeton University,** agreed with Cook-Deegan that the Fink Report offered practical suggestions. Shapiro also agreed that the advancement of knowledge has attendant risks and that a workable process will need to be flexible.

In discussion, the panelists and participants also discussed the forms that public input might take, reasons why scientists were hesitant to talk with journalists, laboratory culture and its effect on public safety, methods of estimating risk, and the possible inclusion of fields of study not typically overseen by institutional biosafety committees.

TRANSPARENCY, PUBLIC PARTICIPATION AND CONFIDENCE, AND THE MEDIA

At the workshop, public participation was a topic of vigorous discussion. Three themes emerged: how and whether to solicit the input of members of the general public in deliberations about regulating life sciences research of concern; how to communicate most effectively with the public about the regulatory oversight of and the review of research; and how to best create regulatory frameworks that warrant and/or inspire public confidence.

Some discussants raised the question of how the public should be involved in the development of regulatory schemes. How, if the public were to serve on regulatory bodies, would individuals be selected and how might a group with admittedly mixed expertise most efficiently deliberate on highly technical issues?

Concerning how to communicate most effectively with the public, an audience member stressed the importance of communicating in calm, rational terms. A panel member added that positive hyperbole—over-optimism—should be avoided as well. Another audience member expressed the opinion that the media should give greater consideration to the use of inflammatory terms such as “doomsday virus,” “biological weapons,” and

"biowarfare" and instead consider using more restrained descriptive terms such as "public health research" and "concern." Zimmer agreed and noted his own frustration with inflammatory headlines. He observed, however, that some of the apocalyptic language used by the media came from quotes by scientists themselves.

Joe Palca, Science Correspondent, National Public Radio, spoke about what scientists think the public wants to know about science and the types of questions the public actually asks. Palca observed that scientists are often accustomed to communicating with a degree of subtlety and nuance lost on the public. He suggested that the scientific community needs to better understand the mechanics of providing accessible answers to questions that the public wants answered. Palca acknowledged that the media is often an intermediary in this process and reminded scientists that journalists often have final responsibility for what is communicated.

Throughout the workshop, a recurring theme involved the question of how best to create a regulatory framework in which the public has confidence. Suggestions included having the public decide which persons or what types of expertise should be represented in regulatory discussions.

THE ROLE OF CORPORATIONS IN LIFE SCIENCES RESEARCH

Dr. Shapiro suggested that the group consider the role of private corporations in life sciences research. Dr. Kevles discussed his experience in 2001 at the 25th anniversary of the Asilomar Conference. He noted that, unlike previously, many life scientists now have ties to industry. He suggested that such relationships would provide a barrier to the creation of a disinterested set of recommendations similar to those put forth at the Asilomar Conference. Kevles noted, however, that it is essential to involve corporations in governance discussions especially in light of many corporations' positions as international actors. Dr. Cook-Deegan agreed and noted that many corporations devote a much more significant portion of their budgets to life sciences research than was the case in 1975.

5

Governance and Oversight

The workshop's final session focused on current regulatory policies and frameworks and their relevance to the H5N1 research controversy. Discussants considered current policies and examples where policies may require modification in order to achieve an acceptable risk/benefit balance. The panel paid considerable attention to the importance of maintaining strong and equitable international relationships and considered which actions may be required to ensure better governance of dual-use research.

Session moderator **Lawrence O. Gostin, University Professor and Director, O'Neill Institute for National and Global Health Law, Georgetown University,** opened the session by remarking on how the policy and regulatory issues raised by the Fouchier and Kawaoka papers related to both existing and potential governance of dual-use research of concern, both nationally and internationally. Gostin framed global health governance as multisectoral rules, institutions, and processes that collectively shape policy and health, both nationally and globally. He emphasized the importance of determining the trigger points at which policy measures can most effectively ensure the public's safety and health. Gostin noted two key decision points: 1) the point at which it is determined whether specific research should be conducted and 2) the point of dissemination of research results. He noted that decisions are often influenced by desired outcomes and emphasized the importance of focused consideration of individual decisions.

Gostin considered three types of potentially applicable regulations. One type is government regulation effected by preconditions placed on research at the moment of funding. Such requirements might be applied at the level of the NIH or more broadly at the level of all research funded by the U.S.

Department of Health and Human Services. Alternatively, the government might enact a government-wide dual-use policy, a proposal which has recently been discussed in Congress. A third type of regulation might be applied in close proximity to the research itself, from within the scientific community and at the level of the institution.

Gostin emphasized the international dimension of regulation of dual-use research of concern in the life sciences. He supported a "thoughtful international process" of regulation that carefully considers the burdens and benefits of regulations for low and middle-income countries that are involved in sharing information and biological specimens.

U.S. POLICY ON DUAL-USE RESEARCH OF CONCERN

Anthony Fauci, Director, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health, discussed both the evolution of U.S. policy on dual-use research of concern and its current status. Fauci described how the U.S. dual-use policy has been in effect, informally, for many years and, as a result of the H5N1 controversy, is in the process of becoming formalized, more transparent, and more proactive.

In decades of funding research with dual-use potential, Fauci stated that the NIH has never had an instance in which funded research was retroactively judged as having been funded or published improperly. In the few instances in which the NSABB had reviewed research of concern, the determination was made that the benefits offered to society by the research outweighed potential harm. However, Fauci asserted that the NIAID recognizes both the importance of carrying out reviews preemptively and of ensuring transparency. Fauci noted that oversight must stretch beyond the researcher and his or her colleagues and emphasized that it is important to recognize that people without a vested interest in the research are a part of the decision-making process.

Fauci discussed the newly issued U.S. government policy that defines dual-use research as "life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technology 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]." [1] The policy defines both the types of experiments that are cause for concern and addresses the question of how potential risks associated with the research should be mitigated. Fauci noted that the need for risk mitigation raises the following questions: Should the experimental

[1]National Institutes of Health, "United States Government Policy for Oversight of Life Sciences Dual Use Research of Concern," March 29, 2012. (See Appendix B.)

design be modified? Does the experiment require increased biosecurity or biosafety? Are there countermeasures available for the microbe in question?

The new U.S. policy will have repercussions at the institutional level—most notably, for the development of risk mitigation plans—and Fauci noted that a draft U.S. government policy for local institutional oversight of dual-use research of concern would be opened for public comment soon.[2] In response to questions from the audience, he clarified that while a framework has been drafted that includes examples of approaches that universities and research institutions might choose to take, the framework is not itself a regulation; rather, it is an invitation for institutions to join a discussion about possible approaches for the implementation of the U.S. policy. Fauci stated that the U.S. government wants to ensure that the draft policy does not place burdensome requirements on institutions. In response to a question from Gostin about the degree to which bright lines are necessary, Fauci replied that when there is real or perceived disagreement within the scientific community about whether certain experiments should be performed, an open, transparent dialogue that includes the media should take place. In response to a question from Gostin about the relative roles of governmental and institutional mechanisms, Fauci emphasized that the weight of oversight should fall at the institutional level, long before publication becomes an issue. He urged that NSABB reviews be reserved for situations when involvement by an outside entity is critical.

ROLE OF RESEARCH INSTITUTIONS

Panelist **Ann Arvin, Lucile Salter Packard Professor of Pediatrics and Microbiology and Immunology, Stanford University School of Medicine and Vice-Provost and Dean of Research, Stanford University,** noted that research universities are all fully committed to partnering with the government and international organizations in order to develop strategies to anticipate and mitigate the possible consequences of dual-use research of concern in the life sciences. She noted the effectiveness of current institutional mechanisms to ensure responsible research on recombinant DNA and select agents and toxins. Arvin noted that institutional biosafety committees review experimental protocols, environmental health and safety staff ensure the safety of research facilities, and researchers receive training in biosafety—all of which create a culture of safety and openness.

As to whether the current practices are robust enough to handle the emerging issues of dual-use research of concern, Arvin believes that additional efforts are necessary at the local level, that institutions need more

[2] The draft policy was released for public comment in February 2013.

resources to meet those needs, and that the expectations placed on universities need to be realistic.

Arvin considered the new U.S. policy on the oversight of dual-use research to be very helpful as a foundation for new educational initiatives for researchers and students. She is in favor, however, of replacing the "dual-use" concept with "life sciences research of concern."

Arvin described several challenges that universities face with regard to life sciences research of concern. She noted that, in any given institution, it will be difficult to maintain awareness of dual-use research of concern, since such research is relatively rare. Arvin observed that institutional biosafety committees are traditionally charged with ensuring safety during the performance of research but lack training to estimate future threat risk. Biosafety committees, she noted, already draw from a much smaller pool than do committees overseeing human and animal subjects (which are themselves built only slowly and over time). Furthermore, she observed, their members may lack necessary specialized knowledge with regard to specific pathogens and host-pathogen interactions. Within an institution, she noted, the expert is often the person proposing the research. In response to a question from the audience, Arvin stated that an institution would, if necessary, seek expertise about risk/benefit from outside experts. She noted the value of assembling specialized knowledge and considering some issues in advance—a topic that is addressed in the new U.S. policy. In response to another audience question, she noted that institutions may want to have resources in place for when a researcher produces concerning results that were unanticipated. Arvin also observed that, as universities will be working more closely with federal agencies on new questions pertaining to mitigation plans and details of scientific studies, openness and transparency are important to insure that valuable research is not unnecessarily impeded and that openness in research is maintained. Arvin reiterated the difficulty of predicting whether research programs could have harmful consequences. She emphasized that these uncertainties "become quite vivid at the point of local control." She pointed out that when it is the researchers themselves stating that it is difficult to judge the risk of future harm, it may appear self-serving. She observed that it is important for others to assist actively when communicating with the public on this matter.

IMPLICATIONS FOR INTERNATIONAL POLICIES

David Franz, Former Commander, U.S. Army Medical Research Institute of Infectious Diseases, spoke about U.S. communication efforts regarding life sciences research of dual-use concern in the context of international relationships.

Franz observed that, since its inception, the NSABB has produced webcasts, video conferences, and workshops and has held three roundtable discussions that involved 47 countries and included a WHO representative as a co-chair. Franz stated that the NSABB international panel seeks to meet its international colleagues as partners and aims to learn about their perspectives and the problems they encounter. Although the group remains small, the panel seeks to expand the network of those wrestling with questions of responsible life sciences research.

Franz noted that, despite advances made in building trust and communicating NSABB's activities and materials, other countries may choose to formulate policies that differ from those in the United States. Franz noted that, at every level, assessments are made of the value versus the cost of regulations and oversight and that global competitiveness is a factor in such assessments. Franz recognized that some level of risk is inherent in every operating infectious disease laboratory but called for leadership and balance in the management of U.S. research laboratories.

The International Arena from a European Perspective

David Heymann, Chairman, Health Protection Agency, United Kingdom, Head, Center on Global Health Security at Chatham House in London, and Professor, Infectious Disease Epidemiology, London School of Hygiene and Tropical Medicine, discussed current international rules and regulations, the degree of consistency that they lend to decision making, and whether there can be true international partnerships.

Heymann spoke of the difficulties encountered in the changing relationship between the concerns for commerce and health. Heymann observed that, prior to the outbreak of H5N1 in poultry in 2007, WHO member states had agreed to freely share virus samples, which were used to identify novel viruses and to create new vaccines. During the 2007 outbreak of H5N1, this agreed-upon mechanism for ensuring the reliable, timely transfer of viral samples was complicated by the Minister of Health of Indonesia, who requested a material transfer agreement for each virus that Indonesia provided to the WHO. The WHO did not invoke the International Health Regulations, which would have required the transfer of the virus samples, but rather addressed Indonesia's request by making a resolution that the issue be addressed by an international working group outside of the WHO governance mechanism. The Pandemic Influenza Preparedness framework, which was formulated in response, included provisions for material transfer agreements. As a second example of an area in which commerce and health regulations come into conflict, Heymann cited export control laws. In the case of the Fouchier manuscript, the Dutch government required, as stipulated by European Union (EU) legislation,

an export license because a portion of the research results were to have been redacted from the published manuscript. The Netherlands and other EU-member countries questioned the EU's regulations and requested the WHO's assistance in resolving this question.

An audience member with experience in the negotiations surrounding the Pandemic Influenza Preparedness Framework at the WHO spoke to the virus-sharing question as an equity issue for the virus-providing countries, noting that the countries stopped freely sharing viral samples because, at least in part, they learned that the WHO was sharing them with the vaccine-development industry, and that the donating countries received nothing in return. The Indonesian request for a material transfer agreement was the result of a sense of inequity. This illustrates the fact that, for developing countries, the conversation goes beyond sharing of information and materials, and encompasses ethical issues about credit, compensation, and overall concerns about mutual work in the interest of public health. As a result of such considerations, biosecurity may be of lower priority in developing countries than in developed countries.

Heymann discussed his view on a way forward for research of dual-use concern. He called for an international framework on the ethical and social benefits of influenza research that would form a foundation for discussions about trade and public health. According to Heymann, such a framework might (1) create peer pressure for researchers to carry out research in socially responsible ways, (2) lead to safer laboratories, and (3) be a map for national governments that want to create their own legislation.

Intersections with and Contributions of the Biological Weapons Convention: A Model of a Multisectoral Approach

Piers Millet, Deputy Head, Implementation Support Unit for the Biological Weapons Convention, United Nations Office for Disarmament Affairs, spoke about the many strong intersections between the issues surrounding dual-use research and concerns that motivate the Biological Weapons Convention (BWC).

The BWC is an international treaty that attempts to strike a balance between the outright ban of bioweapons and the peaceful use of pathogen research in the life sciences. There are 165 nation signatories of the BWC. Millet noted that, at review conferences of the Convention a decade ago, delegates hailed primarily from departments of defense or foreign affairs, whereas today the meetings include representative of departments of health, agriculture, trade, and the sciences.

Signatory states commit to not developing, acquiring, or stockpiling biological agents or toxins other than for prophylactic, protective, or

other peaceful purposes and commit to withholding assistance from other nations or groups in contravention to the BWC, both directly and indirectly. Simultaneously, states are not to interfere with the international exchange of these agents, equipment, etc., for peaceful purposes. Millet discussed how, in 2008, BWC delegates held extensive discussions about the oversight of research. Elements of consensus from those discussions were that: (1) national frameworks need to apply in both the public and private sector and throughout the scientific life cycle; (2) measures should be proportionate to risks and should not unduly restrict permitted activities; (3) stakeholders should be engaged at every point in the process; and (4) national, regional, and international frameworks should be harmonized.

Issues relevant to the BWC bear directly on issues raised by the H5N1 controversy. At the BWC's 7th Review Conference in December 2011, it was decided to continue the practice of holding annual meetings between the Review Conferences held every five years. The 2011 Review Conference established standing agenda items for the annual meetings. These include an examination of relevant advances in science and technology. Each year, delegates will examine issues such as:

- Developments with potential uses both contrary to the BWC and beneficial to the implementation of the BWC;
- Measures to strengthen biorisk management; and
- Codes of conduct and other approaches to raise awareness of the risks and benefits of life science research and biotechnology.

In addition, the science and technology topics slated for discussion in upcoming years are germane to discussions about dual-use research. The topics include enabling technologies such as DNA sequencing and synthesis, synthetic biology, systems biology, and bioinformatics (2012); disease (2013); pathogenicity, virulence, toxicology, immunology, and perhaps transmissibility (2014); and production, dispersal, and delivery technologies for biological agents and toxins (2015).

Discussion

Will the U.S. attempt to create processes and norms be sufficient from an international perspective? Will informal outreach suffice or should the process be formalized?

Dr. Franz suggested that, to date, current efforts for global engagement are sufficient. He noted, however, that "dual-use research of concern" *per se* is too narrow to encompass all of the issues globally, particularly in countries where human survival is a primary concern or where little or

no laboratory research is conducted. Franz suggested that a more globally inclusive term, e.g., "responsible life sciences research" might be more effective in harmonizing discussions.

Professor Gostin asked whether a regulatory body in the U.S. that makes a decision about whether or not to allow the publication of a paper is sufficient or whether the decision should be subject to international oversight. Franz replied by saying that he believes it is important to consider the international community in the decision, especially if the decision leans in the direction of not publishing. He noted that his opinion was rooted in a public health perspective as well as in a perspective that favors international relationships based on mutual trust.

Regarding the role and sufficiency of informal activities, Dr. Millet noted the importance of the direct connections that have been made between scientific communities, e.g., the Inter-Academy Panel, International Union of Microbiological Societies, International Union of Biochemistry and Molecular Biology, and the International Council for Science. On behalf of the Biological Weapons Convention, these groups have addressed dual-use research, education, and other issues. Millet believes that from these informal processes, formal processes will have to emerge. Millet observed that this important, informal process—whereby a critical sense of community is established—is seriously underfunded.

How do we reach a formal international consensus? Dr. Heymann acknowledged the difficulty of achieving an international consensus. He is confident that the United States will continue to play a leadership role in such a discussion, through meetings such as the current workshop. However, he believes that it will be difficult to align a framework created by the United States with a framework that might be developed through consensus by other countries such as Russia, China, India, and Brazil. He believes that we will need to begin with a set of universally accepted principles and then move over the next decade or more to hard norms and possibly a convention.

What can we learn from other international agreements and the activities of people in other scientific disciplines? Dr. Millet mentioned several examples that he believes are worthy of emulation. He noted that the Chemical Weapons Convention has had considerable success in engaging the private sector. He applauded the work being done by the biorisk management community and lauded the model and accomplishments of the Financial Action Task Force (which audits the laws and regulations regarding the financing of terrorism).

Has the NIH's position[3] on the two research papers dampened scientists' willingness to discuss these issues? Dr. Fauci offered clear affirmation

[3] Anthony Fauci, Gary Nabel, and Francis Collins, "A Flu Virus Risk Worth Taking," *Washington Post*, December 30, 2011.

of NIH's support of the two studies in question. When asked whether the NIH is willing to entertain the possibility that approved research will be found, at some later date, to be indefensible, Fauci would only say that, regarding the experiments in question, an impartial panel decided that they were worth doing and worth publishing. He observed that there is reasonable debate taking place about gain-of-function studies and expressed support of studies that aim to remain ahead of what is likely occurring in the natural world. He agreed that discussions about the justifiability of this type of experiment should take place before the fact and be conducted by disinterested parties.

The Moratorium on Research on Laboratory-modified H5N1. Dr. Fauci reminded the audience that the moratorium on laboratory-modified H5N1 was voluntary (though mandatory for intramural NIH research). He supported extending the moratorium until questions related to specific research programs of concern are resolved, i.e., whether there are risks, and, if so, whether the researchers have an appropriate plan for mitigating the risks.[4] Dr. Millet was not as optimistic that the voluntary moratorium would hold in light of the recent NSABB recommendation for full publication of the Fouchier and Kawaoka manuscripts.

Professor Gostin concluded the session by revisiting the discussion's central questions: Should specific research take place? If the research is conducted, should it be published? Who should decide what research is published? What partnerships are critical? What norms should prevail?

Gostin asked the audience to consider a key question in light of their own circumstances: How can we ensure that the international community moves toward a consensus based on true partnerships and a genuine understanding of common values—a consensus that prepares us to face this situation more effectively the next time it arises?

[4] Researchers declared an end to the moratorium on January 23, 2013.

6

Moving Forward

In concluding remarks, workshop planning committee co-chair David Relman recognized the importance of the workshop as a venue wherein individuals from a broad range of interests and perspectives might ask important questions. He suggested that the participants reflect both upon lessons learned from the H5N1 controversy and on where ongoing discussions should occur and among whom. He stated that ongoing conversations should be open, transparent, and frank and might include questions of:

- How general or specific would a regulatory mechanism need to be? To what degree would it be designed to defer to case-by-case considerations? Conversely, to what degree might it apply to a range of disciplines?
- Who should be involved in decisions about risk/benefits and determinations regarding regulations?
- What are possible mechanisms for accomplishing the proper involvement?
- What is the role of universities as it relates to the need for better education of scientists in research ethics?

REGULATORY PATHS, GRAY ZONES, AND AVOIDING UNDUE BURDENS

Throughout the workshop, the absence of "bright lines" and, conversely, the specter of "gray zones" was a recurring theme. If the scientific and biosecurity communities were to consider creating a new mechanism to

address research that falls within a gray zone—i.e., research that is neither freely shared nor classified—what are the appropriate considerations? How should research that, at its inception, is not seen as research of concern but that, in the course of its performance, becomes so, be addressed? Of related interest was the question of appropriate document control or information management measures for research of concern whose findings may have already been dispersed.

Several participants cautioned against focusing on regulatory frameworks to the point of relegating discussions about ethics to a separate sphere. They expressed concerns that a system consumed with checking boxes on regulatory paperwork might draw attention away from the thoughtful ethical considerations that they felt should accompany life sciences research. A related discussion issue was the importance of informal aspects of scientific culture that can play a role in protecting the public health.

DUAL-USE RESEARCH: WHAT'S IN A NAME?

Several workshop participants voiced dissatisfaction with the term "dual-use research of concern."

Dr. Franz suggested that using the term "dual-use" orients a conversation in a narrowly regulatory direction. In Franz's view, this may give rise to a risk that the burden of the regulation outweighs any potential protection. He cautioned that excessive regulation might "hobble the entire enterprise because of the behavior of a few." Franz favored the terms "responsible life sciences research" and "culture of responsibility," as they are oriented toward leadership, honesty, healthy scientific culture, and acceptance of responsibility. Franz suggested that current efforts to oversee the funding and publication of life sciences research are, in their current form, sufficient. Rather than impose additional layers of regulation, Franz advocated a change in behavior and in the scientific culture.

An audience member relayed what she has heard from people in a network of high-containment laboratories funded by the National Institute of Allergy and Infectious Diseases. When scientists are asked to state whether a specific research project is or is not "dual-use research of concern," she remarked, it is difficult for them to provide an answer. However, if the research is simply described and a question posed to the researcher about how to manage the research responsibly, then the researcher will deliberate on the question and give serious thought to the management of the research.

Dr. Brent raised several points about practicality and ethics in the context of dual-use. He noted that it is not possible to know what the near-term or long-term benefits and potential for misuse are, and he expressed the opinion that scientific (or technological) experts tend to underestimate both benefits and risks. He observed that researchers consider themselves to have positive,

well-intentioned motives for conducting research and that the person who misapplies the results of the research is always thought to be someone else. Moreover, Brent observed that "use" is not the only consequence of "dual-use research." He stated that the mere existence of the knowledge about how to create a transmissible, lethal virus may itself constitute harm.

RISK

Many of the workshop discussions were related to the questions of what constitutes risk, how is risk determined, and by whom? Related to these questions were observations that:

- No consensus exists within the scientific community about what constitutes right action.
- It is impossible to predict all of the ways in which a particular piece of research could be utilized for harm.
- It is impossible to predict the ways in which a particular piece of research could be utilized for harm *in the future*.
- Knowledge cannot be unmade; therefore, the estimation of risk (and acting on that estimation) is critically important.

In the specific case of the H5N1 controversy, several questions repeatedly emerged:

- Is "nature" performing these experiments in the wild? If so, does that provide justification for scientists' to perform them in the laboratory?
- Are certain experimental questions particularly high risk?
- Are there some areas of research that should not be pursued?

BROAD AND INCLUSIVE PARTICIPATION

The role of public inclusion in the process of formulating and carrying out an effective mechanism of oversight was a topic of several questions:

- Should non-scientist members of the public be included in the development of oversight mechanisms, and if so, who, how, and at what point(s)?
- What are the characteristics of effective oversight mechanisms that prompt public confidence?

Many participants saw the need for an internationally agreed upon system for the regulation of research of dual-use concern and stated that

while the process may be led, to some degree, by the United States, it cannot be dominated by the United States.

THE NEED TO REVAMP THE EDUCATION OF OUR NEXT GENERATION OF SCIENTISTS

Workshop planning committee co-chair **David Korn, Consultant in Pathology, Massachusetts General Hospital and Professor of Pathology, Harvard Medical School**, concluded the workshop by calling on U.S. universities to be more proactive in educating their students and junior faculty in the ethical conduct of research. Korn placed particular emphasis on the importance of ethics in the sharing of data, disclosures of conflict of interest issues, and the conduct of risk/benefit estimations. In Korn's view, institutional efforts designed to promote the ethical and responsible conduct of research would be much more effective than any oversight that might be imposed from the outside.

Appendixes

Appendix A

Timeline

1918	Outbreak of "Spanish" flu (H1N1)
1957	Outbreak of "Asian" flu (H2N2)
1968	Outbreak of "Hong Kong" flu (H3N2)
1971	Seattle Crown Gall Group begins exploring bacterium-to-plant transmissible plasmid.
1972	Draft of Biological Weapons Convention text
1973	Recombinant DNA methodologies in place
1974	Moratorium on recombinant DNA
1975	Asilomar Conference on Recombinant DNA
1975	Biological Weapons Convention established
1976	National Institutes of Health (NIH) issues initial guidelines for research involving recombinant DNA.
1980	In Diamond v. Chakrabarty, the U.S. Supreme Court rules that "a live, human-made micro-organism is patentable subject matter."

1981	Ability to reverse engineer a plus/positive-strand RNA virus (poliovirus)
1982	Publication of National Research Council (NRC) report *Scientific Communication and National Security* (aka the Corson Report)
1985	National Security Decision Directive-189 (NSDD-189) released
1990	Ability to reverse engineer a negative strand RNA animal virus
2004	Publication of NRC report *Biotechnology Research in an Age of Terrorism* (aka the Fink Report)
2005	First meeting of the National Science Advisory Board on Biosecurity (NSABB)
2006	Publication of NRC report *Globalization, Biosecurity, and the Future of the Life Sciences*
2006-2007	Ability to reverse engineer double-stranded RNA viruses
2007	Publication of NSABB's "Proposed Framework for the Oversight of Dual Use Life Sciences Research: Strategies for Minimizing the Potential Misuse of Research"
	Publication of NRC report *Science and Security in a Post-9/11 World*
2009	Outbreak of "swine" flu (H1N1)
2011	Publication of NSABB's "Recommendations on Communications of Experimental Adaptation of Avian Influenza A/H5N1"
	Publication of World Health Organization's (WHO) "Pandemic Influenza Preparedness Framework"
	7th Review Conference, Biological Weapons Convention

December 20	Publication of National Institutes of Health's (NIH) "Press Statement on the NSABB Review of H5N1 Research" (see Appendix B)
2012	
January 20	Publication of "NIH Statement on H5N1" (see Appendix B)
January 31	Publication of NSABB statement "Adaptations of Avian Flu Virus Are a Cause For Concern" (see Appendix B)
February	Publication of WHO's "Report on Technical Consultation on H5N1 Research Issues" (see Appendix B)
March 29	Publication of "United States Government Policy for Oversight of Life Sciences Dual Use Research of Concern" (see Appendix B)
March 30	Publication of NSABB "Findings and Recommendations" (see Appendix B)
April 14	Publication of NIH's "Statement on NSABB's March 30, 2012 Recommendations to NIH on H5N1 Research" (see Appendix B)
April 20	Publication of NIH's "Statement by NIH Director Francis Collins, M.D., Ph.D., on the NSABB Review of Revised H5N1 Manuscripts" (See Appendix B)
May 2	Publication of the Kawaoka paper (see Appendix C)
June 22	Publication of the Fouchier paper (see Appendix C)

Appendix B

Official Statements

- December 20, 2011, National Institutes of Health (NIH), "Press Statement of the NSABB Review of H5N1 Research."
- December 30, 2011, World Health Organization (WHO), "WHO Concerned that New H5N1 Influenza Research Could Undermine the 2011 Pandemic Influenza Preparedness Framework."
- January 20, 2012, NIH, "NIH Statement on H5N1."
- January 31, 2012, National Science Advisory Board for Biosecurity (NSABB), "Adaptations of Avian Flu Virus Are a Cause for Concern." *ScienceExpress* January 31, 2012. Reprinted with permission from AAAS.
- February 2012, WHO "Report on Technical Consultation on H5N1 Research Issues."
- March 29, 2012, NIH, "United States Government Policy for Oversight of Life Sciences Dual Use Research of Concern."
- March 30, 2012, NSABB, "Findings and Recommendations, March 29-30, 2012."
- April 14, 2012, NIH, "Statement on NSABB's March 30, 2012 Recommendations to NIH on H5N1 Research."
- April 20, 2012, NIH, "Statement by NIH Director Francis Collins, M.D., Ph.D. on the NSABB Review of Revised H5N1 Manuscripts."

U.S. Department of Health and Human Services
NIH News
National Institutes of Health

For Immediate Release
Tuesday, December 20, 2011

Contact:
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Press Statement on the NSABB Review of H5N1 Research

The U.S. government remains concerned about the threat of influenza, for the risks it poses seasonally, as well as its potential to cause a pandemic. Our domestic and global influenza surveillance efforts have become increasingly capable, along with expanded vaccine manufacturing capacity and assistance to other countries in their efforts to detect and respond to a pandemic. To enhance the detection of and response to influenza outbreaks, the U.S. government supports a broad range of domestic and global preparedness and response efforts that include research on better diagnostics, vaccines, and therapeutics.

Currently, H5N1 avian influenza virus — the strain commonly referred to as "bird flu" — rarely infects humans and does not spread easily from person to person. However, many scientists and public health officials are concerned that the virus could evolve in nature into a form that is transmissible among humans — an event that could potentially make this deadly virus an extremely serious global public health threat. Thus research on factors that can affect the transmissibility of the H5N1 virus is critically important to international efforts to prepare and prevent threats to public health.

While the public health benefits of such research can be important, certain information obtained through such studies has the potential to be misused for harmful purposes. The National Science Advisory Board for Biosecurity (NSABB) — an independent expert committee that advises the Department of Health and Human Services (HHS) and other Federal departments and agencies on matters of biosecurity — completed a review of two unpublished manuscripts describing NIH-funded research on the transmissibility of H5N1. These manuscripts — which describe laboratory experiments that resulted in viruses with enhanced transmissibility in mammals – concluded that the H5N1 virus has greater potential than previously believed to gain a dangerous capacity to be transmitted among mammals, including perhaps humans, and describe some of the genetic changes that appear to correlate with this potential.

Following its review, the NSABB decided to recommend that HHS ask the authors of the reports and the editors of the journals that were considering publishing the reports to make changes in the manuscripts. Due to the importance of the findings to the public health and research communities, the NSABB recommended that the general conclusions highlighting the novel outcome be published, but that the manuscripts not include the methodological and other details that could enable replication of the experiments by those who would seek to do harm.

The NSABB also recommended that language be added to the manuscripts to explain better the goals and potential public health benefits of the research, and to detail the extensive safety and security measures taken to protect laboratory workers and the public.

HHS agreed with this assessment and provided these non-binding recommendations to the authors and journal editors.

Recognizing the significant potential benefit of the information about the experimental details to the global influenza surveillance and research communities, the U.S. government is working to establish a mechanism to allow secure access to the information to those with a legitimate need in order to achieve important public health goals. The U.S. government is also developing a proposed oversight policy that would augment existing approaches to evaluating research that has the potential to be misused for harmful purposes.

The NSABB supports the overall goals of the National Institutes of Health, in conducting safe, ethical and informative research to enhance health, lengthen life, and reduce the burdens of illness and disability. To learn more about the NSABB, visit www.biosecurityboard.gov.

About the National Institutes of Health (NIH): NIH, the nation's medical research agency, includes 27 Institutes and Centers and is a component of the U.S. Department of Health and Human Services. NIH is the primary federal agency

conducting and supporting basic, clinical, and translational medical research, and is investigating the causes, treatments, and cures for both common and rare diseases. For more information about NIH and its programs, visit www.nih.gov.

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World Health Organization

Media centre

WHO concerned that new H5N1 influenza research could undermine the 2011 Pandemic Influenza Preparedness Framework

Statement
30 December 2011

The World Health Organization (WHO) takes note that studies undertaken by several institutions on whether changes in the H5N1 influenza virus can make it more transmissible between humans have raised concern about the possible risks and misuses associated with this research. WHO is also deeply concerned about the potential negative consequences. However, WHO also notes that studies conducted under appropriate conditions must continue to take place so that critical scientific knowledge needed to reduce the risks posed by the H5N1 virus continues to increase.

H5N1 influenza viruses are a significant health risk to people for several reasons. Although this type of influenza does not infect humans often, when it does, approximately 60% of those infected die. In addition, because these viruses can cause such severe illness in people, scientists are especially concerned that this type of influenza could one day mutate so it spreads easily between people and causes a very serious influenza pandemic.

Research which can improve the understanding of these viruses and can reduce the public health risk is a scientific and public health imperative. In order to enable those public health gains, countries where these viruses occur should share their influenza viruses for public health purposes while countries and organizations receiving these viruses should share benefits resulting from the virus sharing. Both types of sharing are on equal footing and equally important parts of the collective global actions needed to protect public health.

While it is clear that conducting research to gain such knowledge must continue, it is also clear that certain research, and especially that which can generate more dangerous forms of the virus than those which already exist, has risks. Therefore such research should be done only after all important public health risks and benefits have been identified and reviewed, and it is certain that the necessary protections to minimize the potential for negative consequences are in place.

In May 2011, the new Pandemic Influenza Preparedness (PIP) Framework came into effect. This Framework was adopted by all WHO Member

States as a guide to the sharing of influenza viruses with pandemic potential and the resulting benefits. One specific requirement of this Framework, which pertains to influenza viruses of pandemic potential, and is in keeping with best scientific practice, is for laboratories receiving them through WHO's Global Influenza Surveillance and Response System (GISRS) to collaborate with, and appropriately acknowledge, scientists in countries where the virus originated when initiating research.

WHO recognizes that the scientists who led the work of the new studies received their virus samples from the WHO Global Influenza Surveillance Network (GISN), which preceded GISRS, and before negotiations on the new PIP Framework began. However, now that the Framework has been adopted by all WHO Member States, WHO considers it critically important that scientists who undertake research with influenza viruses with pandemic potential samples fully abide by the new requirements.

Since the PIP Framework represents a major step forward and was agreed upon only after several years of difficult negotiations, WHO stresses that this H5N1 research must not undermine this major public health achievement. WHO will work with Member States and other key parties to ensure scientists understand the new requirements that have been agreed to with the Framework.

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NIH Statement on H5N1

http://www.nih.gov/about/director/01202012_h5n1_statement.htm Februray 13, 2012

The NIH Director

January 20, 2012

Last month, the National Science Advisory Board for Biosecurity (NSABB)—an independent expert committee that advises the Department of Health and Human Services (HHS) and other Federal departments and agencies on matters of biosecurity—completed a review of two unpublished manuscripts describing National Institutes of Health (NIH)-funded research on the transmissibility of H5N1 influenza. The NSABB concluded that publishing the methodological and other details of this work could potentially enable replication of experiments that had enhanced transmissibility of H5N1 influenza (in ferrets) by those who might wish to do harm, and recommended that the manuscripts not be published in full. NSABB members also discussed whether there should be a temporary moratorium on the broad communication of dual-use H5N1 research until the issues raised by the research could be resolved. HHS provided the NSABB's non-binding recommendations to the authors of the manuscripts and the editors of the journals to which the manuscripts had been submitted for publication. To date, the manuscripts have not been published.

Today, the authors of the unpublished manuscripts and other scientists in the H5N1 research community announced that they will voluntarily suspend certain research on the H5N1 virus for 60 days, pending a thorough international discussion about its future directions and parameters for its safe conduct and responsible communication. This suspension applies both to research that enhances the transmissibility of highly pathogenic avian influenza viruses in mammals, as well as any experiments with H5N1 viruses already shown to be transmissible in ferrets. We applaud the decision by these scientists, who have demonstrated great responsibility and flexibility in pausing their work to allow for a full dialogue about the risks and benefits of this research. NIH, the Centers for Disease Control and Prevention, and other U.S. government agencies that conduct or fund such research will also abide by this moratorium. We continue to urge the international scientific community to work toward a consensus on the future directions of such research to improve public health in light of international security implications, while ensuring the global influenza surveillance and research communities can share through appropriate means critical information about the potential transmissibility of H5N1 influenza in humans. Understanding how influenza viruses become human pandemic threats is vitally important to global health preparedness. Such research helps us to understand the ability of the virus to cross between species and enables the development of tools for the prediction, prevention, and treatment of outbreaks.

To this end, officials with the World Health Organization are now working to organize a forum for the international scientific community to discuss these issues in the coming weeks. We look forward to participating in this important dialogue.

Francis S. Collins, M.D., Ph.D.
Director, National Institutes of Health

Anthony S. Fauci, M.D.
Director, National Institute of Allergy and Infectious Diseases
National Institutes of Health

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Sciencexpress Policy Forum

Adaptations of Avian Flu Virus Are a Cause for Concern

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Members of the National Science Advisory Board for Biosecurity explain its recommendations on the communication of experimental work on H5N1 influenza.

We are in the midst of a revolutionary period in the life sciences. Technological capabilities have dramatically expanded, we have a much improved understanding of the complex biology of selected microorganisms, and we have a much improved ability to manipulate microbial genomes. With this has come unprecedented potential for better control of infectious diseases and significant societal benefit. However, there is also a growing risk that the same science will be deliberately misused and that the consequences could be catastrophic. Efforts to describe or define life-sciences research of particular concern have focused on the possibility that knowledge or products derived from such research, or new technologies, could be directly misapplied with a sufficiently broad scope to affect national or global security. Research that might greatly enhance the harm caused by microbial pathogens has been of special concern (*1*–*3*). Until now, these efforts have suffered from a lack of specificity and a paucity of concrete examples of "dual use research of concern" (*3*). Dual use is defined as research that could be used for good or bad purposes. We are now confronted by a potent, real-world example.

Highly pathogenic avian influenza A/H5N1 infection of humans has been a serious public health concern since its identification in 1997 in Asia. This virus rarely infects humans, but when it does, it causes severe disease with case fatality rates of 59% (*4*). To date, the transmission of influenza A/H5N1 virus from human to human has been rare, and no human pandemic has occurred. If influenza A/H5N1 virus acquired the capacity for human-to-human spread and retained its current virulence, we could face an epidemic of substantial proportions. Historically, epidemics or pandemics with high mortalities have been documented when humans interact with new agents for which they have no immunity, such as with *Yersinia pestis* (plague) in the Middle Ages and the introduction of smallpox and measles into the Americas after the arrival of Europeans.

Recently, several scientific research teams have achieved some success in isolating influenza A/H5N1 viruses that are transmitted efficiently between mammals, in one instance with maintenance of high pathogenicity. This information is very important because, before these experiments were done, it was uncertain whether avian influenza A/H5N1 could ever acquire the capacity for mammal-to-mammal transmission.

Now that this information is known, society can take steps globally to prepare for when nature might generate such a virus spontaneously. At the same time, these scientific results also represent a grave concern for global biosecurity, biosafety, and public health. Could this knowledge, in the hands of malevolent individuals, organizations, or governments, allow construction of a genetically altered influenza virus capable of causing a pandemic with mortality exceeding that of the "Spanish flu" epidemic of 1918? The research teams that performed this work did so in a well-intended effort to discover evolutionary routes by which avian influenza A/H5N1 viruses might adapt to humans. Such knowledge may be valuable for improving the public health response to a looming natural threat. And, to their credit and that of the peer reviewers selected by the journals *Science* and *Nature*, the journals themselves, as well as the U.S. government, it was recognized before their publication that these experiments had dual use of concern potential.

The U.S. government asked National Science Advisory Board for Biosecurity (NSABB) (*5*), to assess the dual-use research implications of two as-yet-unpublished manuscripts on the avian influenza A/H5N1 virus, to consider the risks and benefits of communicating the research results, and to provide findings and recommendations regarding the responsible communication of this research.

Risk assessment of public harm is challenging because it necessitates consideration of the intent and capability of those who wish to do harm, as well as the vulnerability of the public and the status of public health preparedness for both deliberate and accidental events. We found the potential risk of public harm to be of unusually high magnitude. In formulating our recommendations to the government, scientific journals, and the broader scientific community, we tried to balance the great risks against the benefits that could come from making the details of this research known. Because the NSABB found that there was significant potential for harm in fully publishing these results and that the harm exceeded the benefits of publication, we therefore recommended that the work not be fully communicated in an open forum. The NSABB was unanimous that communication of the results in the two manuscripts it reviewed should be greatly limited in terms of the experimental details and results.

This is an unprecedented recommendation for work in the life sciences, and our analysis was conducted with careful consideration both of the potential benefits of publication and of the potential harm that could occur from such a precedent. Our concern is that publishing these experiments in detail would provide information to some person, organization, or government that would help them to develop similar mammal-adapted influenza A/H5N1 viruses for harmful purposes. We believe that as scientists and as members of the general public, we have a primary responsibility "to do no harm" as well as to act prudently and with some humility as we consider the immense power of the life sciences to create microbes with novel and unusually consequential properties. At the same time, we acknowledge that there are clear benefits to be realized for the public good in alerting humanity of this potential threat and in pursuing those aspects of this work that will allow greater preparedness and the potential development of novel strategies leading to future disease control. By recommending that the basic result be communicated without methods or details, we believe that the benefits to society are maximized and the risks minimized. Although scientists pride themselves on the creation of scientific literature that defines careful methodology that would allow other scientists to replicate experiments, we do not believe that widespread dissemination of the methodology in this case is a responsible action.

The life sciences have reached a crossroads. The direction we choose and the process by which we arrive at this decision must be undertaken as a community and not relegated to small segments of government, the scientific community, or society. Physicists faced a similar situation in the 1940s with nuclear weapons research, and it is inevitable that other scientific disciplines will also do so.

Along with our recommendation to restrict communication of these particular scientific results, we discussed the need for a rapid and broad international discussion of dual-use research policy concerning influenza A/H5N1 virus with the goal of developing a consensus on the path forward. There is no doubt that this is a complex endeavor that will require diligent and nuanced consideration. There are many important stakeholders whose opinions need to be heard at this juncture. This must be done quickly and with the full participation of multiple societal components.

We are aware that the continuing circulation of the highly pathogenic avian influenza A/H5N1 virus in Eurasia—where it is constantly found to cause disease in animals of particular regions—constitutes a continuing threat to humankind. A pandemic, or the deliberate release of a transmissible highly pathogenic influenza A/H5N1 virus, would be an unimaginable catastrophe for which the world is currently inadequately prepared. It is urgent to establish how best to facilitate the much-needed research as well as minimize potential dual use.

To facilitate and motivate this process, we also discussed the possibility of the scientific community participating in a self-imposed moratorium on the broad communication of the results of experiments that show greatly enhanced virulence

or transmissibility of such potentially dangerous microbes as the influenza A/H5N1 virus. until consensus is reached on the balance that must be struck between academic freedom and protecting the greater good of humankind from potential danger. With proper diligence and rapid achievement of a consensus on a proper path forward, this could have little detrimental effect on scientific progress but significant effect on diminishing risk.

There are many parallels with the situation in the 1970s and recombinant DNA technologies (*6*–*8*). The Asilomar Conference in California in 1975 was a landmark meeting important to the identification, evaluation, and mitigation of risks posed by recombinant DNA technologies. In that case, the research community voluntarily imposed a temporary moratorium on the conduct of recombinant DNA research until they could develop guidance for the safe and responsible conduct of such research. We believe that this is another Asilomar-type moment for public health and infectious-disease research that urgently needs our attention.

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Report on technical consultation on H5N1 research issues

Geneva, 16–17 February 2012

Context

Approximately 60% of persons known to have been infected by the avian influenza A(H5N1) virus have died from their illness. To date, most known human infections have occurred through contact with, or exposure to, infected birds. The prospect that H5N1 viruses circulating in nature might evolve and acquire the ability to spread with ease from person to person is a serious public health concern.

Research on the genetic basis of the transmissibility of H5N1 by two groups (one in the Netherlands and the other a joint Japan/USA group) resulted in laboratory-modified H5N1 viruses capable of respiratory transmission between ferrets. These mammals are often used in influenza research because, in some respects, ferret influenza infection shows similarities to human influenza infection. The results of these two studies demonstrate that relatively few genetic changes in H5N1 viruses can enable transmission via the respiratory route in these animals, and, in turn, suggest that H5N1 viruses could become more easily transmissible from person to person. The findings suggest that such changes could occur in nature, but do not provide an estimate of the likelihood that they will occur.

During the autumn of 2011, after manuscripts describing the research studies and their findings were submitted to scientific journals, the papers were reviewed by the National Science Advisory Board for Biosecurity (NSABB) in the United States, which recommended against publishing some details of the work. Specifically, the NSABB recommended publishing the general conclusions, without details of the research methods used or the specific mutations, to reduce the possibility that anyone seeking to do harm could replicate the experiments.

On January 20, 2012, the researchers who conducted this work and some other research groups announced a 60-day voluntary research moratorium to allow time for organizations and governments to "find the best solutions for opportunities and challenges that stem from the work". The scientific journals to which the papers had been submitted for publication also voluntarily deferred publication.

In light of the global relevance of these issues, WHO convened a preliminary technical consultation on 16–17 February 2012. The purpose was to clarify key facts about the studies and to address the most urgent issues concerning the management of these laboratory-modified viruses, and how access to and dissemination of any findings should be handled.

Twenty-two participants[1] were invited, including those with direct involvement in, or knowledge of, the content, oversight, or potential dissemination of this work. Representatives from countries where H5N1 is currently circulating were also present. Participants reviewed the chronology of the transfer of the H5N1 viruses used in the research studies, from country of origin to the research laboratories; the associated agreements regarding use of the samples; how the research proposals were reviewed; and the oversight of the work. Under conditions of stringent security, they read the full and redacted versions of both unpublished research reports, and also heard brief presentations by the researchers, summarizing their work.

Further, the participants were asked to recognize that while this research had elicited important scientific and social concerns from a number of different perspectives, the purpose of this meeting was not to debate these broader perspectives, but to find

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[1] See http://www.who.int/influenza/human_animal_interface/list_participants/en/index.html for the full list of participants.

practical, feasible, *ad hoc* solutions to the questions of access to research findings and management of the laboratory-modifed viruses.

Overview of the research findings

The studies indicated that different experimental methods can generate viable H5N1 or other influenza viruses with certain H5 characteristics, which demonstrate increased transmissibility in ferrets. In each study, the increase in capacity for transmission by the respiratory route was associated with a group of specific mutations, although these differed between the two studies. Both studies were essentially proof-of-principle experiments, and thus were not designed to elucidate the pathogenicity or degree of transmissibility of the laboratory-modified viruses. It was noted that the research methods used in these studies are not novel and are widely used in biomedical research.

Participants agreed on the public health value of the data on genetic modifications for improving the existing surveillance performed by both the human public health and animal health sectors, so as to monitor for variants that may be indicative of important changes among circulating H5N1 viruses. The findings of these studies provide a valuable complement to the accumulating data on virus evolution occurring in nature, and to ongoing analyses of in-host pathogen evolutionary dynamics.

Participants noted that the research findings had to be considered within a social context. The studies had raised concerns about the potential misuse of the viruses and the research findings. The participants also noted that, if disseminated to the public health and scientific community, the results would offer significant benefits to global health. Specifically, the findings could be used to improve the sensitivity of public health surveillance, facilitate the early detection of potentially pandemic H5N1 strains, and might aid the development of vaccines and the assessment of the potential value of other countermeasures.

Overview of options discussed

Several issues relating to publication were considered:

- If the research were to be published in redacted form, would genetic sequence data and/or the research methods remain completely restricted, or should the information be made available to a limited audience, after a public health justification for use of the information?
- If the latter, what workable mechanism would allow selective access to this information by laboratories involved in public health surveillance and legitimate research?
- What criteria would be required for access, and which organization would exercise governance over access?
- How could dissemination to those permitted access be performed securely?
- Could the confidentiality of the information be maintained?

On the question of limiting access to the results through publication of redacted versions, some participants observed that there was no current practical mechanism to limit access. Further, it would not be difficult for knowledgeable scientists to determine the information that had been removed, as novel methods had not been used. Limiting access to those with a need for the information would pose insurmountable practical problems. Chief among these problems are the development and implementation of a mechanism to disseminate the information to diverse and geographically distributed groups while maintaining the confidentiality of the detail. Therefore, such a mechanism would not realistically resolve concerns about dual-use research. There may be benefit in creating such a mechanism to deal with other dual-use research information in the future. However, this will require thorough consideration of and international agreement on practical issues such as security, access requirements, governance, and liability.

Establishing such a mechanism and implementing it effectively in the very short term was not considered to be feasible based on the information known to this group.

Six questions were explored with regard to the two laboratory-modified viruses:

- After the current moratorium on this research expires, should the viruses be destroyed?
- Should the samples be kept at their current locations?
- Is it necessary to transfer them to locations of increased laboratory biosecurity?
- What biosafety and laboratory biosecurity considerations and standards should be required for any subsequent work?
- If the viruses are not to be destroyed, how could the findings of research be applied towards the development of vaccine-candidate viruses or other countermeasures?
- What further research would be acceptable or desirable, especially in light of the PIP Framework?

It was not believed that any purpose would be served by destroying these laboratory-modified viruses, given their utility for future research and public health surveillance. Although the viruses are currently in facilities that met or exceeded the required biosafety and biosecurity standards, the participants were in agreement that an urgent review is needed to define the conditions under which future research on laboratory-modified H5N1 viruses might take place. The participants noted the need, after the moratorium, for clear guidance on the biosafety and biosecurity standards necessary in other research sites, and for a comprehensive system of monitoring.

Next steps

The next steps will be:

1) to convene a qualified group to define the essential biosafety and laboratory biosecurity standards and practices to be observed in future work with these laboratory-modified viruses;

2) to increase awareness of the nature and objectives of this research and to place the results in the context of the current assessment of the threat posed by wild-type H5N1 viruses and our rapidly increasing understanding of their biology. This situation has also highlighted the continuing need for better communication across all cultural settings about the intrinsic value of research for the protection of global public health and for conveying a sober assessment of the threat posed by H5N1 to human health;

3) to hold a further discussion on the scientific and societal issues raised by this kind of research. Specific topics to be addressed include how to strengthen public safety and security while ensuring that critical scientific research continues, as well as mechanisms to assess and manage sensitive research.

Consensus points[2]

- Recent work discussed at this meeting underscores that influenza A(H5N1) viruses remain an important risk for causing a future pandemic. Therefore, research on these viruses, including on transmissibility and pathogenicity, remains critical to close important gaps in knowledge in order to reduce the danger posed; such research should continue. The PIP Framework,[3] which was

[3] These consensus points were initially posted on the WHO web site immediately following the meeting.
[3] See http://www.who.int/influenza/pip/en/ for details.

adopted by all WHO Member States in 2011, now provides a global framework for the sharing of influenza viruses with human pandemic potential and the sharing of benefits arising from such sharing. Implementation of this Framework is integral to global pandemic preparedness and response. Future research projects should involve countries from which source material were obtained.

- The two studies that were conducted to better understand the transmissibility of H5N1 influenza viruses have shown that these viruses have the potential to become more transmissible among mammals. In light of the continuing evolution of H5N1 viruses, the results of these studies provide an important contribution to public health surveillance of H5N1 viruses and to a better understanding of the properties of these viruses.

- At the same time, these studies have raised important and valid concerns about whether they increase risks to the safety of humans. Concerns which have been raised include the potential misuse of the results or methods as well as potential breaches in biosafety and biosecurity related to pathogens. These concerns highlight how important it is that researchers are aware of such issues, exercise judgment about the conduct of their research, dissemination of the results, and for institutional bodies reviewing such studies to identify and address potential concerns about "dual use". Such safeguards already exist, but continued emphasis should be placed on assuring and reinforcing safety and security.

- The laboratory-modified H5N1 viruses are currently stored in well-established research facilities with high security and high safety (BSL3+).[4] There have been no safety breaches related to the storage of the laboratory-modified H5N1 viruses at these facilities. At the same time, the biosafety and biosecurity conditions under which further research is conducted on the laboratory-modified H5N1 viruses should be fully addressed by relevant authorities. This is a matter of urgency and should be achieved as quickly as possible. In the interim, the laboratory-modified H5N1 viruses should remain in their present locations. In addition, the current moratorium on research to enhance the transmissibility of H5N1 influenza viruses and the further research on the laboratory-modified viruses should continue until the conditions have been determined. Other research on H5N1 viruses should not stop.

- There is a preference, from a public health perspective, for full disclosure of the information in these papers. However, there are significant social concerns surrounding this research. Two critical issues that must be addressed before publication of the papers are: (1) a focused communications plan to increase public awareness and understanding of the significance of these studies and the rationale for their publication, and (2) a review of the essential biosafety and biosecurity aspects of the newly developed knowledge.

- Participants discussed the concept of publication of redacted manuscripts with a mechanism for providing the restricted information to legitimate recipients. The group recognized the difficulty of rapidly creating and regulating such a mechanism in light of the complexity of international and national legislation. A consensus was reached that the redaction option is not viable to deal with the two papers under discussion in view of the urgency of the above mentioned public health needs. The participants noted there may be a need for such a mechanism in the future.

[4] Biosafety level 3(enhanced) containment laboratory.

- Apart from consideration of these two manuscripts, participants acknowledged the existence of broader issues requiring more detailed exploration and advised that these be considered in subsequent consultations involving other stakeholders.

United States Government Policy for Oversight of Life Sciences Dual Use Research of Concern

Section I: Purpose and Principles

1) The purpose of this Policy is to establish regular review of United States Government funded or conducted research with certain high-consequence pathogens and toxins for its potential to be dual use research of concern (DURC) in order to: (a) mitigate risks where appropriate; and (b) collect information needed to inform the development of an updated policy, as needed, for the oversight of DURC. The fundamental aim of this oversight is to preserve the benefits of life sciences research while minimizing the risk of misuse of the knowledge, information, products, or technologies provided by such research.
2) This Policy complements existing United States Government regulations and policies governing the possession and handling of pathogens and toxins. Currently, the Select Agent Regulations ensure appropriate oversight of biosafety and biosecurity of the possession and handling of pathogens and toxins that have the potential to pose a severe threat to human, animal, or plant health, or to animal and plant products. In addition, recommendations from Federal advisory bodies such as the National Science Advisory Board for Biosecurity (NSABB) have helped inform United States Government policies for identifying and managing DURC. This Policy will be updated, as needed, following domestic dialogue, engagement with our international partners, and input from interested communities including scientists, national security officials, and global health specialists.
3) The following principles guide implementation of this Policy:
    a) Life sciences research is essential to the scientific advances that underpin improvements in the health and safety of the public, agricultural crops and other plants, animals, the environment, materiel, and national security. Despite its value and benefits, some research may provide knowledge, information, products, or technologies that could be misused for harmful purposes.
    b) Accordingly, some degree of Federal and institutional oversight of DURC is critical to reducing the risks to public health and safety, agricultural crops and other plants, animals, the environment, materiel, and national security.
    c) Measures that mitigate the risks of DURC should be applied, where appropriate, in a manner that minimizes, to the extent possible, adverse impact on legitimate research, is commensurate with the risk, includes flexible approaches that leverage existing processes, and endeavors to preserve and foster the benefits of research.
    d) The United States Government will facilitate the sharing of the results and products of life sciences research conducted or funded by United States Government agencies, and honor United States Government obligations within relevant international frameworks and agreements, while taking into account United States' national security interests.
    e) In executing this Policy, the United States Government will abide by and enforce all relevant Presidential Directives and Executive Orders, all applicable laws and regulations, and support the implementation of legally binding treaties, commitments, and United Nations Security Council resolutions prohibiting the development and use of biological agents as weapons.

Section II: Definitions

1) For the purpose of this Policy, DURC 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[1].

2) "Life sciences" pertains to living organisms (e.g., microbes, human beings, animals, and plants) and their products, including all disciplines and methodologies of biology such as aerobiology, agricultural science, plant science, animal science, bioinformatics, genomics, proteomics, synthetic biology, environmental science, public health, modeling, engineering of living systems, and all applications of the biological sciences. The term is meant to encompass the diverse approaches for understanding life at the level of ecosystems, organisms, organs, tissues, cells, and molecules.
3) Extramural research is that which is funded by a department or agency under a grant, contract, cooperative agreement, or other agreement and not conducted directly by the department or agency.
4) Intramural research is that which is directly conducted by a department or agency.

Section III: Scope

Under this Policy, review will focus on research that involves one or more of the agents or toxins listed in Section (III.1) below, which pose the greatest risk of deliberate misuse with most significant potential for mass casualties or devastating effects to the economy, critical infrastructure, or public confidence, and produces, aims to produce, or is reasonably anticipated to produce one or more of the effects listed in Section (III.2) below:

1) Agents and toxins[2]:
   a) Avian influenza virus (highly pathogenic)
   b) *Bacillus anthracis*
   c) Botulinum neurotoxin
   d) *Burkholderia mallei*
   e) *Burkholderia pseudomallei*
   f) Ebola virus
   g) Foot-and-mouth disease virus
   h) *Francisella tularensis*
   i) Marburg virus
   j) Reconstructed 1918 Influenza virus
   k) Rinderpest virus
   l) Toxin-producing strains of *Clostridium botulinum*
   m) Variola major virus
   n) Variola minor virus
   o) *Yersinia pestis*
2) Categories of experiments:
   a) Enhances the harmful consequences of the agent or toxin;
   b) Disrupts immunity or the effectiveness of an immunization against the agent or toxin without clinical or agricultural justification;
   c) Confers to the agent or toxin resistance to clinically or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin or facilitates their ability to evade detection methodologies;
   d) Increases the stability, transmissibility, or the ability to disseminate the agent or toxin;
   e) Alters the host range or tropism of the agent or toxin;

[1] This definition of DURC is derived from the NSABB definition, but is modified for purposes of this Policy.
[2] These agents and toxins are regulated by the Select Agent Program under Federal Law (7 C.F.R. part 331, 9 C.F.R. part 121, and 42 C.F.R. part 73), and have the potential to pose a severe threat to human, animal, or plant health, or to animal and plant products.

f) Enhances the susceptibility of a host population to the agent or toxin; or
g) Generates or reconstitutes an eradicated or extinct agent or toxin listed in Section (III.1) above.

Section IV: Department and Agency Responsibilities

1) Federal departments and agencies that conduct or fund life sciences research should implement the following actions:
   a) Conduct a review to identify all current or proposed, unclassified intramural or extramural, life sciences research projects that fall within the scope of Section III. This review will include, at a minimum, initial proposals and any progress reports.
   b) Determine which, if any, of the projects identified in Section (IV.1.a) meet the definition of DURC in Section (II.1) of this document.
   c) Assess the risks and benefits of such projects, including how research methodologies may generate risks and/or whether open access to the knowledge, information, products, or technologies generates risk.
   d) Based on the risk assessment, in collaboration with the institution or researcher, develop a risk mitigation plan to apply any necessary and appropriate risk mitigation measures. In addition:
      i) For DURC that is proposed and not yet funded, departments and agencies will assess whether to incorporate risk mitigation measures in the grant, contract, or agreement.
      ii) For currently funded DURC, funding departments and agencies will consider modifying the grant, contract, or agreement to incorporate risk mitigation measures. If such modifications are not possible or desirable, departments and agencies will seek voluntary implementation of mitigation measures by the institution.
   e) A risk mitigation plan may include, but not be limited to, the following risk mitigation measures:
      i) Modifying the design or conduct of the research.
      ii) Applying specific or enhanced biosecurity or biosafety measures.
      iii) Evaluating existing evidence of medical countermeasures (MCM) efficacy, or conducting experiments to determine MCM efficacy against agents or toxins resulting from DURC, and where effective MCM exist, including that information in publications.
      iv) Referring the institution to available DURC educational tools such as: http://oba.od.nih.gov/biosecurity/biosecurity.html
      v) Regularly reviewing, at the institutional level, emerging research findings for additional DURC.
      vi) Requesting that institutions notify funding departments or agencies if additional DURC is identified, and propose modifications to the risk mitigation plan, as needed.
      vii) Determining the venue and mode of communication (addressing content, timing, and possibly the extent of distribution of the information) to communicate the research responsibly.
      viii) Reviewing annual progress reports from Principal Investigators to determine if DURC results have been generated, and if so, flagging them for institutional attention and applying potential mitigation measures as described above, as necessary.
      ix) If the risks posed by the research cannot be adequately mitigated with the measures above, Federal departments and agencies will determine whether it is appropriate to:
         (a) Request voluntary redaction of the research publications or communications [3];
         (b) Classify the research:
            (i) In accordance with National Security Decision Directive/NSDD-189, departments and agencies will make classification determinations within

[3] Actions taken to restrict the publication of technology may have implications under export control laws and regulations (e.g., 15 CFR parts 730-774 and 22 CFR parts 120-130).

the scope of their classification authorities and appropriate classification guidelines or may consult with other departments and agencies to make these determinations.

(ii) Departments and agencies may consider whether to refer classified research to another department or agency for funding.

(c) Not provide or terminate research funding.

2) Federal departments and agencies are requested to report the following to the Assistant to the President for Homeland Security and Counterterrorism:
   a) Within 60 days of issuance of this Policy, the following results of the review conducted in response to Section (IV.1.a):
      i) Aggregate number of current and proposed unclassified, intramural, and extramural research projects identified that include work with one or more of the agents and toxins in Section (III.1).
      ii) Aggregate number of current and proposed unclassified, intramural, and extramural research projects that include work with one or more of the agents and toxins in Section (III.1) and produces, aims to produce, or are reasonably anticipated to produce one or more of the effects listed in Section (III.2).
   b) Within 90 days of issuance of this Policy, the following results of the review conducted in response to Sections (IV.1. b. c. and d):
      i) Number of unclassified current and proposed DURC projects.[4]
      ii) Number of current projects identified as DURC through initial proposals versus progress reports.[5]
      iii) Summary of risks, mitigation measures already in place that address those risks, any additional mitigation measures that have been proposed or implemented, and number of projects to which each mitigation measure would be applied.
3) Following completion of the reporting requirements in Section (IV.2), Federal departments and agencies are requested to submit periodic reports on items in Section (IV.2.a. and b) biannually.
4) Federal departments and agencies should implement Section IV in accordance with their relevant and applicable authorities, regulations, and statutes.
5) For additional guidance on how to conduct the risk assessment identified in Section (IV. 1.c), departments and agencies may refer to the "Proposed Framework for the Oversight of Dual Use Life Sciences Research: Strategies for Minimizing the Potential Misuse of Research Information," which identifies useful assessment tools and is available at: http://oba.od.nih.gov/biosecurity/biosecurity_documents.html .

Section V: Consultation

As necessary and appropriate, the United States Government will continue to consult with the NSABB (in compliance with provisions of the Federal Advisory Committee Act) or convene the Countering Biological Threats Interagency Policy Committee for guidance on matters relating to the review and conduct of DURC and the mitigation of DURC risks.

[4,5] Report the number of projects by agent and/or toxin plus the category of experiment.

National Science Advisory Board for Biosecurity
Findings and Recommendations
March 29-30, 2012

Summary

On March 29-30, 2012, the National Science Advisory Board for Biosecurity (NSABB or Board) convened to examine two revised manuscripts regarding the transmissibility of highly pathogenic avian influenza A virus H5N1 (H5N1) in ferrets. After careful deliberation, the NSABB unanimously recommended that the revised manuscript submitted by Dr. Yoshihiro Kawaoka be communicated in full. The NSABB also recommended, in a 12-to-6 decision, that the data, methods, and conclusions presented in the revised manuscript submitted by Dr. Ron Fouchier be communicated after appropriate scientific review and revision.

Background

In the Fall of 2011, the NSABB reviewed manuscripts from Dr. Ron Fouchier, Erasmus Medical Center, and Dr. Yoshihiro Kawaoka, the University of Wisconsin, reporting the transmissibility of H5N1 in mammals. The manuscripts, submitted for publication in *Science* and *Nature* respectively, described the generation of mutations in H5N1 that enable the airborne transmission of the virus between ferrets. Ferrets are commonly used as an animal model for influenza transmissibility in humans. At that time, the Board recognized the importance of the research in advancing knowledge of influenza transmission and supporting public health efforts. Specifically, the Board recognized that the experiments confirmed that H5N1 had the potential to become mammalian transmissible and thus posed a threat of a future pandemic. This information was significant because until then it had been uncertain whether this virus had the evolutionary capacity to adapt to mammalian transmissibility. The Board understood, however, that the specific findings would enable others to synthesize and express an H5N1 strain with mammal-to-mammal airborne transmissibility, and thus it had significant concerns that the information in the manuscripts could be misused to endanger public health and national security. Given these dual use concerns,[1] the Board recommended that the information in these manuscripts be published in a redacted form with the omission of certain details that could enable the direct misuse of the research by those with malevolent intent. The goal was to deliver the critical information about the H5N1 potential for pandemic spread while minimizing the possible risk that the information could be used for nefarious purposes.

In February 2012, the World Health Organization (WHO) convened a technical consultation to "clarify key facts about the studies and to address the most urgent issues concerning the management of these laboratory-modified viruses, and how access to and dissemination of any findings should be handled."[2] At this meeting, additional non-public data were presented and discussed, and key clarifications were made by the authors, who subsequently revised the manuscripts. In light of this, the United States Department of Health and Human Services convened the NSABB in a closed session March 29-30, 2012, to review the newly revised manuscripts and to recommend whether and/or how the information

[1] In the *Proposed Framework for the Oversight of Dual Use Life Sciences Research: Strategies for Minimizing the Potential Misuse of Research information*, the NSABB defined "dual use research" as "[r]esearch yielding new technologies or information with the potential for both benevolent and malevolent applications."
[2] WHO Report on Technical Consultation on H5N1 Research Issues
http://www.who.int/influenza/human_animal_interface/mtg_report_h5n1.pdf

should be communicated. Taking into account the additional information in the revised manuscripts, new non-public epidemiological information, and security information to be presented in a classified briefing, the NSABB was charged with:

- Assessing the dual use research implications of two unpublished, revised manuscripts on the transmissibility of highly pathogenic avian influenza A virus H5N1;
- Considering the risks and benefits of communicating the research results; and
- Developing findings and recommendations regarding whether the information should be communicated, and if so, to what extent.

NSABB Approach

On March 29-30, 2012, the NSABB members read the revised copies of the manuscripts, heard presentations, and discussed the findings with the authors. The Board also engaged public health officials, influenza experts, journal editors, security experts, and individuals involved in the oversight of H5N1 research both from the United States and from the international communities. The Board's discussions were informed by the analytical frameworks[3] that it previously developed for considering the risks and benefits associated with the communication of dual use research of concern.[4]

Findings

The NSABB strongly supports the unrestricted communication of research information unless that information could be directly misused to pose a significant and near-term risk to public health and safety or if the risks associated with misuse of the information are so significant that no amount of potential benefits can justify the risks. The Board concluded that the communication of the information in these revised manuscripts still presents dual use research concerns. The risks and benefits associated with communicating, or not communicating, these findings were considered in light of additional information and key clarifications. The majority of the members of the NSABB concluded that:

- ***The data are not immediately enabling.*** As currently written, the revised manuscripts do not appear to provide information that would enable the near-term misuse of the research in ways that would endanger public health or national security. The mutations described in the manuscripts do not appear to result in H5N1 viruses that are both highly pathogenic and transmissible between ferrets through the air. The Board emphasized that if additional information were included that would enable the construction of an H5N1 virus that was both highly pathogenic and transmissible between mammals through the air, then the information in the manuscripts would have more implications for misuse and would require additional consideration regarding communication.

[3] www.biosecurityboard.gov, see the *Proposed Framework for the Oversight of Dual Use Life Sciences Research: Strategies for Minimizing the Potential Misuse of Research Information* in the NSABB Documents link.
[4] In the *Proposed Framework for the Oversight of Dual Use Life Sciences Research: Strategies for Minimizing the Potential Misuse of Research Information*, the NSABB defined "dual use research of concern" as "research that, based on current understanding, can be reasonably anticipated to provide knowledge, products, or technologies that could be directly misapplied by others to pose a threat to public health and safety, agricultural crops and other plants, animals, the environment or materiel."

- ***These data may benefit public health and surveillance efforts.*** New information regarding epidemiology and the natural evolution of the virus in the field has emerged that underscores the fact that understanding specific mutations and the biologic properties associated with these mutations may improve international surveillance and public health efforts. While more research needs to be conducted to validate these ideas, potential public health benefits may include enhanced surveillance of viruses in birds and humans and other mammals (e.g., possible reassortment viruses in pigs) and improved risk assessment of circulating strains. The information in the manuscripts also may help inform public health decisions regarding pandemic preparedness (e.g., maintenance or strengthening of vaccine stockpiles and strain selection for vaccine development). The revised manuscripts provided a greater appreciation of the direct applicability of the information to ongoing and future influenza surveillance efforts.

- ***Global cooperation is essential for pandemic influenza preparedness.*** The Board recognizes that international cooperation is critical to ensuring public health and safety on a global scale and that such cooperation is predicated upon the free exchange of information. The Board's discussions underscored the risks associated with not sharing the information, which could jeopardize pandemic influenza preparedness efforts. Specifically, there was concern that the United States would be perceived as redacting information with potential public health benefits and that this could undermine valuable international collaborations. The information in these manuscripts will help public health officials prepare for influenza outbreaks in parts of the world where the virus is endemic.

- ***The research was conducted under appropriate conditions.*** The NSABB noted during its review of the initial and revised manuscripts that both studies were conducted under rigorous biosafety conditions, including appropriate biosafety containment, practices, training, and occupational-health programs. Because the research involved the use of a select agent, the research also was conducted under the oversight of the Select Agent Program, including periodic inspection of the facilities and biosecurity review by the United States Centers for Disease Control and Prevention (CDC) and/or the United States Department of Agriculture (USDA).

  However, the Board recognized that biosafety requirements might be different if the engineered viruses had greatly altered properties. A review of the biosafety regulations would be prudent, should be performed by qualified professionals, and should be based upon a risk assessment of the work environment and the altered viruses.

- ***There is an urgent need for effective United States and international policies for the oversight and communication of dual use research of concern.*** The NSABB has noted previously that it is important that dual use research issues are identified and managed early in the research process rather than after the research has been conducted, let alone when a manuscript is ready for publication. The newly released *United States Government Policy for Oversight of Life Sciences Dual Use Research of Concern*[5] was based upon this principle and the urgency of the recent deliberations. This policy applies to life sciences research funded by the United States and will ensure that dual use concerns are addressed during the evaluation of ongoing and

[5] http://oba.od.nih.gov/oba/biosecurity/PDF/United_States_Government_Policy_for_Oversight_of_DURC_FINAL_version_032812.pdf

future federally funded research on H5N1 influenza virus. The Board's discussion was informed by this new policy initiative.

The Board also noted the need for guidelines to aid in the determination of how/whether certain types of "gain-of-function" experiments with influenza should be conducted or communicated. These two H5N1 studies both used well-known techniques to change the mode of transmission of H5N1 avian influenza virus from fecal-oral to respiratory and from avian-avian transmission to mammal-mammal transmission. Further gain-of-function experiments of this type are likely to be contemplated by these and other laboratories around the world. Experiments that change the mode of transmission or host range of a zoonotic agent are of particular concern and require detailed analyses of risks and benefits before they are conducted or communicated. At the present time, no specific guidelines exist to aid in these analyses for future studies of influenza virus.

Since scientific research and protecting public health are global endeavors, the Board urges the U.S. Government to closely engage the international community during the policy-development process so that scientific information can be shared between and among appropriate global partners. To this end, the Board will soon consider the findings and recommendations of its Working Group on Global Engagement, which has been charged with addressing the communication and other challenges presented by H5N1 dual use research of concern, challenges that are inherently international in scope.

- ***There is a critical need for a mechanism for disseminating sensitive scientific information.*** There remains a pressing need for an effective and feasible mechanism to provide controlled access to scientific information that has potential public health benefits but poses a significant risk for misuse if broadly disseminated. There are complex questions involved in developing such a mechanism, many of them legal issues. Nonetheless, a feasible, secure mechanism for sharing sensitive scientific information with individuals who have a legitimate need to know in order to support public health, safety, and security efforts is essential.

In contrast, a minority of members of NSABB concluded that:

- ***The data in the newly-revised Fouchier manuscript are immediately and directly enabling.*** As currently written, the revised Fouchier manuscript provides information that would enable the near-term misuse of the research in ways that would endanger public health or national security. The mutations described in this manuscript appear to result in modified H5N1 viruses that are transmissible between ferrets by respiratory route, as claimed by the authors, and in modified viruses that appear to be as pathogenic as the parental H5N1 strain, which in nature is known to be highly pathogenic in humans. The data in the Kawaoka paper, however, are less immediately and directly enabling because the approach involved the use of less virulent viral strains.

- ***While the data in the two manuscripts may benefit public health and surveillance efforts, these data may not be directly relevant or immediately helpful to the current public health or surveillance infrastructure.*** The evolutionary paths taken by naturally occurring H5N1 viruses may not be similar to those selected under these laboratory conditions. The relevance of the laboratory-derived mutations and their meaning for the evolution of H5N1 viruses in natural environments are unclear. Excessive attention to these mutations may in fact distract

surveillance efforts from what might be the naturally occurring mutations of greater interest. Furthermore, the current surveillance infrastructure is ill-equipped to detect the emergence of highly transmissible influenza viruses in real-time prior to their dissemination in nature. While there may be benefits to the dissemination of the mutation data in the Fouchier manuscript and global cooperation is essential for pandemic influenza preparedness, it is unlikely that the benefits will be fully realized in the near-term.

These Board members agreed with the rest of the Board about the general importance of global cooperation, the urgent need for effective policies for the oversight and communication of dual use research of concern, and the critical need for a mechanism for disseminating sensitive scientific information.

Recommendations

The Board considered the manuscripts separately and after careful deliberation made the following recommendations:

- ***The revised Kawaoka manuscript should be communicated in full.*** The NSABB unanimously recommended the full communication of this revised manuscript.

- ***The data, methods, and conclusions presented in the revised Fouchier manuscript should be communicated, but not as currently written.*** In a 12-to-6 decision, the NSABB recommended communicating the data, methods, and conclusions presented in this revised manuscript. However, the Board identified a number of scientific clarifications that should be made prior to publication of the manuscript. Importantly, the Board also noted that additional information that would enable the construction of an H5N1 virus that is both highly pathogenic and transmissible between mammals through the air should not be included in the manuscript. Such information could conceivably be directly misused to threaten public health or national security and additional considerations regarding communication would be necessary. Six of the 18 voting members felt that the data, methods, and conclusions presented in the revised Fouchier manuscript should not be communicated.

- ***The U.S. Government should continue to develop national, and participate in the development of international, policies for the oversight and communication of dual use research of concern.*** The newly released *United States Government Policy for Oversight of Life Sciences Dual Use Research of Concern* is an important first step in ensuring that dual use concerns associated with federally funded life sciences research will be addressed and managed early in and continuously during the research process. This policy will apply to H5N1 research as well as other agents and toxins that pose the greatest risk of misuse. In implementing this policy, the U.S. Government should monitor how effectively it facilitates the identification and timely management of dual use research of concern. However, it is essential that such oversight does not unduly burden or slow the progress of life sciences research. The oversight process should be periodically and robustly reviewed and modified as necessary to address these issues.

  The U.S. Government should also provide guidance on how to deal with "gain-of-function" studies that increase pathogenesis of zoonotic agents, particularly avian influenza viruses. Experiments that change the mode of transmission or host range of a zoonotic agent are of

particular concern and require a detailed analysis of risks and benefits before they are conducted or communicated.

Scientific research and protecting public health and safety are global endeavors. It is therefore critical that the U.S. Government continue to work with its international partners to develop, enforce and continually review consistent policies for the oversight of dual use research that enable the effective management and sharing of sensitive research information.

- ***The U.S. Government should expeditiously develop a mechanism to provide controlled access to sensitive scientific information.*** The majority of the NSABB recommends that the information contained in these revised H5N1 manuscripts should be communicated in full, but the Board also recognizes that research findings will likely emerge in the very near future that should not be widely disseminated because of a high risk of misuse but that nevertheless should be made available to certain researchers and public health officials around the world who have a legitimate need to know. The need for an effective, practical, and feasible mechanism for selectively sharing sensitive scientific information has never been more apparent. In order to manage the risks posed by communicating future cases of dual use research of concern, the Board strongly urges the U.S. Government to develop in an expeditious manner a practical and secure mechanism for sharing sensitive scientific information in order to support public health, safety, and security efforts.

Statement on NSABB's March 30, 2012 Recommendations to NIH on H5N1 Research

April 14, 2012

On March 29-30, 2012, the National Science Advisory Board for Biosecurity (NSABB) was convened to examine two revised manuscripts regarding the transmissibility of the H5N1 avian flu virus in ferrets.

The NSABB is an independent federal advisory committee chartered to provide advice and guidance to the Secretary of the Department of Health and Human Services, the Director of the National Institutes of Health, and all Federal entities that conduct, support, or have an interest in life sciences research regarding biosecurity oversight of dual use research, defined as biological research with legitimate scientific purpose that may be misused to pose a biologic threat to public health and/or national security.

After careful deliberation, the NSABB unanimously recommended that the revised manuscript submitted by Dr. Yoshihiro Kawaoka be communicated in full. The NSABB also recommended, in a 12-to-6 decision, that the data, methods, and conclusions presented in the revised manuscript submitted by Dr. Ron Fouchier be communicated after appropriate further scientific review and revision. A final recommendation of these two revised manuscripts regarding the transmissibility of the H5N1 avian flu virus in ferrets will be made by the HHS Secretary and brought to the broader U.S. government.

In addition, in their final recommendations submitted to NIH yesterday, the NSABB also made two other thoughtful recommendations about future approaches to the challenges presented by oversight of dual use research. Those recommendations are being carefully reviewed and considered. HHS will continue to work with scientific and national security experts, the public, and the international community regarding the long term recommendations on dual use research.

I want to take this occasion to express my sincere gratitude to the NSABB members, who have worked tirelessly to study the issue carefully, hear directly from the experts, and weigh the benefits and risks of making the research data public.

The full NSABB recommendations can be found at
http://www.nih.gov/about/director/03302012_NSABB_Recommendations.pdf. (PDF - 268 KB)

Francis S. Collins, M.D., Ph.D.
Director, National Institutes of Health

Statement by NIH Director Francis Collins, M.D., Ph.D. on the NSABB Review of Revised H5N1 Manuscripts

April 20, 2012

On March 29 and 30, the National Science Advisory Board for Biosecurity (NSABB), an independent expert committee that advises the National Institutes of Health (NIH), the Department of Health and Human Services (HHS) and other Federal departments and agencies on matters of biosecurity, convened to review unpublished revised manuscripts describing NIH-funded research on the transmissibility of H5N1 influenza virus—the strain commonly referred to as "bird flu." One manuscript, "Aerosol transmission of avian influenza A/H5N1 virus," contained research findings by Dr. Ron Fouchier. The other manuscript, "Haemagglutinin mutations that confer human-type receptor recognition and support respiratory droplet transmission of H5N1 influenza A virus in ferrets," contained research findings by Dr. Yoshihiro Kawaoka. To clarify the results of their research findings, both authors revised their manuscripts from versions reviewed earlier by the NSABB. The NSABB reviewed the revised manuscripts to make recommendations as to whether, and if so how, they should be communicated.

This line of research is critically important because it will help public health officials understand, detect, and defend against the emergence of H5N1 virus as a human threat, a development that could pose a pandemic scenario. The value of this research notwithstanding, certain information obtained through such studies has the potential to be misused for harmful purposes—a characteristic associated with what is referred to as "dual use research of concern." These particular manuscripts include the important finding that the H5N1 virus has greater potential than previously believed to gain the capacity to be transmitted among mammals, as assessed by experiments with ferrets. The manuscripts describe some of the genetic changes that appear to correlate with this potential.

During its March meeting, the NSABB took into account the new and clarified information in the manuscripts, additional perspectives provided by influenza biology experts, highly pertinent but as yet unpublished epidemiologic data, and relevant security information.

After careful deliberation, the NSABB unanimously recommended the revised manuscript by Dr. Yoshihiro Kawaoka be communicated in full. The NSABB also recommended, in a 12-to-6 decision, that the data, methods, and conclusions presented in the revised manuscript by Dr. Ron Fouchier be communicated fully after a number of further scientific clarifications are made in the manuscript. The recommendation to communicate the research was based on the observation that the information in the revised manuscripts has direct applicability to ongoing and future influenza surveillance efforts and does not appear to enable direct misuse of the research in ways that would endanger public health or national security.

The HHS Secretary and I concur with the NSABB's recommendation that the information in the two manuscripts should be communicated fully and we have conveyed our concurrence to the journals considering publication of the manuscripts. This information has clear value to national and international public health preparedness efforts and must be shared with those who are poised to realize the benefits of this research.

The Secretary's decision takes account of relevant U.S. law, international obligations, and a rigorous analysis of the benefits and risks of publication. The work in the Netherlands by Ron Fouchier is subject also to laws and regulations of the Netherlands, and the Dutch government is conducting its own review of Dr. Fouchier's work. We respect that process and value the dialogue we have with Dutch authorities toward our common goals of encouraging scientific inquiry, advancing global health, and protecting the safety and security of our populations and the wider global community.

In addition, the recently released Federal policy on dual use research of concern is an important step in enhancing the oversight of federally funded life sciences research going forward. Through implementation of this policy, the U.S. Government aims to preserve the benefits of vitally important life sciences research that holds the promise of enhancing quality of life for all of us, while minimizing the possibility that the knowledge, information, products, or technologies provided by such research could be misused for harm.

I am grateful to the NSABB members for the time and effort they have dedicated to considering the complex issues pertinent to dual use research generally, and for working so tirelessly on developing the most thoughtful recommendations possible regarding these two manuscripts.

Francis S. Collins, M.D., Ph.D.
Director, National Institutes of Health

Appendix C

The Two Published H5N1 Papers

"Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets," Masaki Imai, et al., *Nature* 420 (486). Copyright 2012. Mcmillan Publishers Limited. All rights reserved.

"Airborne Transmission of Influenza A/H5N1 Virus Between Ferrets," Sander Herfst, et al., *Science* 336 (June 22, 2012):1543. Reprinted with permission from AAAS.

LETTER

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Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets

Masaki Imai[1], Tokiko Watanabe[1,2], Masato Hatta[1], Subash C. Das[1], Makoto Ozawa[1,3], Kyoko Shinya[4], Gongxun Zhong[1], Anthony Hanson[1], Hiroaki Katsura[5], Shinji Watanabe[1,2], Chengjun Li[1], Eiryo Kawakami[2], Shinya Yamada[5], Maki Kiso[5], Yasuo Suzuki[6], Eileen A. Maher[1], Gabriele Neumann[1] & Yoshihiro Kawaoka[1,2,3,5]

Highly pathogenic avian H5N1 influenza A viruses occasionally infect humans, but currently do not transmit efficiently among humans. The viral haemagglutinin (HA) protein is a known host-range determinant as it mediates virus binding to host-specific cellular receptors[1–3]. Here we assess the molecular changes in HA that would allow a virus possessing subtype H5 HA to be transmissible among mammals. We identified a reassortant H5 HA/H1N1 virus—comprising H5 HA (from an H5N1 virus) with four mutations and the remaining seven gene segments from a 2009 pandemic H1N1 virus—that was capable of droplet transmission in a ferret model. The transmissible H5 reassortant virus preferentially recognized human-type receptors, replicated efficiently in ferrets, caused lung lesions and weight loss, but was not highly pathogenic and did not cause mortality. These results indicate that H5 HA can convert to an HA that supports efficient viral transmission in mammals; however, we do not know whether the four mutations in the H5 HA identified here would render a wholly avian H5N1 virus transmissible. The genetic origin of the remaining seven viral gene segments may also critically contribute to transmissibility in mammals. Nevertheless, as H5N1 viruses continue to evolve and infect humans, receptor-binding variants of H5N1 viruses with pandemic potential, including avian–human reassortant viruses as tested here, may emerge. Our findings emphasize the need to prepare for potential pandemics caused by influenza viruses possessing H5 HA, and will help individuals conducting surveillance in regions with circulating H5N1 viruses to recognize key residues that predict the pandemic potential of isolates, which will inform the development, production and distribution of effective countermeasures.

Although H5N1 viruses continue to cause outbreaks in poultry and there are cases of human infection in Indonesia, Vietnam, Egypt and elsewhere (http://www.who.int/influenza/human_animal_interface/H5N1_cumulative_table_archives/en/index.html), they have not acquired the ability to cause human-to-human transmission. Investment in H5N1 vaccines has therefore been questioned. However, because humans lack immunity to influenza viruses possessing an H5 HA, the emergence of a transmissible H5-HA-possessing virus would probably cause a pandemic. To prepare better for such a scenario, it is critical that we understand the molecular changes that may render H5-HA-possessing viruses transmissible in mammals. Such knowledge would allow us to monitor circulating or newly emerging variants for their pandemic potential, focus eradication efforts on viruses that already have acquired subsets of molecular changes critical for transmission in mammals, stockpile antiviral compounds in regions where such viruses circulate, and initiate vaccine generation and large-scale production before a pandemic. Therefore, we studied the molecular features that would render H5-HA-possessing viruses transmissible in mammals.

Previous studies suggested that HA has a major role in host-range restriction of influenza A viruses[1–3]. The HA of human isolates preferentially recognizes sialic acid linked to galactose by α2,6-linkages (Siaα2,6Gal), whereas the HA of avian isolates preferentially recognizes sialic acid linked to galactose by α2,3-linkages (Siaα2,3Gal)[3]. A small number of avian H5N1 viruses isolated from humans show limited binding to human-type receptors, a property conferred by several amino acid changes in HA[4–9]. None of the H5N1 viruses tested transmitted efficiently in a ferret model[10–13], although, while our paper was under review, one study[14] reported that a virus with a mutant H5 HA and a neuraminidase (NA) of a human virus in the H5N1 virus background caused respiratory droplet transmission in one of two contact ferrets.

To identify novel mutations in avian H5 HAs that confer human-type receptor-binding preference, we introduced random mutations into the globular head (amino acids 120–259 (H3 numbering), which includes the receptor-binding pocket) of A/Vietnam/1203/2004 (H5N1; VN1203) HA (Supplementary Fig. 1). Although this virus was isolated from a human, its HA retains avian-type receptor-binding properties[6,15]. We also replaced the multibasic HA cleavage sequence with a non-virulent-type cleavage sequence, allowing us to perform studies in biosafety level 2 containment (http://www.who.int/csr/resources/publications/influenza/influenzaRMD2003_5.pdf). The mutated polymerase chain reaction (PCR) products were cloned into RNA polymerase I plasmids[16] containing the VN1203 HA complementary DNA, which resulted in *Escherichia coli* libraries representing the randomly generated HA variants. Sequence analysis of 48 randomly selected clones indicated an average of 1.0 amino acid changes per HA globular head (data not shown). To generate an H5N1 virus library, plasmids for the synthesis of the mutated HA gene and the unmodified NA gene of VN1203 were transfected into human embryonic kidney (293T) cells together with plasmids for the synthesis of the six remaining viral genes of A/Puerto Rico/8/34 (H1N1; PR8), a laboratory-adapted human influenza A virus.

Turkey red blood cells (TRBCs; which possess both Siaα2,6Gal and Siaα2,3Gal on their surface (data not shown)) were treated with *Salmonella enterica* serovar Typhimurium LT2 sialidase, which preferentially removes α2,3-linked sialic acid (that is, avian-type receptors), creating TRBCs that predominantly possess Siaα2,6Gal on the cell surface (Siaα2,6-TRBCs; Supplementary Fig. 2). The virus library was then adsorbed to Siaα2,6-TRBCs at 4 °C and extensively washed to remove nonspecifically or weakly bound viruses. Bound viruses were eluted by incubation at 37 °C for 30 min, and then diluted to approximately ~0.5 viruses per well (on the basis of a pilot experiment that

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assessed the approximate number of eluted viruses). We screened one-third of the library (that is, 2.1×10^6 viruses) in three separate selection experiments (that is, 0.7×10^6 viruses per experiment) and isolated 370 viruses that bound to Siaα2,6-TRBCs (Supplementary Fig. 2). Individual viruses were then grown in Madin-Darby canine kidney (MDCK) cells modified to overexpress Siaα2,6Gal (AX4 cells[17]), and screened again for their ability to agglutinate Siaα2,6-TRBCs (Supplementary Fig. 2). The parental control virus (designated VN1203/PR8) with avian-type receptor-binding specificity agglutinated untreated TRBCs (which possess both human- and avian-type receptors on their surface), but not TRBCs possessing predominantly human-type receptors (Siaα2,6-TRBCs; Supplementary Table 1). By contrast, of the 370 viruses originally isolated, nine agglutinated Siaα2,6-TRBCs, albeit with different efficiencies (Supplementary Table 1). All nine viruses possessed mutations in the region targeted for random mutagenesis; one mutant also possessed an additional mutation (E119G) in an area that was not targeted for mutation. Most of the mutations clustered around the receptor-binding pocket (Fig. 1a). Several of the selected viruses possessed mutations known to increase binding to human-type receptors, including N186K (ref. 9), S227N (ref. 5) and Q226L (which confers human-type receptor binding together with G228S)[15] (all shown in blue in Fig. 1a). The identification of known determinants of human-type receptor-binding specificity from a library of random mutants validates our approach. Notably, our screen also identified mutations not previously associated with receptor-binding specificity.

Although viruses were diluted to ~0.5 viruses per well for amplification in AX4 cells, we cannot exclude the possibility that some wells were infected with more than one virus, resulting in mixed populations. To confirm the significance of the identified mutations in HA for human-type receptor binding, the mutations were engineered into a VN1203/PR8 virus (possessing an avirulent HA cleavage site sequence, as described earlier). All nine mutants were generated; however, after two passages in MDCK cells, the S136N mutation reverted to the wild-type sequence. This mutant was excluded from further evaluation.

First, we confirmed the binding of the remaining eight variants to Siaα2,6-TRBCs (Supplementary Table 1). For comparison, we included a VN1203/PR8 virus with two changes in its HA (Q226L and G228S) previously shown to have increased binding to Siaα2,6Gal[6,15]. Indeed, compared to the wild-type VN1203/PR8 virus, the Q226L/G228S mutant displayed an increased ability to bind to human-type receptors. For the recreated variants, haemagglutination titres were higher and slightly different from the initial characterization, which we attribute to biological differences (the initial characterization was carried out with non-concentrated cell culture supernatant and potentially mixed virus populations, whereas the recreated viruses were concentrated and purified) and to experimental differences (that is, differences between the TRBC batches or the efficiency of α2,3-sialidase treatment, or both). Collectively, however, these experiments demonstrate that this random mutagenesis approach allows the identification of hitherto unrecognized amino acid substitutions that permit avian virus HAs to bind to human-type receptors.

To characterize further the receptor-binding properties of the selected variants, we used solid-phase binding assays in which sialylglycopolymers were absorbed to plates, which were then incubated with virus (Fig. 2a). A virus possessing the HA and NA genes of the seasonal human A/Kawasaki/173/2001 (H1N1; K173) virus and the remaining genes from PR8 (K173/PR8) served as a control virus with typical human-type receptor specificity. Indeed, K173/PR8 preferentially bound to Siaα2,6Gal. In contrast, VN1203/PR8 bound to only Siaα2,3Gal. As reported elsewhere[6,15], the Q226L/G228S mutations led to increased binding to Siaα2,6Gal. Variants I202T/R220S, W153R/T160I, N169I/H184L/I217M and H130Q/K157E resembled VN1203/PR8 in their binding to glycans, despite the fact that these mutants weakly agglutinated Siaα2,6-TRBCs (see Supplementary Table 1). These viruses may have bound to glycans on TRBCs that were different from Siaα2,6Galβ1,4GlcNAc used in this study. However, variants N186K/M230I, S227N/G228A and Q226L/E231G showed an appreciable increase in binding to Siaα2,6Gal but also retained binding capacity for Siaα2,3Gal. Of all of the variants tested, only E119G/V152I/N224K/Q226L exhibited specificity for only Siaα2,6Gal. Thus, only one H5 HA variant with receptor-binding capability akin to that of seasonal influenza viruses was isolated from the library screen of 2.1×10^6 viruses. To identify the amino acid change(s) responsible for the conversion from Siaα2,3Gal to Siaα2,6Gal recognition in the E119G/V152I/N224K/Q226L virus HA, we tested the amino acid changes at positions 119, 152, 224 and 226 individually and in various combinations. Solid-phase binding assays demonstrated that the N224K/Q226L combination is critical for the shift from Siaα2,3Gal to Siaα2,6Gal recognition (Fig. 2b); Q226L in combination with V152I also conferred weak binding to α2,6-glycans.

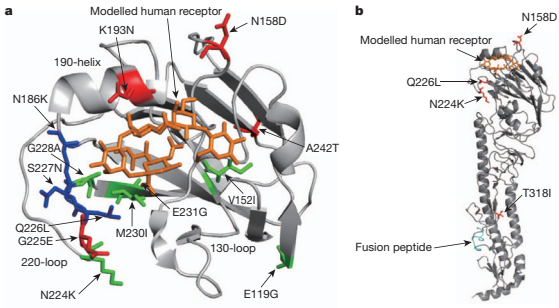

Figure 1 | Localization of amino acid changes identified in this study on the three-dimensional structure of the monomer of VN1203 HA (Protein Data Bank accession 2FK0)[15]. **a**, Close-up view of the globular head of VN1203 HA. Mutations known to increase affinity to human-type receptors are shown in blue. Amino acid changes not previously known to affect receptor binding are shown in green. Additional mutations that occurred in the HA of H5 avian–human reassortant viruses during replication and/or transmission in ferrets are shown in red. **b**, The positions of four mutations in the HA of H5 transmissible reassortant mutant virus, HA(N158D/N224K/Q226L/T318I)/CA04, are highlighted in red. The fusion peptide of HA is shown in cyan. All mutations are shown with H3 numbering. Images were created with MacPymol (http://www.pymol.org/).

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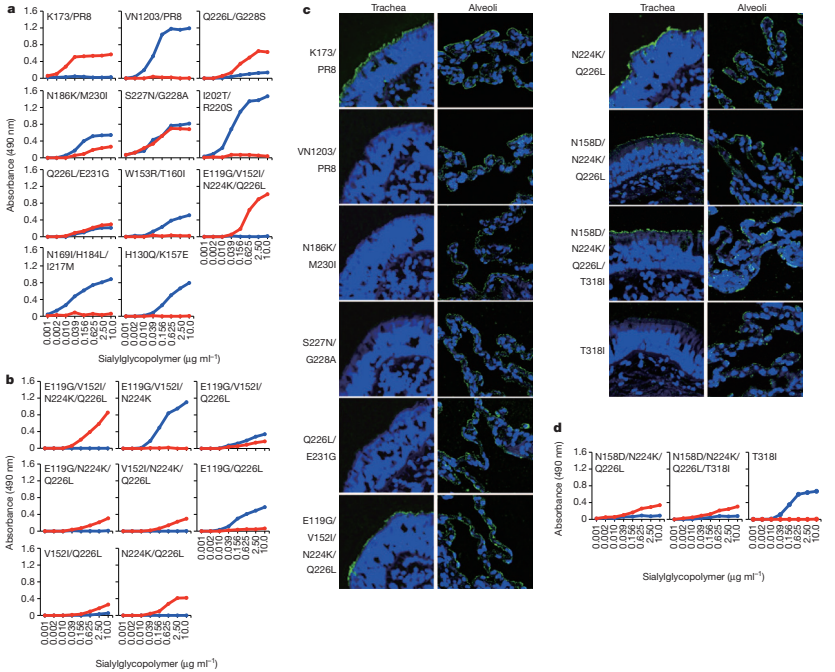

Figure 2 | Characterization of the receptor-binding properties of isolated viruses. **a**, Binding of VN1203 mutants to sialylglycopolymers in solid-phase binding assays. A human virus (K173/PR8), an avian virus (VN1203/PR8) and mutant VN1203/PR8 viruses were compared for their ability to bind to sialylglycopolymers containing either α2,3-linked (blue) or α2,6-linked (red) sialic acids. **b**, Identification of mutations that confer binding to human-type receptors. **c**, Binding of VN1203 mutant viruses to human respiratory tissues. K173/PR8, VN1203/PR8 and mutant VN1203/PR8 viruses were incubated with human tissue sections and then stained with either anti-K173 antiserum (green) or anti-VN1203 HA antibodies (green). All sections were subsequently incubated with labelled secondary antibodies and Hoechst dye (blue). **d**, Characterization of the receptor-binding properties of N158D/N224K/Q226L, N158D/N224K/Q226L/T318I and T318I viruses. The direct binding of virus to sialylglycopolymers containing either α2,3-linked (blue) or α2,6-linked (red) sialic acids was determined as described in panel **a**.

To assess the effect of enhanced α2,6-glycan recognition on the attachment of viruses to human respiratory tracts, sections of tracheal and lung tissues were exposed to K173/PR8 (human-type receptor binder), VN1203/PR8 (avian-type receptor binder) and mutant VN1203/PR8 viruses (Fig. 2c). Because the N186K/M230I, S227N/G228A, Q226L/E231G, E119G/V152I/N224K/Q226L and N224K/Q226L mutants exhibited appreciable binding to Siaα2,6Gal (Fig. 2a, b), the attachment of these mutants was also tested. On tracheal sections, the K173/PR8 virus bound extensively to ciliated epithelial cells (Fig. 2c and Supplementary Fig. 3), whereas the VN1203/PR8 virus bound poorly. By contrast, on lung sections, both viruses bound extensively to the alveolar epithelial surface (both type I and II pneumocytes; Fig. 2c and Supplementary Fig. 4). The binding patterns of these viruses correlate with the distribution of Siaα2,3Gal (that is, avian-type receptors; present in lung epithelia) and Siaα2,6Gal (that is, human-type receptors; present in both trachea and lung epithelia) on the tissues, as observed with lectin staining[18] (Supplementary Fig. 5). Like the human K173/PR8 virus, the E119G/V152I/N224K/Q226L and N224K/Q226L mutants exhibited strong binding to the ciliated epithelial cells of the trachea (Fig. 2c and Supplementary Fig. 3). By contrast, the N186K/M230I, S227N/G228A and Q226L/E231G mutants displayed little-to-no binding to tracheal epithelia (Fig. 2c), despite their binding to Siaα2,6Gal (Fig. 2a). A number of sialylated oligosaccharides with differing branching patterns and chain lengths are thought to be present on the cell surface[19]. We therefore speculate that the mutants can recognize a short glycan structure such as Siaα2,6Galβ1,4GlcNAc, but may not recognize longer, more complex glycan structures, which are possibly required for binding to human tracheal epithelium. On the other hand, all mutants bound to alveolar epithelial cells (both type I and II pneumocytes; Fig. 2c and Supplementary Fig. 4). When the tissue sections were pre-treated with *Arthrobacter ureafaciens* sialidase (which cleaves all non-reducing terminally branched and unbranched sialic acids), virus binding to the tissues was substantially reduced (Supplementary Fig. 6a–c), confirming the sialic acid binding specificity of the virus. These data indicate that alterations in the receptor specificity of the E119G/V152I/N224K/Q226L and N224K/Q226L

mutants have profound effects on virus attachment to human respiratory epithelium.

In an avian H3 HA, the Q226L mutation changed the binding preference from avian- to human-type[20]. A previous study found that the Q226L mutation on an H5 HA does not confer efficient binding to α2,6-glycans in a glycan array[15]; however, when tested in combination with G228S, increased binding to human-type receptors, but not a complete switch from avian- to human-type receptor-binding specificity, was observed[15]. By contrast, here we found that Q226L in combination with N224K resulted in a switch from Siaα2,3Gal to Siaα2,6Gal binding in an H5 HA and allowed virus binding to human tracheal epithelia (Fig. 2c). The receptor-binding domain of HA is formed by the 190-helix at the top of HA, the 220-loop at the edge of the globular head, and the 130-loop at the other edge of the globular head (Fig. 1a). Crystal structure analysis revealed that the 220-loop of avian H5 HA is closer to the opposing 130-loop than in human H3 HA, indicating that a wider binding site for human H3 HA, compared to that of avian H5 HA, may be required to optimize contacts with the larger Siaα2,6-glycans[21]. N224 lies on the turn leading into the 220-loop, adjacent to position 226 (Fig. 1a). Replacement of N224 may alter the orientation of the 220-loop and thus optimize contacts between L226 and Siaα2,6Gal-containing receptors, thereby increasing the preference for α2,6 linkages.

Recent studies reported that 2009 pandemic H1N1 and H5N1 viruses show high genetic compatibility[22,23]. These two viruses have been isolated from pigs[24–28], which have been considered as 'mixing vessels' for the reassortment of avian, swine and human strains. Thus, the coexistence of H5N1 and 2009 pandemic H1N1 viruses could provide an opportunity for the generation of transmissible H5 avian–human reassortants in mammals. Therefore, we generated reassortant viruses possessing the mutant VN1203 HAs generated above, and the seven remaining gene segments from a prototype 2009 pandemic H1N1 virus (A/California/04/2009, CA04). Experiments with viruses possessing the wild-type HA cleavage site were performed in enhanced biosafety level 3 (BSL3+) containment laboratories approved for such use by the Centers for Disease Control and Prevention (CDC) and the United States Department of Agriculture (USDA). Because efficient human-to-human transmission is a critical feature of pandemic influenza viruses, we examined the growth and transmissibility of reassortant viruses in ferrets, which are widely accepted as an animal model for influenza virus transmissibility and pathogenesis studies. Because the E119G/V152I/N224K/Q226L and N224K/Q226L variants bound extensively to human tracheal epithelia (Fig. 2c), we generated by reverse genetics (rg) three H5 reassortant viruses possessing the VN1203 HA or mutant HAs (all with the wild-type multibasic cleavage site) and the remaining genes from the CA04 virus. The VN1203 HA mutants tested included the one containing four mutations, E119G, V152I, N224K and Q226L (designated rg(E119G/V152I/N224K/Q226L)/CA04), and another containing two mutations, N224K and Q226L (designated rg(N224K/Q226L)/CA04).

To determine whether the introduced HA mutations affected the replication of the H5 reassortant viruses, six ferrets were inoculated intranasally with 10^6 plaque-forming units (p.f.u.) of virus. On day 3 after infection, a recombinant virus whose genes all came from CA04, rgCA04, replicated efficiently in the respiratory organs of infected animals, and was isolated from the colon, but not from any other organs tested (Fig. 3 and Supplementary Table 2). A virus possessing H5 VN1203 HA and the remaining genes from CA04 (designated rgVN1203/CA04) replicated to titres comparable to those of rgCA04 in nasal turbinates, but substantially less in the lungs. By contrast, the two H5 reassortant viruses with HA mutations (rg(E119G/V152I/N224K/Q226L)/CA04 and rg(N224K/Q226L)/CA04) were severely limited in their replicative ability in trachea. Although virus titres in nasal turbinates and lung were not statistically different between rg(N224K/Q226L)/CA04 and rgCA04, the virus titre in nasal turbinates was significantly lower in animals inoculated with rg(E119G/V152I/N224K/Q226L)/CA04 than in animals inoculated with rgCA04 (Dunnett's test; $P = 0.0002$; Fig. 3). Notably, rgVN1203/CA04 (avian-type receptor binder) replicated efficiently in nasal turbinates of ferrets, which have a similar sialic acid receptor distribution pattern to that of the human respiratory tract[29,30]. The reason for this discrepancy is unclear; however, replication of avian H5N1 viruses in ferret nasal turbinates has been reported[12,13].

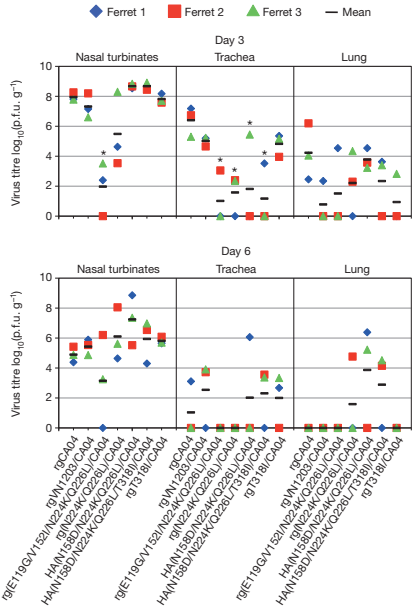

Figure 3 | Virus replication in respiratory organs. Ferrets were infected intranasally with 10^6 p.f.u. of virus. Three ferrets per group were killed on days 3 and 6 after infection for virus titration. Virus titres in nasal turbinates, trachea and lung were determined by use of a plaque assay on MDCK cells. Horizontal bars show the mean. Asterisks indicate virus titres significantly different from that of rgCA04 (Dunnett's test; $P < 0.05$).

Although virus titres in respiratory organs were generally lower on day 6 after infection than on day 3 after infection, rg(N224K/Q226L)/CA04 still showed high levels of replication at day 6 after infection; titres in nasal turbinates ranged from $10^{4.6}$ to $10^{8.1}$ p.f.u. g^{-1} (Fig. 3). Sequence analysis of viruses in nasal turbinates on day 6 after infection revealed that viruses in ferret 2 and ferret 3 possessed N158D and N158K mutations in their HA (in addition to the original two mutations), respectively, leading to the loss of the glycosylation site at position 158 (that is, 158N-S-T to 158D-S-T or 158K-S-T; Fig. 1a and Supplementary Table 3). In nasal turbinates on day 6 after infection, the titre of the virus with the N158D/N224K/Q226L mutations ($10^{8.1}$ p.f.u. g^{-1}; see Fig. 3, ferret 2 of rg(N224K/Q226L)/CA04) was approximately four orders of magnitude higher than that of the original rg(N224K/Q226L)/CA04 ($10^{4.6}$ p.f.u. g^{-1}; Fig. 3, ferret 1 of rg(N224K/Q226L)/CA04), whereas the virus with the N158K/N224K/Q226L mutations ($10^{5.6}$ p.f.u. g^{-1}; Fig. 3, ferret 3 of rg(N224K/Q226L)/CA04) grew to one order of magnitude higher than

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the original mutant. These data indicate that the additional mutation N158D improved the replication of rg(N224K/Q226L)/CA04 in ferrets. To test the effect of this mutation on the replication of H5 reassortant viruses in ferrets, we examined the replicative ability of a virus with the triple N158D/N224K/Q226L HA substitutions in ferrets. This HA(N158D/N224K/Q226L)/CA04 virus replicated efficiently in infected animals, except in the trachea (Fig. 3 and Supplementary Table 2). On day 3 after infection, this virus was isolated from the brain of two of the three animals tested, although we did not observe neurological signs in these animals. These results indicate that the N158D mutation contributed to the efficient growth in the nasal turbinates of ferrets of an H5 reassortant virus with the N224K/Q226L mutations. Removal of the glycosylation site at position 158 has been reported to result in enhanced binding of H5N1 viruses to human-type receptors in combination with the Q226L/G228S mutations[7]. A previous study showed that H5N1 viruses lacking this glycosylation site transmit efficiently by direct contact among guinea-pigs[31]. By contrast, H5N1 viruses that acquire this glycosylation site lose the ability to transmit among guinea-pigs. Therefore, we speculated that the loss of the glycosylation site in HA(N158D/N224K/Q226L)/CA04 virus may affect its transmissibility in ferrets.

To assess the ability of H5 reassortant viruses with human-type receptor specificity to transmit between ferrets, we placed naive ferrets in wireframe cages next to ferrets inoculated with 10^6 p.f.u. of rgCA04, rgVN1203/CA04, rg(N224K/Q226L)/CA04, or HA(N158D/N224K/Q226L)/CA04 (Supplementary Fig. 7). Similar to previous experiments[32], rgCA04 was efficiently transmitted via respiratory droplets to all three contact ferrets, as evidenced by the detection of virus in nasal washes and haemagglutination inhibition (HI) antibody in these animals (Table 1 and Fig. 4). By contrast, rgVN1203/CA04 and rg(N224K/Q226L)/CA04 were not transmitted; neither virus shedding nor seroconversion was detected in any contact animals, despite the binding of the latter to Siaα2,6Gal. This result was consistent with that of previous studies in which human-type receptor recognition was shown to be necessary but not sufficient for respiratory droplet transmission of an H5N1 virus in a ferret model[12,14]. In the HA(N158D/N224K/Q226L)/CA04-inoculated group, virus was recovered from two of the six contact ferrets (pairs 1 and 2) between days 5 and 7 after contact. Moreover, seroconversion was detected in five animals including those from which virus was recovered. No animals died in the course of these transmission experiments. This finding demonstrates the generation of an H5 HA that supports virus transmission by respiratory droplets among ferrets.

To determine whether additional mutations occurred in the HA of HA(N158D/N224K/Q226L)/CA04 during transmission, viral RNA was analysed from nasal washes of inoculated and contact ferrets (Fig. 4 and Supplementary Table 4). On day 5 after infection, the A242S and T318I mutations in HA were present in five (pairs 1, 3, 4, 5 and 6) and one (pair 2) of the six inoculated animals, respectively. Viruses derived from the contact animals of pair 1 on day 7 after contact had two changes in HA (K193N and A242S) (Fig. 1a), whereas those derived from the contact animals of pair 2 contained a single change in HA (T318I) (Fig. 1b), indicating that additional changes in HA occurred during the infection of ferrets with HA(N158D/N224K/Q226L)/CA04. No mutations in the remaining genes were detected in any of these viruses from nasal washes compared with the CA04 virus sequences.

Because HA(N158D/N224K/Q226L)/CA04 was isolated from only one-third of the contact animals, we isolated a virus from the nasal wash of the contact ferret that shed a high titre ($10^{7.5}$ p.f.u. ml^{-1}) of virus on day 7 after contact (pair 2) (Fig. 4d) to evaluate the replication and transmissibility of that virus in ferrets. This mutant virus, designated HA(N158D/N224K/Q226L/T318I)/CA04, replicated efficiently in the nasal turbinates and was isolated from brain tissue (Fig. 3 and Supplementary Table 2). In the transmission study, four of the six contact ferrets were positive for virus between days 3 and 7 after contact, and all contact animals were seropositive; no animals died in the course of the transmission experiments (Table 1; Fig. 4e and Supplementary Fig. 8). Notably, this transmission pattern is comparable to that of the 1918 pandemic H1N1 virus when tested under the same experimental conditions; the 1918 pandemic virus was recovered from the nasal wash of two of three contact animals (our own unpublished data). Sequence comparison of viruses from inoculated and contact animals identified mutations at positions 225 and 242 as well as a reversion at position 224 (Fig. 1a and Supplementary Table 5) (in addition to the original four mutations) although the 224 reversion was found only in viruses from inoculated ferrets. Collectively, these findings demonstrate that four amino acid substitutions (N158D/N224K/Q226L/T318I) in H5 HA confer efficient respiratory droplet transmission in ferrets to a virus possessing an H5 HA in a 2009 pandemic H1N1 backbone. We also confirmed that recombinant viruses possessing the three HA mutations N158D, N224K and Q226L, or the four HA mutations N158D, N224K, Q226L and T318I, and the NA of VN1203 in a PR8 backgrand (designated N158D/N224K/Q226L or N158D/N224K/Q226L/T318I, respectively) preferentially bind to Siaα2,6Gal and attach to human tracheal epithelia (Fig. 2c, d).

HA(N158D/N224K/Q226L/T318I)/CA04 transmitted by respiratory droplet more efficiently than HA(N158D/N224K/Q226L)/CA04, raising the possibility that the T318I mutation is involved in the efficient transmission of avian H5N1/pandemic H1N1 reassortants. To explore the functional role of this mutation in respiratory droplet transmission, we generated an H5 reassortant expressing the H5 HA with the T318I mutation and examined its receptor-binding specificity and transmissibility. This reassortant (designated rgT318I/CA04) bound to only Siaα2,3Gal and showed little binding to human tracheal epithelia (Fig. 2c, d). rgT318I/CA04 did not transmit via respiratory droplet among ferrets (Table 1 and Fig. 4f), although it replicated in nasal turbinates and trachea as efficiently as rgCA04 (Fig. 3 and Supplementary Table 2). These results indicate that the T318I mutation alone is not sufficient for H5 reassortant viruses to transmit efficiently among ferrets.

Table 1 | Transmission in ferrets inoculated with H5 avian–human reassortant viruses

Virus	Inoculated ferrets			Contact ferrets	
	Weight loss (%)*	Peak virus titre in nasal wash (mean $\log_{10}$(p.f.u. ml^{-1})) (days after inoculation)	Seroconversion (positive and total numbers) (HI titre)†	Virus detection in nasal wash (positive and total numbers)	Seroconversion (positive and total numbers) (HI titre)
rgCA04	3 of 3 (15.1)	7.5 (1)	3 of 3 (≥1,280, ≥1,280, ≥1,280)	3 of 3	3 of 3 (≥1,280, ≥1,280, ≥1,280)
rgVN1203/CA04	3 of 3 (5.9)	5.3 (5)	3 of 3 (80, 40, 80)	0 of 3	0 of 3 (<10, <10, <10)
rg(N224K/Q226L)/CA04	2 of 3 (7.8)‡	3.9 (5)	3 of 3 (≥1,280, ≥1,280, ≥1,280)	0 of 3	0 of 3 (<10, <10, <10)
HA(N158D/N224K/Q226L)/CA04	6 of 6 (5.7)	6.7 (3)	6 of 6 (640, ≥1,280, ≥1,280, 640, ≥1,280, ≥1,280)	2 of 6	5 of 6 (160, 320, 20, 160, 40, <10)
HA(N158D/N224K/Q226L/T318I)/CA04	6 of 6 (9.8)	6.1 (5)	6 of 6 (≥1,280, ≥1,280, 640, ≥1,280, ≥1,280, ≥1,280)	4 of 6	6 of 6 (640, 640, ≥1280, 80, ≥1,280, 320)
rgT318I/CA04	3 of 5 (1.5)§	5.6 (3)	5 of 5 (40, 20, 20, 40, 40)	0 of 5	0 of 5 (<10, <10, <10, <10, <10)

* Maximum percentage weight loss is shown.
† Haemagglutination inhibition (HI) assays were carried out with homologous virus and turkey red blood cells.
‡ One animal did not lose any body weight.
§ Two animals did not lose any body weight.

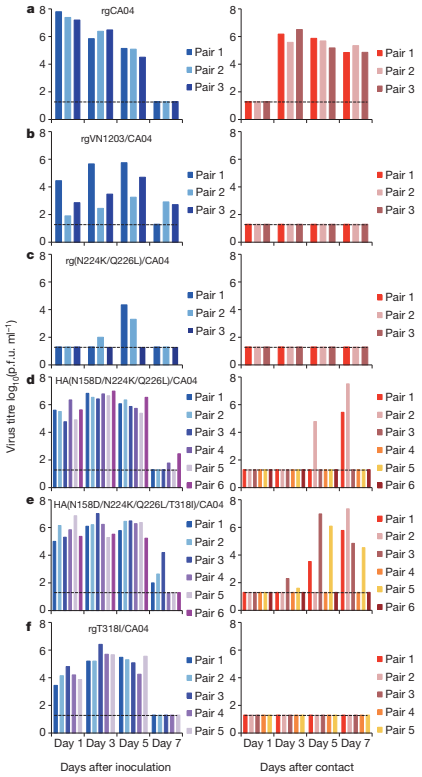

Figure 4 | Respiratory droplet transmission of H5 avian–human reassortant viruses in ferrets. **a**–**f**, Groups of three, five, or six ferrets were inoculated intranasally with 10^6 p.f.u. of rgCA04 (**a**), rgVN1203/CA04 (**b**), rg(N224K/Q226L)/CA04 (**c**), HA(N158D/N224K/Q226L)/CA04 (**d**), HA(N158D/N224K/Q226L/T318I)/CA04 (**e**), or rgT318I/CA04 (**f**). One day after infection, three, five, or six naive ferrets were placed in adjacent cages. Nasal washes were collected every other day from both inoculated (left panel) and contact (right panel) animals for virus titration. Virus titres in organs were determined by using a plaque assay on MDCK cells. The lower limit of detection is indicated by the horizontal dashed line.

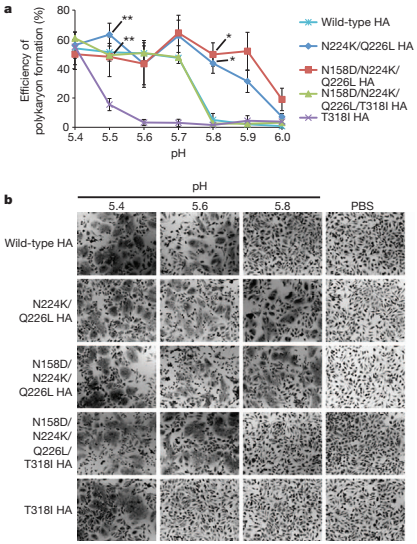

Figure 5 | Polykaryon formation by HeLa cells expressing wild-type or mutant HAs after acidification at low pH. **a**, The efficiency of polykaryon formation over a pH range of 5.4–6.0 was estimated from the number of nuclei in polykaryons divided by the total number of nuclei in the same field. The mean and standard deviations determined from five randomly chosen fields of cell culture are shown. Single asterisks indicate values significantly different between the wild-type HA and the N224K/Q226L or N158D/N224K/Q226L HA (Tukey test; $P < 0.05$). The double asterisk indicates values significantly different between the T318I HA and the N224K/Q226L or N158D/N224K/Q226L HA (Tukey test; $P < 0.05$). **b**, Representative fields of cells expressing the indicated HAs and exposed to pH 5.4, 5.6, or 5.8 are shown. Images were taken at ×10 magnification.

Influenza virus HA protein has membrane-fusion as well as receptor-binding activity. Notably, in the three-dimensional model of influenza A virus HA, residue 318 is located proximally to the fusion peptide (Fig. 1b), which has key roles in the membrane fusion process. To assess the effect of HA mutations on low-pH-induced membrane fusion activity, we examined the pH at which the fusion activity of wild-type and mutant HA was activated (Fig. 5). The wild-type HA had a threshold for membrane fusion of pH 5.7; the N224K/Q226L and N158D/N224K/Q226L mutations raised the threshold for fusion to >pH 5.9, whereas the T318I mutation reduced the threshold for fusion to pH 5.5. The N158D/N224K/Q226L/T318I mutations showed wild-type fusogenic properties (that is, a threshold at pH 5.7). The HA of influenza virus undergoes a low-pH-dependent conformational change, which is required for fusion of the viral envelope with the target membrane[33]. Such a conformational change to a fusion-active form can also lead to viral inactivation. Therefore, sustained and efficient human-to-human transmission of virus may require a certain level of stability of the HA protein in an acidic environment, as the pH of human nasal mucosa, where human influenza viruses replicate primarily, is approximately pH 5.5–6.5 (ref. 34). Our findings suggest that an increase in the pH threshold for fusion as a result of the N224K/Q226L mutations that shift the HA receptor recognition from avian-type to human-type may reduce HA protein stability; however, the T318I mutation decreases the pH threshold for fusion activity, resulting in a stable mutant HA.

Because heat treatment at neutral pH is also known to promote a fusogenic form of HA protein[35,36] and serve as a surrogate assay for HA stability[37], we next tested whether the HA mutations described above affect the heat stability of the HA protein. Wild-type and mutant HA viruses were incubated at 50 °C for various times, after which the loss of infectivity and haemagglutination activity were determined. The wild-type and N224K/Q226L viruses lost most of their infectivity by heating for 60 min (>5.5-$\log_{10}$ decrease in titre; Fig. 6a), whereas the

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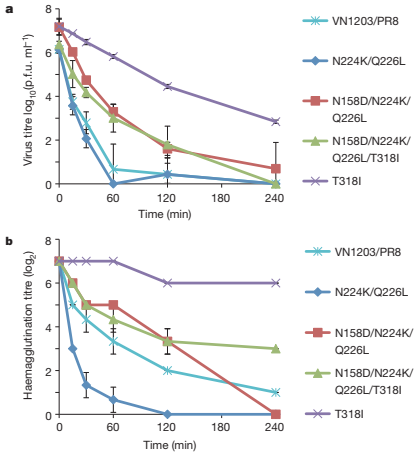

Figure 6 | Effect of heat treatment on the infectivity and haemagglutination activity of viruses. Aliquots of a virus stock containing 128 HA units were incubated for the times indicated at 50 °C. **a**, Virus titres in heat-treated samples were determined by plaque assays on MDCK cells. **b**, Haemagglutination titres in heat-treated samples were determined by using haemagglutination assays with 0.5% TRBCs. Each point represents the mean ± standard deviation from triplicate experiments.

N158D/N224K/Q226L and N158D/N224K/Q226L/T318I mutants exhibited considerable tolerance to high temperature (3.9- and 3.4-$\log_{10}$ decrease after a 60-min incubation, respectively) and the T318I mutant was most resistant (only a 1.4-$\log_{10}$ decrease under the same conditions). In haemagglutination assays, the N224K/Q226L mutant HA lost activity more rapidly than did the wild-type HA, and N158D/N224K/Q226L lost activity more rapidly than did the N158D/N224K/Q226L/T318I mutant (Fig. 6b). Thus, addition of the N158D mutation to the N224K/Q226L HA increased HA stability and subsequent addition of the fourth mutation, T318I, rendered the HA protein even more stable. Taken together, these results suggest that the addition of the T318I mutation to H5 HAs that preferentially recognize human-type receptors restores HA protein stability, thereby allowing a virus carrying the N158D/N224K/Q226L/T318I mutations in HA to transmit efficiently via respiratory droplet among ferrets. In conclusion, a fine balance of mutations affecting different functions in HA (such as receptor-binding specificity and HA stability) may be critical to confer transmissibility in ferrets.

We next compared the pathogenicity in ferrets of H5 avian–human reassortants with that of the pandemic H1N1 virus CA04 (Fig. 7, Supplementary Information and Supplementary Figs 9–11). The control virus, rgCA04, caused substantial body weight loss (15.1%) (Table 1 and Supplementary Fig. 9). By contrast, the four reassortant viruses caused only modest weight loss (<10%) in most of the animals. However, no statistically significant differences in body weight loss were found between the reassortant viruses and rgCA04. Pathological examination revealed similar histological changes and levels of viral antigens in the nasal mucosa of rgCA04-, HA(N158D/N224K/Q226L)/CA04- and HA(N158D/N224K/Q226L/T318I)/CA04-infected ferrets (Fig. 7a, b). In the rgVN1203/CA04 and rg(N224K/Q226L)/CA04 groups, however, less tissue damage was found in the nasal mucosa compared with the rgCA04 group on day 3 after infection (Dunnett's test; $P = 0.0057$ and 0.0175, respectively; Fig. 7b). In addition, all three

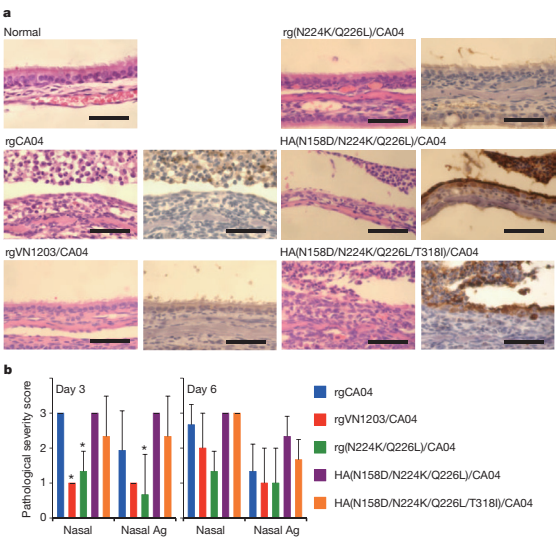

Figure 7 | Pathological analyses of H5 avian–human reassortant viruses. **a**, Representative histological changes in nasal turbinates from influenza-virus-infected ferrets. Three ferrets per group were infected intranasally with 10^6 p.f.u. of virus, and tissues were collected on day 3 after infection for pathological examination. Uninfected ferret tissues served as negative controls (normal). Left panel, haematoxylin-and-eosin staining. Right panel, immunohistochemical staining for viral antigen detection (brown staining). Scale bars, 50 μm. **b**, Pathological severity scores in infected ferrets. To represent comprehensive histological changes, respiratory tissue slides were evaluated by scoring the pathological changes and viral antigen expression levels. The pathological scores were determined for each animal in each group ($n = 3$ per group on days 3 and 6 after infection) using the following scoring system: 0, no pathological change/antigen negative; 1, affected area (<30%) or only interstitial lesion/rare viral antigens; 2, affected area (<80%, ≥30%)/moderate viral antigens; 3, severe lesion (≥80%)/many viral antigens. Nasal, pathological changes in the nasal mucosa; nasal Ag, viral antigens in the nasal mucosa. Asterisks indicate virus pathological scores significantly different from that of rgCA04 (Dunnett's test; $P < 0.05$). Error bars denote standard deviation.

viruses caused lung lesions (Supplementary Information and Supplementary Figs 10 and 11).

To assess whether current control measures may be effective against the H5 transmissible reassortant mutant virus, we examined the reactivity of sera from individuals vaccinated with an H5N1 prototype vaccine[38] against a virus possessing the N158D/N224K/Q226L/T318I mutations in HA. We found that pooled human sera from individuals immunized with this vaccine reacted with the virus possessing the mutant H5 HA (N158D/N224K/Q226L/T318I) at a higher titre than with a wild-type H5 HA virus (VN1203/PR8; Supplementary Table 6), indicating that current H5N1 vaccines would be efficacious against the H5 transmissible reassortant mutant virus. In addition, the H5 transmissible reassortant mutant virus (HA(N158D/N224K/Q226L/T318I)/CA04) was highly susceptible to a licensed NA inhibitor, oseltamivir (Supplementary Table 7). These experiments show that appropriate control measures would be available to combat the transmissible virus described in this study.

Currently, we do not know whether the mutations that we identified in this study that allowed the HA(N158D/N224K/Q226L/T318I)/CA04 virus to be transmissible in ferrets would also support sustained human-to-human transmission. In particular, we wish to emphasize that the transmissible HA(N158D/N224K/Q226L/T318I)/CA04 virus possesses seven segments (all but the HA segment) from a human pandemic 2009 H1N1 virus. Human-virus-characteristic amino acids in these seven segments may have critically contributed to the respiratory droplet transmission of the HA(N158D/N224K/Q226L/T318I)/CA04 virus in ferrets. Examples include amino acids in the PB2 polymerase protein that confer efficient replication in mammalian, but not avian, cells[39–43]. As the PB2 gene of the HA(N158D/N224K/Q226L/T318I)/CA04 virus is of human virus origin, the virus possesses high replicative ability in mammalian cells. In contrast, most avian virus PB2 proteins lack these human-type amino acids, although one of these changes (a glutamic-acid-to-lysine mutation at position 627) is found in highly pathogenic avian H5N1 viruses circulating in the Middle East[44]. As a second example, the viral NA gene may contribute to viral transmissibility. The NA protein cleaves α-ketosidic linkages between a terminal sialic acid and an adjacent sugar residue, an activity that balances the sialic-acid-binding activity of HA. A recent study found that a human virus NA gene was critical to confer limited transmissibility to a mutant H5 avian-human reassortant virus[14]. In general, a human-type receptor recognizing H5 HA alone may not be sufficient to confer transmissibility in mammals, but may have to act together with other human-virus-characteristic traits (in PB2, NA, and/or other viral proteins). Therefore, at this point we cannot predict whether the four mutations in the H5 HA identified here would render a wholly avian H5N1 virus transmissible.

Three of the residues identified here (N224, Q226 and T318) have been strictly conserved among H5 HA proteins isolated since 2003. However, as H5N1 viruses continue to evolve and infect people, receptor-binding variants of H5N1 viruses, including avian–human reassortant viruses as tested here, may emerge. One of the four mutations we identified in our transmissible virus, the N158D mutation, results in loss of a glycosylation site. Many H5N1 viruses isolated in the Middle East, Africa, Asia and Europe do not have this glycosylation site. Therefore, only three nucleotide changes are needed for the HA of these viruses to support efficient transmission in ferrets. In addition, the H5N1 viruses circulating in these geographic areas also possess a glutamic-acid-to-lysine mutation at position 627 in the PB2 protein, which promotes viral replication in certain mammals, including humans[40,45]. Therefore, these viruses may be several steps closer to those capable of efficient transmission in humans and are of concern.

Our study highlights the pandemic potential of viruses possessing an H5 HA. Although current vaccines may protect against a virus similar to that tested here, the continued evolution of H5N1 viruses reinforces the need to prepare and update candidate vaccines to H5 viruses. The amino acid changes identified here will help individuals conducting surveillance in regions with circulating H5N1 viruses (for example, Egypt, Indonesia, Vietnam) to recognize key residues that predict the pandemic potential of isolates. Rapid responses in a potential pandemic situation are essential in order to generate appropriate vaccines and initiate other public health measures to control infection. Furthermore, our findings are of critical importance to those making public health and policy decisions.

Our research answers a fundamental question in influenza research: can H5-HA-possessing viruses support transmission in mammals? Moreover, our findings have suggested that different mechanisms (that is, receptor-binding specificity and HA stability) may act in concert for efficient transmissibility in mammals. This knowledge will facilitate the identification of additional mutations that affect viral transmissibility; the monitoring of this expanded set of changes in natural isolates may improve our ability to assess the pandemic potential of H5N1 viruses. Thus, although a pandemic H5N1 virus may not possess the amino acid changes identified in our study, the findings described here will advance our understanding of the mechanisms and evolutionary pathways that contribute to avian influenza virus transmission in mammals.

METHODS SUMMARY

Viruses. All recombinant viruses were generated by using reverse genetics essentially as described previously[16]. All experiments with the viruses possessing the wild-type HA cleavage site were performed in an enhanced biosafety level 3 (BSL3+) containment laboratory approved for such use by the CDC and the USDA.

Infection and transmission in ferrets. Six–ten-month-old female ferrets (Triple F Farms) were intramuscularly anaesthetized and intranasally inoculated with 10^6 p.f.u. (500 μl) of virus. On days 3 and 6 after infection, ferrets were killed for virological and pathological examinations. The virus titres in various organs were determined by use of plaque assays in MDCK cells.

For transmission studies in ferrets, animals were housed in adjacent transmission cages that prevented direct and indirect contact between animals but allowed spread of influenza virus through the air (Showa Science; Supplementary Fig. 7). Ferrets were intranasally inoculated with 10^6 p.f.u. (500 μl) of virus (inoculated ferrets). Twenty-four hours after infection, naive ferrets were each placed in a cage adjacent to an inoculated ferret (contact ferrets). To assess viral replication in the nasal turbinates, we determined viral titres in nasal washes collected from virus-inoculated and contact ferrets on day 1 after inoculation or co-housing, respectively, and then every other day. Animal studies were performed in accordance with Animal Care and Use Committee guidelines of the University of Wisconsin-Madison.

Biosafety and biosecurity. All recombinant DNA protocols were approved by the University of Wisconsin-Madison's Institutional Biosafety Committee after risk assessments were conducted by the Office of Biological Safety, and by the University of Tokyo's Subcommittee on Living Modified Organisms, and, when required, by the competent minister of Japan. In addition, the University of Wisconsin-Madison Biosecurity Task Force regularly reviews the research program and ongoing activities of the laboratory. The task force has a diverse skill set and provides support in the areas of biosafety, facilities, compliance, security and health. Members of the Biosecurity Task Force are in frequent contact with the principal investigator and laboratory personnel to provide oversight and assure biosecurity. Experiments with viruses possessing the wild-type HA cleavage site were performed in enhanced BSL3 containment laboratories approved for such use by the CDC and the USDA. Ferret transmission studies were conducted by three scientists with both DVM and PhD degrees who each had more than a minimum of 6 years of experience with highly pathogenic influenza viruses and animal studies with highly pathogenic viruses. Our staff wear powered air-purifying respirators that filter the air, and disposable coveralls; they shower out on exit from the facility. The containment facilities at University of Wisconsin-Madison were designed to exceed standards outlined in Biosafety in Microbiological and Biomedical Laboratories (5th edition; http://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf). Features of the BSL3-enhanced suites include entry/exit through a shower change room, effluent decontamination, negative air-pressure laboratories, double-door autoclaves, double HEPA-filtered exhaust air, and gas decontamination ports. The BSL3-Agriculture suite features include all those listed for BSL3-enhanced plus HEPA-filtered supply and double-HEPA-filtered exhaust air, double-gasketed watertight and airtight seals, airtight dampers on all ductwork, and the structure was pressure-decay tested during commissioning. The University of Wisconsin-Madison facility has a dedicated alarm system that monitors all building controls and sends alarms (~500 possible alerts). Redundancies and emergency resources are built-in to the facility including two

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air handlers, two compressors, two filters each place filters are needed, two effluent sterilization tanks, two power feeds to the building, an emergency generator in case of a power failure and other physical containment measures in the facility that operate without power. Biosecurity monitoring of the facility is ongoing. All personnel undergo Select Agent security risk assessment by the United States Criminal Justice Information Services Division and complete rigorous biosafety, BSL3 and Select Agent training before participating in BSL3-level experiments. Refresher training is scheduled on a regular basis. The principal investigator participates in training sessions and emphasizes compliance to maintain safe operations and a responsible research environment. The laboratory occupational health plan is in compliance with the University of Wisconsin-Madison Occupational Health Program. Select agent virus inventory is checked monthly and submitted to the University of Wisconsin-Madison Research Compliance Specialist. Virus inventory is submitted 1–2 times per year to the file holder in the Select Agent branch of the CDC. The research program, procedures, occupational health plan, documentation, security and facilities are reviewed annually by the University of Wisconsin-Madison Responsible Official and at regular intervals by the CDC and the Animal and Plant Health Inspection Service (APHIS) as part of the University of Wisconsin-Madison Select Agent Program.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Author Contributions M.I., T.W., M.H., S.C.D., M.O., K.S., G.Z., A.H., H.K., S.W., C.L., S.Y., M.K., Y.S., E.A.M., G.N. and Y.K. designed the experiments; M.I., T.W., M.H., S.C.D., M.O., K.S., G.Z., A.H., H.K., S.W., C.L., S.Y. and M.K. performed the experiments; M.I., T.W., M.H., S.C.D., M.O., K.S., G.Z., A.H., H.K., S.W., C.L., E.K., S.Y., M.K., Y.S., E.A.M., G.N. and Y.K. analysed the data; M.I., T.W., M.H., S.C.D., K.S., E.A.M., G.N. and Y.K. wrote the manuscript; M.I., T.W. and M.H. contributed equally to this work.

METHODS

Cells. Madin–Darby canine kidney (MDCK) cells and MDCK cells overexpressing Siaα2,6Gal (AX4 cells[17]) were maintained in Eagle's minimal essential medium (MEM) containing 5% newborn calf serum. Human embryonic kidney 293T cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (FBS). HeLa cells were maintained in MEM containing 10% FBS. All cells were maintained at 37 °C in 5% CO_2.

Plasmid construction and reverse genetics. Plasmid constructs for viral RNA production (pPolI)—containing the genes of the A/Vietnam/1203/2004 (H5N1; VN1203), A/Puerto Rico/8/34 (H1N1; PR8), A/Kawasaki/173/2001 (H1N1; K173) and A/California/04/2009 (H1N1; CA04) viruses flanked by the human RNA polymerase I promoter and the mouse RNA polymerase I terminator—were constructed as described[16]. The multibasic amino acids at the haemagglutinin (HA) cleavage site (RERRRKKR↓G) of the reassortant viruses between VN1203 and PR8 were changed to RETR↓G by site-directed mutagenesis. All transfectant viruses were generated by using reverse genetics essentially as described previously[16]. Recombinant viruses were amplified in MDCK or AX4[17] cells and stored at −80 °C until use. The HA segment of all viruses was sequenced to ensure the absence of unwanted mutations. All experiments with the reassortant viruses between VN1203 and CA04 were performed in enhanced biosafety level 3 containment laboratories approved for such use by the CDC and the USDA.

To introduce random mutations into the globular head of the VN1203 HA protein, a 143-amino-acid region spanning residues 120–259 (H3 numbering) was selected. This region was subjected to PCR-based random mutagenesis by use of the GeneMorph II kit (Stratagene) following the manufacturer's instructions. The targeted mutation rate (1–2 amino acid replacements per molecule) was achieved through optimization of the template quantity, and was confirmed by sequence analysis of 48 individual clones. By using a PCR-based cloning strategy, we inserted the mutagenized region into its respective vector containing the VN1203 HA gene between the human RNA polymerase I promoter and mouse RNA polymerase I terminator sequences. The composition of the plasmid library was confirmed by sequencing. The plasmid library was then used to generate an influenza virus library, essentially as described[16]. The size of the virus library was 7×10^6 p.f.u.

Preparation of sialidase-treated TRBCs. Turkey red blood cells (TRBCs) were washed three times with phosphate-buffered saline (PBS), and diluted to 20% (vol/vol) in PBS. TRBCs (1 ml) were incubated with 500 U of α2,3-sialidase from *Salmonella enterica* serovar Typhimurium LT2 (NEB) for 20–24 h at 37 °C, washed three times in PBS, and re-suspended in PBS or MEM containing 1% bovine serum albumin (BSA) (MEM/BSA).

Haemagglutination assay. Viruses (50 µl) were serially diluted with 50 µl of PBS in a microtitre plate. An equal volume (that is, 50 µl) of a 0.5% (vol/vol) TRBC suspension was added to each well. The plates were kept at room temperature and haemagglutination was assessed after a 1-h incubation.

Virus library screening. To select VN1203 HA variants that had acquired the ability to recognize human-type receptors, three parallel experiments were carried out, each with 0.7×10^6 viruses. The virus library was first incubated with 0.1 ml of 10% (vol/vol) α2,3-sialidase-treated TRBCs for 10 min at 4 °C. After this incubation, the TRBCs and bound viruses were pelleted at 1,000 r.p.m. for 1 min, and the pellets then washed ten times in MEM/BSA containing 313 mM NaCl. Bound viruses were eluted by incubation at 37 °C for 30 min and then diluted to approximately 0.5 virus per well (determined by virus titration in a pilot study). Individual viruses were then amplified in AX4 cells, which overexpress Siaα2,6Gal[17]. Individual viruses were re-screened by using haemagglutination assays with α2,3-sialidase-treated TRBCs.

Solid-phase binding assay. Viruses were grown in MDCK cells, clarified by low-speed centrifugation, laid over a cushion of 30% sucrose in PBS, and ultracentrifuged at 25,000 r.p.m. for 2 h at 4 °C. Virus stocks were aliquoted and stored at −80 °C. Virus concentrations were determined by using haemagglutination assays with 0.5% (vol/vol) TRBCs. The direct receptor-binding capacity of viruses was examined by use of a solid-phase binding assay as previously described[9]. Microtitre plates (Nunc) were incubated with the sodium salts of sialylglycopolymers (poly-L-glutamic acid backbones containing *N*-acetylneuraminic acid linked to galactose through either an α2,3 (Neu5Acα2,3Galβ1,4GlcNAcβ1-pAP) or an α2,6 (Neu5Acα2,6Galβ1,4GlcNAcβ1-pAP) bond) in PBS at 4 °C overnight. After the glycopolymer solution was removed, the plates were blocked with 0.15 ml of PBS containing 4% BSA at room temperature for 1 h. After four successive washes with ice-cold PBS, the plates were incubated in a solution containing influenza virus (8–32 HA units in PBS) at 4 °C overnight. After washing as described above, the plates were incubated for 2 h at 4 °C with rabbit polyclonal antiserum to either K173 or VN1203 virus. The plates were then washed again as before and incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG antiserum for 2 h at 4 °C. After washing, the plates were incubated with *O*-phenylenediamine (Sigma) in PBS containing 0.01% H_2O_2 for 10 min at room temperature, and the reaction was stopped with 0.05 ml of 1 M HCl. The optical density at 490 nm was determined in a plate reader (Infinite M1000; Tecan).

Virus binding to human airway tissues. Paraffin-embedded normal human trachea (US Biological) and lung (BioChain) tissue sections were deparaffinized and rehydrated. Sections were then blocked by using 4% BSA in PBS and covered with virus suspensions (64 HA units in PBS) at 4 °C overnight. After being washed four times in ice-cold PBS, the sections were incubated with primary antibodies for 3 h at 4 °C. The primary antibodies used were as follows: a pool of mouse anti-VN1203 HA monoclonal antibodies (15A3, 3G2, 7A11, 8A3, 14C5 and 18E1; Rockland); rabbit anti-K173 polyclonal antibody; rabbit anti-surfactant protein A polyclonal antibody (Millipore); and mouse anti-surfactant protein A monoclonal antibody (Abcam). Antibody binding was detected by using an IgG secondary antibody conjugated with Alexa Fluor 488 or Alexa Fluor 633 (Molecular Probes). Sections were also counterstained with Hoechst 33342, trihydrochloride, trihydrate (Molecular Probes). The samples were examined by using confocal laser scanning microscopy (model LSM 510; Carl Zeiss).

To confirm sialic-acid-specific virus binding, tissue sections were treated, before incubation with viruses, with *Arthrobacter ureafaciens* sialidase (Sigma) for 3 h at 37 °C. Viruses bound to tissue were detected as described above.

Experimental infection of ferrets. Animal studies were performed in accordance with the Animal Care and Use Committee guidelines of the University of Wisconsin-Madison. We used 6–10-month-old female ferrets (Triple F Farms) that were serologically negative by haemagglutination inhibition (HI) assay for currently circulating human influenza viruses. Six ferrets per group were anaesthetized intramuscularly with ketamine and xylazine (5–30 mg and 0.2–6 mg kg^{-1} of body weight, respectively) and inoculated intranasally with 10^6 p.f.u. (500 µl) of viruses. On days 3 and 6 after infection, three ferrets per group were killed for virological and pathological examinations. The virus titres in various organs were determined by use of plaque assays in MDCK cells.

Excised tissue samples of nasal turbinates, trachea, lungs, brain, liver, spleen, kidney and colon from euthanized ferrets were preserved in 10% phosphate-buffered formalin. Tissues were then trimmed and processed for paraffin embedding and cut into 5-µm-thick sections. One section from each tissue sample was stained by using a standard haematoxylin-and-eosin procedure, whereas another one was processed for immunohistological staining with a mixture of two anti-influenza virus rabbit antibodies (1:2,000; R309 and anti-VN1203; both prepared in our laboratory) that react with CA04 and VN1203, respectively. Specific antigen–antibody reactions were visualized by using an indirect two-step dextran-polymer technique (Dako EnVision system; Dako) and 3,3′ diaminobenzidine tetrahydrochloride staining (Dako).

Ferret transmission study. For transmission studies in ferrets, animals were housed in adjacent transmission cages that prevented direct and indirect contact between animals but allowed spread of influenza virus through the air (Showa Science; Supplementary Fig. 7). Three, five, or six ferrets were inoculated intranasally with 10^6 p.f.u. (500 µl) of virus (inoculated ferrets). Twenty-four hours after infection, three, five, or six naive ferrets were each placed in a cage adjacent to an inoculated ferret (contact ferrets). The ferrets were monitored for changes in body weight and the presence of clinical signs. To assess viral replication in nasal turbinates, we determined viral titres in nasal washes collected from virus-inoculated and contact ferrets on day 1 after inoculation or co-housing, respectively, and then every other day.

Serological tests. Serum samples were collected between days 14 and 20 after infection, treated with receptor-destroying enzyme, heat-inactivated at 56 °C for 30 min, and tested by use of an HI assay with 0.5% TRBCs (http://www.wpro.who.int/entity/emerging_diseases/documents/docs/manualonanimalaidiagnosisandsurveillance.pdf). Viruses bearing homologous HA were used as antigens for the HI tests.

Polykaryon formation representing membrane fusion activity. Monolayers of HeLa cells grown in 12-well plates were transfected with the protein expression vector pCAGGS[46] encoding wild-type or mutant HA. At 24 h after transfection, cells transiently expressing HA protein were treated with trypsin (1 µg ml^{-1}) in MEM containing 0.3% BSA for 30 min at 37 °C to cleave the HA into its HA1 and HA2 subunits. Polykaryon formation was induced by exposing the cells to low-pH buffer (145 mM NaCl, 20 mM sodium citrate (pH 6.0–5.4)) for 2 min at 37 °C. After this exposure, the low-pH buffer was replaced with MEM containing 10% FBS and the cells were incubated for 3 h at 37 °C. The cells were then fixed with methanol and stained with Giemsa's solution and photographed with a digital camera mounted on an inverted microscope (Nikon, Eclipse Ti). For quantitative analyses, cell nuclei were counted in five randomly chosen fields of cell culture. Polykaryon formation activity was calculated from the number of nuclei in polykaryons divided by the total number of nuclei in the same field.

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Thermostability. Viruses (128 HA units in PBS) were incubated for the times indicated at 50 °C. Subsequently, infectivity and haemagglutination activity were determined by use of plaque assays in MDCK cells and haemagglutination assays using 0.5% TRBCs, respectively.

Neuraminidase (NA) inhibition assay. To assess the sensitivity of viruses to the NA inhibitor oseltamivir, NA inhibition assays were performed as described previously[32].

Statistical analysis. All statistical analyses were performed using JMP 9.0.0 (SAS Institute Inc.). The statistical significance of differences between rgCA04 and H5 avian/human reassortant viruses was determined by using a Dunnett's test. Comparisons of polykaryon formation between wild-type and mutant HAs were done using Tukey's test. P values of <0.05 were considered significant.

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H5N1

REPORT

Airborne Transmission of Influenza A/H5N1 Virus Between Ferrets

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Highly pathogenic avian influenza A/H5N1 virus can cause morbidity and mortality in humans but thus far has not acquired the ability to be transmitted by aerosol or respiratory droplet ("airborne transmission") between humans. To address the concern that the virus could acquire this ability under natural conditions, we genetically modified A/H5N1 virus by site-directed mutagenesis and subsequent serial passage in ferrets. The genetically modified A/H5N1 virus acquired mutations during passage in ferrets, ultimately becoming airborne transmissible in ferrets. None of the recipient ferrets died after airborne infection with the mutant A/H5N1 viruses. Four amino acid substitutions in the host receptor-binding protein hemagglutinin, and one in the polymerase complex protein basic polymerase 2, were consistently present in airborne-transmitted viruses. The transmissible viruses were sensitive to the antiviral drug oseltamivir and reacted well with antisera raised against H5 influenza vaccine strains. Thus, avian A/H5N1 influenza viruses can acquire the capacity for airborne transmission between mammals without recombination in an intermediate host and therefore constitute a risk for human pandemic influenza.

Influenza A viruses have been isolated from many host species, including humans, pigs, horses, dogs, marine mammals, and a wide range of domestic birds, yet wild birds in the orders Anseriformes (ducks, geese, and swans) and Charadriformes (gulls, terns, and waders) are thought to form the virus reservoir in nature (*1*). Influenza A viruses belong to the family Orthomyxoviridae; these viruses have an RNA genome consisting of eight gene segments (*2*, *3*). Segments 1 to 3 encode the polymerase proteins: basic polymerase 2 (PB2), basic polymerase 1 (PB1), and acidic polymerase (PA), respectively. These proteins form the RNA-dependent RNA polymerase complex responsible for transcription and replication of the viral genome. Segment 2 also encodes a second small protein, PB1-F2, which has been implicated in the induction of cell death (*4*, *5*). Segments 4 and 6 encode the viral surface glycoproteins hemagglutinin (HA) and neuraminidase (NA), respectively. HA is responsible for binding to sialic acids (SAs), the viral receptors on host cells, and for fusion of the viral and host cell membranes upon endocytosis. NA is a sialidase, responsible for cleaving SAs from host cells and virus particles. Segment 5 codes for the nucleocapsid protein (NP) that binds to viral RNA and, together with the polymerase proteins, forms the ribonucleoprotein complexes (RNPs). Segment 7 codes for the viral matrix structural protein M1 and the ion-channel protein M2 that is incorporated in the viral membrane. Segment 8 encodes the nonstructural protein NS1 and the nucleic-export protein (NEP) previously known as NS2. NS1 is an antagonist of host innate immune responses and interferes with host gene expression, whereas NEP is involved in the nuclear export of RNPs into the cytoplasm before virus assembly (*2*, *3*).

Influenza A viruses show pronounced genetic variation of the surface glycoproteins HA and NA (*1*). Consequently, the viruses are classified based on the antigenic variation of the HA and NA proteins. To date, 16 major antigenic variants of HA and 9 of NA have been recognized in wild birds and are found in numerous combinations designated as virus subtypes (for instance, H1N1, H5N1, H7N7, and H16N3), which are used in influenza A virus classification and nomenclature (*1*, *6*). This classification system is biologically relevant, as natural host antibodies that recognize

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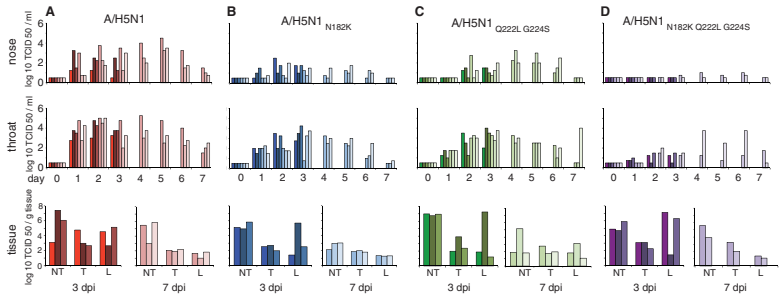

Fig. 1. In experiment 1, we inoculated groups of six ferrets intranasally with 1×10^6 $TCID_{50}$ of (**A**) influenza $A/H5N1_{wildtype}$ virus and the three mutants (**B**) $A/H5N1_{HA\ N182K}$, (**C**) $A/H5N1_{HA\ Q222L,G224S}$, and (**D**) $A/H5N1_{HA\ N182K,Q222L,G224S}$. Three animals were euthanized at day 3 for tissue sampling and at day 7, when this experiment was stopped. Virus titers were measured daily in nose swabs (top) and throat swabs (middle) and also on 3 and 7 dpi in respiratory tract tissues (bottom) of individual ferrets. Virus titers in swabs and nasal turbinates (NT), trachea (T), and lungs (L) were determined by end-point titration in MDCK cells. [One animal inoculated with $A/H5N1_{HA\ N182K,Q222L,G224S}$ died at 1 dpi due to circumstances not related to the experiment (D).] (Top two rows) Virus shedding from the URT as determined by virus titers in nasal and throat swabs was highest in $A/H5N1_{wildtype}$-inoculated animals. The mutant that yielded the highest virus titers during the 7-day period was $A/H5N1_{HA\ Q222L,G224S}$, but titers were ~1 log lower than for the $A/H5N1_{wildtype}$-inoculated animals. In the first 3 days, when six animals per group were present, no significant differences were observed between $A/H5N1_{HA\ N182K}$- and $A/H5N1_{HA\ Q222L,G224S}$-inoculated animals, as calculated by comparing the viral titer (Mann-Whitney test, $P = 0.589$ and 0.818 for nose and throat titers, respectively). (Bottom row) No marked differences in virus titers in respiratory tissues were observed between the four groups. Each bar color denotes a single animal.

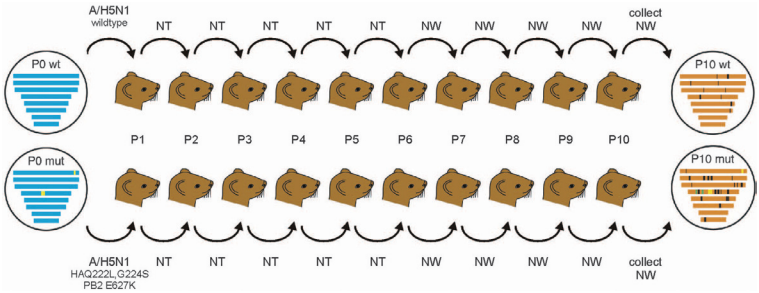

Fig. 2. Experiment 3, virus passaging in ferrets (P1 to P10, passages 1 to 10). Because no airborne transmission was observed in experiment 2, A/H5N1$_{wildtype}$ and A/H5N1$_{HA\ Q222L,G224S\ PB2\ E627K}$ were serially passaged in ferrets to allow adaptation for efficient replication in mammals. Each virus was inoculated intranasally with 1×10^6 $TCID_{50}$ in one ferret (2×250 μl, divided over both nostrils). Nose and throat swabs were collected daily. Animals were euthanized at 4 dpi, and nasal turbinates and lungs were collected. Nasal turbinates were homogenized in virus-transport medium, and this homogenate was used to inoculate the next ferret, resulting in passage 2 (fig. S6). Subsequent passages 3 to 6 were performed in the same way. From passage six onward, nasal washes (NW) were collected at 3 dpi in addition to the nasal swabs. To this end, 1 ml of PBS was delivered drop wise into the nostrils of the ferrets, thereby inducing sneezing. Approximately 200 μl of the sneeze was collected in a Petri dish, and PBS was added to a final volume of 2 ml. For passages 7 through 10, the nasal-wash sample was used for the passages in ferrets. The passage-10 nasal washes were subsequently used for sequence analyses and transmission experiments to be described in experiment 4. For details, see the supplementary materials.

one HA or NA subtype will generally not cross-react with other HA and NA subtypes.

On the basis of their virulence in chickens, influenza A viruses of the H5 and H7 subtypes can be further classified into highly pathogenic avian influenza (HPAI) and low-pathogenic avian influenza (LPAI) viruses. Viruses of subtypes H1 to H4, H6, and H8 to H16 are LPAI viruses. The vast majority of H5 and H7 influenza A viruses are also of the LPAI phenotype. HPAI viruses are generally thought to arise in poultry after domestic birds become infected by LPAI H5 and H7 viruses from the wild-bird reservoir (*7*, *8*). The HA protein of influenza A viruses is initially synthesized as a single polypeptide precursor (HA0), which is cleaved into HA1 and HA2 subunits by trypsin-like proteases in the host cell. The switch from LPAI to HPAI virus phenotype occurs upon the introduction of basic amino acid residues into the HA0 cleavage site, also known as the multibasic cleavage site (MBCS). The MBCS in HA can be cleaved by ubiquitously expressed host proteases; this cleavage facilitates systemic virus replication and results in mortality of up to 100% in poultry (*9*, *10*).

Since the late 1990s, HPAI A/H5N1 viruses have devastated the poultry industry of numerous countries in the Eastern Hemisphere. To date, A/H5N1 has spread from Asia to Europe, Africa, and the Middle East, resulting in the death of hundreds of millions of domestic birds. In Hong Kong in 1997, the first human deaths directly attributable to avian A/H5N1 virus were recorded (*11*). Since 2003, more than 600 laboratory-confirmed cases of HPAI A/H5N1 virus infections in humans have been reported from 15 countries (*12*). Although limited A/H5N1 virus transmission between persons in close contact has been reported, sustained human-to-human transmission of HPAI A/H5N1 virus has not been detected (*13*–*15*). Whether this virus may acquire the ability to be transmitted via aerosols or respiratory droplets among mammals, including humans, to trigger a future pandemic is a key question for pandemic preparedness. Although our knowledge of viral traits necessary for host switching and virulence has increased substantially in recent years (*16*, *17*), the factors that determine airborne

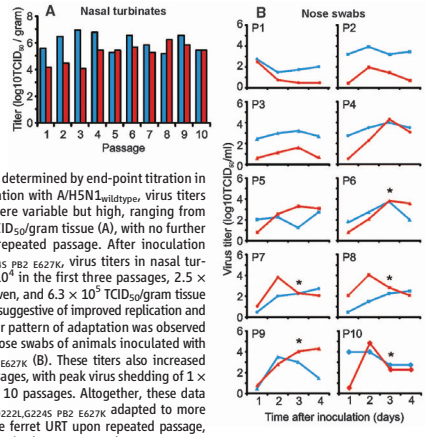

Fig. 3. Virus titers in (**A**) the nasal turbinates collected at day 4 and (**B**) nose swabs collected daily until day 4, from ferrets inoculated with A/H5N1$_{wildtype}$ (blue) and A/H5N1$_{HA\ Q222L,G224S\ PB2\ E627K}$ (red) throughout the 10 serial passages described in Fig. 2. Virus titers were determined by end-point titration in MDCK cells. After inoculation with A/H5N1$_{wildtype}$, virus titers in the nasal turbinates were variable but high, ranging from 1.6×10^5 to 7.9×10^6 $TCID_{50}$/gram tissue (A), with no further increase observed with repeated passage. After inoculation with A/H5N1$_{HA\ Q222L,G224S\ PB2\ E627K}$, virus titers in nasal turbinates averaged 1.6×10^4 in the first three passages, 2.5×10^5 in passages four to seven, and 6.3×10^5 $TCID_{50}$/gram tissue in the last three passages, suggestive of improved replication and virus adaptation. A similar pattern of adaptation was observed in the virus titers in the nose swabs of animals inoculated with A/H5N1$_{HA\ Q222L,G224S\ PB2\ E627K}$ (B). These titers also increased during the successive passages, with peak virus shedding of 1×10^5 $TCID_{50}$ at 2 dpi after 10 passages. Altogether, these data indicate that A/H5N1$_{HA\ Q222L,G224S\ PB2\ E627K}$ adapted to more efficient replication in the ferret URT upon repeated passage, with evidence for such adaptation by passage number 4. In contrast, analyses of the virus titers in the nose swabs of the ferrets collected at 1 to 4 dpi throughout the 10 serial passages with A/H5N1$_{wildtype}$ revealed no changes in patterns of virus shedding. Asterisks indicate that a nose wash was collected before the nose swab was taken, which may influence the virus titer that was detected.

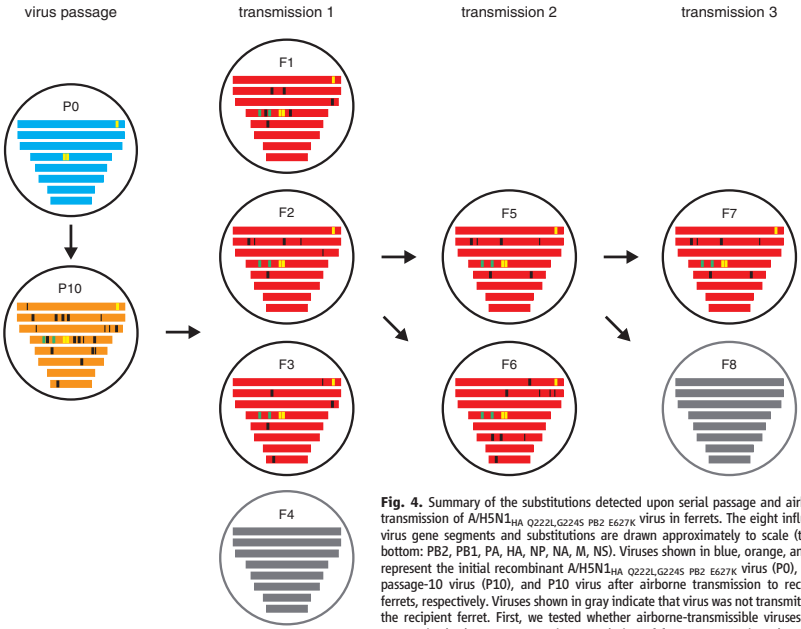

Fig. 4. Summary of the substitutions detected upon serial passage and airborne transmission of A/H5N1$_{HA\ Q222L,G224S\ PB2\ E627K}$ virus in ferrets. The eight influenza virus gene segments and substitutions are drawn approximately to scale (top to bottom: PB2, PB1, PA, HA, NP, NA, M, NS). Viruses shown in blue, orange, and red represent the initial recombinant A/H5N1$_{HA\ Q222L,G224S\ PB2\ E627K}$ virus (P0), ferret passage-10 virus (P10), and P10 virus after airborne transmission to recipient ferrets, respectively. Viruses shown in gray indicate that virus was not transmitted to the recipient ferret. First, we tested whether airborne-transmissible viruses were present in the heterogeneous virus population of ferret P10. We inoculated four donor ferrets intranasally, which were then housed in transmission cages and paired with four recipient ferrets. Transmissible viruses were isolated from three out of four recipient ferrets (F1 to F3). Next, we took a throat-swab sample from F2 (this sample contained the highest virus titer among the positive recipient ferrets), and this sample was used to inoculate two more donor ferrets intranasally. In a transmission experiment, these donors infected two recipient ferrets via airborne transmission (F5 and F6). Virus isolated from F5 was passaged once in MDCK cells and was subsequently used in a third transmission experiment in which two intranasally inoculated donor ferrets transmitted the virus to one of two recipient ferrets (F7). The genetic composition of the viral quasi-species present in the nasal wash of ferret P10 was determined by sequence analysis using the 454/Roche GS-FLX sequencing platform. Conventional Sanger sequencing was used to determine the consensus sequence in one high-titer nasal- or throat-swab sample for each ferret. Thick and thin black vertical bars indicate amino acid and nucleotide substitutions, respectively; substitutions introduced by reverse genetics are shown in yellow; substitutions detected in passage 10 and all subsequent transmissions are shown in green.

transmission of influenza viruses among mammals, a trait necessary for a virus to become pandemic, have remained largely unknown (*18*–*21*). Therefore, investigations of routes of influenza virus transmission between animals and on the determinants of airborne transmission are high on the influenza research agenda.

The viruses that caused the major pandemics of the past century emerged upon reassortment (that is, genetic mixing) of animal and human influenza viruses (*22*). However, given that viruses from only four pandemics are available for analyses, we cannot exclude the possibility that a future pandemic may be triggered by a wholly avian virus without the requirement of reassortment. Several studies have shown that reassortment events between A/H5N1 and seasonal human influenza viruses do not yield viruses that are readily transmitted between ferrets (*18*–*20*, *23*). In our work, we investigated whether A/H5N1 virus could change its transmissibility characteristics without any requirement for reassortment.

We chose influenza virus A/Indonesia/5/2005 for our study because the incidence of human A/H5N1 virus infections and fatalities in Indonesia remains fairly high (*12*), and there are concerns that this virus could acquire molecular characteristics that would allow it to become more readily transmissible between humans and initiate a pandemic. Because no reassortants between A/H5N1 viruses and seasonal or pandemic human influenza viruses have been detected in nature and because our goal was to understand the biological properties needed for an influenza virus to become airborne transmissible in mammals, we decided to use the complete A/Indonesia/5/2005 virus that was isolated from a human case of HPAI A/H5N1 infection.

We chose the ferret (*Mustela putorius furo*) as the animal model for our studies. Ferrets have been used in influenza research since 1933 because they are susceptible to infection with human and avian influenza viruses (*24*). After infection with human influenza A virus, ferrets develop respiratory disease and lung pathology similar to that observed in humans. Ferrets can also transmit human influenza viruses to other ferrets that

serve as sentinels with or without direct contact (fig. S1) (*25*–*27*).

Host restriction of replication and transmission of influenza A viruses is partly determined by specific SA receptors on the surface of susceptible cells. The affinity of influenza viruses for these receptors varies according to the species from which they are isolated. Influenza viruses of avian origin preferentially bind to α-2,3–linked SA receptors, whereas human influenza viruses recognize α-2,6–linked SA receptors. The receptor distribution in ferrets resembles that of humans in that the α-2,6–linked SA receptors are predominantly present in the upper respiratory tract (URT), and the α-2,3–linked SA receptors are mainly present in the lower respiratory tract. In chickens and other birds, α-2,3–linked SAs predominate, but both α-2,3–linked and α-2,6–linked SA are present throughout the respiratory and enteric tracts (fig. S2) (*28*). The differences in receptor distribution between humans and avian species are thought to determine the host restriction of influenza A viruses. A switch in receptor specificity from avian α-2,3–SA to human α-2,6–SA receptors, which can be acquired by specific mutations in the receptor binding site (RBS) of the HA, is expected to be necessary for an avian virus to become transmissible and, thus, gain the potential to become pandemic in humans.

Besides a switch in receptor specificity to facilitate infection of cells in the URT, increased virus production in the URT and efficient release of virus particles from the respiratory tract to yield airborne virus may also be required (*22*). Such traits are likely to be determined by the viral surface glycoproteins and the proteins that form the viral polymerase complex. Amino acid substitutions in the polymerase proteins have already been shown to be major determinants of host range and transmission, including for pandemic influenza viruses (*29*–*31*). Whereas avian viruses, in principle, replicate at temperatures around 41°C (the temperature in the intestinal tract of birds), for replication in humans the viruses need to adapt to 33°C (the temperature of the human URT). The amino acid substitution Glu^{627}→Lys^{627} (E627K) in the polymerase complex protein PB2 has been associated with increased virus replication in mammalian cells at such lower temperatures (*16*, *17*, *32*).

In addition, when newly formed virus particles bud from the host cell membrane after virus replication, the NA present on the virus membrane facilitates the release of particles. For A/H5N1, this process is rather inefficient, and released particles tend to form virus aggregates (*22*). Therefore, a balance between the properties endowed by HA and NA may be required to generate single particles. These established effects were thus used as the basis for the initial substitutions chosen in the current study.

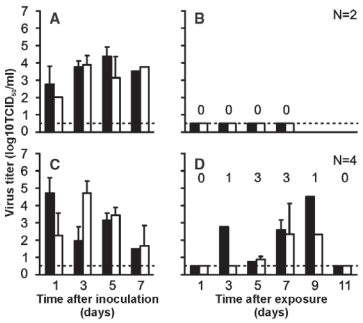

Fig. 5. Airborne transmission of A/H5N1 viruses in ferrets. Transmission experiments are shown for $A/H5N1_{wildtype}$ (**A** and **B**) and $A/H5N1_{HA\ Q222L,G224S\ PB2\ E627K}$ (**C** and **D**) after 10 passages (P10) in ferrets. Two or four ferrets were inoculated intranasally with nasal-wash samples collected from P10 virus of $A/H5N1_{wildtype}$ and $A/H5N1_{HA\ Q222L,G224S\ PB2\ E627K}$, respectively, and housed individually in transmission cages (A and C). A naïve recipient ferret was added to each transmission cage adjacent to a donor ferret at 1dpi (B and D). Virus titers in throat (black bars) and nose swabs (white bars) were determined by end-point titration in MDCK cells. Geometric mean titers and SDs (error bars) of positive samples are shown. The number of animals infected via airborne transmission is indicated in (D) for each time point after exposure; the drop from three animals infected at day 7 to one animal at day 9 and no animals at day 11 is explained by the fact that the animals that became infected via airborne transmission had cleared the virus by the end of the experiment and, therefore, detectable amounts of virus were no longer present. The dotted lines indicate the lower limit of virus detection.

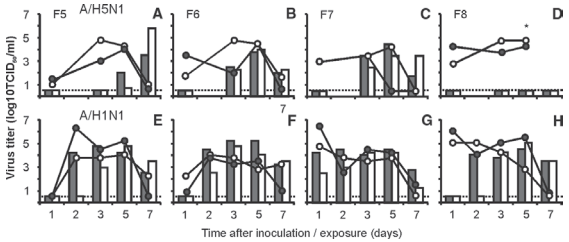

Fig. 6. Comparison of airborne transmission of experimental passaged A/H5N1 and 2009 pandemic A/H1N1 viruses in individual ferrets. A throat-swab sample from ferret F2 at 7 days postexposure (dpe) (Fig. 5D) was used for the transmission experiments shown in (**A**) and (**B**), and a virus isolate obtained from a nose swab collected from ferret F5 at 7 dpi (Fig. 6A) was used for the experiments in (**C**) and (**D**). For comparison, published data on transmission of 2009 pandemic A/H1N1 virus between ferrets is shown in (**E**) to (**H**) (*27*). Data for individual transmission experiments is shown in each panel, with virus shedding in inoculated and airborne virus–exposed animals shown as lines and bars, respectively. For the transmission experiments with airborne-transmissible A/H5N1 (A to D), nose or throat swabs were not collected at 2 dpi and 2 dpe. White circles and bars represent shedding from the nose; black circles and bars represent shedding from the throat. The asterisk indicates the inoculated animal that died 6 days after intranasal inoculation.

Human-to-human transmission of influenza viruses can occur through direct contact, indirect contact via fomites (contaminated environmental surfaces), and/or airborne transmission via small aerosols or large respiratory droplets. The pandemic and epidemic influenza viruses that have circulated in humans throughout the past century were all transmitted via the airborne route, in contrast to many other respiratory viruses that are exclusively transmitted via contact. There is no exact particle size cut-off at which transmission changes from exclusively large droplets to aerosols. However, it is generally accepted that for infectious particles with a diameter of 5 μm or less, transmission occurs via aerosols. Because we did not measure particle size during our experiments, we will use the term "airborne transmission" throughout this Report.

Biosafety and biosecurity concerns have remained foremost in our planning for this research program. The details are explained in the supplementary materials and are summarized here: The enhanced Animal Biosafety Laboratory level 3 (ABSL3+) facility at Erasmus Medical Center

H5N1

Table 1. Lethality of WT and airborne-transmissible A/H5N1 virus in ferrets upon inoculation via different routes. *n*, number of animals; N.A., not applicable.

Inoculation route	Virus	Dead or moribund (no. dead/no. tested)	Day of death postinoculation (no.)
Intratracheal	A/H5N1$_{wildtype}$	6/6*	2 (n = 2), 3 (n = 4)
	A/H5N1/F5	6/6	3 (n = 6)
Intranasal	A/H5N1$_{wildtype}$/P10	2/2†	6 (n = 2)
	A/H5N1$_{HA\ Q222L,G224S\ PB2\ E627K}$/P10	0/4	N.A.
	A/H5N1/F2	0/2	N.A.
	A/H5N1/F5	1/2	6 (n = 1)
Airborne	A/H5N1$_{wildtype}$	N.A.	N.A.
	A/H5N1$_{HA\ Q222L,G224S\ PB2\ E627K}$/P10	0/3	N.A.
	A/H5N1/F2	0/2	N.A.
	A/H5N1/F5	0/1	N.A.

*These data refer to a published study (*45*). †These ferrets were inoculated with P10 H5N1$_{wildtype}$ virus, but data are consistent with previous studies that used larger groups of animals inoculated with the original strain (*39*, *40*).

Table 2. Receptor specificity of the different mutant A/H5N1 viruses, as determined by a modified TRBC hemagglutination assay. Introduction of Q222L and G224S in the A/H5N1 HA resulted in a receptor binding preference switch from the avian α-2,3– to the human α-2,6–linked SA receptor. Subsequent substitution of H103Y and T156A resulted in an increased affinity for α-2,3– and α-2,6–linked SA, in agreement with glycan array studies (*51*). For details, see supplementary experiment 9. HAU, hemagglutination units.

Virus	Subtype	HA titer (HAU/50 μl)		
		TRBC	α-2,3–linked TRBC	α-2,6–linked TRBC
A/Netherlands/213/03	H3N2	64	0	64
A/Vietnam/1194/04	H5N1	64	64	0
A/H5N1$_{PB2\ E627K}$	H5N1	64	16	0
A/H5N1$_{HA\ H103Y,T156A\ PB2\ E627K}$	H5N1	64	48	0
A/H5N1$_{HA\ Q222L,G224S\ PB2\ E627K}$	H5N1	64	0	24
A/H5N1$_{HA\ H103Y,T156A,Q222L,G224S\ PB2\ E627K}$	H5N1	64	4	32

(MC) Rotterdam, the Netherlands, was constructed for the specific purpose of containing pathogenic and transmissible influenza viruses and other pathogens of concern. The facility consists of a negatively pressurized laboratory with an interlock room. All in vivo and in vitro experimental work is carried out in negatively pressurized class 3 isolators or class 3 biosafety cabinets, respectively. The facility is secured by procedures recognized as appropriate by the institutional biosafety officers and facility management at Erasmus MC, as well as Dutch and U.S. government inspectors.

Before and during the research, biosafety officers of Erasmus MC and inspectors from the Dutch government, as well as from the U.S. Centers for Disease Control and Prevention, approved the facilities and procedures. Explicit permits for research on genetically modified airborne-transmissible A/H5N1 virus were obtained from the Dutch government. The research was performed strictly in accordance with the Dutch Code of Conduct for Biosecurity (*33*). All personnel were instructed and trained extensively for working in the ABSL3+ facility, handling (highly pathogenic) influenza virus, and controlling incidents (such as spills). To further prevent occupational risks, research personnel used protective equipment and were offered seasonal and A/H5N1 influenza vaccines (*25*). For emergency purposes, Erasmus MC holds supplies of oseltamivir and has quarantine hospital rooms.

Using a combination of targeted mutagenesis followed by serial virus passage in ferrets, we investigated whether A/H5N1 virus can acquire mutations that would increase the risk of mammalian transmission (*34*). We have previously shown that several amino acid substitutions in the RBS of the HA surface glycoprotein of A/Indonesia/5/2005 change the binding preference from the avian α-2,3–linked SA receptors to the human α-2,6–linked SA receptors (*35*). A/Indonesia/5/2005 virus with amino acid substitutions N182K, Q222L/G224S, or N182K/Q222L/G224S (numbers refer to amino acid positions in the mature H5 HA protein; N, Asn; Q, Gln; L, Leu; G, Gly; S, Ser) in HA display attachment patterns similar to those of human viruses to cells of the respiratory tract of ferrets and humans (*35*). Of these changes, we know that together, Q222L and G224S switch the receptor binding specificity of H2 and H3 subtype influenza viruses, as this switch contributed to the emergence of the 1957 and 1968 pandemics (*36*). N182K has been found in a human case of A/H5N1 virus infection (*37*).

Our experimental rationale to obtain transmissible A/H5N1 viruses was to select a mutant A/H5N1 virus with receptor specificity for α-2,6–linked SA shed at high titers from the URT of ferrets. Therefore, we used the QuickChange multisite-directed mutagenesis kit (Agilent Technologies, Amstelveen, the Netherlands) to introduce amino acid substitutions N182K, Q222L/G224S, or N182K/Q222L/G224S in the HA of wild-type (WT) A/Indonesia/5/2005, resulting in A/H5N1$_{HA\ N182K}$, A/H5N1$_{HA\ Q222L,G224S}$, and A/H5N1$_{HA\ N182K,Q222L,G224S}$. Experimental details for experiments 1 to 9 are provided in the supplementary materials (*25*). For experiment 1, we inoculated these mutant viruses and the A/H5N1$_{wildtype}$ virus intranasally into groups of six ferrets for each virus (fig. S3). Throat and nasal swabs were collected daily, and virus titers were determined by end-point dilution in Madin Darby canine kidney (MDCK) cells to quantify virus shedding from the ferret URT. Three animals were euthanized after day 3 to enable tissue sample collection. All remaining animals were euthanized by day 7 when the same tissue samples were taken. Virus titers were determined in the nasal turbinates, trachea, and lungs collected postmortem from the euthanized ferrets. Throughout the duration of experiment 1, ferrets inoculated intranasally with A/H5N1$_{wildtype}$ virus produced high titers in nose and throat swabs—up to 10 times more than A/H5N1$_{HA\ Q222L,G224S}$, which yielded the highest virus titers of all three mutants during the 7-day period (Fig. 1). However, no significant difference was observed between the virus shedding of ferrets inoculated with A/H5N1$_{HA\ Q222L,G224S}$ or A/H5N1$_{HA\ N182K}$ during the first 3 days when six animals per group were present. Thus, of the viruses with specificity for α-2,6–linked SA, A/H5N1$_{HA\ Q222L,G224S}$ yielded the highest virus titers in the ferret URT (Fig. 1).

As described above, amino acid substitution E627K in PB2 is one of the most consistent host-range determinants of influenza viruses (*29*–*31*). For experiment 2 (fig. S4), we introduced E627K into the PB2 gene of A/Indonesia/5/2005 by site-directed mutagenesis and produced the recombinant virus A/H5N1$_{HA\ Q222L,G224S\ PB2\ E627K}$. The introduction of E627K in PB2 did not significantly affect virus shedding in ferrets, because virus titers in the URT were similar to those seen in A/H5N1$_{HA\ Q222L,G224S}$-inoculated animals [up to 1×10^4 50% tissue culture infectious doses ($TCID_{50}$)] (Mann-Whitney U rank-sum test, $P = 0.476$) (Fig. 1 and fig. S5). When four naïve ferrets were housed in cages adjacent to those with four inoculated animals to test for airborne transmission as described previously (*27*), A/H5N1$_{HA\ Q222L,G224S\ PB2\ E627K}$ was not transmitted (fig. S5).

Because the mutant virus harboring the E627K mutation in PB2 and Q222L and G224S in HA did not transmit in experiment 2, we designed an experiment to force the virus to adapt to replication in the mammalian respiratory tract and to select virus variants by repeated

passage (10 passages in total) of the constructed A/H5N1$_{HA\ Q222L,G224S\ PB2\ E627K}$ virus and A/H5N1$_{wildtype}$ virus in the ferret URT (Fig. 2 and fig. S6). In experiment 3, one ferret was inoculated intranasally with A/H5N1$_{wildtype}$ and one ferret with A/H5N1$_{HA\ Q222L,G224S\ PB2\ E627K}$. Throat and nose swabs were collected daily from live animals until 4 days postinoculation (dpi), at which time the animals were euthanized to collect samples from nasal turbinates and lungs. The nasal turbinates were homogenized in 3 ml of virus-transport medium, tissue debris was pelleted by centrifugation, and 0.5 ml of the supernatant was subsequently used to inoculate the next ferret intranasally (passage 2). This procedure was repeated until passage 6.

From passage 6 onward, in addition to the samples described above, a nasal wash was also collected at 3 dpi. To this end, 1 ml of phosphate-buffered saline (PBS) was delivered dropwise to the nostrils of the ferrets to induce sneezing. Approximately 200 μl of the "sneeze" was collected in a Petri dish, and PBS was added to a final volume of 2 ml. The nasal-wash samples were used for intranasal inoculation of the ferrets for the subsequent passages 7 through 10. We changed the source of inoculum during the course of the experiment, because passaging nasal washes may facilitate the selection of viruses that were secreted from the URT. Because influenza viruses mutate rapidly, we anticipated that 10 passages would be sufficient for the virus to adapt to efficient replication in mammals.

Virus titers in the nasal turbinates of ferrets inoculated with A/H5N1$_{wildtype}$ ranged from ~1×10^5 to 1×10^7 $TCID_{50}$/gram tissue throughout 10 serial passages (Fig. 3A and fig. S7). In ferrets inoculated with A/H5N1$_{HA\ Q222L,G224S\ PB2\ E627K}$ virus, a moderate increase in virus titers in the nasal turbinates was observed as the passage number increased. These titers ranged from 1×10^4 $TCID_{50}$/gram tissue at the start of the experiment to 3.2×10^5 to 1×10^6 $TCID_{50}$/gram tissue in the final passages (Fig. 3A and fig. S7). Notably, virus titers in the nose swabs of animals inoculated with A/H5N1$_{HA\ Q222L,G224S\ PB2\ E627K}$ also increased during the successive passages, with peak virus shedding of 1×10^5 $TCID_{50}$ at 2 dpi after 10 passages (Fig. 3B).These data indicate that A/H5N1$_{HA\ Q222L,G224S\ PB2\ E627K}$ was developing greater capacity to replicate in the ferret URT after repeated passage, with evidence for such adaptation becoming apparent by passage number 4. In contrast, virus titers in the nose swabs of the ferrets collected at 1 to 4 dpi throughout 10 serial passages with A/H5N1$_{wildtype}$ revealed no changes in patterns of virus shedding.

Passaging of influenza viruses in ferrets should result in the natural selection of heterogeneous mixtures of viruses in each animal with a variety of mutations: so-called viral quasi-species (*38*). The genetic composition of the viral quasi-species present in the nasal washes of ferrets after 10 passages of A/H5N1$_{wildtype}$ and A/H5N1$_{HA\ Q222L,G224S\ PB2\ E627K}$ was determined by sequence analysis using the 454/Roche GS-FLX sequencing platform (Roche, Woerden, the Netherlands) (tables S1 and S2). The mutations introduced in A/H5N1$_{HA\ Q222L,G224S\ PB2\ E627K}$ by reverse genetics remained present in the virus population after 10 consecutive passages at a frequency >99.5% (Fig. 4 and table S1). Numerous additional nucleotide substitutions were detected in all viral gene segments of A/H5N1$_{wildtype}$ and A/H5N1$_{HA\ Q222L,G224S\ PB2\ E627K}$ after passaging, except in segment 7 (tables S1 and S2). Of the 30 nucleotide substitutions selected during serial passage, 53% resulted in amino acid substitutions. The only amino acid substitution detected upon repeated passage of both A/H5N1$_{wildtype}$ and A/H5N1$_{HA\ Q222L,G224S\ PB2\ E627K}$ was T156A (T, Thr; A, Ala) in HA. This substitution removes a potential N-linked glycosylation site (Asn-X-Thr/Ser; X, any amino acid) in HA and was detected in 99.6% of the A/H5N1$_{wildtype}$ sequences after 10 passages. T156A was detected in 89% of the A/H5N1$_{HA\ Q222L,G224S\ PB2\ E627K}$ sequences after 10 passages, and the other 11% of sequences possessed the substitution N154K, which removes the same potential N-linked glycosylation site in HA.

In experiment 4 (see supplementary materials), we investigated whether airborne-transmissible viruses were present in the heterogeneous virus population generated during virus passaging in ferrets (fig. S4). Nasal-wash samples, collected at 3 dpi from ferrets at passage 10, were used in transmission experiments to test whether airborne-transmissible virus was present in the virus quasi-species. For this purpose, nasal-wash samples were diluted 1:2 in PBS and subsequently used to inoculate six naïve ferrets intranasally: two for passage 10 A/H5N1$_{wildtype}$ and four for passage 10 A/H5N1$_{HA\ Q222L,G224S\ PB2\ E627K}$ virus.

The following day, a naïve recipient ferret was placed in a cage adjacent to each inoculated donor ferret. These cages are designed to prevent direct contact between animals but allow airflow from a donor ferret to a neighboring recipient ferret (fig. S1) (*27*). Although mutations had accumulated in the viral genome after passaging of A/H5N1$_{wildtype}$ in ferrets, we did not detect replicating virus upon inoculation of MDCK cells with swabs collected from naïve recipient ferrets after they were paired with donor ferrets inoculated with passage 10 A/H5N1$_{wildtype}$ virus (Fig. 5, A and B). In contrast, we did detect virus in recipient ferrets paired with those inoculated with passage 10 A/H5N1$_{HA\ Q222L,G224S\ PB2\ E627K}$ virus. Three (F1 to F3) out of four (F1 to F4) naïve recipient ferrets became infected as confirmed by the presence of replicating virus in the collected nasal and throat swabs (Fig. 5, C and D). A throat-swab sample obtained from recipient ferret F2, which contained the highest virus titer among the ferrets in the first transmission experiment, was subsequently used for intranasal inoculation of two additional donor ferrets. Both of these animals, when placed in the transmission cage setup (fig. S1), again transmitted the virus to the recipient ferrets (F5 and F6) (Fig. 6, A and B). A virus isolate was obtained after inoculation of MDCK cells with a nose swab collected from ferret F5 at 7 dpi. The virus from F5 was inoculated intranasally into two more donor ferrets. One day later, these animals were paired with two recipient ferrets (F7 and F8) in transmission cages, one of which (F7) subsequently became infected (Fig. 6, C and D).

We used conventional Sanger sequencing to determine the consensus genome sequences of viruses recovered from the six ferrets (F1 to F3 and F5 to F7) that acquired virus via airborne transmission (Fig. 4 and table S3). All six samples still harbored substitutions Q222L, G224S, and E627K that had been introduced by reverse genetics. Surprisingly, only two additional amino acid substitutions, both in HA, were consistently detected in all six airborne-transmissible viruses: (i) H103Y (H, His; Y, Tyr), which forms part of the HA trimer interface, and (ii) T156A, which is proximal but not immediately adjacent to the RBS (fig. S8). Although we observed several other mutations, their occurrence was not consistent among the airborne viruses, indicating that of the heterogeneous virus populations generated by passaging in ferrets, viruses with different genotypes were transmissible. In addition, a single transmission experiment is not sufficient to select for clonal airborne-transmissible viruses because, for example, the consensus sequence of virus isolated from F6 differed from the sequence of parental virus isolated from F2.

Together, these results suggest that as few as five amino acid substitutions (four in HA and one in PB2) may be sufficient to confer airborne transmission of HPAI A/H5N1 virus between mammals. The airborne-transmissible virus isolate with the least number of amino acid substitutions, compared with the A/H5N1$_{wildtypes}$, was recovered from ferret F5. This virus isolate had a total of nine amino acid substitutions; in addition to the three mutations that we introduced (Q222L and G224S in HA and E627K in PB2), this virus harbored H103Y and T156A in HA, H99Y and I368V (I, Ile; V, Val) in PB1, and R99K (R, Arg) and S345N in NP (table S3). Reverse genetics will be needed to identify which of the five to nine amino acid substitutions in this virus are essential to confer airborne transmission.

During the course of the transmission experiments with the airborne-transmissible viruses, ferrets displayed lethargy, loss of appetite, and ruffled fur after intranasal inoculation. One of eight inoculated animals died upon intranasal inoculation (Table 1). In previously published experiments, ferrets inoculated intranasally with WT A/Indonesia/5/2005 virus at a dose of 1×10^6 $TCID_{50}$ showed neurological disease and/or

H5N1

death (*39*, *40*). It should be noted that inoculation of immunologically naïve ferrets with a dose of 1×10^6 $TCID_{50}$ of A/H5N1 virus and the subsequent course of disease is not representative of the natural situation in humans. Importantly, although the six ferrets that became infected via respiratory droplets or aerosol also displayed lethargy, loss of appetite, and ruffled fur, none of these animals died within the course of the experiment. Moreover, previous infections of humans with seasonal influenza viruses are likely to induce heterosubtypic immunity that would offer some protection against the development of severe disease (*41*, *42*). It has been shown that mice and ferrets previously infected with an A/H3N2 virus are clinically protected against intranasal challenge infection with an A/H5N1 virus (*43*, *44*).

After intratracheal inoculation (experiment 5; fig. S9), six ferrets inoculated with 1×10^6 $TCID_{50}$ of airborne-transmissible virus F5 in a 3-ml volume of PBS died or were moribund at day 3. Intratracheal inoculations at such high doses do not represent the natural route of infection and are generally used only to test the ability of viruses to cause pneumonia (*45*), as is done for vaccination-challenge studies. At necropsy, the six ferrets revealed macroscopic lesions affecting 80 to 100% of the lung parenchyma with average virus titers of 7.9×10^6 $TCID_{50}$/gram lung (fig. S10). These data are similar to those described previously for $A/H5N1_{wildtype}$ in ferrets (Table 1). Thus, although the airborne-transmissible virus is lethal to ferrets upon intratracheal inoculation at high doses, the virus was not lethal after airborne transmission.

To test the effect of the mutations in HA in the airborne-transmissible virus on its sensitivity to antiviral drugs, we used virus isolated from F5 (experiment 6). This airborne-transmissible virus with nine amino acid substitutions displayed a sensitivity to the antiviral drug oseltamivir similar to that of $A/H5N1_{wildtype}$ (table S4).

In experiment 7, we evaluated the recognition of the airborne-transmissible virus by antisera raised against potential A/H5N1 vaccine strains. Because only HA recognition by antibodies is evaluated in this assay, chimeric viruses were generated based on six gene segments of the mouse-adapted A/Puerto Rico/8/34 (PR8) virus with the HA and PB2 genes of the transmissible virus harboring amino acid substitutions H103Y, T156A, Q222L, and G224S in HA and E627K in PB2. We replaced the MBCS of the HA by a monobasic cleavage site, allowing us to do these experiments under BSL2 conditions. The chimeric PR8/H5 virus reacted well with ferret antisera raised against A/Indonesia/5/2005 and several other prepandemic vaccine strains (table S5). In fact, the presence of the four HA mutations increased the reactivity with H5 antisera by twofold or more.

We subsequently used the same PR8/H5 chimeric virus in experiment 8 to evaluate the presence of existing immunity against the airborne-transmissible virus in sera obtained from human volunteers more than 70 years of age. The introduction of receptor-binding site mutations Q222L/G224S and the mutations H103Y and T156A in HA, acquired during ferret passage, did not result in increased cross-reactivity with human antisera (table S6), indicating that humans do not have antibodies against the HA of the airborne-transmissible A/H5N1 virus that was selected in our experiments.

Substitutions Q222L and G224S have previously been shown to be sufficient to switch receptor-binding specificity of avian influenza strains (i.e., α-2,3–linked SA) to that of human strains (i.e., α-2,6–linked SA) (*20*, *35*, *46*, *47*). Amino acid position 103 is distal from the RBS, forms part of the trimer interface, and is unlikely to affect receptor specificity (fig. S8). T156 is part of a N-glycosylation sequon, and T156A (as well as N154K) would delete this potential glycosylation site (fig. S8); amino acid T156 is proximal but not immediately adjacent to the RBS. Loss of N-glycosylation sites at the tip of HA has been shown to affect receptor binding of A/H1 (*48*, *49*) and the virulence of A/H5 virus (*50*). We evaluated the impact of the HA mutations that emerged during passaging in ferrets in a modified turkey red blood cell (TRBC) assay (Table 2). In this assay, the binding of influenza viruses, with a mutated HA, to normal TRBCs (expressing both α-2,3–linked SA and α-2,6–linked SA) and modified TRBCs with either α-2,3–linked SA or α-2,6–linked SA on the cell surface was evaluated and compared to two reference viruses with known receptor binding preference: avian A/H5N1 and human A/H3N2 viruses. As expected and shown before, introduction of the Q222L and G224S mutations in the HA of A/H5N1 changed the receptor binding preference from α-2,3–linked SA to α-2,6–linked SA (*35*). Furthermore, in our hands, the introduction of substitutions H103Y and T156A not only enhanced binding of $A/H5N1_{HA\ Q222L,G224S\ PB2\ E627K}$ to α-2,6–linked SA, as expected from glycan array studies (*51*), but also increased the affinity for α-2,3–linked SA. When these two mutations were introduced in the $A/H5N1_{wildtype}$ HA, the affinity for α-2,3–linked SA also increased.

Substitutions Q222L and G224S have previously emerged in avian A/H2 and A/H3 viruses in nature (*36*, *52*), and mutations associated with similar changes in receptor binding specificity have been detected repeatedly in A/H5 viruses—for instance, substitution N182K has been reported nine times (*37*, *51*), which is why we initially selected it for our investigations. The other three substitutions we found consistently in airborne-transmissible viruses have all previously been detected in HPAI A/H5N1 viruses circulating in the field (*53*). Only a minor fraction of the A/H5N1 viruses that have circulated in outbreaks have been sequenced (estimated to be <0.001%) (*53*, *54*). Yet the individual substitutions we obtained, as well as combinations of T156A and H103Y or T156A and E627K, have already been reported in public sequence databases (*53*); thus, we conclude that these mutations do not appear to have a detrimental effect on virus fitness. Substitution H103Y has only been found once, in combination with T156A in a duck in China (*53*). Substitution E627K in PB2 has been found in ~27% of avian A/H5N1 virus sequences and in ~29% of human A/H5N1 viruses (*53*). Substitution T156A in HA has been reported in >50% of the viruses sequenced and was detected in 100% of the viruses from human cases in Egypt (*53*).

Investigations of viral quasi-species during a massive avian influenza A/H7N7 virus outbreak in the Netherlands indicated that viruses with human adaptation markers, including HA mutations that alter receptor specificity and mutations in polymerase proteins that increase polymerase activity like E627K in PB2, emerged rapidly in poultry (*55*–*57*). Given the large numbers of HPAI A/H5N1 virus-infected hosts globally, the high viral mutation rate, and the apparent lack of detrimental effects on fitness of the mutations that confer airborne transmission, it may simply be a matter of chance and time before a human-to-human transmissible A/H5N1 virus emerges.

The specific mutations we identified in these experiments that are associated with airborne transmission represent biological traits that may be determined by a set of different amino acid substitutions. For example, amino acid substitutions D701N (D, Asp) or S590G/R591Q in PB2 yield a similar phenotype to E627K (*29*). N182K and other substitutions in the RBS of HA may yield a similar phenotype to Q222L/G224S (*35*). Such mutations should be considered for A/H5N1 surveillance studies in outbreak areas. Imai *et al*. recently identified different RBS changes (N220K, Q222L) along with N154D (affecting the same N-glycosylation sequon as T156A) and T314I in HA as determinants of airborne transmission of an A/H5 virus (*58*). This airborne virus contained seven genes of the 2009 pandemic A/H1N1 virus (which has S590G/R591Q in PB2 rather than E627K), with the HA of A/H5N1 virus A/Vietnam/1203/2004 (*58*). These data indicate that different lineages of A/H5N1 virus and different amino acid substitutions that affect particular biological traits (receptor binding, glycosylation, replication) can yield airborne-transmissible A/H5N1 viruses.

Although our experiments showed that A/H5N1 virus can acquire a capacity for airborne transmission, the efficiency of this mode remains unclear. Previous data have indicated that the 2009 pandemic A/H1N1 virus transmits efficiently among ferrets and that naïve animals shed high amounts of virus as early as 1 or 2 days after exposure (*27*). When we compare the A/H5N1 transmission data with that of reference (*27*), keeping in mind that our experimental design for studying transmission is not quantitative, the data shown in Figs. 5 and 6 suggest that A/H5N1 airborne transmission was less robust, with less

and delayed virus shedding compared with pandemic A/H1N1 virus.

Airborne transmission could be tested in a second mammalian model system such as guinea pigs (*59*), but this would still not provide conclusive evidence that transmission among humans would occur. The mutations we identified need to be tested for their effect on transmission in other A/H5N1 virus lineages (*60*), and experiments are needed to quantify how they affect viral fitness and virulence in birds and mammals. For pandemic preparedness, antiviral drugs and vaccine candidates against airborne-transmissible virus should be evaluated in depth. Mechanistic studies on the phenotypic traits associated with each of the identified amino acid substitutions should provide insights into the key determinants of airborne virus transmission. Our findings indicate that HPAI A/H5N1 viruses have the potential to evolve directly to transmit by aerosol or respiratory droplets between mammals, without reassortment in any intermediate host, and thus pose a risk of becoming pandemic in humans. Identification of the minimal requirements for virus transmission between mammals may have prognostic and diagnostic value for improving pandemic preparedness (*34*).

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Supplementary Materials

www.sciencemag.org/cgi/content/full/336/6088/1534/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S10
Tables S1 to S6
References (*61*–*72*)

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Appendix D

Workshop Agenda

Issues Raised, Lessons Learned, and Paths Forward for Dual-Use Research in the Life Sciences: The H5N1 Research Controversy—A Workshop
20 F Street, NW Conference Center
Washington, DC 20001
May 1, 2012

8:00 **Continental Breakfast**

8:30 **Welcome:** Harvey Fineberg, Institute of Medicine

8:35 **Introduction:** Workshop Planning Committee Co-Chairs

David Relman, Stanford University and VA Palo Alto Health Care System
David Korn, Massachusetts General Hospital and Harvard Medical School

8:40 **Session 1: The Ongoing Revolution in the Life Sciences and Associated Technologies**

Moderator: David Baltimore, California Institute of Technology

Speaker: Roger Brent, Fred Hutchinson Cancer Research Center

Commentators:

Lawrence D. Kerr, National Counterproliferation Center, Office of the Director of National Intelligence

Joe Palca, National Public Radio

9:30 Discussion with Participants

10:00 Break

10:15 Session 2: Two Case Studies from Conceptualization to Dissemination of Findings with Consideration of Plausible Points of Intervention and Decision-Making

Moderator: Alice Huang, California Institute of Technology

Speakers: 1918 Spanish Flu Reconstruction:

Jeffery K. Taubenberger, National Institute of Allergy and Infectious Diseases, National Institutes of Health

H5N1 Avian Influenza:

Robert G. Webster, St. Jude Children's Research Hospital

11:15 Discussion with Participants

12:00 Lunch

1:15 Session 3: Roundtable Discussion on the Nature of the Social Contract

Moderator: Harold T. Shapiro, Princeton University

Speakers: Ruth L. Berkelman, Emory University
Robert Cook-Deegan, Duke University
Gregory E. Kaebnick, The Hastings Center
Daniel J. Kevles, Yale University
Carl Zimmer, Frequent Contributor, The *New York Times*

2:15 Discussion with Participants

3:00 Break

3:15 Session 4: Roundtable Discussion on Governance, Oversight, and the Path Forward

Moderator: Lawrence O. Gostin, Georgetown University

Speakers: Ann Arvin, Stanford University
Anthony S. Fauci, National Institute of Allergy and Infectious Diseases, National Institutes of Health
David Franz, United States Army Medical Research Institute for Infectious Diseases (retired)
David Heymann, U.K. Health Protection Agency
Piers Millet, United Nations Office for Disarmament Affairs' Implementation Support Unit

4:15 Discussion with Participants

5:00 Wrap-Up: Workshop Planning Committee Co-Chairs

David Relman, Stanford University and VA Palo Alto Health Care System
David Korn, Massachusetts General Hospital and Harvard Medical School

5:15 Adjourn

Appendix E

Workshop Attendees

Issues Raised, Lessons Learned, and Paths Forward for Dual-Use Research in the Life Sciences: The H5N1 Research Controversy—A Workshop
20 F Street, NW Conference Center
Washington, DC 20001
May 1, 2012

Kevin Anderson, U.S. Department of Homeland Security
Lida Anestidou, National Academy of Sciences
Jessica Appler
Ronald Atlas, American Society for Microbiology
Patrick Ayscue, National Academy of Sciences
Tali Bar-Shalom, Executive Office of the President
Gaymon Bennett, Fred Hutchinson Cancer Research Center
Frazier Benya, National Academy of Engineering
Sharon Bergquist, American Association for the Advancement of Science
Matt Bisenius, U.S. House of Representatives
Carol Blum, Council on Governmental Relations
Catherin Borden, Federal Judicial Center
Shayne Brannman, U.S. Department of Health and Human Services
Erica Carroll, Defense Threat Reduction Agency
Sara Carter, J. Craig Venter Institute
Joe Cecil, Federal Judicial Center
Christopher Chadwick, George Washington University
Daniel Chertow, National Institutes of Health

Parag Chitnis, National Science Foundation
Anita Cicero, Center for Biosecurity of UPMC
Limor Darash, Stanford Univeristy
Dan Davis, National Institutes of Health
Sally Davis, National Institutes of Health
Gregory Deye, National Institutes of Health
Diane Dieuliis, U.S. Department of Health and Human Services
Dennis Dixon, National Institutes of Health
Laurie Doepel, National Institutes of Health
Joseph Dudley, Science Applications International Corporation
Gerald Epstein, U.S. Department of Homeland Security
Kevin Finneran, National Academy of Sciences
Pat Fitch, National Biodefense Analysis & Countermeasures Center
Bart Forsyth, U.S. House of Representatives
Maggie Fox, *National Journal*
Frank Gottron, Congressional Research Service
Rebecca Gurba, United States Strategic Command
Mireille Guyader, Embassy of France
Peter Hale, The Foundation for Vaccine Research
Edward Hammond, Third World Network
Jaydee Hanson, International Center for Technology Assessment
Robert Hardy, Council on Governmental Relations
Dennis Harris, The National Academies
Jahna Hartwig, Williams Mullen
Hillery Harvey, National Institutes of Health
Erin Heath, American Association for the Advancement of Science
James Holt, U.S. Department of Health and Human Services Office of General Counsel
Alison Hottes
Richard Johnson, Global Helix LLC
Peter Jutro, U.S. Environmental Protection Agency
Rebecca Katz, U.S. Department of State
Bettyann Kevles
Veronique Kiermer, Nature Publishing Group
Jane Knisely, National Institutes of Health
Lori Knowles, University of Alberta, Canada
Gregory Koblentz, George Mason Univesity
Todd Kuiken, Woodrow Wilson Center International Center for Scholars
Laura Kwinn, U.S. Department of Health and Human Services
Linda Lambert, National Institutes of Health
Helen Lawrence, Federal Bureau of Investigation
Theresa Lawrence, U.S. Department of Health and Human Services
Ori Lev, National Institutes of Health

Yaling Liu, Lehigh University
Daniel Lucey, Georgetown University Medical Center
Brendan Maher, *Nature*
David Malakoff, *Science*
James Matthews, Sanofi Pasteur
James Meegan, National Institutes of Health
Julie Meeks, Federal Judicial Center
Amy Nevel, U.S. Department of Health and Human Services
Pauline Newman, United States Court of Appeals
Stuart Nightingale, National Institutes of Health
LeighAnne Olsen, Institute of Medicine
Paula Olsiewski, Alfred P. Sloan Foundation
Terry O'Sullivan, University of Akron
Chris Park, U.S. Department of State
Andreea Paulopol, U.S. Department of State
Eleonore Pauwels, Woodrow Wilson International Center for Scholars
Alan Pearson, U.S. Department of Agriculture
Dana Perkins, U.S. Department of Health and Human Services
Diane Post, National Institutes of Health
Nair Prashant, *Proceedings of the National Academy of Sciences*
David Proctor, National Academy of Sciences
Jean Richards, U.S. Department of Health and Human Services
Corinne Ringholz, Analytic Services, Inc
Tina Saey, *Science News*
Kate Saylor, American Association for the Advancement of Science
William Schulz, *Chemical & Engineering News*
Glenn Schweitzer, National Academy of Sciences
Vivian B. Shapiro
Dana Shea, Congressional Research Service
Toby Smith, Association of American Universities
Amy Smithson, Center for Nonproliferation Studies
Erik Stemmy, National Institutes of Health
Earl Stoddard, University of Maryland Center for Health and Homeland Security
David Tatel, U.S. Court of Appeals for the D.C. Circuit
Ronald Taylor, The National Academies
Terence Taylor, International Council for the Life Sciences
Eric Toner, Center for Biosecurity, UPMC
Nancy Touchette, National Institutes of Health
Jessica Tucker, U.S. Department of Health and Human Services
Ewelina Tunia, U.S. Department of Defense
Richard Tyner, U.S. Department of Agriculture
Serina Vandegrift, U.S. Department of Health and Human Services

Ashok Vaseashta, Institute of Electrical and Electronics Engineers
Kathleen Vogel, Cornell University
Ava Walker, Defense Threat Reduction Agency
Ruixue Wang, National Institutes of Health
Ross White, The Hastings Center
Amali Wijeweera, U.S. Department of Homeland Security
Marcienne Wright, U.S. Department of Health and Human Services

Appendix F

Biographical Information of Committee and Staff

CO-CHAIRS

David Korn, B.A., scl, M.D., cl, Harvard University, is presently Consultant in Pathology at the Massachusetts General Hospital and Professor of Pathology at Harvard Medical School. From November 15, 2008 to June 30, 2011, he was the inaugural Vice-Provost for Research at Harvard University. Prior to joining Harvard, Dr. Korn had served as the Chief Scientific Officer of the Association of American Medical Colleges (AAMC) in Washington, D.C. since January 15, 2007, and before that as the Senior Vice President for Biomedical and Health Sciences Research at the Association since September 1, 1997.

Dr. Korn served as Carl and Elizabeth Naumann Professor and Dean of the Stanford University School of Medicine from October 1984 to April 1995, and as Vice President of Stanford University from January 1986 to April 1995. Previously, he had served as Professor and Chairman of the Department of Pathology at Stanford, and Chief of the Pathology Service at the Stanford University Hospital, since June 1968. Dr. Korn has been Chairman of the Stanford University Committee on Research; President of the American Association of Pathologists (now the American Society for Investigative Pathology), from which he received the Gold-Headed Cane Award for lifetime achievement in 2004; President of the Association of Pathology Chairman, from which he received the Distinguished Service Award in 1999; a member of the Board of Directors and of the Executive Committee of the Federation of American Societies for Experimental

Biology; and a member of the Board of Directors of the Association of Academic Health Centers.

Dr. Korn was a founder and Chairman of the Board of Directors of the California Transplant Donor Network, one of the nation's largest Organ Procurement Organizations. Later, he was a founder of the nonprofit Association for the Accreditation of Human Research Protection Programs, created to enhance and standardize the protection of human research participants. He has been a member of National Academies' Institute of Medicine (IOM) since 1989, has served on many National Academy of Sciences and IOM committees, was a founder of the IOM's Clinical Research Roundtable, and is currently co-chair of the NAS Committee on Science, Technology, and Law. In 1996-1997 Dr. Korn chaired a Special Subcommittee of the Science Board of the Food and Drug Administration to Review the FDA's Intramural Research Program, for which he received the Commissioner's Special Citation and the Harvey W. Wiley Medal. From 1984 to 1991 he served as Chairman of the National Cancer Advisory Board, a position to which he was appointed by President Reagan. Dr. Korn is a Fellow of the AAAS and has served on its Council, and he was a member of the University Grants Committee of Hong Kong from 1998-2004, where he was Chairman of the Medical Subcommittee.

Dr. Korn served on the Boards of Directors of the Stanford University Hospital from October 1982 to April 1995, the Children's Hospital at Stanford from October 1984 to its closure, and the Lucile Salter Packard Children's Hospital at Stanford from October 1984 to April 1995. He was a member of the Board of Directors of the California Society of Pathologists from 1983-1986.

Dr. Korn has been a member of the editorial boards of the *American Journal of Pathology*, *The Journal of Biological Chemistry*, and *Human Pathology*, and for many years was an Associate Editor of the latter. He has sat on many Society Councils and Boards. His more than 250 publications range from bacteriophage biochemistry and genetics to the biochemistry and molecular biology of DNA replication in human cells, and more recently, concern issues of academic values and integrity, research integrity, health and science policy, and financial conflicts of interest in academic medicine.

David A. Relman is the Thomas C. and Joan M. Merigan Professor in the Departments of Medicine and of Microbiology and Immunology at Stanford University, and Chief of Infectious Diseases at the VA Palo Alto Health Care System in Palo Alto, California. He received an S.B. (Biology) from MIT (1977) and M.D. (magna cum laude) from Harvard Medical School (1982), completed his clinical training in internal medicine and infectious diseases at Massachusetts General Hospital, served as a postdoctoral fellow in micro-

biology at Stanford University, and joined the faculty at Stanford in 1994. Dr. Relman's current research focus is the human indigenous microbiota (microbiome), and in particular the nature and mechanisms of variation in patterns of microbial diversity in the human body as a function of time (microbial succession) and space (biogeography within the host landscape) and in response to perturbation, e.g., antibiotics (community robustness and resilience). One of the goals of this work is to define the role of the human microbiome in health and disease. This research integrates theory and methods from ecology, population biology, environmental microbiology, genomics, and clinical medicine. During the past few decades, his research directions have also included pathogen discovery and the development of new strategies for identifying previously unrecognized microbial agents of disease. This work helped to spearhead the application of molecular methods to the diagnosis of infectious diseases in the 1990s. His research has emphasized the use of genomic approaches for exploring host-microbe relationships. Past scientific achievements include the description of a novel approach for identifying previously unknown pathogens, the identification of a number of new human microbial pathogens, including the agent of Whipple's disease, and some of the most extensive and revealing analyses to date of the human indigenous microbial ecosystem. Dr. Relman advises the U.S. government as well as nongovernmental organizations in matters pertaining to microbiology, emerging infectious diseases, and biosecurity. He currently serves as Chair of the Institute of Medicine's Forum on Microbial Threats, a member of the National Science Advisory Board for Biosecurity (NSABB), and a member of the Physical and Life Sciences Directorate Review Committee for Lawrence Livermore National Laboratory, and advises several U.S. government departments and agencies on matters related to pathogen diversity, the future life sciences landscape, and the nature of present and future biological threats. Dr. Relman co-chaired a three-year National Research Council study that produced a widely cited report entitled *Globalization, Biosecurity, and the Future of the Life Sciences* (2006). He is a Fellow of the American Academy of Microbiology, a member of the Association of American Physicians, and currently the President of the Infectious Diseases Society of America. Dr. Relman was the recipient of both the NIH Director's Pioneer Award and the Distinguished Clinical Scientist Award from the Doris Duke Charitable Foundation in 2006. He was elected to the Institute of Medicine in 2011.

MEMBERS

Ruth Berkelman is Rollins Professor and Director, Center for Public Health Preparedness and Research at Emory University and the director of the Emory Preparedness and Emergency Response Research Center. She began

her career in public health as an Epidemic Intelligence Service (EIS) Officer at the Centers for Disease Control and Prevention (CDC). At CDC, she served as Deputy Director of the National Center for Infectious Diseases, and as a Senior Advisor to the Director; she retired from the U.S. Public Health Service in 2000 as Assistant Surgeon General. She is nationally and internationally recognized in infectious diseases and disease surveillance. She has taken leadership roles with national organizations and is currently serving as Chair of the Public and Scientific Affairs Board, American Society of Microbiology. She has been elected to the Institute of Medicine, and was appointed in 2007 to the National Biodefense Science Board.

Gail Cassell is Visiting Professor, Department of Global Health and Social Medicine, Harvard Medical School and former vice president, Scientific Affairs and Distinguished Lilly Research Scholar for Infectious Diseases, Eli Lilly and Company in Indianapolis. She is the former Charles H. McCauley Professor and chair of the Department of Microbiology at the University of Alabama Schools of Medicine and Dentistry at Birmingham, a department that ranked first in research funding from the National Institutes of Health during the decade of her leadership. She obtained her bachelor's from the University of Alabama in Tuscaloosa and in 1993 was selected as one of the top 31 female graduates of the 20th century. She obtained her doctorate in microbiology from the University of Alabama at Birmingham and was selected as its 2003 Distinguished Alumnus.

She is a past president of the American Society for Microbiology (the oldest and single largest life sciences organization with a membership of over 42,000). She was a member of the National Institutes of Health Director's Advisory Committee and a member of the Advisory Council of the National Institute of Allergy and Infectious Diseases of NIH. She was named to the original Board of Scientific Councilors of the Center for Infectious Diseases, Centers for Disease Control and served as chair of the board. She recently served a three-year term on the Advisory Board of the Director of the Centers for Disease Control and Prevention and as a member of the Secretary of Health and Human Services Advisory Council of Public Health Preparedness. Currently she is a member of the Science Board of the Federal Food and Drug Administration. Since 1996, she has been a member of the U.S.-Japan Cooperative Medical Science Program responsible for advising the respective governments on joint research agendas (U.S. State Department/Japan Ministry of Foreign Affairs). She has served on several editorial boards of scientific journals and has authored more than 250 articles and book chapters. Cassell has received national and international awards and an honorary degree for her research in infectious diseases. She is a member of the Institute of Medicine of the National Academy of Sciences and is currently serving a three-year term on the IOM Council, the governing board.

Cassell has been intimately involved in establishment of science policy and legislation related to biomedical research and public health. For nine years she was chair of the Public and Scientific Affairs Board of the American Society for Microbiology; has served as an adviser on infectious diseases and indirect costs of research to the White House Office of Science and Technology Policy; and has been an invited participant in numerous Congressional hearings and briefings related to infectious diseases, antimicrobial resistance, and biomedical research. She has served two terms on the LCME, the accrediting body for U.S. medical schools, as well as other national committees involved in establishing policies in training in the biomedical sciences. Currently she is a member of the Board of Directors of the Burroughs Wellcome Fund, the Leadership Council of the School of Public Health of Harvard University and the Advisory Council of the School of Nursing of Johns Hopkins.

Stanley Falkow is Professor Emeritus, Department of Microbiology and Immunology, Stanford University. He formulated molecular Koch's postulates, which have guided the study of the microbial determinants of infectious diseases since the late 1980s. Dr. Falkow received his B.S. from the University of Maine and went on to earn his Ph.D. from Brown University. He discovered that infectious microorganisms use genes that are activated only inside host cells. Dr. Falkow has published numerous articles and has served on the editorial boards of several professional publications. In addition, he has received numerous awards for his achievements in science, including the Bristol-Myers Squibb Award for Distinguished Achievement in Infectious Disease Research, the Altemeier Medal from the Surgical Infectious Diseases Society of America, the Howard Taylor Ricketts Award Lecture at the University of Chicago, and the Paul Ehrlich–Ludwig Darmstaedter Prize. In 2003, he received the Abbott Lifetime Achievement Award from the American Society for Microbiology and the Selman A. Waksman Award in Microbiology from the National Academy of Sciences (NAS). He received the Robert Koch Award in 2000. Dr. Falkow was president of the American Society for Microbiology in 1997-1998. He was elected to the Institute of Medicine in 1997 and received the Maxwell-Finland Award from the National Foundation for Infectious Diseases in 1999. Also in 1999, he was named an honorary doctor of science by the University of Guelph, Canada, and received the University of Maine Alumni Career Award. He has received honorary doctorates in Europe and the United States. Dr. Falkow is a member of NAS and the National Academy of Arts and Sciences. He is also an elected fellow of the American Association for the Advancement of Science and a foreign member of the U.K. Royal Society. Dr. Falkow was nominated twice for a Nobel Prize in physiology or medicine. In 2008, Dr. Falkow received the Lasker Award for medical research.

David P. Fidler is James Louis Calamaras Professor of Law at Indiana University. Professor Fidler specializes in international law. He is one of the world's leading experts on international law and global health and is an internationally recognized expert on biosecurity threats posed by biological weapons and bioterrorism, the international legal and policy implications of "non-lethal" weapons, counterinsurgency and rule of law operations, and the globalization of baseball.

In addition to his teaching and scholarly activities, Professor Fidler has served as an international legal consultant to the World Bank (on foreign investment in Palestine), the World Health Organization and the U.S. Centers for Disease Control and Prevention (on global health issues), the U.S. Department of Defense's Defense Science Board (on bioterrorism), the Scientists Working Group on Biological and Chemical Weapons of the Center for Arms Control and Non-Proliferation, U.S. Joint Forces Command (on rule of law issues in complex operations), the Interagency Afghanistan Integrated Civilian-Military Pre-Deployment Training Course organized by the Departments of Defense, State, Agriculture, and the U.S. Agency for International Development, and various initiatives undertaken by non-governmental organizations in the areas of global health and arms control. He was also the editor for the Insights publication series of the American Society of International Law from 2007-2009.

Richard J. Roberts, F.R.S., is Chief Scientific Officer at New England Biolabs in Beverly, Massachusetts. He is the winner, with Phillip A. Sharp, of the 1993 Nobel Prize for Physiology or Medicine for his independent discovery of "split genes." Roberts attended the University of Sheffield where he obtained a B.Sc. in chemistry in 1965 and a Ph.D. in organic chemistry in 1968. His postdoctoral research was carried out in Professor J.L. Strominger's laboratory at Harvard University, where he studied the RNAs that are involved in the biosynthesis of bacterial cell walls. After postdoctoral research at Harvard, he took a post at Cold Spring Harbor Laboratory in New York in 1972. In 1992, he joined New England Biolabs.

STAFF

Anne-Marie Mazza is the Director of the Committee on Science, Technology, and Law. Dr. Mazza joined the National Academies in 1995. She has served as Senior Program Officer with both the Committee on Science, Engineering and Public Policy and the Government-University-Industry Research Roundtable. In 1999 she was named the first director of the Committee on Science, Technology, and Law, a newly created activity designed to foster communication and analysis among scientists, engineers, and members of the legal community. Dr. Mazza has been the study director on

numerous Academy reports including, *Reference Manual on Scientific Evidence*, 3rd Edition (2011); *Review of the Scientific Approaches Used During the FBI's Investigation of the 2001 Anthrax Letters* (2011); *Managing University Intellectual Property in the Public Interest* (2010); *Strengthening Forensic Science in the United States: A Path Forward* (2009); *Science and Security in A Post 9/11 World* (2007); *Reaping the Benefits of Genomic and Proteomic Research: Intellectual Property Rights, Innovation, and Public Health* (2005); and *Intentional Human Dosing Studies for EPA Regulatory Purposes: Scientific and Ethical Issues* (2004). Between October 1999 and October 2000, Dr. Mazza divided her time between the National Academies and the White House Office of Science and Technology Policy, where she served as a Senior Policy Analyst responsible for issues associated with a Presidential Review Directive on the government-university research partnership. Before joining the Academy, Dr. Mazza was a Senior Consultant with Resource Planning Corporation. Dr. Mazza was awarded a B.A., M.A., and Ph.D., from The George Washington University.

Eileen Choffnes is Scholar and Director of the Institute of Medicine's (IOM) Forum on Microbial Threats. Established in 1996, the Forum on Microbial Threats is the premier convening activity of the Institute of Medicine. Her work focuses on emerging, reemerging, and novel infectious disease threats of humans, plants, and animals—domestically and globally—and the interplay of host/environment/microorganism interactions on disease emergence, establishment, and spread. Her previous appointment was as the Study Director of a National Research Council/IOM Ad Hoc Committee on Advances in Technology and the Prevention of Their Applications to Next Generation Biowarfare Threats. The Committee's reports—*Globalization, Biosecurity and the Future of the Life Sciences* and *An International Perspective on Advancing Technologies and Strategies for Managing Dual-Use Risks* were released in 2006 and 2005, respectively. Many of the findings and recommendations in these reports have now become official U.S. government policy in the USG *National Strategy for Countering Biological Threats* (November 2009). She is an internationally recognized expert on technological convergence in the life and physical sciences as well as the security challenges posed by emerging, reemerging, or novel diseases on health, ecological, and economic well-being. She has held senior technical and science policy positions within the Executive and Legislative branches of the United States government concerned with identification of and responses to infectious disease security concerns. She regularly advises governmental and non-governmental organizations on infectious disease policies and practices. She is a member of the Infectious Diseases Society of America, the American Association for the Advancement of Science, and was elected to the Council on Foreign Relations in 2008.

Jo L. Husbands is a Scholar/Senior Project Director with the Board on Life Sciences of the National Academy of Sciences (NAS), where she manages studies and projects to help mitigate the risks of the misuse of scientific research for biological weapons or bioterrorism. She represents the NAS on the Biosecurity Working Group of IAP: The Global Network of Science Academies, which also includes the academies of Australia, China, Cuba, Egypt, India, Nigeria, Poland (chair), Russia, and the United Kingdom. From 1991-2005 she was Director of the NAS Committee on International Security and Arms Control (CISAC) and its Working Group on Biological Weapons Control. Before joining the National Academies, she worked for several Washington, DC-based nongovernmental organizations focused on international security. Dr. Husbands is currently an adjunct professor in the Security Studies Program at Georgetown University. She is a member of the International Studies Association, the Honor Roll of Women in International Security, the Global Agenda Council on Nuclear, Chemical, and Biological Weapons of the World Economic Forum, and the Temporary Working Group on Education and Outreach of the Organization for the Prohibition of Chemical Weapons. She is also a Fellow of the International Union of Pure and Applied Chemistry. She holds a Ph.D. in Political Science from the University of Minnesota and a Masters in International Public Policy (International Economics) from the Johns Hopkins University School of Advanced International Studies.

Steven Kendall is Associate Program Officer for the Committee on Science, Technology, and Law. Dr. Kendall has contributed to numerous Academy reports including the *Reference Manual on Scientific Evidence*, 3rd Edition (2011), *Review of the Scientific Approaches Used During the FBI's Investigation of the 2001 Anthrax Mailings* (2011), *Managing University Intellectual Property in the Public Interest* (2010); and *Strengthening Forensic Science in the United States: A Path Forward* (2009). Dr. Kendall completed his Ph.D. in the Department of the History of Art and Architecture at the University of California, Santa Barbara, where he wrote a dissertation on 19th century British painting. Dr. Kendall received his M.A. in Victorian Art and Architecture at the University of London. Prior to joining the National Research Council in 2007, he worked at the Smithsonian American Art Museum and the Huntington in San Marino, California.

Karin Matchett is a freelance writing consultant who works on topics in science, technology, and medicine; food and agriculture; and energy and climate. Her work spans all phases of documents' development—from a sharp outline to the first draft to rounds of revision. Dr. Matchett has done developmental evaluations and substantive editing for well over 200 research grants in academic settings. She has written strategic visioning

documents, summaries of expert panels in academia, the occasional summary of the photovoltaics industry, and proposals for academic program development and research. She also works with nonprofit organizations to develop reports, proposals, and web content.

Dr. Matchett has a Ph.D. in the history of science from the University of Minnesota, with an emphasis on 20th century life sciences and agriculture in the United States and Mexico. She completed a postdoctoral fellowship under the mentorship of Daniel Kevles at Yale University in which she did research and writing on topics at the intersection of the life sciences and law. Her current research focus is in energy and climate issues as they relate to human psychology and American society and culture.