vulnerability that affects every individual copy of Windows is not as bad as a vulnerability that affects every individual person. Still, the lessons from computer security are valuable to anyone considering policies intended to encourage life-saving research in virology while at the same time prevent that research from being used to cause harm. This debate will not go away; it will only get more urgent.

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POLICY FORUM

Evolution, Safety, and Highly Pathogenic Influenza Viruses

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Experience with influenza has shown that predictions of virus phenotype or fitness from nucleotide sequence are imperfect and that predicting the timing and course of evolution is extremely difficult. Such uncertainty means that the risk of experiments with mammalian-transmissible, possibly highly virulent influenza viruses remains high even if some aspects of their laboratory biology are reassuring; it also implies limitations on the ability of laboratory observations to guide interpretation of surveillance of strains in the field. Thus, we propose that future experiments with virulent pathogens whose accidental or deliberate release could lead to extensive spread in human populations should be limited by explicit risk-benefit considerations.

In response to two sets of experiments on mammalian-transmissible, modified influenza A/H5N1 viruses (*1*, *2*) the U.S. Department of Health and Human Services has promulgated a new policy on dual-use research of concern (*3*). This policy, and other statements from U.S. and international bodies, identify the need for risk mitigation in future studies of mammalian-transmissible variants of highly pathogenic influenza virus and certain other infectious agents (*4*, *5*), raising an important new question: How should funders, regulators, and researchers evaluate what future experiments should be done with such viruses? The answer to this question depends on the relative magnitude of risks and benefits of such experiments. For influenza, useful evaluations of either risks or benefits depend in part on what we know about virus evolution. Proponents of continued research in this area suggest that knowing the mutations involved in mammalian airborne transmission will aid surveillance, allowing us to see whether there is evolution in the direction of a pandemic virus. Mitigating the risk of accidental or deliberate release of these strains, as directed by the new policy (*3*), also depends on how well we can predict the virulence, transmissibility, and evolutionary trajectory of influenza viruses.

We contend that predictions about how particular influenza strains will behave in humans or, even more important, how they will evolve, remain highly speculative. The most striking examples of influenza's unpredictability are in the area of drug resistance. Animal models, in vitro studies, and mathematical models have all contributed to our understanding of drug-resistant strains. Despite that knowledge, aspects of the spread of adamantane-resistant A/H3N2 (2003 to the present) and the spread of oseltamivir-resistant A/H1N1 (2007 to 2009) took the influenza community by surprise.

Adamantanes were used to treat influenza starting in the late 1960s. Animal models showed essentially no "fitness cost" or virulence reduction in strains with mutations conferring adamantane resistance (*6*), which commonly emerge during treatment (*7*) and can spread within families (*7*). These observations would have predicted a high likelihood that adamantane resistance would spread widely in populations where adamantanes were used, but such spread did not occur for decades, for reasons that remain unclear. Then, in 2003 to 2004, adamantane-resistant viruses emerged to rapidly become the dominant influenza A/H3N2 isolates globally (*8*, *9*). Although adamantane use likely played a role in the genesis and initial spread of the resistant lineage (*8*), the near-fixation of this lineage in global A/H3N2 isolates may have been due to the presence of immune escape mutations (*9*), a selection pressure whose importance in spreading this virus could not have been confidently predicted. Also unanticipated was the persistence of resistant viruses to the present day, despite minimal use of adamantanes (*9*).

Resistant strains emerge de novo in several percent of influenza patients treated with the neuraminidase inhibitor oseltamivir (*10*). However, extensive spread of resistance was not reported before 2007. At the time, this situation was explainable by the large fitness costs observed in animal models for the most common oseltamivir-resistance mutation (*11*). Public health officials and virologists were therefore surprised when, in winter 2007 to 2008, resistance to oseltamivir in A/H1N1 viruses carrying the H275Y mutation arose and spread widely. Even more surprising was the finding that this rise was not explained by geographic patterns of antiviral use, and high prevalence of the resistant strain was first detected in Norway, where oseltamivir use was negligible (*12*). Only after the global spread of this drug-resistant A/H1N1 lineage have we begun to understand the additional enabling mutations (*13*–*15*) that led to its rapid emergence. Although drug-resistant strains were obviously anticipated under drug pressure, these experiences serve as a warning against overconfidence in predicting the timing and direction of influenza evolution.

Antigenic drift of influenza A hemagglutinin is probably the best-studied evolutionary process in any virus, yet both the timing and amino acid location of changes that lead to important antigenic changes (*16*) in any particular strain remain difficult to predict (*17*). As a result, in about half of all influenza seasons, one or more components of the trivalent vaccine are poorly matched to the circulating strains of flu (*18*). Predictions of virus properties from sequence information are likewise imprecise, highlighting how much more needs to be learned about the effects of genetic environment on properties related to individual mutations. In the 2009

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pandemic, for example, the E627K mutation in the PB2 gene was expected to enhance virulence and promote transmission, but it did not spread widely, and it did not enhance virulence in animal models when introduced in the genetic background of the 2009 pandemic virus (*19*).

Contentions that H5N1 mutant strains might not be highly transmissible or as pathogenic as wild-type H5N1 strains in humans (*20*) may therefore be falsely reassuring. The evidence from ferrets that these viruses are transmissible between mammals by the airborne route, coupled with the demonstrated human virulence of wild-type avian A/H5N1 strains, indicate that H5N1 and its mutants should be taken seriously (*21*).

By emphasizing the limits of predictability, we do not mean to suggest that molecular studies of influenza transmissibility and virulence, or surveillance of animal virus populations, lack value. Viral sequences contain important information; studying the behavior of novel variants in the laboratory and adding precision to surveillance in the field are necessary if we are ever to improve our predictive powers. But because we cannot predict how these strains will behave in humans or, critically, how they will evolve, such studies on transmissible versions of dangerous zoonotic pathogens are particularly risky and deserve special treatment.

Indeed, great care was taken in the experiments now being reported (*1*, *2*), and the World Health Organization (WHO) specifically noted that the "laboratory-modified H5N1 viruses are currently stored in well-established research facilities with high security and high safety (BSL-3+)" (*5*) (see Fig. 1). However, it is not a given that all future experiments will be conducted with such care, and WHO also called it "a matter of urgency" to define "the biosafety and biosecurity conditions under which further research is conducted on the laboratory-modified H5N1 viruses" (*5*). In doing so, we must remember that accidents happen even under high-level containment conditions. Accidental infections of laboratory workers with severe acute respiratory syndrome (SARS) coronavirus (*21*) and with smallpox (*22*), and the accidental release of foot-and-mouth disease virus from a laboratory in the United Kingdom (*23*), indicate the serious and real risk of laboratory accidents, a risk that increases with the number of laboratories and workers involved. The 1977 reemergence of the A/H1N1 influenza virus after decades of absence contributed to seasonal influenza for three decades. This virus's genetic similarity to 1950 A/H1N1 viruses suggests that it descended from a laboratory strain (*24*), although the exact circumstances remain unclear.

Exactly how to translate this high level of concern into the current framework of biological safety is not straightforward. U.S. government guidelines (*25*) indicate the current highest biosafety level (BSL), BSL-4, "for work with dangerous and exotic agents that pose a high individual risk of life-threatening disease, which may be transmitted via the aerosol route and for which there is no available vaccine or therapy." In practice, these guidelines are not followed literally: Smallpox virus, for which there are substantial vaccine stockpiles, is limited to only two BSL-4 laboratories, and Ebola virus, which transmits only by blood contact, is classified as BSL-4, whereas the SARS coronavirus, which has proven epidemic potential and no widely available vaccine, seems to meet all of the BSL-4 criteria yet is handled in BSL-3 laboratories. As of 2009, there were at least 14 "entities" maintaining registered BSL-4 laboratories in the United States (*26*), at least 24 BSL-4 laboratories worldwide (*27*), and at least 1495 BSL-3 laboratories registered in the United States and working with select agents (*27*), although the total number of BSL-3 laboratories in the United States is unknown (*27*). Comprehensive data from intramural laboratories at the National Institute of Allergy and Infectious Diseases estimate the risk of exposure as 2 per 100,000 operator-hours; only 1 of the 12 exposures involved in this calculation involved a clinical infection (*28*). Data from a larger set of U.S. laboratories indicates 26 incidents, 8 of which resulted in documented infections, in BSL-3 and BSL-4 laboratories (not including agricultural pathogens). Another five, all involving infections, are known in non-U.S. BSL-3 and BSL-4 laboratories between 2002 and 2007; in the United States and elsewhere, reporting of such incidents is incomplete (*28*).

The modified H5N1 strains do not fit neatly into the categories established in the biosafety guidelines: Aerosol transmissibility among humans is possible (and is a key reason for interest in their study) but unproven; vaccines may protect laboratory workers but are not available on a scale sufficient for the general population, which might be at risk if a laboratory worker were infected and were able to transmit the infection to others. Although we are not in a position to resolve the debate about the proper biosafety level, we do believe that funding agencies and regulators should limit the number of laboratories and individuals working on these modified strains. Each additional laboratory and individual worker adds to the risk of accidental or malicious release, so there should be a strong presumption against increasing these numbers unless the benefits of an additional study are very well justified scientifically. Biological safety depends not only on the physical barriers of BSL-3 or BSL-4 facilities but also, importantly, on the experience, training, and skill of laboratory workers; these factors should also be considered when deciding who is permitted to work with these agents. At present, U.S. law requires specific safety and security

Fig. 1. Scientists working in a BSL-4 laboratory. CDC scientists connect to a supportive air hose to breathe air and to maintain positive pressure inside the protective BSL-4 lab suit.

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measures for work on select agents (including certain highly pathogenic influenza viruses). Principles for biosafety are laid out in the widely used *Biosafety in Microbiological and Biomedical Laboratories* (*25*). We recommend that compliance with these measures and principles be actively monitored by the Centers for Disease Control and Prevention (CDC) on a basis more frequent than the current requirement for federal inspection of BSL-3 laboratories, which is every 3 years and when a new select agent is added (*27*, *29*). These requirements apply only within the United States; we share the concern expressed by many experts about variation in biosafety practices worldwide (*27*). For experiments on the evolution of viral or bacterial transmissibility to mammals, there should be an explicit requirement to justify why the research must be done with virulent strains.

Traditional peer-reviewed funding decisions evaluate scientific merit first and then undertake risk mitigation if it is considered necessary; the dual-use research of concern (DURC) policy in the United States (*3*) does not specify how, if at all, this approach would change. We propose that the decision about whether research on mammalian-transmissible H5N1 viruses or agents with similar potential for damage to public health should be funded or should proceed with restrictions should not be left to each department or agency, because some may lack the relevant expertise to evaluate risks and benefits in light of the overall portfolio of studies already approved or under way. A single interagency committee, including experts in fields such as evolutionary microbiology and biodefense as well as virology, needs to review the small number of proposals identified by grant administrators or scientific review committees that involve pathogens whose accidental or deliberate release would represent a major threat to public health. In contrast to the National Science Advisory Board on Biosecurity, which is only advisory, this committee should have decision-making authority. The U.S. government is actively considering options to strengthen DURC governance, including a possible review group to provide independent assessments of research proposals. Similar considerations should motivate policies outside the United States (*27*).

Each additional study of mammalian-transmissible, highly pathogenic influenza will improve our understanding and may move us closer to an ability to control such viruses, but will also increase the risk of an accident that could trigger a global public health disaster, especially if evolution proceeds in an unfavorable direction. This exceptional level of risk should motivate exceptional caution by scientists, funders, and regulators worldwide.

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POLICY FORUM

Influenza: Options to Improve Pandemic Preparation

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Science and society have been struggling to find a way to protect humankind from recurring epidemics and pandemics of influenza. Here, we review the options available in the short term and also briefly address the solutions that research may make available in the long term.

Every year, seasonal influenza causes several hundred million cases and 250,000 to 500,000 deaths, of which 90 million cases and 28,000 to 111,500 deaths occur in children (*1*, *2*). Pandemic influenza strikes periodically, infecting billions of people and potentially causing millions of deaths. Recently, two studies showing that, in the laboratory, pathogenic H5N1 influenza strains can evolve to be transmissible in ferrets divided the scientific community between those saying that the studies should not have been done and/or should not be published in their entirety and those saying that the studies are useful and should be published in their entirety (*3*–*6*). Because influenza in ferrets so closely models the disease in humans, the findings of H5N1 transmissibility between ferrets suggest that transmission of such strains between humans also could occur. The controversy continues, and it is evident that the studies have reminded us that a deadly H5N1 pandemic is not impossible. Therefore, it is important to discuss what options

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