Mammalian-Transmissible H5N1 Influenza: Facts and Perspective

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ABSTRACT Two recently submitted (but as yet unpublished) studies describe success in creating mutant isolates of H5N1 influenza A virus that can be transmitted via the respiratory route between ferrets; concern has been raised regarding human-to-human transmissibility of these or similar laboratory-generated influenza viruses. Furthermore, the potential release of methods used in these studies has engendered a great deal of controversy around publishing potential dual-use data and also has served as a catalyst for debates around the true case-fatality rate of H5N1 influenza and the capability of influenza vaccines and antivirals to impact any future unintentional or intentional release of H5N1 virus. In this report, we review available seroepidemiology data for H5N1 infection and discuss how case-finding strategies may influence the overall case-fatality rate reported by the WHO. We also provide information supporting the position that if an H5N1 influenza pandemic occurred, available medical countermeasures would have limited impact on the associated morbidity and mortality.

Life science is currently at a critical crossroads. Two recently submitted manuscripts to *Science* and *Nature* raise serious questions regarding research censorship and a grave concern for global biosecurity, biosafety, and public health. These two studies describe success in creating mutant isolates of H5N1 influenza A virus that can be transmitted via the respiratory route between ferrets. The National Science Advisory Board for Biosecurity (NSABB) was asked by the U.S. government to assess the dual-use research implications of these manuscripts. The NSABB review concluded that a significant potential for harm existed in fully publishing the methods and results; thus, the NSABB recommended that details of the work not be fully communicated in an open forum (1, 2). However, the World Health Organization (WHO) has stated a preference from a public health perspective for full disclosure of the information in these two studies (3).

The recommendation from the NSABB has resulted in a flurry of commentaries regarding both the merits and flaws of this decision (4–15). There have been calls for "solid science and not speculation" and for decisions to be based on "sound scientific principles" (12, 14). Some of these same commentaries have provided selective data, not the entire body of published literature, to address specific issues related to the NSABB decision (12, 14). The issues are (i) the determination of the human case-fatality rate for H5N1 infection and its implication for a future possible pandemic and (ii) the capability of our current influenza vaccines to impact any future unintentional or intentional release of H5N1 virus.

H5N1 VIRUS INFECTION CASE-FATALITY RATE

The WHO reports that 345 of the confirmed 584 cases of human H5N1 infection have died; the cumulative case-fatality rate is 59% and ranges from 30 to 80% depending on the country (16). One group of investigators recently concluded that "the case-fatality rate that is offered by the WHO, and that is driving this controversy, is likely orders of magnitude too high" (14). Their conclusion was based on 10 published studies of H5N1 seroepidemiology in potentially exposed persons. The WHO has established criteria for the serologic identification of H5N1 in humans (17). Using literature search strategies similar to those of a previous review of H5N1 seroepidemiology (18), we identified 24 studies published to date that evaluate the seroepidemiology of H5N1 infection in humans (19–42). This analysis includes three follow-up studies

related to the 1997 outbreak of H5N1 influenza in Hong Kong; therefore, we excluded those three studies from further analysis since current H5N1 viruses are not similar to the strain that caused that outbreak (43). This is consistent with the findings and actions of the WHO: the 1997 cases are not currently included in the case count for H5N1 infections, and the 1997 isolate is not recommended for inclusion in current H5N1 vaccines (16, 44). The remaining 21 studies were conducted and published after 2004; 13 meet the WHO criteria for serologic confirmation (titer of ≥1:80) (19, 24, 26, 27, 33–41). Three of the 13 studies reported serologic evidence of H5N1 infection (24, 39, 41). In total, 26 (0.47%) of 5,487 participants in these studies were seropositive for H5N1.

All 13 studies that used the WHO serologic screening criteria were conducted within 4 months of the occurrence of human H5N1 cases or within 6 months of H5N1 poultry outbreaks in the area from which participants were enrolled. The timing of the participant surveys maximized the possibility that these studies would detect recent H5N1 infections had they occurred (45). Most of the individuals tested were exposed to sick poultry and/or a symptomatic human case of H5N1 infection.

One seroepidemiology study of H5N1 infection in Thai villagers conducted by Khuntirat et al. (29) has been cited as documentation of an increased rate of H5N1 subclinical infections (12, 14). In that study, approximately 6% and 3.5% of participants were reported to have elevated antibody levels to one of two H5N1 viruses, respectively. Serologic samples were obtained from villagers more than 2 years after sporadic H5N1 outbreaks were reported in poultry and one confirmed and two possible human H5N1 cases occurred in the area. This study was not included in the 13 studies detailed above because serologic results did not meet the WHO criteria for serologic confirmation; the investigators used a low threshold antibody titer (≥1:10) as evidence of

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previous infection. This study has been criticized, since using such a low threshold could lead to overestimation of the true seroprevalence because of the increased likelihood of false-positive results (46, 47). In addition, the study was not initiated until 2 years after H5N1 infections in humans or poultry had been reported in the area.

Of the 13 studies that used the WHO criteria, five reported the range of serologic titers detected (26, 27, 33, 34, 37). These studies were conducted within 4 months of the occurrence of human cases and within 6 months of poultry outbreaks. The five studies, which included 2,629 participants, found that no participants had evidence of previous infection based on the WHO criteria for serologic confirmation and only 13 (0.49%) had neutralization titers between 1:10 and 1:40. One study documented that most participants had detectable H5N1 titers below 1:80; however, a reevaluation of the dilutions used in the study led the authors to conclude that these serologic titers did not represent detectable antibody (33; J. Katz, personal communication). The results of these five studies, which involved sampling of participants more contemporary to evidence of circulating H5N1 viruses than the study by Khuntirat et al., demonstrated at least a 10-fold-lower prevalence of intermediate serologic results (i.e., neutralization titers between 1:10 and 1:40) than to those found in the latter study. We believe that these data support the concern regarding false-positive results in the Khuntirat et al. study (29).

Some researchers have stated that, because of the specificity of the WHO case definition, milder or asymptomatic H5N1 cases have been missed by traditional case-based surveillance and therefore a small fraction of the total number of infected cases has been accounted for under the WHO surveillance system (12, 14). When population-based seroepidemiology studies are used to supplement clinical-based surveillance, a more complete picture of the epidemiology of that infection is generated than that by use of clinical case-based surveillance alone. Mild or asymptomatic cases can be detected by serologic evidence of prior infection even if such cases are missed by traditional surveillance at the time of their infection. Another case detection strategy is to follow exposed persons over time to identify any influenza-like illnesses in such groups. Exposed persons can include persons with known exposure to confirmed or suspected human H5N1 cases, persons with occupational or household exposure to sick birds, or persons living in the same locations as human and/or avian cases. Periods of observation of exposed persons by health authorities have lasted for up to 6 months. To date, this type of targeted surveillance has not uncovered additional cases of mild influenza-like illness caused by H5N1 infection (48). As with any surveillance system, case ascertainment for H5N1 infections certainly has not captured 100% of the cases. However, all of the data presented above suggest that the number of mild infections that have been missed is likely relatively small.

Up to this point, we have discussed the likelihood of missing mild cases of H5N1 infection; however, it is also important to consider the potential to miss fatal cases of H5N1 infection. Investigators have shown that current case-based surveillance for H5N1 infection in countries with ongoing avian H5N1 transmission does not always document fatal cases, with such cases being missed either because the diagnosis was not considered or virologic confirmation was lacking (46, 47, 49). A more complete ascertainment of fatal cases of H5N1 infection would increase the current H5N1 case-fatality rate. While this is also likely an infre-

quent occurrence, the phenomenon of missing cases of fatal infectious illnesses has been documented in other situations. For example, one would expect that fatal cases of human rabies would rarely escape detection; however, instances of previously fatal human rabies cases have been uncovered only after recipients of their donated organs subsequently developed rabies and died (50, 51).

The available seroepidemiologic data for human H5N1 infection support the current WHO-reported case-fatality rates of 30% to 80% (16). While some have suggested that concern about such high case-fatality estimates was a major factor in the NSABB decision, such estimates were only one of a number of factors considered by the NSABB (12). In fact, if H5N1 virus does become a pandemic virus, the virulence (as measured by the case-fatality rate) could decrease 10- to 20-fold from what is currently documented and the virus would still generate a more severe pandemic than the 1918 pandemic, where the overall case-fatality rate was probably about 2%. Given the global population and the current dynamics of population movement around the world, an H5N1 pandemic, even with a relatively low case-fatality rate, would be a truly catastrophic event.

ROLE OF VACCINES AND ANTIVIRAL AGENTS IN MITIGATING AN H5N1 INFLUENZA PANDEMIC

The primary public health response to an influenza pandemic is a pandemic vaccine. Secondary to the pandemic vaccine is the use of antivirals. If an H5N1 strain, regardless of its origin, becomes readily transmissible between humans and begins to spread in the population, it likely will result in an influenza pandemic. The proposal that viable vaccines and available antivirals will make a substantial difference in the global morbidity and mortality associated with the pandemic is not supported by data from the previous three pandemics. The time required to manufacture both egg-based and cell culture-based influenza vaccines has resulted in "too little, too late" vaccine responses for the 1957, 1968, and 2009 pandemics on a worldwide scale.

For example, by 28 October 2009, only 16.8 million doses of pandemic 2009 A(H1N1)pmd09 vaccine had been shipped by the U.S. federal government to states (52). An ample supply of the vaccine was not available until after the second wave had subsided in early October; by that time, demand for the vaccine had dropped dramatically. The Centers for Disease Control and Prevention (CDC) estimated that the 2009 A(H1N1)pdm09 pandemic vaccine prevented only 200 to 520 deaths in the United States because of delay in availability (53).

Mammalian cell-based pandemic vaccines were licensed for use in the European Union in 2009 and were used there during the pandemic response. As in the United States, both the egg-produced and the cell culture-produced influenza vaccines arrived too late and in too little quantity to have a significant impact on the pandemic in the European Union. According to the date of marketing authorization in Europe, a mammalian cell-based vaccine was available only after three adjuvanted egg-based influenza vaccines were already in distribution (54). The European experience with the availability of a pandemic cell-based vaccine did not demonstrate a measurable improvement in vaccine production speed nor was it sufficient to alter the overall public health impact of the pandemic in Europe.

Given the need to distribute pandemic vaccines globally and the fact that not all countries have the financial assets to purchase sufficient quantities of vaccine for their populations during a pan-

demic, the WHO coordinates a program for donation and distribution of pandemic influenza vaccines. As of 10 November 2010, the last WHO update for pandemic vaccine distribution, only 78 million doses of A(H1N1)pdm09 vaccine had been distributed to 77 countries (55). All of these vaccine doses were distributed well after the second wave of the pandemic, months after developed countries had started their vaccine campaigns.

From a historical perspective, influenza vaccine also arrived in quantities too small and too late to have a significant public health impact in the United States for both the 1957 and 1968 pandemics. Given the experience of the three previous pandemics, unless newer and more effective influenza vaccine technologies are developed that facilitate substantially faster production and generate far greater numbers of doses in much shorter time frames, it is unlikely that influenza vaccine will have a significant public health impact during the next pandemic. The technology behind our current influenza vaccines is simply not sufficient to address the complex challenges associated with an influenza pandemic in the 21st century.

Currently there are limited H5N1 influenza vaccine stockpiles around the globe, including one in the United States. These vaccines are not designed to protect the general population but are to be targeted to a small subset of critical-asset individuals, until a pandemic-specific vaccine can be produced. Furthermore, it is unclear as to how effective the currently stockpiled H5N1 vaccines would be against an emergent pandemic strain, given the diversity of existing H5N1 clades.

Antivirals are the only other pharmaceutical intervention available for influenza. As with influenza vaccines during a pandemic, the global capacity for antiviral manufacturing falls far short of global needs. While there are global and national stockpiles of antivirals devoted to pandemic response, during the 2009–2010 pandemic, there were significant disparities regarding use and availability of antivirals around the world (56). Current antiviral stockpiles and antiviral manufacturing capacity support the conclusion that should another influenza pandemic occur in the foreseeable future, the impact of antiviral use on human morbidity and mortality will be no better than was documented in the 2009 pandemic.

SUMMARY

In summary, we believe that the debate about the case-fatality rate of H5N1 influenza in humans and the suggested important role of currently available antivirals and vaccines in mitigating an H5N1 pandemic are without merit. Furthermore, we do not believe that continued focus on these issues helps to address how best to manage research involving influenza viruses, such as H5N1, that are transmissible between mammals and have the potential to be highly virulent in humans. Future discussions specific to the current controversy need to resolve critical questions such as how we safely conduct H5N1 virus transmission studies in mammals, how we share critical methods and results with those who have a need to know, and how we ensure that laboratory-generated H5N1 viruses do not escape controlled environments. Resolution of these issues with regard to H5N1 influenza viruses has the potential to serve as a template for similar situations involved existing or emergent pathogens. It is our belief that the current controversy provides a valuable opportunity for scientists and public policy experts to work together in creating this road map for the future.

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