Reply to "Studies on Influenza Virus Transmission between Ferrets: the Public Health Risks Revisited"

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We are gratified that Ron Fouchier has joined (1) the important effort to quantify the risks (2–5) of the creation of potential pandemic pathogens, including ferret-transmissible variants of influenza A/H5N1. However, we disagree with many aspects of his assessment.

As in our article and Fouchier's letter, here we proceed through the calculation, starting with probability of laboratory-acquired infections and the conditional probability of sparking a pandemic given such an infection and concluding with the consequences thereof. We then discuss some more general considerations.

PROBABILITY OF A LABORATORY-ACQUIRED INFECTION (LAI) IN A LAB WORKING ON PATHOGENS WITH PANDEMIC POTENTIAL

Fouchier bases his calculations on one of the sources we also used, the tabulation by Henkel et al. of reports of accidents involving select agents in the United States between 2004 and 2010 (6). However, he argues that the risk in his laboratory is considerably lower than the lower bound obtained from these reports of 0.2% per laboratory-year in a biosafety level 3 (BSL3) laboratory. He states, "These estimates, however, do not take into account specific pathogen types or research settings. This is crucial, because working practices in, e.g., virology and microbiology laboratories are different and because each biosafety laboratory is unique" (1). He proposes an alternative calculation based on 0 viral laboratory-acquired infections (LAI) in BSL3 labs over 2,044 lab-years in BSL2, -3, and -4 labs with select agents (6) and suggests that the proper value is $<1/2{,}044$ lab-years, or $<5 \times 10^{-4}$/lab-year.

These numbers are both conceptually and statistically invalid. While bacteriology and virology labs certainly perform some different activities, neither the references cited by Fouchier nor any other evidence of which we are aware justifies the relevant claim: that BSL3 bacteriology labs are more accident-prone than BSL3 virology labs over a given time span. Absent any such evidence, the proper comparison would be BSL3 LAI/BSL3 lab-years. Unfortunately, BSL3 lab-years are not publicly available. Therefore, in our original calculation, we used 2,044 lab-years in BSL2, -3, and -4 labs as a denominator to calculate a lower bound on the risk, with LAI in BSL3 as the numerator. Fouchier's suggestion to use the same (too-large) denominator to form an upper bound is inappropriate and is made more so by excluding bacterial LAI from the numerator but keeping bacterial lab-years in the denominator.

If one does choose to use 0 viral LAIs as the numerator, one would need to specify the number of viral BSL3 lab-years for a proper denominator. In this case, given the uncertainty surrounding a rare event, the proper way to account for 0 observed events is not to say that the true rate is less than one divided by the number of lab-years, but that the true rate has a 95% confidence interval between 0 and the value obtained by dividing 3 by the number of observed lab-years (7).

Moreover, while U.S. labs working with select agents show no reports of accidental viral infections in 2004 to 2010, accidental LAIs have occurred in or from BSL3, BSL3 agricultural (BSL3Ag), or BSL4 laboratories in China, Singapore, the United Kingdom, Russia, and Taiwan (8). Finally, underreporting of LAI is internationally the rule rather than the exception, in part because serosurveillance is not routinely performed in many high-containment labs, and in part because reporting systems, if they exist, are inadequate. The Netherlands has been singled out as notable for inadequate surveillance and reporting of LAI (9). Also, as a general principle, it is self-evident that the number of potentially serious laboratory exposures is greater than the number of actual confirmed laboratory infections. For example, the CDC has reported a number of potentially serious laboratory exposures this year, but none of them would have been factored into LAI calculations. At the time of this writing, it is unclear whether the exposure of a CDC technician to Ebola virus due to an error of switching live and inactivated samples has resulted in infection; whatever the outcome, this incident reinforces the idea that accidental exposures are possible in the best virologic laboratories.

Fouchier proposes another measure of accident rate, the number of accidents per worker-year, where a worker is defined as a person with access approval to a BSL2, -3, or -4 lab that handles select agents. From these data he calculates a rather low risk of <1 per 70,000 worker-years, using the Henkel et al. (6) denominator, including all agents at all biosafety levels, and the numerator of known viral infections. This suffers from the same conceptual and statistical problems noted above and from the additional problem that the individuals "approved" to have access to a select agent facility will be highly heterogeneous in the amount of time they actually spend there. A more relevant metric, we suggest, is that estimated for the intramural labs in NIAID, which experienced 3 known LAIs in 634,500 person-hours of actually work in a BSL3 lab, or about a 1% risk for every 2,000 h of work in a BSL3 lab (8).

Fouchier lists a number of enhancements to standard BSL3 practices that are in place in the Erasmus Medical Center facility and proposes that these provide an increase in safety of at least a

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factor of 10 above that of standard BSL3 labs. This factor of 10 is arbitrary. Using a factor of 10 to represent an unknown value in the absence of data to support this strikes us as inconsistent with the rules of caution that apply to dealing with unknown hazards of high-consequence events. We agree that it is difficult to quantify the impact of these practices, and we agree that some account should be taken of these enhancements, for laboratories that use them. We leave it to impartial risk assessors to decide how these enhancements should be accounted for.

Any risk assessment, however, must explicitly account for the possibility of human error or malicious removal of the agents from the source lab, circumventing these enhanced safeguards (10). As observed by Kimman et al., "in the majority of cases of LAIs a direct cause could not be assigned. . ., suggesting that a failure was not noticed in many cases or that containment may have been insufficient" (9). In relation to the gain-of-function risk assessment, Gavin Huntley-Fenner, a speaker at the National Academy of Sciences Symposium on Gain-of-Function, wrote "we need to plan as though human error is inevitable. Research suggests that even the most experienced and knowledgeable workers sometimes cut corners and that everyone is susceptible to distraction, fatigue and faulty reasoning" (10). In this regard, it is notable that all three recently publicized CDC laboratory incidents (11, 12) involved removal of infectious material from the BSL3 containment facilities in which they were handled, in the false belief that the material was not infectious (the anthrax agent and Ebola virus) or in the false belief that the material did not contain a highly pathogenic avian influenza (HPAI) contaminant (influenza virus). Under such circumstances, even the highest-functioning mechanical systems and best-trained personnel in the source lab cannot prevent accidents in the destination lab.

Fouchier states that vaccination of laboratory workers in his laboratory reduces the risk of infection for such workers and that heightened surveillance of such workers and provision of antiviral drugs in case of an exposure will reduce their risk further. The benefits of vaccination and antivirals in preventing infection are overstated for several reasons. (i) Vaccination of workers and heightened surveillance and treatment can be effective if the accidental exposure involves a member of that laboratory, but not necessarily if it involves personnel from other laboratories, as in the three CDC incidents (11, 12). Probability calculations must separate exposures in the source laboratory from exposures in other laboratories.

(ii) The availability and effectiveness of vaccines are not certain. In general, vaccine efficacy even against well-matched seasonal influenza virus varies widely (13). Some ferret gain-of-transmission experiments involve subtypes for which there are (at the time of the experiment) no licensed vaccines (14–16). Moreover, the effectiveness of vaccines and antivirals against laboratory-engineered strains in these experiments is uncertain, even when they are effective against the starting strain; this is why the initial report of ferret-transmissible strains described assays of vaccine neutralization (experiment 7) and antiviral susceptibility (experiment 6) (17). After performing the experiment, one can retrospectively infer that these protections would have been effective, but at the time of proposing an experiment, neither is certain. Indeed, even after obtaining in vitro results, Fouchier stated that the effectiveness of drugs and vaccines in vivo against the strains produced in his experiments was in doubt and needed testing (18).

(iii) An additional crucial point is that the rate of laboratory-acquired infections in BSL3 labs, which we cite as at least 0.2% per laboratory-year, already reflects the routine use of vaccination and prompt treatment of suspected exposures for many of the pathogens considered. Hence, the 0.2%/lab-year figure is, to a large degree, the rate at which breakthrough, detectable infections occur in laboratory workers who have immunologic (19) and pharmacological (20) protection, and therefore, for many pathogens it is not a rate pertaining to unprotected workers. To adjust downwards from this rate double-counts the protective benefits of those vaccines and drugs.

PROBABILITY OF ONWARD TRANSMISSION GIVEN AN LAI

Fouchier further argues that the risk of onward transmission from an LAI would be reduced relative to those we posited, a range of 5 to 60%. He suggests that prophylaxis and vaccination should not only reduce the probability of infection (discussed above) but also reduce the probability of onward transmission by a factor of 100. The effect of prophylaxis and vaccination should be accounted for, as we noted in our article (5), but again only if the infection occurs in the source laboratory where workers are prepared; moreover, the factor of 100 reduction is much too optimistic, for the following reasons. (i) It assumes the infection is detected, which may or may not occur before spread. (ii) It assumes that vaccines and antivirals are given and effective against the lab-engineered virus, which is not guaranteed or in some cases even likely for the reasons noted above. (iii) If infection is detected before spread, and if vaccines and antivirals are given to the exposed person rapidly, and if the strain is susceptible to the antiviral, the reduction in infectiousness of a vaccinated, antiviral-treated case is probably closer to a factor of 5 to 8 than a factor of 100. This estimate is based on an assumption of multiplicative effects of antivirals and vaccination, using clinical data to estimate that oseltamivir reduces infectiousness approximately 5× (by 80%) (21) and a meta-analysis of published studies showing no reduction in infectiousness (22) yet suggesting a "best guess" of a 1.7-fold (40%) reduction in infectiousness from a well-matched inactivated vaccine (22). If infection is not detected before spread, or if vaccines and antivirals are not given to the infected person(s) rapidly enough to prevent spread, or if the strain is not susceptible to the antiviral or vaccine, then there is no reduction in risk.

Fouchier suggests that quarantine of laboratory workers would reduce transmission by another factor of 100. Using a factor of 100 to represent an unknown value in the absence of data to support this again strikes us as inconsistent with the rules of caution that apply to dealing with unknown hazards of high-consequence events. Once again, this assumes that any exposure or infection is detected before transmission, something that has not occurred in a number of past LAIs. It also assumes that the exposures occur inside his laboratory, which is not guaranteed due to the possibility of erroneous or malicious removal of the strain from the lab. Notably, the one published study designed to estimate risk of uncontrolled spread given an LAI incorporated the assumption that detected LAIs would be subjected to nonpharmaceutical interventions, which would be somewhat effective against the first few cases of a flu-like agent. The risk of uncontrolled spread in that study came from scenarios in which such measures were not taken, for example, because the infection was not detected (23) (around a 5 to 15% chance depending on parameters), as well as from scenarios in which it was detected but not successfully con-

trolled, particularly for viruses with basic reproductive numbers exceeding 1.5. Of course, no effects of quarantine should be included in scenarios involving accidents outside the source lab.

Moreover, Fouchier suggests that the viruses made transmissible in ferrets would be less transmissible in humans than ordinary pandemic or seasonal viruses, because they transmit less well in ferrets than do human seasonal or pandemic viruses and because their adaptation is to ferrets, not humans. We have two points to make in response.

(i) These claims run counter to the original rationale for ferret-gain-of-transmission studies. That rationale was to predict pandemic potential of natural isolates, which Fouchier earlier argued was associated with "airborne transmission" best studied in mammalian nonhuman hosts (24). Likewise, Yoshihiro Kawaoka described the purpose of his ferret gain-of-transmission studies as "[t]o determine whether H5N1 viruses could be transmitted between humans" (25), and the original reports of ferret transmission experiments say that pandemic potential is associated with the changes observed. For example: "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 . . . Identification of the minimal requirements for virus transmission between mammals may have prognostic and diagnostic value for improving pandemic preparedness" (17). Similarly, in another ferret transmission study on synthetic 1918-like viruses from the Kawaoka lab, mutations conferring ferret transmissibility are specifically called "human-adaptive mutations" (26). The CDC considers ferret transmissibility and human alpha-2,6 receptor binding to be 2 of the top 3 predictors of the threat of emergence of influenza viruses (27). A recent publication by CDC influenza virologists suggests that they interpret specific mutations found in the ferret gain-of-transmission studies as signaling human adaptation, not specifically ferret adaptation (28).

(ii) The claim of lack of transmission in humans is a sharp departure from earlier claims. Fouchier initiated public discussion of these studies by claiming he had created "probably one of the most dangerous viruses you can make" (29). Current denials that this is possible seem to be designed to reduce perceived risk from the experiments rather than to describe new scientific data or understanding. Paul Keim, the Chair of the National Science Advisory Board on Biosecurity who reviewed the original submission of the H5N1 paper, stated, "I can't think of another pathogenic organism that is as scary as this one" (29). The reaction of another member of the NSABB, Michael Imperiale, was as follows: "[Imperiale] also says it was news to him that the mutated virus did not spread between ferrets via the aerosol route as readily as seasonal strains, as Fouchier showed at the ASM meeting. 'That really didn't come across to me in the paper,' he says. 'I didn't see that kind of comparison.'" (30). Without understanding why this change in interpretation has occurred, it is difficult to incorporate into a risk analysis a speculation that ferret adaptation reduces human adaptation, as Fouchier argues in his paper. Moreover, whether or not this occurred in the particular experiment involving H5N1 in the Fouchier lab, it cannot be assumed to be a reliable outcome of future studies.

CONSEQUENCE OF ONWARD TRANSMISSION OF AN LAI

Fouchier argues that the consequences of onward transmission would be less than assumed in the upper-bound estimate we use for the case fatality ratio (60%), though he does not indicate what estimate he thinks would be more appropriate.

Assertions that wild-type H5N1 is much less than 60% lethal are not well founded. The estimate that several percent of persons in large areas of Asia were asymptomatically infected with H5N1, used to support a lower estimate of wild-type H5N1 lethality, comes from work by Wang et al. (31) which has been directly refuted by influenza serology experts and epidemiologists (32) and further refuted by a separate analysis that was similarly critical of the data used by Wang et al. (33). We do not regard the case fatality risk (CFR) of H5N1 in naturally exposed humans as a settled issue, and well-conducted serosurveys may support the idea that asymptomatic or subclinical infections are more common than previously estimated, at least in some populations (34). Yet for the moment there is little evidence that the observed ~60% CFR in humans for H5N1 is the result of missing large numbers of milder infections, in contrast to the situation, for example of H7N9, where detected cases are thought to be a small fraction of the total (35).

Fouchier further cites evidence of human attenuation when other viruses have been passaged in nonhuman hosts and implies that the viruses passaged in ferrets in his laboratory are attenuated, stating, "[i]n addition, it is important to note that fatalities in ferrets infected with A/H5N1 virus via respiratory droplets or aerosols have not occurred, contrary to when ferrets received large dosages of A/H5N1 virus directly in the (lower) airways" (1). Our response to this point is threefold.

(i) There is no direct evidence that the ferret-passaged variants of H5N1 from Fouchier's laboratory are less virulent for humans, or indeed for ferrets, than wild-type H5N1. Such a comparison would require lower mortality from the ferret-transmissible strain following inoculations of the same doses by the same route. In Table 1 of reference 17, it is shown that 6/6 ferrets died from wild-type or ferret-transmissible virus when exposed by the intratracheal route: in this assay, their virulence was indistinguishable. Table 1 gives no data on wild-type H5N1 administered by the intranasal route, suggesting that ferret-passaged (but nontransmissible) wild-type H5N1 can be used as a stand-in. Even this suboptimal comparison, using four different isolates, is not statistically significant (2/2 versus 1/8; $P = 0.07$). When the engineered viruses were transmitted by aerosol to ferrets, 0/6 died, consistent with a 95% confidence interval for the probability of lethality in ferrets of 0 to 46%. We do not know the inoculum in these transmission experiments and how it compares to inocula in humans if they were infected by aerosol, or how this translates into fatality risk in humans. From a risk assessment perspective, the conservative assumption that human lethality of evolved strains is similar to that of the starting strains is well justified.

(ii) As with transmissibility, reduced ferret lethality of ferret-transmissible strains is a falsifiable hypothesis only when the experiment is undertaken, not a known result. It might occur or it might not occur, and one cannot tell without doing the experiments. It is certainly not a law of nature that transmissibility brings reduced lethality; such reduction did not occur, for example, when H7N1 viruses were made transmissible in ferrets (15).

(iii) As with reduced transmissibility, the assertions of reduced lethality are inconsistent with early statements about the experiments. NSABB member Michael Imperiale was quoted in Science as saying in 2012, "What Ron [Fouchier] is saying now is not what was in the paper. We were led to believe by the paper that aerosol

transmission is also lethal." (30). This view was shared by at least one reporter who attended the Malta presentation of the results (36).

RISKS AND BENEFITS

Fouchier asserts that his claims of the likely low human transmissibility and lethality of the ferret-adapted strains should not be interpreted as reducing the likely benefits of the work for public health. We disagree. Given the uncertainties about whether the strains created in any given laboratory are indeed transmissible and virulent for humans, there should indeed be some probability assigned to the scenarios in which they are not and some probability assigned to those in which they are. This does reduce the overall risk by some factor, though for reasons stated above, we believe the reduction would be modest, rather than the orders-of-magnitude reduction suggested by Fouchier.

While the impact on risk assessment might be to assign less than 100% weight to the scenario of a virulent, pandemic-like strain being released, we believe the same uncertainty negates or even reverses the principal public health benefits claimed for this work. These purported benefits depend on the assumption that mutations found in ferret passage experiments reliably predict pandemic risk. CDC experts state that they have deployed teams to Cambodia based on the presence in H5N1 isolates there of mutations identified in ferret passage experiments (28) and relied on these markers for pandemic threat assessment of H7N9: "Early detection of these molecular markers in H7N9 viruses isolated from humans gave public health authorities evidence that these viruses posed an immediate pandemic threat" (28). Yet there is no evidence that this reliance has improved decisions by CDC or other public health officials, because we do not know if the strains they identified as high risk actually are higher risk than average. This condition of ignorance stems from the fact that there is no validated predictive algorithm for pandemic risk (37).

To take a simplified example, suppose it were the case that 25% of the time, strains produced in ferret passage experiments were highly lethal and transmissible in humans, and 75% of the time they were attenuated. We would not know which instances are which, but suppose we knew these are the overall frequencies. In this case, it would be appropriate to multiply our pandemic risk calculations by about 25%, because 3 out of 4 ferret passage experiments would produce strains not very harmful to humans. Twenty-five percent of the risk we estimated (5) is still exceptionally high. Yet now consider the use of this information by public health authorities. At best, three out of every four times they identified a veterinary or zoonotic isolate as high risk, they would actually be targeting a strain with features that make it attenuated in humans. They would be deploying resources to contain a strain that is, unbeknownst to them, human attenuated. One in four times, they might identify a strain with somewhat increased risk for humans, albeit not necessarily the strain most deserving of attention. In fact, because the prediction of mutational effects becomes more uncertain with changes in the genetic background, the predictive power of such targeting activities is even lower. In summary, while the possibility that ferret gain-of-transmission strains are attenuated in humans modestly reduces the risk estimate associated with producing and using them, it may nullify and even reverse the utility of such studies for public health.

Considering a particular sequence change may help to further illuminate this issue. The CDC team's description of the public health benefits of GOF experiments refers to the lysine mutation at PB2 position 627 as an important factor in raising the level of concern for animal or zoonotic human virus isolates (28). The H1N1 strain of 2009 created a pandemic that caused over 100,000 to 200,000 respiratory deaths globally (38, 39) despite lacking this mutation. Had there been surveillance in place for the viruses giving rise to that pandemic, the lack of this mutation might have misled experts into thinking the virus carried a lower risk and focusing attention on other viruses—a false negative. Indeed, Fouchier's lab was the first to demonstrate that in that genetic background, there was no detectable effect of the mutation (40). This is just one anecdote—though arguably the most pertinent, as it is the only modern pandemic—supporting the general fact that interpreting surveillance through the lens of particular mutations remains an unproven and error-prone technique (37).

WAYS FORWARD

Fouchier repeatedly describes his adjustments to the probability estimates we proposed as "conservative," implying that the actual risk is even less than his figures show. His analysis is not conservative. His estimate of one LAI per ~700,000 worker-years is dramatically lower than that currently estimated for any category of laboratory, and current estimates themselves are too low due to underreporting (9). Moreover, describing the estimates as conservative is at odds with the use of large factors to stand for unknown effects of safety enhancements, inconsistent use of numerators and denominators to favor lower probabilities, and the assumption that safety enhancements used in the Erasmus MC laboratory will be effective in the face of evidence that many laboratory infections have no traceable cause and that many mishaps involving infectious exposures may occur outside the "home laboratory." The assumptions of antiviral and vaccine effectiveness and reduced human transmissibility and virulence of selected strains range from uncertain (in the case of much of the published work) to unknowable (in the case of experiments not yet done) and false (in the case of reduced virulence and vaccine availability in examples such as H7N1) (15). Such assumptions are "anticonservative," giving too-optimistic predictions. Further problems include unsupported claims that the implementation of the select agent program necessarily strengthens biosafety. For example, in the CDC report on the lab accident involving H5N1, the description of the event indicates that scientists were making their decisions in reference to the select agent rule, as opposed to whether there was a biosafety breach (41). The quality of "targeted risk assessments" undertaken before each study is performed is unclear; such assessments have not been quantitative to date (42, 43).

Some of the disagreements discussed here could be clarified by a clearer understanding of the data. It would be extremely helpful for CDC to tabulate incidents with select agents, including LAIs, by the biosafety level of the laboratory involved, so that proper denominators can be used for calculations rather than having to rely on bounding arguments (6). Critical evaluation of claims about the safety of particular laboratories—not only the Erasmus MC laboratory discussed by Fouchier but others where potential pandemic pathogen experiments are proposed or conducted—is impossible without transparent reporting of potential loss, release, and theft events at these particular facilities and in laboratories more generally (9). If the CDC incidents of 2014 have any lesson, it is that state-of-the-art biosafety and biosecurity in highly respected facilities are no guarantee against human error, so there

is a limit to the reassurance one should take from lists of preventive measures in place at any particular facility.

Finally, there are previously published general recommendations regarding risk analysis and catastrophic events. Ord et al. have noted that when one performs a risk analysis and estimates an exceptionally low probability (*P*) of a catastrophic outcome, it is crucial to consider the probability *q* (which may exceed *P*) that the model used to derive that probability is itself wrong, in a way that understates the true probability of the outcome (44). In such a circumstance a correction is needed, adjusting the estimate upward to account for this uncertainty. The combination of an implausibly low estimate of LAI risk with assumptions that are difficult to defend, in a field where underreporting of accidents is thought to be routine (9), would seem to make the assessment suggested by Fouchier's letter (1) a prime candidate for such adjustment.

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