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

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Lipsitch and Inglesby recently estimated the potential public health risks associated with research on influenza virus transmission via respiratory droplets or aerosols between ferrets, leading them to conclude that such research is too risky to be conducted (1). The authors of that and other publications (2–4) estimated the probability of laboratory-acquired infections (LAIs) and onward transmission of the viruses under investigation, as well as the potential consequence to public health if such events were to occur. Given the weight assigned to these risk estimates, it is important that potential pitfalls in the underlying assumptions in these analyses be rigorously scrutinized. Importantly, the published estimates were based on historical data and did not take into account the numerous risk reduction measures that are in place in the laboratories where the research is conducted. Here, I provide a critical appraisal of the published work, discussing, challenging, and modifying the estimates based on the specific conditions under which the work is performed and the properties of the viruses under investigation. By doing so, the outcome of the risk assessment changes from serious risks to negligible risks for humans and the environment. As a consequence, a more balanced debate about the research on influenza virus transmission via respiratory droplets or aerosols between ferrets is warranted, in particular given the substantial public health benefits assigned to this type of research (5, 6).

PROBABILITY OF LABORATORY-ACQUIRED INFECTIONS

Initial calculations of the potential risks associated with research on influenza virus transmission via respiratory droplets or aerosols between ferrets (1–4) used reports on select agent theft, loss, and release collected by the U.S. Centers for Disease Control and Prevention (CDC) from 2004 to 2010 (7) to calculate the probability of occurrence of LAIs. Although these reports have limitations (1, 4, 7), they provide the most recent account of LAIs in the United States and probably reflect the current state of the art in biosafety and biosecurity practices better than older studies on laboratory incidents (8, 9), e.g., as a consequence of the implementation of the U.S. select agent program and best practices developed in biosafety and biosecurity in general over the last decades. From 2004 to 2010, 11 LAIs in total were reported to the U.S. CDC, 4 of which occurred in biosafety level 3 (BSL3) facilities. During this 7-year period, on average 10,000 individuals per year had access to select agents in an average of 292 laboratories per year, thus totaling 2,044 laboratory-years and 70,000 person-years of follow-up (7). From these data, the probability of occurrence of LAIs under BSL3 conditions was calculated as 4/2,044 (or 2×10^{-3}) per laboratory-year, or 4/70,000 (or 5.7×10^{-5}) per person-year (1–4). 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 (10, 11). Research facilities and the experiments that are conducted are therefore appraised through targeted risk assessments, in which the planned studies are scrutinized before any experiment is started. On this note, it is important that none of the LAIs reported to the U.S. CDC from 2004 to 2010 involved viruses (7), and the risks of LAIs associated with work on viral pathogens should thus be estimated as less than 1 per 2,044 ($<5 \times 10^{-4}$ per laboratory-year), or less than 1 per 70,000 ($<1.4 \times 10^{-5}$ per person-year). Unfortunately, the report by Henkel et al. (7) does not specify how many of the 2,044 laboratory-years and 70,000 person-years were related to BSL3 facilities versus BSL2 and BSL4 facilities. Thus, using 2,044 and 70,000 as the denominators yields an underestimation of the true probability of LAIs under BSL3 conditions, as discussed previously (1, 4).

SOME KEY BIOSAFETY MEASURES AND RISK MITIGATION STRATEGIES AT ERASMUS MC

Research on influenza virus transmission via respiratory droplets or aerosols between ferrets is performed in facilities and under conditions that are specifically designed for the purpose of such studies (12–16). In ordinary BSL3 laboratories, including diagnostic laboratories, work is performed in open-front class 2 biosafety cabinets with directional airflow, aimed at protecting the environment from release of pathogens and protecting laboratory workers from exposure. Contrary to ordinary BSL3 conditions for work with viruses, all *in vivo* and *in vitro* experimental work on influenza virus transmission in the Erasmus MC facility is carried out in class 3 isolators or class 3 biosafety cabinets, which are airtight boxes with negative pressure (<-200 Pa), to ensure inward flow in case of leakage (12, 16). Handling is done through airtight gloves fitted to the front of these cabinets. Air released from the class 3 units is filtered by high efficiency particulate air (HEPA) filters and then leaves directly via the facility ventilation system, again via HEPA filters. Only authorized and experienced personnel that have received extensive training can access the facility. For animal handling, personnel always work in pairs to reduce the chance of human error. Although the laboratory is considered "clean" because all experiments are conducted in closed class 3 cabinets and isolators, special personal protective equipment, including laboratory suits, gloves, and FFP3 (class 3 filtering face piece) facemasks, are used, and all personnel are vaccinated with the homologous A/H5N1 vaccine. All equipment in the fa-

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cilities is monitored electronically, and alarm systems are employed to ensure that workers do not enter the facilities if equipment is malfunctioning. All personnel have been instructed and trained how to act in case of incidents, which are handled upon consultation between a senior staff member, a clinical microbiologist, the institutional biosafety officers, and the facility management. Antiviral drugs (oseltamivir or zanamivir) are used immediately in the event that an incident should occur. Every incident in the laboratory is followed up by actions to prevent such incidents from happening again. The facilities, personnel, and procedures are inspected by the U.S. CDC every 3 years, in agreement with the U.S. select agent regulations for overseas laboratories and by the Dutch government (Inspectie Leefomgeving en Transport [ILT] inspection) (12, 16).

The biosafety conditions in the Erasmus MC facility thus extend well beyond "normal" BSL3 conditions for working with viruses, and a number of these biosafety measures should be considered when the probability of LAIs is inferred from the U.S. CDC report. Unfortunately, an exact number for the effectiveness of individual biosafety measures is not available (9). However, it is reasonable to assume that the effectiveness of the physical separation of personnel from the viruses they work with through the use of class 3 isolator units and class 3 biosafety cabinets, the use of personnel protective equipment, the extensive training program, the use of experienced personnel only, and the application of a two-person rule to reduce human error during animal experiments would yield a decrease in the probability of LAIs. Although the magnitude of this increase in safety is not known, I assume that it is at least a factor of 10. Using the risk analysis done by others and this assumption of reduced risk, the probability of a LAI in the Erasmus MC facility would be reduced to below $0.1 \times (1.4 \times 10^{-5})$, or $<1.4 \times 10^{-6}$ per person-year. The quantitative risk assessment to be performed upon request of the U.S. government (17) will have the challenging task of a better quantitative assessment of the effectiveness for each of the biosafety measures in individual laboratories, to yield exact numbers instead of the conservative estimates used here.

The vaccination of laboratory personnel against the homologous A/H5N1 virus under investigation produces another layer of safety that results in further risk mitigation. Given the generally accepted efficacy of influenza vaccine of ~65% for laboratory-confirmed influenza in healthy adults (18), vaccination reduces the probability of an LAI that results in viral escape to below $0.35 \times (1.4 \times 10^{-6})$, or $<5 \times 10^{-7}$ per person-year. The 65% value is almost certainly an underestimate, because this number is taken from general population studies that include individuals with impaired immunity and nonexact matches between the vaccine antigen and the circulating viruses. It is important to note that the antibody titers in our vaccinated laboratory workers are high (geometric mean titer, 987; range, 160 to 10,240) compared to the titers generally accepted as protective against seasonal influenza ($\geq$40) (19) and that individuals are revaccinated if and when their antibody titers decrease (12).

As a consequence of the monitoring of equipment both electronically and by visual inspection, potential exposures to virus are unlikely to go unnoticed. Upon any potential exposures, personnel receive oseltamivir treatment upon consultation with various specialists as indicated above. Such early treatment with drugs has been reported to have ~80% efficacy against human influenza virus infection (20) and avian influenza virus infection (21). Here, it is important to note that viruses under study in the Erasmus MC facility are evaluated for their sensitivity to oseltamivir (12). The immediate treatment of laboratory personnel with oseltamivir upon any potential exposure to virus is thus expected to reduce the probability of LAI further, to below $0.2 \times (5 \times 10^{-7})$, or $<1 \times 10^{-7}$ per person-year, given the average 80% efficacy in preventing laboratory-confirmed influenza.

From this analysis, the conservative estimate is that when research is performed on transmission of influenza viruses via respiratory droplets or aerosols between ferrets in the Erasmus MC facility, to which 10 persons have access, 1 LAI would be expected to occur less frequently than once every 1 million years.

PROBABILITY OF ONWARD TRANSMISSION FROM A CASE OF LAI

The second factor in the equation of the previous risk assessments is the probability of onward transmission from each case of LAI. Previous studies used 5 to 60% as the probability of onward transmission (1, 2), which is based on the unlimited spread of a pandemic influenza virus in the general population. It is important to note that onward transmission from LAIs has so far been uncommon (7–9). In the case of research on influenza virus transmission via respiratory droplets or aerosols between ferrets, a substantial, scientifically justified reduction from the probability of 0.05 to 0.6 of onward transmission from an LAI can be made, based on the above-mentioned biosafety measures and risk mitigation strategies that are in place (12).

The first factor that needs to be considered is that laboratory personnel that acquired the LAI were vaccinated against the homologous A/H5N1 virus and treated with oseltamivir upon any incident with potential exposure to the virus. Although the vaccination and treatment may have been insufficient to prevent infection altogether (hence the occurrence of the LAI at a frequency of less than once every 1 million years), the virus shedding in H5-vaccinated and oseltamivir-treated individuals is likely to be reduced substantially, compared to the onward transmission in times of spread during an influenza pandemic from untreated immunologically naive individuals. If we assume a conservative 2-log reduction in virus excretion in immunized and treated individuals (20–24) compared to untreated immunologically naive individuals, the range of probability of onward transmission from a case of LAI would be reduced to $<5 \times 10^{-4}$ to 6×10^{-3}.

As an important risk mitigation strategy to reduce onward transmission upon any potential LAI, Erasmus MC policy dictates enforcement of quarantine of any laboratory personnel that are potentially virus exposed. This policy would reduce the exposure of nonlaboratory personnel to one (the partner of the laboratory worker) or nil, rather than the ~100 contacts human adults would ordinarily have during a 5-day time frame (25). As a consequence, the transmission probability can be further reduced ~100-fold, yielding a probability of onward transmission from the case of LAI of $<5 \times 10^{-6}$ to 6×10^{-5}.

A final factor to consider in the calculation of the probability of onward transmission from each case of LAI is the basic reproduction number (R0) of the influenza virus under investigation. As indicated above, the previous risk assessments were based on R0 of pandemic influenza virus. However, laboratory experiments have shown that the efficiency of transmission of the laboratory-derived influenza viruses was lower than that of the transmission of pandemic and seasonal influenza viruses in ferrets, as could be

expected (12, 16, 26). Moreover, given that the viruses are ferret adapted rather than human adapted, even an extremely conservative adjustment of the transmissibility parameter by a factor of 2 would yield a "final" estimation of the probability of onward transmission from a case of LAI of $<2.5 \times 10^{-6}$ to 3×10^{-5}.

PROBABILITY OF AN LAI FOLLOWED BY ONWARD TRANSMISSION

Multiplying the probability of occurrence of an LAI by the probability of onward transmission from each case of LAI, one can estimate that the probability of an LAI resulting in onward transmission would range between $(1 \times 10^{-7}) \times (2.5 \times 10^{-6})$ (or 2.5×10^{-13}) and $(1 \times 10^{-7}) \times (3 \times 10^{-5})$ (or 3×10^{-12}). From this analysis, the estimate is that when research is performed on transmission of influenza viruses via respiratory droplets or aerosols between ferrets in the Erasmus MC facility, to which 10 persons have access, 1 LAI with onward transmission would be expected to occur far less frequently than once every 33 billion years. This probability could be assigned the term "negligible," given that the age of our planet is only 5 billion years.

THE CONSEQUENCE OF AN LAI FOLLOWED BY ONWARD TRANSMISSION

In previous work (1–4), it was assumed that if a ferret-adapted avian influenza virus caused an LAI and onward transmission, it could cause a pandemic with an attack rate of 24 to 38%, as deduced from previous pandemics, and a case fatality rate ranging from 1 to 60% in a population of 7 billion people, thus leading to millions of, or more than a billion, fatalities. However, I consider an attack rate and case fatality rate of this magnitude to be unrealistic. Given that the avian influenza viruses under investigation are ferret adapted rather than human adapted, it is unlikely that these viruses would spread as efficiently between humans. Of note, this does not mean that the ferret model is therefore useless for studies to increase our fundamental knowledge about airborne virus transmission; it simply means that—just like when the mouse model is used to address fundamentals in immunology—we need to carefully validate any results obtained in animals before extrapolation to humans. Throughout the history of virology, scientists have adapted viruses to cells, chicken embryos, or animal species in order to yield viruses that have increased replication properties in these specific hosts or cells but at the same time lose replication capacity and virulence in others (27). Examples are the passaging of vaccinia virus in chicken embryo fibroblasts to yield modified vaccinia virus Ankara (MVA), which is now in use as a safe vaccine vector (28), the passaging of measles virus, mumps virus, and rubella virus in various cells to yield the live-attenuated MMR vaccine (29), and the passaging of influenza viruses in mice, ferrets, and eggs to yield the vaccine strain A/PR/8/34 (30), all of which are highly attenuated in humans.

The higher bound of the range of case fatality rates of 60% stems from the number of deaths recorded among laboratory-confirmed cases of A/H5N1 influenza reported to the WHO (31). Since mild cases of infection—those individuals that do not consult a physician or remain untested—are not recorded, the true case fatality rate of A/H5N1 virus infections in humans is unknown. Due to intrinsic difficulties associated with serology data to estimate the numbers of previously infected individuals, there is no consensus on the incidence of A/H5N1 infections in Southeast Asia (32, 33), but case fatality rates orders of magnitude lower than

60% have been inferred (27). In 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 (12, 13, 16).

CONCLUDING REMARKS

On the topic of intentional or accidental releases of viruses from laboratories involved in influenza virus transmission studies, it is important to note that during a decade of transmission studies on pandemic and epidemic strains derived from the 1918, 1957, 1968, and 2009 pandemics and on various wild-type and laboratory-adapted zoonotic viruses of subtypes H1, H2, H5, H7, and H9 (summarized in reference 16), no LAIs have been recorded. There have also been no recorded intentional or accidental releases during more than a century of research with human and animal influenza viruses, including highly pathogenic avian influenza viruses, even at times when biocontainment measures were largely nonexistent. Some have argued that the 1977 Russian influenza epidemic was the result of a laboratory accident (2), but in 1977, influenza research was done under conditions of limited biocontainment, and attenuated and wild type strains were tested in humans. We do not know what happened in 1977, but we cannot conclude that the virus escaped a BSL3(+) laboratory.

Since natural influenza pandemics have occurred on average every 30 years over the last century, the probability that the next pandemic will emerge in nature is orders of magnitude larger than emergence from a laboratory. Given the recently summarized immediate and short-term benefits of research on influenza viruses that are transmitted via respiratory droplets or aerosols between ferrets (5, 6) and the longer-term aims to fully understand and predict and prevent pandemics, combined with the extremely low risk to humans and the environment associated with properly conducted experiments, I conclude that it is sensible and acceptable to restart the research, provided that any laboratory participating in this research adopt biosafety and biosecurity conditions comparable to those that are currently in place (12–16).

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