WHERE SHOULD THE RED LINES BE DRAWN ?

1. **Making Ebola, Lassa or other hemorraghic fever viruses transmissible by coughing or sneezing**

2. **Making HIV transmissible by coughing, sneezing or skin contact**

3. **Making Ebola or rabies transmissible by mosquitos**

4. **Making highly-pathogenic avian influenza viruses transmissible between humans**

5. **Increasing the transmissibility of SARS and MERS viruses between humans**

6. **Making influenza viruses resistant to vaccines and antiviral drugs**

7. **Creating chimeric viruses that could be anticipated to have pandemic potential**

8. **Recreating extinct or eradicated viruses**

9. **Making drug-susceptible bacteria resistant to antibiotics**

10. **Making group A streptococcus (*S. pyogenes*) resistant to penicillin**

11. **Making malaria (*P. falciparum*) resistant to artemisinin combination treatment**

12. **Increasing toxin production of *Pertussis* or *Clostridium difficile***

HI-PATH AVIAN INFLUENZA VIRUSES

SOME IMMEDIATE RED LINES CAN BE DRAWN

1. **GOF experiments *to obtain mammalian transmissibility* of HPAI viruses by respiratory droplets**

Strain	Known human cases (dead-end infections)	Deaths	Mortality
H5N1	826	440	53%
H7N9	>640	224	35%

Other dead-end infection strains (121 cases):
H5N6, H6N1, H7N2, H7N3, H7N7, H9N2 & H10N8

2. **GOF experiments with chimeric influenza viruses**

H1N1 1918-like and analogs

3. **Human pandemic influenza viruses**

Engineering H1N1, H2N2, H3N2 *to totally escape vaccine control*

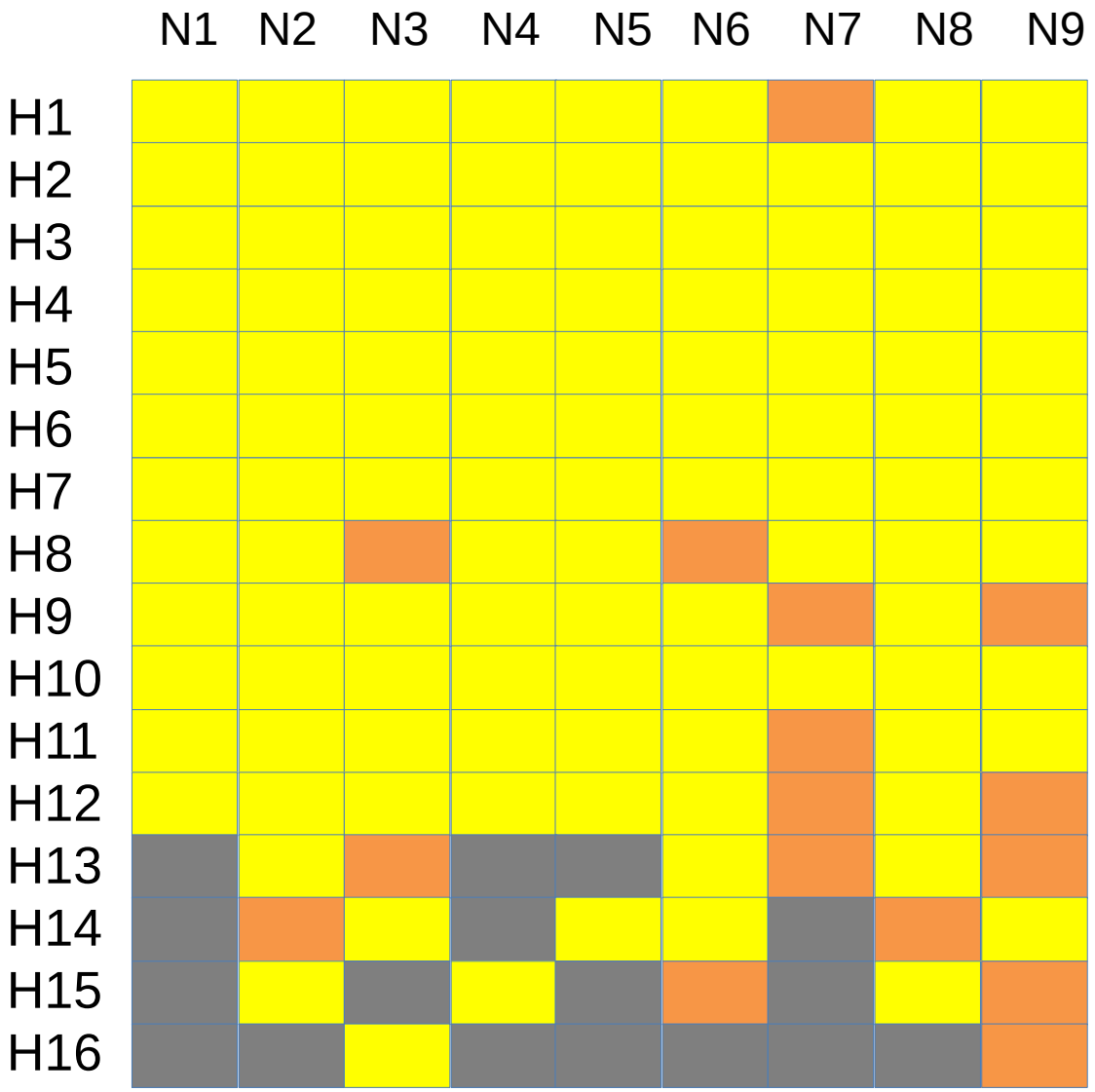
4. **GOF experiments *to increase transmissibility or pathogenesis* of human respiratory viruses**

SARS-CoV	8422	916	11%

Foundation for Vaccine Research
NSABB Meeting, May 5, 2015

Sources WHO & CDC

Aquatic bird reservoir of 127 H,N flu combinations

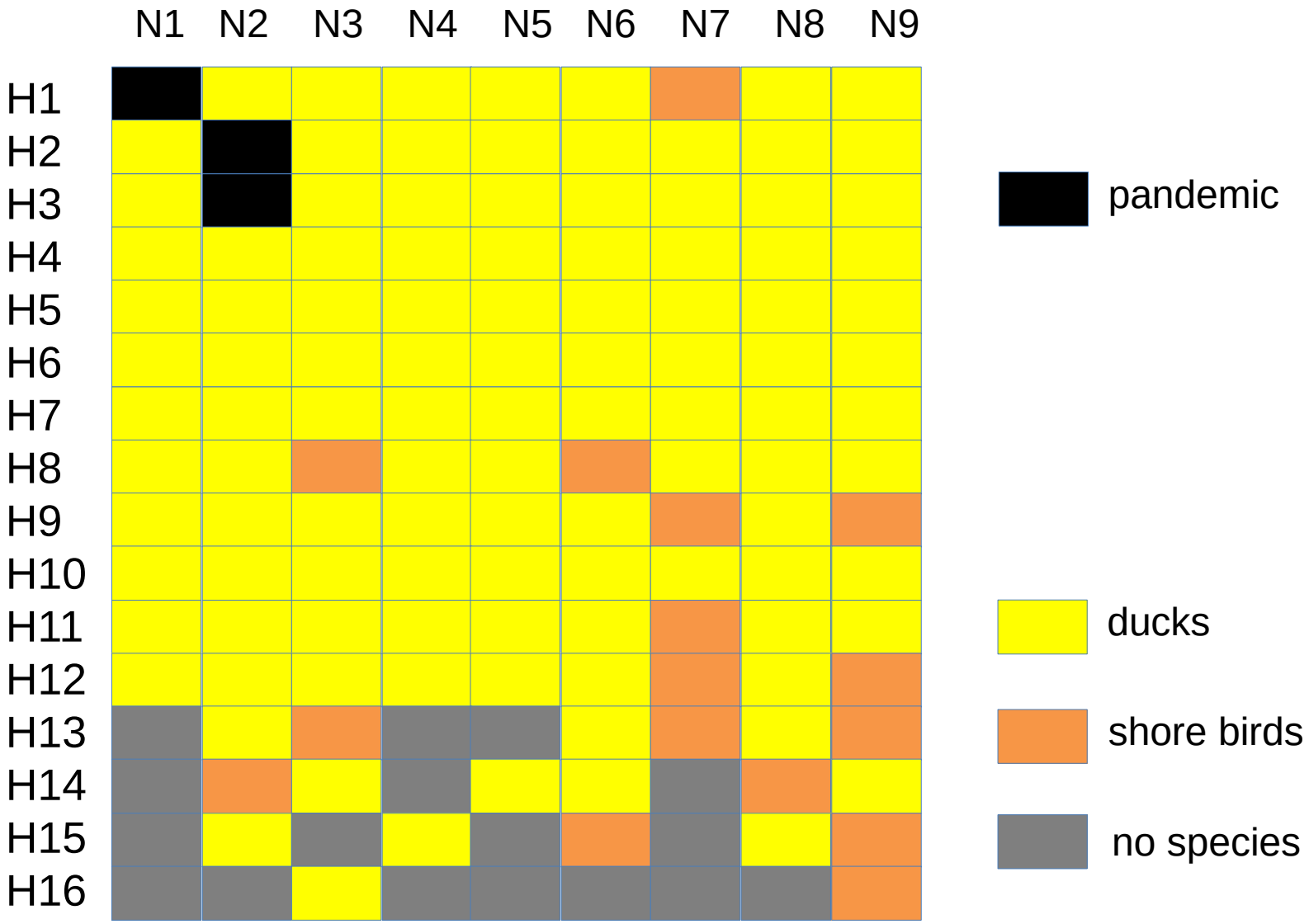


ducks

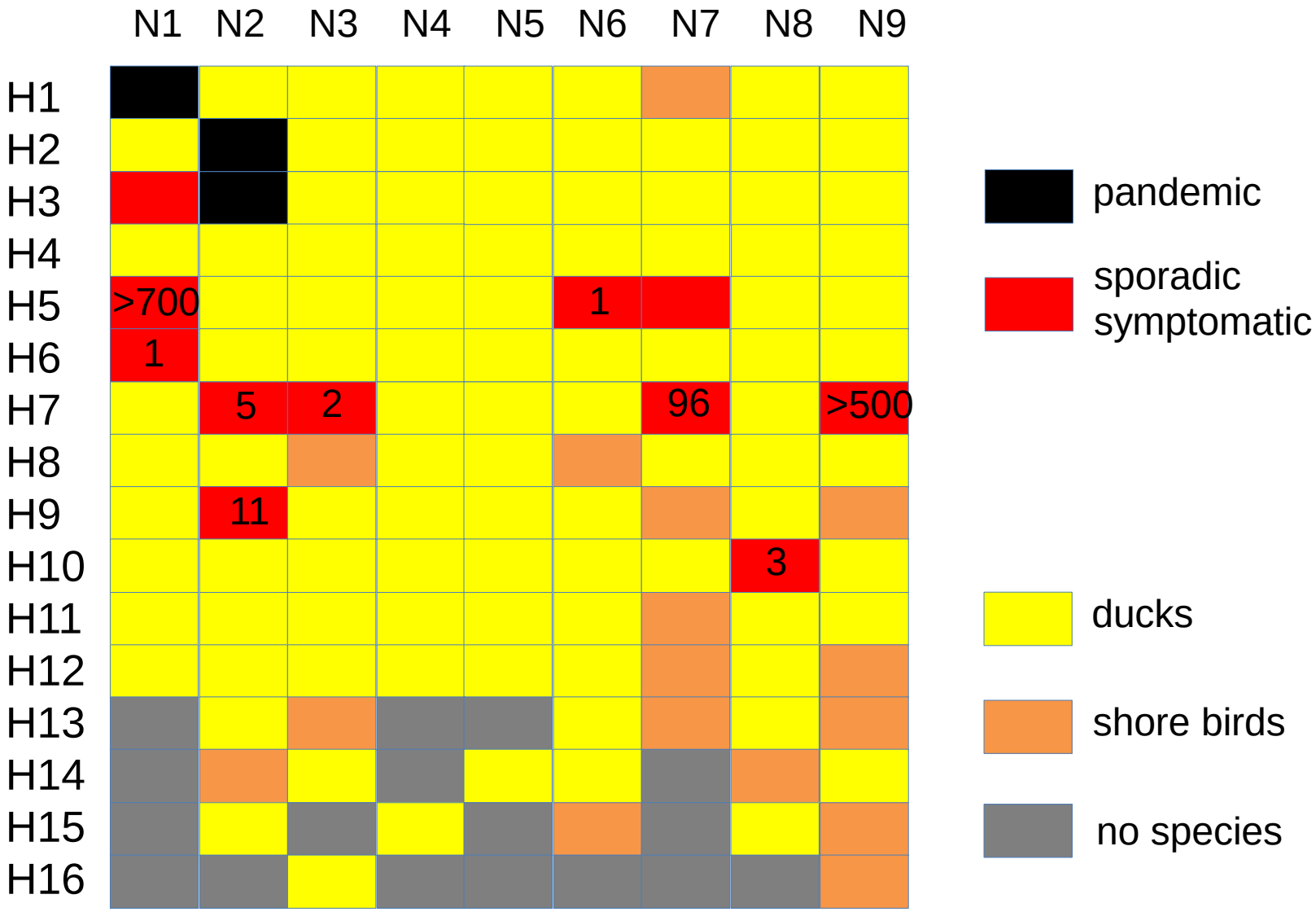
shore birds

no species

Only 3 pandemic H,N combinations in 100 years



Spillovers occur – all dead-end infections



Detailed response to Fouchier's criticism of my mBio comments

To: *The National Science Advisory Board for Biosecurity, in advance of the May 5, 2015 meeting*

From: *Lynn C. Klotz, PhD*
Senior Science Fellow
Center of Arms Control and Non-proliferation,
Washington D.C., USA

My comments on Dr. Fouchier's calculation that "1 LAI would be expected to occur less frequently than once every 1 million years" were published on April 14 2015 in mBio. Fouchier's response to my comments was also published there.

His response is problematic in several ways. In addressing the problems, I will quote frequently from my comments and from his response to make sure it is clear what was said.

The biggest problem is that Dr. Fouchier does not once address my calculation of potential fatalities and fatality burden that employs his low probability of an undetected or unreported LAI escaping from his laboratory. Instead, he chooses to argue against my peripheral comments that his probability is likely much too low. His focus unfortunately pulls attention away from my calculation that finds intolerable potential fatalities and fatality burden.

Detailed comments on Fouchier's criticism

I am numbering the comments to keep each point separate.

1. Dr. Fouchier seems to misunderstand my arguments that his formula, $y=1/P_1$, is too simple. I questioned the meaning of his calculation:

> "Does it give us the elapsed time for a 10% chance that an LAI occurs? Does it give us elapsed time for a 50% chance, or an 80% chance? In this regard, the elapsed time for a 100% chance is infinite, as we can never be absolutely certain that an LAI will occur."

Solving the equation $E = 1 - (1-P_1)^{yn}$ for y, gives $y = (1/n) \times \log(1-E) / \log(1-P_1)$, a better equation for calculation of elapsed time when likelihood or chance of an escape must be considered. Derivations of these equations may be found in the Appendix at the end. The equations and derivations are not necessary to understand the arguments here. They are included only to further document some of my arguments for those who have a basic understanding of algebra and elementary probability.

In reply, Fouchier writes:

> "In calculations of the probability of a community LAI ("E"), Dr. Klotz further assumes that transmission studies in the Erasmus MC facility will be performed for a period ("y") of 1 million years. I am hopeful that our research enterprise will have reached solid conclusions on determinants of airborne transmission a bit sooner."

Rhetorical quip aside, neither his one-million-year calculation result nor my questioning of it implies or assumes in any way that research must be performed for a given number of years. My questioning and my alternative equation are simply a comment on his methodology. It was he who brought up this time frame in the first place: "1 LAI would be expected to occur less frequently than once every 1 million years." Elsewhere in my comments, I assume the research enterprise will be concluded in ten years, as does he.

2. I agree with Fouchier that probability of escape from a laboratory "is the key challenge in this debate." Acknowledging this uncertainty, I use the words 'arguments' and 'likely': "arguments as to why the Fouchier value for P_1 is likely much too low."

Dr. Fouchier writes:

> "Dr. Klotz suggests that incidents at the U.S. CDC laboratories and the long history of escape of LAI agents and other escapes from laboratories show that my estimates of the likelihood of LAIs occurring at the Erasmus MC facility are too low."

The CDC's shipping of an H5N1 contaminated sample to USDA and similar incidents shows the importance of not underestimating human error, especially if one considers the influenza lab at the CDC to be one of the top federal labs in the country. Although biosecurity measures have improved greatly over the years, human nature has not. Laboratory accidents will happen and laboratory workers will get infected, not realize it or not admit it, and take the infection home. The Achilles' heel in Fouchier's argument is that no number of safety procedures can provide for human error.

While the history of escapes should make us worry that the probability may be much higher, the difference between Fouchier and me is moot here since I employ his low probability in my calculations.

3. Dr. Fouchier writes:

> "Dr. Klotz proposes to multiply the low likelihood of LAIs by 300, based on an estimated 30 laboratories involved in the "whole research enterprise" for 10 years, and assumes that part of this research enterprise may lack the rigorous safety practices in place at Erasmus MC. Both assumptions are wrong, to the best of my knowledge; just over a handful of laboratories have worked on airborne transmission of avian influenza viruses, each of which has rigorous safety practices in place."

Our disagreement here is because we define "whole research enterprise" differently. I define it as research on pathogens subject to the NIH funding pause (influenza and SARS category pathogens). He defines it as only influenza research. I implied that the whole research enterprise includes the other pathogens by picking the number 15 for NIH's 15 projects subject to the pause. Perhaps I should have been explicit by listing the pathogen categories as I have just done. In addition, some of the laboratories throughout the world conducting this research that are not funded by NIH may have lax safety standards.

Thus, both of my assumptions are likely correct.

4. Dr. Fouchier writes:

> "Another key aspect is that Dr. Klotz estimates the likelihood of onward transmission from a case of LAI as 0.1 (10%), in contrast to my justification for an adjusted likelihood of $<1 \times 10^{-5}$, based on the specific conditions under which the research is performed, without providing a rationale for that important deviation"

I certainly do provide a rationale for the 10% through references (8) and (9) to risk assessment studies:

> "Summarizing the literature, Lipsitch and Inglesby estimate the probability that a community LAI leads to a global spread (pandemic) to be 5 to 60%. This range is consistent with the 5 to 15% range found by Merler and coworkers (8) and with the 1 to 30% range found in a focused risk assessment (9) for infection spread beginning on crowded public transportation."

Furthermore, there is a rather arcane subject in probability theory, branching theory, which allows prediction of the likelihood of uncontrolled spread of any pathogen based on its R_o value and the variance to mean of the R_o. A large variance to mean would occur due to super spreaders, for instance some people infected with SARS. For a wide range of R_o values, Lipsitch and coworkers have calculated the probability of uncontrolled spread (see figure 4a in their study). For a single infected individual with $R_o = 2$, the probability ranges from 10% (spread of R_o's) to 80% (uniform R_o).

Thus, the pandemic likelihood from a single infected individual is potentially large. I suspect that future risk assessments will confirm that once a highly contagious potential pandemic pathogen escape occurs, the probability of an uncontrolled outbreak is significant.

Fouchier mentions vaccination and antivirals as factors that reduce onward transmission. Antivirals would not be prescribed for undetected LAIs. Vaccines may reduce viral replication in the index case, but active virus may still be present when the infected person leaves the laboratory potentially infecting unvaccinated persons. The annual flu vaccine is sometimes less than 50% effective, so it is unclear if vaccinated laboratory workers are protected by the laboratory vaccine strain.

I would classify vaccination and antivirals, effective or not, as inside laboratory measures. But if an LAI escape occurs, clearly these measures were not effective in preventing the undetected or unreported LAI.

Again, we come back to the probability of escape from a laboratory as the key challenge in this debate.

Once an undetected or unreported LAI from a highly contagious pathogen escapes from the laboratory, it is out of Fouchier's control. Its global spread will depend on the reproductive number, R_o, and other factors external to Fouchier's laboratory.

Fouchier claims that "the viruses are ferret-adapted rather than human adapted," which could lead to a lower R_o in humans. Among the different mutated viruses presumably under development in his

laboratory, some could be highly transmissible and deadly in humans. We will never know for testing them on humans is, fortunately, unethical. Of course if one escaped…

The argument of being ferret adapted and not human adapted is misleading. First, it cannot be proved. Secondly, Fouchier's own work may have already brought an avian H5N1 virus far closer to successful replication in humans. If such a virus escaped from his laboratory, it may well adapt within the individuals in the early transmission chain and then take off in a big way. Dr. Fouchier and the field do not have the knowledge to know just how short of a successful virus they have engineered. That is why they are doing this work.

5. Dr. Fouchier concludes

> "Finally, Dr. Klotz describes the (apocalyptic) scenario of an influenza pandemic with 140 million fatalities based on a 10% case-fatality rate in 20% of the world's population. These numbers not only ignore the scientifically justifiable counterarguments raised before (2) but also are at odds with the documented influenza pandemics of the past. In my view, the "gain-of-function" debate has suffered from the apocalyptic scenarios that are provided as factual whereas they provide estimates that are far beyond the observed worst cases (8)."

It is estimated that the 2009 pandemic influenza infected 20% of the world population. The 1918 H1N1 "Spanish" flu killed perhaps 2% of its victims. The H5N1 avian influenza virus, the subject of Fouchier's research, kills about 50% of those who are infected through direct contact with poultry. The scenario I use as an example represents a combination of these three <u>real</u> events. While this scenario has not yet and may never occur in nature, it is a possible scenario perhaps more likely from a laboratory escape.

Since the consequences of most scenarios, even one on a par with seasonal influenza– several hundred thousand deaths – would be catastrophic and unacceptable, it behooves us to be exceedingly careful in deciding which potential pandemic pathogen research should be allowed. For much of this research, the potential risk far outweighs the potential benefits.

APPENDIX

Derivation of equations for years to a lab escape

Let P_1 be the yearly probability of escape of a pathogen from a single lab. The first question to be asked is "What is the probability of at least one escape from one of the n labs conducting research on the pathogen for y years.

The probability of no escapes in y years for a single lab is

$$\text{prob (no escape)} = (1-P_1)^y \qquad (1)$$

For y years *and* n labs

$$\text{prob (no escape)} = (1-P_1)^{yxn} \qquad (2)$$

The probability, E, of at least one escape in y years from one of the n labs is

$$E = 1 - (1-P_1)^{yxn} \qquad (3)$$

How much risk are we willing to tolerate; that is, what value of E is too high a risk? E=1%, E=10%? E=50%? E=80%? The level of risk we are willing to tolerate is subjective. A related question is: At our risk tolerance level, how many years y of research in the N labs will it take to exceed our risk toleration? Solving equation (3) for y, will allow this question to be answered.

$$\log (1-E) = \log(1-P_1)^{yxn} = y \times n \times \log(1-P_1)$$

$$y = (1/n) \times \log(1-E) / \log(1-P_1) \qquad (4)$$

Checking the limit for equation (4): If there is no likelihood of escape $P_1=0$, log(1)=0, and as expected $y=\infty$.

Some examples of the use of equation (4):

N =	30						
					y		
			E =		**E =**		**E =**
p_1	**p_N**		**0.01**		**0.5**		**0.99**
0.1	0.958		0.0		0.22		1.46
0.01	0.260		0.0		2.3		15.3
0.002	0.058		0.2		11.5		76.7
0.001	0.030		0.3		23.1		153.4
0.0001	0.003		3.3		231.0		1,535
1.00E-06	3.000E-05		335.0		23,105		153,506

TABLE A. Some sample values for N=30 labs. The body of the Table is years to at least one escape.

For instance, if the probability of escape from a single lab in a single year is 0.0001 or 0.01% (a reasonable estimate), and we will tolerate only a 1% chance of escape, E=0.01, over the 30-lab research enterprise, the number of years for at least one escape is only 3.3 years.

Another observation about equation (4), the number of years of research, y, which must elapse before we reach our risk-tolerance level is inversely proportional to the number of labs, n.

COMMENTS ON THE NSABB DRAFT FRAMEWORK DATED 6 APRIL 2015

Marc Lipsitch, DPhil
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Comments dated April 24, 2015

Overall I believe that the Draft Framework dated 6 April 2015 contains much that is of value, that it makes mostly appropriate recommendations for the structuring of the risk and benefit assessment, and that it appropriately mentions alternative approaches, human error, and the importance of including scenarios where countermeasures may and may not be effective, as well as both scenarios involving accidents and those involving malevolent action.

However one essential element appears to be missing and to suffer from vague and contradictory directions. This is the question of *what exactly* is being assessed for its risks and benefits, what are the *components* of those risks and benefits, and *in comparison to what* are they being assessed?

1. What is being assessed? To calculate, say, the risks of GOF experimentation, it is necessary to specify which pathogen(s), investigated by how many laboratories, for what period of time, at what biosafety level, among other inputs. The risk presented by one laboratory for one year will be multiplied by approximately a factor of 6 if, say, 2 laboratories work for 3 years under the same conditions. Evidence about the rate of laboratory-acquired infections (LAI) is obtainable with denominators of laboratory-years or full-time laboratory-worker-years [1].

RECOMMENDATION: Most importantly, the Framework should specify some unit of research. Specifically, because LAI are the precipitating events for most of the scenarios of greatest concern, I recommend that the unit of analysis be the high-containment laboratory-worker-year or laboratory-year, to facilitate data assimilation. Biosafety conditions should also be specified.

2. What are the components of these risks? An essential aspect of a risk assessment on this topic is to clearly separate the two components: (a) probability of an adverse outcome, and (b) magnitude or consequence of this outcome. For GOF, the probabilities of LAI are not extraordinary, but the consequences may be in certain cases.

RECOMMENDATION: Analysis would be clarified greatly by specifying that these calculations should be described separately and then appropriately combined to estimate risk.

3. In comparison to what are these being assessed? The RA/BA process is intended to aid the USG in making a decision: whether to fund GOF research, and under what conditions. Two possible decisions would be to resume GOF funding using ordinary biosafety review and no additional review, or to stop funding such work for a defined period of time or permanently. In the event of the latter decision, the USG research portfolio on influenza would not be expected to change in overall size, but only in composition.

Note: This issue is particularly confused and contradictory in the current Draft and thus

needs clarifying. Guiding principle 1 in the draft states "The possible risks and benefits of not doing this work also need to be thoroughly examined." As I understand this instruction, it involves either a trivial point (risks of doing = benefits of not doing, and vice-versa) or more likely an instruction to compare GOF to no GOF, without considering the alternatives that would be undertaken. That however contradicts Guiding Principle 2. This needs to be clarified.

Note: This decision is highly consequential. In a medical context, where risk-benefit analysis is commonly employed, very different conclusions would follow from evaluating an antibiotic treatment for a life-threatening condition, where the antibiotic carries a 1/10,000 risk of causing liver failure if the alternatives were (a) no treatment or (b) treatment with another antibiotic of similar efficacy without the risk of liver failure. Similarly, an unrealistic comparison of GOF research to "not doing GOF research" might have a very different risk-benefit profile from the actual comparison, which is replacing GOF in the research portfolio with other approaches, holding the budget constant.

RECOMMENDATION: I recommend that the Framework specify that the RA/BA should compare the risks and benefits of

(1) A USG influenza research portfolio of a fixed budget including GOF and non-GOF research, with composition determined by peer review and other existing mechanisms

vs.

(2) A USG influenza research portfolio of the same budget including only non-GOF research, with composition determined in the same way apart from the removal of GOF research.

Specific comments

1) lines 49-50: SARS and MERS are no longer at issue. Same on lines 262-4. They are not still in the funding pause. They should perhaps be included in the RA/BA but this is not accurate.

2) Overall comments on guiding principles: apart from the comment above, these are sensible and comprehensive.

3) lines 268-279 All of these are important considerations but of particular concern are experiments reasonably anticipated to result in a virus that is readily transmissible, not known to be currently circulating in humans, and virulent in humans. Increasing one of these in the absence of others may not be of such concern, but the three properties together are of special concern. This was much discussed at the NAS meeting, and in particular David Relman's remarks emphasized the cruciality of the combination.

4) lines 282-334 This list is in general quite appropriate and comprehensive, with exceptions described here and in the next comment. The comment "Opportunity costs might also be considered" (l. 333) is ambiguous. If it means opportunity costs of doing GOF as opposed to other, alternative (and generally much safer) approaches, the alternatives MUST be considered (line 180 ff.). If it means something else, it should be spelled out.

5) One category of risk not included and very important is reputational and credibility risk for science. If in the face of ongoing laboratory mishaps at the nation's most prestigious laboratories, the US Government decides to fund and approve experiments to create novel pathogens with pandemic potential, and there is an accident involving serious outcomes following accidental infection, the credibility of science as a whole will suffer, leading the public to question the quality of public stewardship of biomedical funding, and indeed to question the reliability of scientific and medical advice regarding risk. This should be explicitly considered as an independent category of harm that could result from an accident. In an era of science skepticism related to issues from climate change to vaccine safety, this could be harmful to science's ability to inform policy, not to mention to science funding.

6) lines 347-352. Scientific knowledge is a benefit of all scientific research, including GOF and alternative approaches to virology. Scientific knowledge has appropriately been characterized as having unpredictable outcomes. This is a reason to do science, but not a reason to choose one (risky) scientific approach in preference to other (low-risk) approaches. The question of what unique scientific knowledge can be generated is only appropriate if it is asked both of GOF and of alternative types of scientific study that would be foregone -- that is, opportunity costs must be properly accounted for.

7) lines 354-385. The emphasis on comparison against alternatives mentioned in lines 373 and following is welcome, but should cover all of these points.

8) lines 387-391 Informing policy decisions. There is an important distinction between research that can *inform* policy decisions and research that can *uniquely improve* policy

decisions. Policy makers may well use information in decisions that does not make those decisions better. Evidence for a benefit should be evidence that GOF results *uniquely improve* policy decisions. The term uniquely is important because the phenotypes and in most cases the mutations found in the GOF influenza experiments to date were all known to be important for mammalian adaptation of influenza viruses before the GOF studies. Improvement vs. informing is crucial because the ability to predict influenza pandemic risk is agreed by a wide range of scientists with varying views on GOF to be a long-term future aspiration without evidence that such predictions can be validated by experience to date [2].

9) Line 441: accurate is a strange term to use for a hypothetical scenario. A critic could say that some aspect of a hypothetical scenario is not "accurate" because exactly that condition does not exist, but this would be crippling as a constraint on scenario generation. Credible is a good word; plausible or realistic might be other appropriate modifiers.

10) lines 410-480. Again this list is appropriate and comprehensive. Point 14 is ambiguous, and should read "For comparison against the risks of GOF research, scenarios should be generated involving the above categories (where appropriate) involving alternative, non-GOF approaches.

11) line 534-76. This list is appropriate and comprehensive. It should be emphasized that these should be applied to both GOF and alternative approaches.

13) lines 543-4. I this instance (as in all, but especially here) citation counting may be misleading. This work has been extremely controversial and therefore unusually visible. That has prompted acceptance of multiple papers by prestigious journals and much commentary (including criticism of the safety and security aspects) which has significantly contributed to citation counts. For papers as new as these one might argue that citation counts are not good indicators of scientific importance, but rather (in the short term) of visibility and controversy.

1. Lipsitch M, Inglesby TV (2014) Moratorium on research intended to create novel potential pandemic pathogens. MBio 5.
2. Russell CA, Kasson PM, Donis RO, Riley S, Dunbar J, et al. (2014) Improving pandemic influenza risk assessment. Elife 3: e03883.

These references are included for convenience of the NSABB and form part of my formal comments.

Moratorium on Research Intended To Create Novel Potential Pandemic Pathogens

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Research on highly pathogenic organisms is crucial for medicine and public health, and we strongly support it. This work creates a foundation of new knowledge that provides critical insights around the world's most deadly infectious diseases, and it can lay groundwork for the future development of new diagnostics, medicines, and vaccines. Almost all such research can be performed in ways that pose negligible or no risk of epidemic or global spread of a novel pathogen. However, research that aims to create new potential pandemic pathogens (PPP) (1)—novel microbes that combine likely human virulence with likely efficient transmission in humans—is an exception to that rule. While this research represents a tiny portion of the experimental work done in infectious disease research, it poses extraordinary potential risks to the public.

Experiments that create the possibility of initiating a pandemic should be subject to a rigorous quantitative risk assessment and a search for safer alternatives before they are approved or performed. Yet a rigorous and transparent risk assessment process for this work has not yet been established. This is why we support the recently announced moratorium on funding new "gain-of-function" (GOF) experiments that enhance mammalian transmissibility or virulence in severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and influenza viruses. This realm of work roughly corresponds with the work we have termed PPP above. Because the term "gain of function" in other contexts can be used to describe techniques of scientific research that have nothing to do with the creation of novel potential pandemic pathogens, we think the term can be too broad and can mislead. Throughout this commentary, we focus on research designed to create PPP strains of influenza virus, the type of research that initially attracted attention, leading to the moratorium and for which the most discussion has already occurred. Other types of gain-of-function research on influenza and studies intended to enhance pathogenicity or transmissibility of MERS and SARS coronaviruses may or may not fit the definition of PPP research and further clarification is needed and ongoing. As we discuss near the end of this article, it will be essential to clarify the different risks and benefits entailed by different types of experiments covered by the funding pause (2).

The purpose of this research funding pause is to complete "a robust and broad deliberative process . . . that results in the adoption of a new [U.S. Government] gain-of-function research policy" (3). The moratorium would stop new funding for the following:

> . . . research projects that may be reasonably anticipated to confer attributes to influenza, MERS, or SARS viruses such that the virus would have enhanced pathogenicity and/or transmissibility in mammals via the respiratory route. The research funding pause would not apply to characterization or testing of naturally occurring influenza, MERS, and SARS viruses, unless the tests are reasonably anticipated to increase transmissibility and/or pathogenicity. (3)

The new U.S. Government (USG) policy also encourages the currently funded U.S. Government and nongovernment research community to join in adopting a voluntary pause on research that meets this gain-of-function definition. Some 18 NIH research projects that possibly meet that definition have been identified (2). The moratorium does not apply to the larger infectious disease research portfolio supported by the U.S. Government. In particular, it does not affect disease surveillance or vaccine development programs. During the moratorium, a deliberative process will occur that will be led by the National Science Advisory Board for Biosecurity and the National Academy of Sciences. This process is intended to produce "recommendations for risk mitigation, potential courses of action in light of this assessment, and propose methodologies for the objective and rigorous assessment of risks and potential benefits that might be applied to the approval and conduct of individual experiments or classes of experiments" (3).

In this commentary, we discuss key elements of risk analysis and offer an example of an approach that could be taken. We describe benefit analysis, offering an account of the kinds of benefits that are relevant and our own view of those at this point. We note other factors that are important to consider. And we argue that a moratorium is the right approach until a rigorous, objective, and credible risk assessment process can be established.

RISK ANALYSIS

Risk assessment for GOF work should be quantitative, objective, and credible. Extensive qualitative arguments have been made on both sides of this issue, and these arguments have not provided sufficient clarity or evidence to resolve concerns or identify a consensus path forward. Quantitative assessments should now be performed so as to provide specific calculations and information to inform decisions. It is also important for these risk assessments to

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be objective. Given the stakes in this process, the risk assessment process should be directed by those without a clear personal stake in the outcome, just as peer review of science is performed by those without a direct interest in the outcome. The credibility of the risk assessment will depend both on the rigor of the quantitative process and the perceived objectivity of the process.

The record of laboratory incidents and accidental infections in biosafety level 3 (BSL3) laboratories provides a starting point for quantifying risk. Concentrating on the generation of transmissible variants of avian influenza, we provide an illustrative calculation of the sort that would be performed in greater detail in a fuller risk analysis. Previous publications have suggested similar approaches to this problem (1, 4).

Insurers and risk analysts define risk as the product of probability times consequence. Data on the probability of a laboratory-associated infection in U.S. BSL3 labs using select agents show that 4 infections have been observed over <2,044 laboratory-years of observation, indicating at least a 0.2% chance of a laboratory-acquired infection (5) per BSL3 laboratory-year. An alternative data source is from the intramural BSL3 labs at the National Institutes of Allergy and Infectious Diseases (NIAID), which report in a slightly different way: 3 accidental infections in 634,500 person-hours of work between 1982 and 2003, or about 1 accidental infection for every 100 full-time person-years (2,000 h) of work (6).

A simulation model of an accidental infection of a laboratory worker with a transmissible influenza virus strain estimated about a 10 to 20% risk that such an infection would escape control and spread widely (7). Alternative estimates from simple models range from about 5% to 60%. Multiplying the probability of an accidental laboratory-acquired infection per lab-year (0.2%) or full-time worker-year (1%) by the probability that the infection leads to global spread (5% to 60%) provides an estimate that work with a novel, transmissible form of influenza virus carries a risk of between 0.01% and 0.1% per laboratory-year of creating a pandemic, using the select agent data, or between 0.05% and 0.6% per full-time worker-year using the NIAID data.

Readily transmissible influenza, once widespread, has never before been controlled before it spreads globally, and influenza pandemics historically have infected about 24 to 38% of the world's population (8, 9). The case-fatality ratio of a novel strain is of course unpredictable. The worst case might be a case-fatality ratio similar to that of avian H5N1 influenza virus in people, which approaches 60% (10). A greatly attenuated version of the same virus might have a case-fatality ratio of "only" 1%.

Again, multiplying the pandemic attack rate (24% to 38%) times the global population (~7 billion) times the case-fatality ratio (1% to 60%) would produce an estimate of between 2 million and 1.4 billion fatalities from a pandemic of a highly virulent influenza virus strain.

Putting all these numbers together, the select agent data suggest that a laboratory-year of experimentation on virulent, transmissible influenza virus might have an 0.01% to 0.1% chance of killing 2 million to 1.4 billion, or an expected death toll of 2,000 to 1.4 million fatalities per BSL3-laboratory-year. From the NIAID data, for each full-time person-year of BSL-3 work, we might expect a toll of between 10,000 and over 10 million.

These numbers should be discussed, challenged, and modified to fit the particularities of specific types of PPP experiments. For creation of novel, transmissible, virulent influenza virus strains, they may overstate the risk for the following reasons: (i) most such work is done in BSL3+ labs, which may be safer than BSL3; (ii) control measures, including vaccination and antiviral prophylaxis of laboratory workers, might reduce the risk of infection and of spread, although none of these is perfect; (iii) the human case-fatality ratio of an avian influenza virus strain that gains transmissibility could be below 1%; (iv) transmissibility in laboratory animals does not necessarily indicate transmissibility in humans (11, 12); and (v) novel strategies of molecular biocontainment (13), if employed, might reduce the risk of human transmission of a strain used in transmission experiments in other mammals.

On the other hand, these numbers may understate the risk because (i) the select agent calculation includes in its numerator only BSL3 labs, but in the denominator, BSL3 as well as BSL2 and BSL4 "registered entities" as separate figures for BSL3 are not publicly available (5); (ii) the rate of accidents is calculated for U.S. labs, while GOF experiments are performed in many countries; if this work expands to some of the many countries with less stringent standards than those in the United States (14), risks could be higher; and (iii) the costs of an accidental pandemic considered here are deaths only, but additional losses would include scientific credibility, nonfatal health outcomes, economic and educational losses, etc.

The illustrative calculations above show that approximate risk estimates are possible for creation of PPP strains of influenza virus. During the deliberative process initiated with this moratorium, the risk assessment approach that is established should be able to provide calculations that reflect these and other available probability and consequence estimates and take into account the range of modifying factors, including those just described. The risk assessment process should also be able to provide calculations related to PPP experiments where the risks are harder to calculate given more limited data, such as enhancement of coronavirus pathogenicity in small mammals.

BENEFIT ANALYSIS

On the surface, analyzing the benefits of PPP experimentation would seem more difficult. In the cumulative process of knowledge acquisition that is science, it is hard to see far ahead where a particular type of research may lead. On the other hand, scientists make judgments about the relative merits of experimental approaches on a daily basis in their roles as investigators and grant reviewers. Doing and funding science constitute a process of severe winnowing (especially severe in today's tight funding climate) in which we choose to pursue one approach and not to pursue others based on judgments of which approaches are expected to have the lowest cost, highest probability of success, and greatest yield of valuable findings, among other considerations. Implicit in this process is the idea of opportunity cost. In prioritizing the week's or the year's research work, we do not judge in isolation whether a particular experiment should be done or not done. We decide how to allocate our time and funding among possible approaches, devoting resources to the portfolio of efforts that seems most promising. Similar prioritizations are made by funders when they decide which kinds of research will be funded and which research will not.

The analysis of benefits of PPP experiments should follow this familiar approach. The choice is not between doing PPP experiments and doing nothing. Rather, the appropriate question is, within a portfolio of scientific and public health activities designed

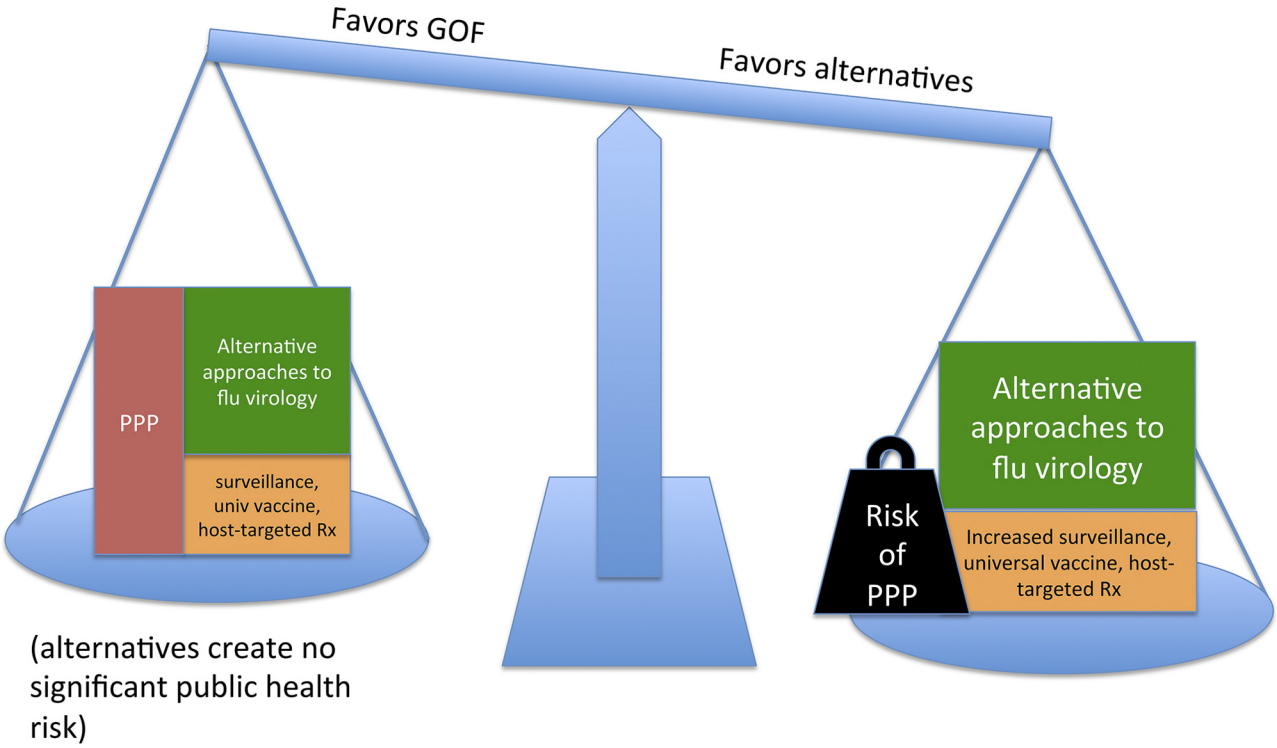

FIG 1 Weighing risks and benefits. The benefits (squares) of spending a fixed quantity of resources on a portfolio of activities, including PPP research (red), other approaches to influenza virus virology (green), and other public health activities to defeat influenza (yellow), should be weighed against the benefits of a portfolio in which the other activities are expanded to use the resources freed by not supporting PPP activities, reflecting the opportunity cost of the PPP research. If there are net benefits to including PPP activities in the portfolio, then they should be weighed against the net risks created by PPP experiments, which in the case of influenza transmissibility enhancement, we have argued (see the main text, Risk Analysis) are exceptionally high. The balance may differ for other activities, but this comparison of benefits of portfolios with and without gain-of-function experiments is the appropriate comparison, with any net benefits weighed against net risks. univ, universal.

to understand and combat influenza or a coronavirus (or, perhaps, in our portfolio of infectious disease countermeasures more broadly), what are the benefits of including PPP approaches compared to the benefits of expanding other parts of the portfolio to use the resources in another way? From the perspective of public health and the practical goal of preventing and treating flu, alternative approaches include those which, like PPP experiments, seek to enhance our scientific understanding of biology, pathogenesis, and transmission. Alternatives also include efforts to develop treatments and prevention measures, including surveillance, through means other than improving our basic biological understanding of influenza (4). This approach is shown graphically in Fig. 1, which also depicts the risks of PPP research. Such risks should be weighed against the risks of alternatives, which are typically much smaller or even negligible. Figure 1 embodies the idea that PPP research should be a component of our research portfolio only if devoting resources to PPP studies at the expense of alternatives has net benefits that outweigh the unique risks of PPP studies.

This comparative approach to benefits should be informed by a hard-nosed look at the benefits that are readily achievable by PPP experiments, not hypothetical outcomes that could someday lead to unspecified benefits. We acknowledge the possibility that PPP experiments may lead to benefits we cannot today envision. But so could the experiments that are done in their place if support for PPP is reallocated to other scientific approaches. The possibility of unanticipated benefits is surely a reason to do science, but it is not a reason to favor PPP approaches over others, unless some specific case can be made for the unique yet unanticipated benefits of PPP work. Such a case seems hard to imagine for benefits that are by assumption unanticipated.

For example, it has been suggested that mutations or phenotypes identified through PPP experiments could be used to sort through the massive diversity of nonhuman influenza virus strains to prioritize those that should trigger countermeasures, including prepandemic vaccine manufacturing. While this is possible in principle, there are many practical barriers to achieving public health benefits of this sort from PPP studies (15). Lists of mutations, and even phenotypes, associated with PPP studies can be compiled and compared against isolates of influenza viruses from birds and other nonhuman sources (16). We know that these lists are unreliable and can even be misleading: the mutations in hemagglutinin identified by two prominent PPP experiments with H5N1 do not reliably confer human receptor specificity even for other H5N1 viruses (17). The E627K mutation in the PB2 gene, known as a virulence and transmissibility determinant before GOF experiments (16, 18, 19), found repeatedly in GOF experiments in H5N1 (20, 21), and used for pandemic risk assessment in H7 viruses (16), was found in some isolates of the H1N1pdm strain in 2009, leading to concern about possible increased virulence and transmissibility. Yet it conferred neither trait in this genetic background (22).

At this time, the high levels of epistasis—dependence of phenotype on the genetic background in which a mutation is found—make prediction of pandemic risk for any given strain more of an art than a science. Indeed, the very presumption that we will see human cases of an incipient pandemic before that pandemic occurs has never been met in practice (23): we have never observed zoonotic cases of any flu virus before it caused a pandemic. This is not to deny that PPP experiments provide any useful data for surveillance and prioritization. Rather, it is to say that other approaches can also identify such predictors (as in the case of the PB2 mutation [11, 13, 14]) and that the ability to use markers of putative transmissibility or virulence to make reliable predictions remains far in the future (23). The fact that some analysts consider mutations identified in PPP experiments when assessing threats of viruses found in surveillance does not mean that the use of such mutations improves the predictions, a claim for which we have no evidence because no pandemic strain has ever been identified in advance. The analysis of benefits of PPP creation should reflect this state of science.

According to some proponents, the most valuable scientific finding of experiments to make ferret-transmissible mutants of influenza A/H5N1 is the definitive proof that such variants could be produced with a small number of mutations. This could not be definitively proven without doing the PPP experiment to manufacture a potentially pandemic variant of H5N1 (24). While it is now undeniable that ferret-transmissible mutants of influenza A/H5N1 can be created experimentally, the impact on scientific opinion about the risk of a pandemic from H5N1 has been hard to gauge. Prior to the gain-of-function experiments, there was a wide range of expert opinion on the likelihood of an H5N1 pandemic (25). Some influenza experts questioned whether H5N1 was a major pandemic threat. After the publication of the experiments producing potentially pandemic H5N1, one prominent member of this group, Peter Palese, noted the shortcomings of the ferret model for humans and correctly concluded that the question of whether H5N1 can transmit efficiently in people remains unsettled (11), as it must until the phenomenon is directly observed in nature. From a practical perspective, responsible policy makers and public health leaders should have been planning for the possibility of an H5N1 pandemic before PPP experiments on H5N1 were undertaken. In some countries of the world, they were stockpiling vaccines against H5N1 (26, 27) and making plans for nonpharmaceutical (8) interventions in the event of a pandemic. The same remains true after the experiments. We have observed no discernible influence of the H5N1 PPP experiments on H5N1 policy preparations.

CALCULATING OTHER FACTORS

During the moratorium, progress should also be made in calculating the risks associated with potential deliberate misuse of PPP strains and with potential deliberate misuse of the information that is created and published following PPP experimental work. This calculation should take into account the possibility of deliberate theft and dissemination by either persons working within a lab or theft by those outside the lab. While the probability of this is likely to be very low for most scientists and most laboratories, it is not zero. There is a precedent of scientists using pathogens from their own labs to cause harm. And as with potential accidents, while the probability may be very low, the consequences could be very high.

This assessment should also take into account the possibility that scientists may deliberately misuse the knowledge gained and published following the experiments by recreating the novel PPP strains in another laboratory using methods from published papers and then purposefully disseminating it. This possibility is typically dismissed out of hand by many scientists. But before dismissing that possibility, an analysis by an assembly of experts in the best position to make that judgment should be conducted. What is the possibility that individuals or groups who would seek to carry out such an act would develop the capacity and skill to carry it out? Given that once knowledge is published, it will be available forever, these questions are not just about the possibility of this happening in today's world but also anytime in the future. Despite the inherent uncertainties in trying to answer these questions, they should be answered with the best possible expertise.

Similarly, the moratorium should be used as a time to answer, or at least be addressing, another major issue as well: the international approach to funding, authorizing, and overseeing PPP. An accident or deliberate act involving PPP anywhere in the world could conceivably impact the public around the world. Therefore, the community of nations has an abiding interest to set common rules for how this work will be pursued. However, at this point, few countries have begun any kind of deliberative process on an approach to research with these unique dangers. Country X should have the right to know if this work is going on in country Y, and if so, what is being done to ensure it is done with the greatest safety and security. But currently, the way country X finds out about PPP work being done elsewhere in the world is when it is published in a science journal. Given the prestige that some scientists have received for pursuing PPP research, it would be surprising if scientists from countries around the world did not increasingly pursue it. As comparatively less experienced labs decided to pursue this work, this will increase potential dangers.

A MORATORIUM IS THE RIGHT STEP

There are prominent scientists who agree that there are potential serious dangers to this work and agree that a risk assessment process is needed but who are opposed to a moratorium being imposed while such a risk assessment process is undertaken. They believe that a moratorium should be avoided for reasons that include the potential damage it can do to the funding and work of that lab and to the careers of those involved in the work.

We have a different view. A substantial number of scientists agree that there are extraordinary potential consequences of the work (15). There is no rigorous, objective, credible risk assessment process to judge the risks and benefits of proceeding with it. We believe that the responsible course is to take a research pause until such a risk assessment process is established, which creates a stronger basis for decisions and actions. This is not solely a scientific issue. It is a scientific and public health and safety issue, and it is an issue in which the public itself has an abiding interest.

We have no interest in stopping scientists from doing their work or preventing laboratories from receiving funding. The narrow and defined area of GOF research intended to create novel potential pandemic strains should be put on pause until the risk assessment process is completed. The same laboratories and scientists whose work has been stopped by the moratorium are free and able to pursue all other avenues of infectious disease research except for that narrowly defined by the GOF definition in the new policy; to the extent that other activities not meeting the narrow

definition in the pause have been included in letters to principal investigators ordering or requesting work stoppage, the boundaries of the funding pause should be quickly clarified to allow important alternative work on flu to continue. We note that there are more than 250 NIH-funded projects listed as active with titles containing MERS, SARS, coronavirus, or influenza (28), of which 18 have been affected by the funding pause. The number that remain on pause may be further reduced by negotiations between investigators and the NIH, which are now under way, that will define which projects truly are within the scope of the moratorium and which do not meet its terms and can resume.

The character and scope of the risk assessments that are applied are important. To establish methodologies and approaches for risk assessment and risk mitigation for this context, it would be valuable to start with a global assessment of the risks and benefits of this realm of research, identifying the common aspects of risk and benefit within PPP experiments and other approaches covered in the funding pause. For example, any risk assessment should include estimates of the probabilities of accidental infection and extensive spread, as well as estimates of the impacts of these events should they occur. The specific values of these estimated parameters will differ for different types of experiments. It will then be necessary to set standards and expectations for the quality and characteristics of risk-benefit assessments for individual experiments, for example, to distinguish coronavirus research from influenza research, enhancements of pathogenicity from enhancements of transmissibility, and other important distinctions. Given that the term "risk assessment" is used to mean different things by different people, an agreement on an approach to individual risk assessments would be needed to ensure rigor and credibility. Once this kind of analytic structure is established, individual risk assessments for GOF experiments that meet the definition in the new USG policy (3) should become the norm before such experiments are funded. Crucially, this process should be quantitative, rather than relying on unquantified and unverifiable assurances that particular laboratories are safe.

CONCLUSIONS

The results of this risk assessment process are important not only to the U.S. Government, which had been a major funder of PPP experiments, but also to other funders, regulators, and investigators worldwide who consider such experiments. Our support for the funding pause and associated deliberative process does not indicate that we would support a permanent end to all experiments subject to the pause. There may be research endeavors that are subject to the moratorium that have a risk-benefit profile sufficiently favorable to justify their resumption once risks and benefits have been explicitly set forth. After 2 years of debate, we think the balance is evidently unfavorable for experiments to enhance avian influenza virus transmissibility, but other classes of experiments may be different. In the meantime, the moratorium is an appropriate and responsible step while dedicated and rigorous efforts are made to understand the risks and benefits of this work.

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Improving pandemic influenza risk assessment

Abstract Assessing the pandemic risk posed by specific non-human influenza A viruses is an important goal in public health research. As influenza virus genome sequencing becomes cheaper, faster, and more readily available, the ability to predict pandemic potential from sequence data could transform pandemic influenza risk assessment capabilities. However, the complexities of the relationships between virus genotype and phenotype make such predictions extremely difficult. The integration of experimental work, computational tool development, and analysis of evolutionary pathways, together with refinements to influenza surveillance, has the potential to transform our ability to assess the risks posed to humans by non-human influenza viruses and lead to improved pandemic preparedness and response.
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Influenza pandemics arise when antigenically novel influenza viruses enter and spread extensively in the human population. By this definition, there have been five influenza pandemics in the last 100 years, the worst of which cost 50 million lives worldwide (***Johnson and Mueller, 2002***). Of these pandemics, three likely arose from the introduction of genes from avian viruses into the human population (1918—H1N1, 1957—H2N2, 1968—H3N2 (***dos Reis et al., 2009***; ***Neumann et al., 2009***, ***Worobey et al., 2014***)), one arose from the introduction of a swine virus (2009—H1N1 (***Smith et al., 2009***)), and one was likely due to the unintended reintroduction of a previously widespread human virus that had not been seen in humans for two decades (1977—H1N1 (***dos Reis et al., 2009***, ***Nakajima et al., 1978***, ***Palese, 2004***)). However, the viruses responsible for these pandemics represent only a tiny fraction of the total diversity of influenza A viruses that exist in nature (***Webster et al., 1992***). Assessing which viruses pose the greatest risk of causing the next human pandemic is an enormous challenge.

Pandemic influenza risk assessment faces a fundamental problem: a paucity of empirical data on the differences between pandemic viruses and their immediate ancestors from non-human hosts. The challenge was clearly articulated by Harvey Fineberg in his analysis of the US government's response to the 1976 swine influenza scare (***Fineberg, 2009***): 'The first lesson is to avoid over-confidence about scientific insights. Major flu pandemics arise on average only about three times every century, which means scientists can make relatively few direct observations in each lifetime and have a long time to think about each observation. That is a circumstance that is ripe for over-interpretation.'

Core elements of current approaches to pandemic preparedness and mitigation, such as the development of vaccines and stockpiling of antiviral drugs, require detailed virological and

immunological data on viruses with perceived pandemic potential and ample lead time for production (***Jennings et al., 2008***, ***Keitel and Piedra, 2014***). The substantial diversity of known influenza viruses in non-human hosts, and the frequent identification of new viruses, makes extensive experimental testing and development of pandemic preparedness measures against all viruses unfeasible. Thus, there is a need for continuing attempts to assess the pandemic risks posed by non-human viruses in order to prioritize viruses of concern for pandemic preparedness planning. Currently, influenza pandemic risk assessment is largely driven by a simple idea: animal viruses that cause sporadic human infections are thought to pose a greater pandemic risk than viruses that have not been documented to infect humans (***Figure 1***). This intuitively attractive idea does not have direct empirical support, as none of the viruses that caused the 1918, 1957, 1968, or 2009 pandemics was detected in humans before they emerged in their pandemic form (***Smith et al., 2009***). This is largely due to a lack of surveillance (1918, 1957, and 1968 pandemics) and to the mistaken assumption that virus subtypes already circulating in humans were unlikely to cause pandemics (2009 pandemic) (***Peiris et al., 2012***). However, increased surveillance has probably improved the chance that the next pandemic virus will be identified prior to sustained human-to-human transmission.

If it is true that influenza surveillance has the possibility of identifying potential pandemic viruses before they begin to spread extensively between humans, then improving the basis for assessment of the risks posed by those viruses is an important goal. The level of public health concern about identified non-human influenza viruses should be a function of the potential of each virus to gain the ability to transmit efficiently from human to human and the severity of disease that such a virus would cause should it become pandemic. These two high-level phenotypes are each determined by the interaction of a number of biochemical traits of the virus during human infection (***Figure 2***) (***Chou et al., 2011***, ***Hatta et al., 2001***, ***Kobasa et al., 2004***, ***Labadie et al., 2007***, ***Yen et al., 2011***), the state of immunity to that influenza virus in human populations at the time of emergence (***Miller et al., 2010***, ***Xu et al., 2010***), and by environmental factors such as temperature and humidity (***Shaman et al., 2011***).

Currently, the primary tool that uses multiple data streams for assessing pandemic risk is the Influenza Risk Assessment Tool (IRAT) (***Cox et al., 2014***, ***Trock et al., 2012***). The IRAT integrates existing knowledge, including information on virus transmissibility and disease severity, with expert opinion about potential pandemic viruses to assign relative risk scores to those viruses. The IRAT is useful for identifying key gaps in knowledge,

Figure 1. Evidence for concern and actions to mitigate influenza pandemics. Types of evidence that have been, or could be, used to justify specific preparedness or mitigation actions prior to evidence of sustained human-to-human transmission, largely based on the authors' interpretation of national and international responses to H5N1, H7N9, and H3N2v outbreaks (***Epperson et al., 2013***, ***WHO, 2011***). Red indicates largely sufficient, orange partly sufficient, yellow minimally sufficient, gray insufficient. * high pathogenicity phenotype as defined by the World Organization for Animal Health (OIE) (***OIE, 2013***).

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focusing risk management efforts, and providing clear documentation of decision rationales. However, to be used optimally, the IRAT requires a substantial amount of experimental data about virus phenotypes including information on receptor binding, transmissibility in laboratory animals, and antiviral treatment susceptibility. In the absence of phenotype data, preliminary assessments with the IRAT must rely on extrapolations from related viruses, which are prone to subjective interpretation.

The biochemical traits that determine virus phenotypes are themselves determined by the genetic sequence of the virus (***Figure 2***). In theory, it might eventually be possible to predict virus phenotype directly from virus sequence data. However, the complexities of the relationships between sequences and traits and from traits to disease phenotypes, make the prediction of pandemic potential from genomic sequence a tremendous challenge. Here, we discuss ways in which laboratory experiments, together with computational and theoretical developments, could improve genotype-to-phenotype prediction and, in conjunction with enhanced surveillance, improve assessment of the risks posed to humans by non-human influenza viruses.

Experimental approaches

One goal of experimental studies on non-human influenza viruses is to identify general virus traits that are likely to affect transmissibility between humans, and then relate those traits to specific virus sequence changes. For obvious reasons, direct experimental assessment of human-to-human transmission of potential pandemic viruses is not feasible. However, influenza viruses that have caused pandemics in humans have been shown to transmit efficiently in animal models (most commonly ferrets) (***Chou et al., 2011***, ***Yen et al., 2011***), thus animal models are thought to be useful for examining the genetic changes in viruses that facilitate human-to-human transmission. For example, several studies have shown that genetic changes in the neuraminidase (NA) and matrix (M) gene segments acquired by the virus lineage responsible for the 2009 H1N1 pandemic increased transmissibility in animal models (***Chou et al., 2011***, ***Lakdawala et al., 2011***, ***Yen et al., 2011***), suggesting that these changes may have played a role in enhancing the virus's transmissibility in humans and hence paved the way for pandemic emergence. When animal experiments provide quantitative measures of virus traits, these can be integrated into quantitative measures of risk assessment such as the IRAT (***Trock et al., 2012***).

Recently, several high-profile and controversial gain-of-function (GoF) studies have attempted to go beyond the characterization of existing viruses to prospectively identify new mutations in avian H5N1 viruses that enhance the ability of these viruses to transmit between ferrets by the airborne route (***Chen et al., 2012***, ***Herfst et al., 2012***, ***Imai et al., 2012***, ***Zhang et al., 2013***). Important questions about the relative risks and benefits of these studies have been debated extensively elsewhere (***Fauci, 2012***; ***Fouchier et al., 2013***; ***Lipkin, 2012***; ***Casadevall and Imperiale, 2014***; ***Lipsitch and Galvani, 2014***); here, we focus on scientific considerations.

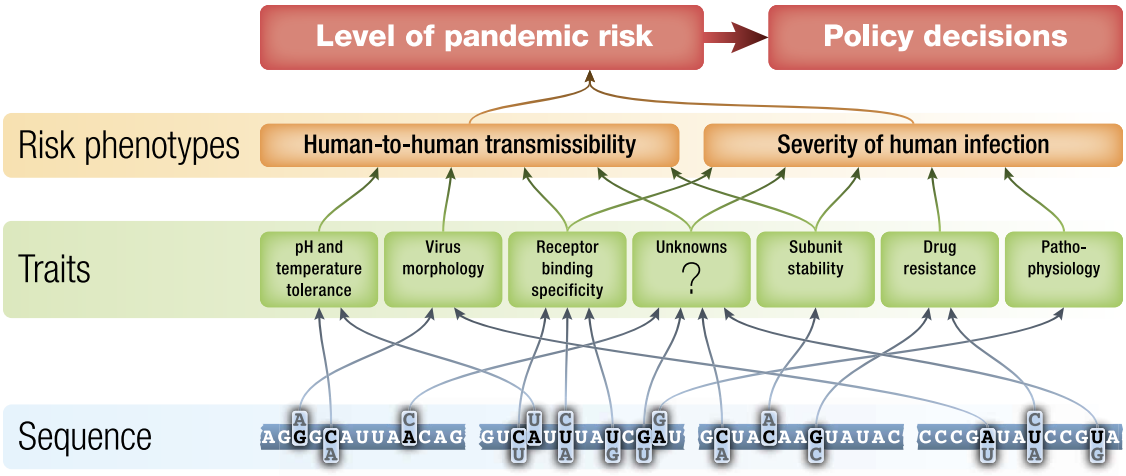

Figure 2. Schematic of potential relationships from virus genetic sequence to level of public health concern/pandemic risk. Pandemic risk is a combination of the probability that a virus will cause a pandemic and the human morbidity and mortality that might result from that pandemic. Arrows represent possible relationships between levels and are not intended to summarize current knowledge.

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Because of the vast size of genetic space, such studies cannot possibly delineate all genetic variants of a virus that might be transmissible—after all, there are more than 10^{18} different possible five-mutation variants of any given hemagglutinin (HA), which is more than what can reasonably be assayed experimentally and the vast majority will not facilitate transmissibility. A more modest goal is to attempt to associate classes of genetic or phenotypic traits with transmissibility. Transmissibility traits identified by GoF studies to date include some that were already known (such as switching receptor binding from avian-like α2,3 sialic acid to human-like α2,6 sialic acid linkages (***Yamada et al., 2006***) and lowering the optimal temperature for viral polymerase activity (***Massin et al., 2001***)), as well as some that are new, such as increasing HA stability and reducing glycosylation on HA's globular head (***Herfst et al., 2012***, ***Imai et al., 2012***). Whether these traits are either necessary or sufficient for transmissibility among humans or even other mammalian animal models remains unclear. For example, a recent study of an avian H5N1 virus found that by reassorting its internal genes with those of a 2009 pandemic virus, the virus could be rendered transmissible in guinea pigs (which have both α2,6 and α2,3 sialic acid in the upper respiratory tract) despite retaining a preference for binding α2,3 sialic acid. However, when mutations identified in earlier ferret GoF experiments were used to switch the receptor specificity to α2,6 sialic acid, transmissibility was lost (***Zhang et al., 2013***).

A key question for efforts to assess pandemic risk of non-human viruses is the degree to which certain substitutions are general markers for a phenotype, or whether the impacts of those mutations are dependent on genetic context and/or specific non-human host. Some mutations have been shown to be strong markers for phenotype for well-defined collections of viruses—for instance, the NA mutation H275Y consistently confers oseltamivir resistance on N1 neuraminidases (although the impact of the mutation on surface expression of NA, and thus virus fitness, varies dramatically) (***Baz et al., 2010***, ***Bloom et al., 2010***). Similarly, the PB2 E627K substitution adapts the viral polymerase to mammalian cells in some viruses (***Long et al., 2013***) but not others (***Herfst et al., 2010***), while other viruses have adapted to mammals via different substitutions in PB2 (***Jagger et al., 2010***, ***Mehle and Doudna, 2009***; ***Zhu et al., 2010***). In many cases, the effect of mutations can be highly sensitive to genetic context—for instance, the effects of cytotoxic T-lymphocyte escape mutations on nucleoprotein (NP) function depend on the stability of the parent protein, which can be affected by at least dozens of other mutations (***Gong et al., 2013***). Similar patterns of context dependence have recently been shown for receptor binding specificity substitutions in H5N1 viruses (***Tharakaraman et al., 2013***). Therefore, even when phenotypic traits of interest can be identified, clear genetic markers for these traits are only present in some cases.

The utility of experimental studies for informing surveillance for higher-risk viruses hinges on the question of whether virus traits associated with risk of infection and transmission in humans possess clear genetic markers. If a trait only arises from a limited number of specific mutations or combination of mutations, then experimentally delineating these mutations would be helpful for surveillance. For these cases, it is important and useful for the community to have access to collections of interpretable genotype to phenotype traits such as in the H5N1 genetic changes inventory (http://www.cdc.gov/flu/avianflu/h5n1-genetic-changes.htm) as well as computational tools to quickly connect new sequences to the body of available mutation annotation knowledge (FluSurver: http://flusurver.bii.a-star.edu.sg/). On the other hand, if a trait can be conferred by a large number of different mutations or combinations of mutations, then it will be less effective to monitor specific mutations. In such cases, it may be more beneficial to focus on the broader biochemical properties of viruses or their proteins. Developing laboratory capacity for rapid phenotype assessment would therefore be a valuable complement to high-throughput sequencing of new viruses. Moving forwards, if such biochemical traits can be clearly delineated and reliably modeled, then computational simulation of proteins could be used to predict phenotype from sequence, even for sequences from viruses that have never been experimentally tested.

Computational predictions

Computational methods present an attractive adjunct to experimental studies because they have higher throughput, have shorter turnaround times, are cheaper, and are safer than experimental work with whole virulent viruses. The main drawback of computational methods is the largely unknown accuracy of their predictions—a drawback that is exacerbated by the lack of an established framework for validating the accuracy of the numerous computational prediction methods that populate the literature.

The elements of influenza pandemic risk assessment that are most amenable to computational prediction are those that correspond to well-defined, quantifiable molecular-scale traits such as receptor-binding preference, antiviral susceptibility, antigenicity of HA and NA, and possibly T-cell epitopes. Higher-level phenotypes such as transmissibility, that integrate phenomena at a range of scales, are not yet sufficiently well understood to be reasonable targets for computational predictions. A variety of computational methods shows promise for genotype-to-phenotype prediction including molecular dynamics simulations that combine high- and low-fidelity models (***Amaro et al., 2009***) and statistical learning approaches that use protein structure, dynamics, and sequence data to predict the phenotypic consequences of mutation (***Kasson et al., 2009***). However, better prospective validation of these tools against experimental data, particularly for exploring context dependency of genetic changes, is essential before these tools can be reliably used for informing public health decisions or policymaking (***Figure 1***).

Making substantial progress in the development of computational tools and the assessment of their accuracy will require collaboration between experimental and computational scientists to produce consistent testing and validation data. One possible mechanism to spur cooperation would be a series of regular community assessment exercises similar to Critical Assessment of protein Structure Prediction (CASP) (***Moult et al., 2011***). In a CASP-like exercise, one or more experimental groups would generate quantitative phenotype data for a set of viruses, for example the relative binding of α2,3-sialoglycans and α2,6-sialoglycans, pH profile of viral activation, or sensitivity to oseltamivir, and challenge computational groups to predict that virus phenotype data from the genetic sequences of the viruses tested. The quantitative experimental data would be held under embargo while the exercise runs. Computational groups would complete predictions for these targets, the experimental data set would then be released, and a meeting would be held to assess the performance of different methods to define avenues for improvement.

Ideal experimental data sets for CASP-like exercises include thermophoretic or interferometric measurements of HA binding affinities to α2,3- and α2,6-sialoglycans (***Xiong et al., 2013***) and multi-method characterizations of viral pH activation shifts for sets of point mutants in HA (***Galloway et al., 2013***, ***Thoennes et al., 2008***). Reliable computational prediction of biochemical traits from genetic data would be a major accomplishment. However, it should be recognized that further major developments, particularly computational prediction of total virus fitness in new hosts, would still be required for realizing the utility of computational tools in policymaking.

Evolutionary theory and modeling

In addition to the genotype–phenotype relationship itself, there is a need for better understanding of the evolutionary mechanisms and pathways that allow adaptive mutations controlling host range to appear and rise in frequency. These mechanisms act in reservoir hosts, in intermediate hosts (if any), and in humans or other potential hosts; they also act at multiple scales, as viruses compete for replication within hosts and transmission between hosts (***Park et al., 2013***, ***Russell et al., 2012***). Developing better phylodynamic model frameworks (***Grenfell et al., 2004***) for modeling virus host transfer and adaptation will require collaboration between theoreticians and experimentalists.

Specific goals would be to determine realistic parameters for mutation/selection processes (***Illingworth et al., 2014***) and virus population bottlenecks at transmission (***Wilker et al., 2013***) and to generate high-resolution data sets to test and train mechanistic models. Such data-driven mechanistic models could shed light on additional constraints to virus genetic change, such as fitness valleys that separate virus genotypes adapted to one species or another, or conflicts in selection acting at different biological scales. For example, at the most simple level of understanding of the role of receptor binding, avian to mammalian host switching is often assumed to only require a binary change in receptor specificity from α2,3 to α2,6 sialic acid and to be directly related to binding affinity. However, in addition to the α2,3 and α2,6 linkages, there is a tremendous variety in the structures of oligosaccharides displaying the sialic acids and in the structure of the sialic acids in different avian hosts (***Gambaryan et al., 2012***, ***Jourdain et al., 2011***). The binding specificity for each receptor variant form may affect the potential for different viruses to cross the species barrier or make the difference between causing severe or only mild disease. Rich experimental data sets that provide insights on such factors will improve the power of evolutionary models to interpret experimental and field data.

Surveillance methodology

Detection of the genetic changes and phenotypes of concern relies on systematic characterization

of influenza viruses circulating in wild and domesticated animal populations. If there are virus traits that correlate with genetic markers observed to increase risk in humans, or that can be computationally inferred from genetic sequence data, it could be possible to monitor those markers in surveillance and adjust risk assessments prior to emergence in humans. However, the acquisition of samples entering existing surveillance networks is largely ad hoc, exhibits substantial variation by host and geographical region, and only a small proportion of the data end up in the public domain (***Figure 3***). Making non-human influenza surveillance more systematic by using statistical analysis to determine appropriate levels of coverage by geographic region and host species would facilitate the early detection of viruses of concern and also have the potential to facilitate detection of evolutionary and epidemiological patterns of virus activity that warn of potential emergence events.

There are large regions of the world and many animal populations for which little or no surveillance is performed but where significant animal influenza diversity can be inferred to exist. Systematic assessments of surveillance by geographic area and host species, similar to efforts for malaria (***Gething et al., 2012***, ***2011***, ***Hay et al., 2010***, ***Sinka et al., 2012***) and dengue (***Bhatt et al., 2013***), would help to identify major gaps where surveillance is either non-existent or unlikely to be sufficient for timely detection of viruses of concern. For enhancing surveillance, prioritizing among these gaps will require substantial improvements in understanding animal host ecology to identify hotspots for virus transmission within and among animal species. Similar efforts are required to better understand what aspects of the human–animal interface facilitate transmission of viruses between animals and humans, particularly in animal production and domestic animal settings, and the human biological and epidemiological factors that promote chains of transmission of newly introduced viruses.

One motivation for changing existing surveillance systems is to increase their power to rapidly detect changes in patterns of non-human influenza virus activity. Substantial changes, such as

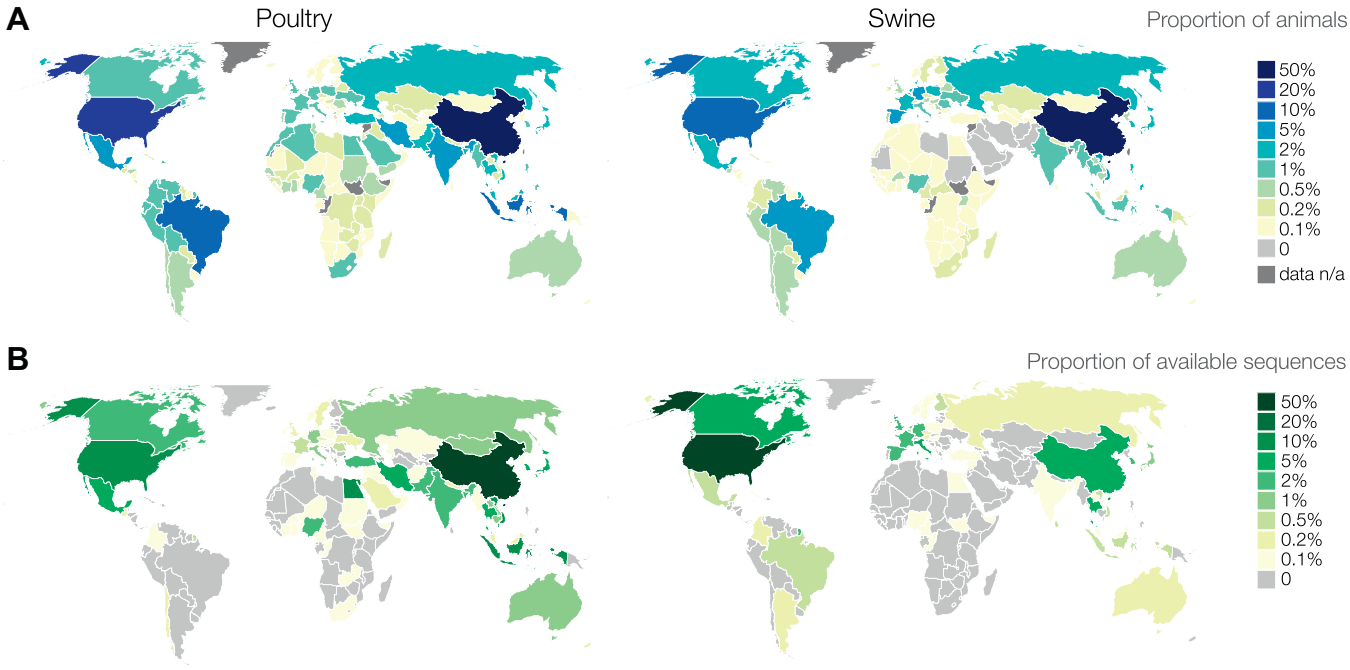

Figure 3. Geographic distribution of publicly available influenza virus genetic sequence data in comparison to poultry and swine populations.
(**A**) Proportions of worldwide animal population by country (data from the Food and Agriculture Organization of the United Nations).
(**B**) Number of unique influenza viruses for which sequence data exists in public databases from poultry or swine by country. Numbers of influenza virus sequences are not representative of influenza virus surveillance activities. Information regarding surveillance activities is not readily available. Virological surveillance, even if robust, may result in negative findings and is not captured in these figures. Most countries do not sequence every influenza virus isolate and some countries conduct virological surveillance without sharing sequence data publicly. Sequences deposited in public databases can reflect uneven geographic distribution and interest regarding viruses of concern such as H5N1 and H9N2.
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the sudden proliferation of a previously rare virus subtype or of a virus with an H9N2 internal gene cassette (**Gao et al., 2013**, **GarcÌa-Sastre and Schmolke, 2014**; **Guan et al., 1999**), could indicate the emergence of new viral variants in non-human hosts that should be prioritized for further study even before the detection of human infections of zoonotic origin (**Vijaykrishna et al., 2011**). To be useful from a human health perspective such detection systems would require sampling of animals with no obvious signs of infection, routine assessment of particular genetic signatures or full genome sequencing, and near real-time sharing of these data; these activities all present potential financial, political, and logistical constraints.

Further development of surveillance infrastructure in some geographic locations and host species is likely to be unpopular or unfeasible due to economic disincentives for disease detection. However, the geographic movements of many non-human influenza hosts, via migration or trade, make it possible to identify surrogate sources of information. For example, by linking virological and serological data, it has been possible to make inferences about swine influenza virus activity in some parts of mainland China based only on the data from Hong Kong (**Strelioff et al., 2013**).

A systematic, open, and timely global surveillance system based on viral sequence data would be a powerful tool in pandemic risk assessment. Viral sequences, with associated metadata and systematic recording of virus negative sample results, provide a rich source of information beyond the simple presence or absence of particular strains. Phylodynamic reconstructions from even a relatively small number of samples are capable of revealing lineages that are proliferating (**Grenfell et al., 2004**, **Pybus and Rambaut, 2009**). Phylogenetic methods can be used to reveal gaps in surveillance (**Smith et al., 2009**, **Vijaykrishna et al., 2011**). Genetic similarity between viruses in different locations or host species can identify drivers of transmission between populations (**Faria et al., 2013**, **Lemey et al., 2014**).

Data on negative samples would provide valuable denominators for estimating the prevalence of infection: tracking infection rates through time would give a window into transmission dynamics and allow investigation of mechanisms underlying virus circulation. The Influenza Research Database (IRD) (http://www.fludb.org) includes an animal surveillance database that contains negative test data but the amount of data is extremely limited compared to the global scale of ongoing surveillance activities. Standards should be developed for consistently recording these relevant associated metadata, so that the number of animals tested, the setting in which sampling took place, and the motivation for sampling associated with genetic data can be submitted in a consistent form to public data repositories, along with all sequence submissions.

Conclusions

It is currently not possible to predict which non-human influenza A virus will cause the next pandemic. Reducing the impact of the next pandemic will rely on early detection and mitigation strategies that slow the early spread to allow more preparatory work to be done. The integration of further experimental data with computational methods and mathematical models in conjunction with refinements to surveillance methodology will increase the feasibility of genotype-to-phenotype based assessments, increase the power of tools for more objectively assessing pandemic risk and decrease the time required for assessing the pandemic threat posed by extant non-human influenza A viruses—all of which can inform strategies to help mitigate the impact of the next pandemic.

Even as risk assessment capabilities improve, scientific insights into non-human influenza viruses must not give way to complacency that the most substantial threats have been identified and characterized. Despite the perceived risks of highly pathogenic H5N1 viruses, the emergence of the 2009 H1N1 pandemic virus in humans, the increasing incidence of human infection with H7N9 viruses in China since 2013, and the first documented human infections with H6N1 (**Wei et al., 2013**) and H10N8 (**Chen et al., 2014**) viruses highlight the importance of remaining vigilant against as-yet unrecognized high-risk viruses and the value of surveillance for influenza viruses in humans. Beyond further scientific investigations and refinement of surveillance capacity, the development of local surveillance-based outbreak response capacity worldwide remains essential. The first wave of the 2013 H7N9 outbreak in China demonstrated the value of swift coordinated action, including the timely dissemination of surveillance data, to limit further incursions of new viruses into the human population. Without developing similar response capacities in other areas at high risk of new virus introductions, we are only building expensive systems for watching the next pandemic unfold.

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From: Charles Stack [mailto:cstack3@uic.edu]
Sent: Thursday, April 23, 2015 1:17 PM
To: National Science Advisory Board for Biosecurity (NIH/OD)
Cc: Marc Lipsitch
Subject: Comments for NSABB Meeting

Dear Madam or Sir:

I have watched the Gain of Function (GOF) controversy for years and became a charter member of the Cambridge Working Group in order to have some influence upon this research.

I agree with Dr. Marc Lipstich and colleagues that the risks of GOF experiments are far too grave considering the marginal usefulness of the discoveries that might be made.

University of Wisconsin Prof. Kawaoka and others claim that GOF research will help to develop new vaccine strains, but this is only true if a pandemic virus in the wild emerges with the same genetic profile of the GOF strain. The odds against this happening are astronomical. Vaccine manufacturers have already said that it would not be economical to produce vaccines unless a strain is circulating and identified in the wild.

Public health is undergoing wrenching financial changes, so we must be prudent with how research dollars are spent. Instead of shot-in-the-dark GOF research, I advocate for increased field surveillance for emerging viruses and other pathogens. Such surveillance may have shown that the recent Ebola virus outbreak in West Africa could have been predicted, based upon seroepidemiology of the human population and culture testing of the indigenous biota. We are performing this surveillance after the fact and need to be out in front of emerging threats.

Thank you for your consideration of my comments,

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