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SYMPOSIUM

What About the Ducks? An Alternative Vaccination Strategy

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Like most emerging disease threats, avian influenza is a zoonotic disease maintained in nature by wildlife. In this case, the reservoir of infection is migratory waterfowl, primarily ducks. Rather than trying to vaccinate most of the world's human population in response to the threat of an avian influenza pandemic, it might be more prudent to vaccinate key reservoir wildlife species from which pandemic strains evolve. This strategy would require a much more intensive research effort to understand the evolution of avian influenza viruses in nature, but it would be far less costly than any of the alternatives. Research priorities for emerging zoonoses, such as new strains of avian influenza viruses, should be re-evaluated with an emphasis on ways to intervene at their source, the natural reservoir hosts from which they originate, rather than focusing upon human-based interventions, which are too often too late.

INTRODUCTION

The previous presentations in this symposium have addressed important issues concerning how we might respond to the threat of an influenza pandemic in humans. I would like to address the topic of how this and future threats of pandemic influenza might be avoided through an understanding of the avian origins of influenza viral genotypes that have potential for causing human pandemics. I believe this is an important topic worthy of consideration in this symposium on bioethics, and it is relevant to the subject of vaccines, as you will see in a moment. But first, I would like to put things into perspective regarding the origins of human disease with pandemic potential.

ZOONOTIC ORIGINS OF HUMAN DISEASE

In a recent review of all human infections [1], a total of 1,415 known human pathogenic agents was compiled from a thorough search of the medical literature. The taxonomic groups represented by these 1,415 human pathogens include helminths (32 percent), bacteria (including rickettsia) (31 percent), viruses and prions (19 percent), fungi (13 percent), and protozoa (5 percent). Of this total, the majority (868, or 61 percent) are considered to be zoonotic pathogens. These are pathogens that we share with other animals, either directly or through intermediate hosts such as arthropod vectors. Many more human pathogens have zoonotic

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evolutionary origins, but have since adapted exclusively to humans with no existing connection to non-human animal reservoirs.

Among these 1,415 known human pathogens, about 12 percent (175) are causing what we consider to be emerging diseases — new diseases — that either we haven't seen before or previously were rare. These include most of the important diseases that threaten human health today, and 75 percent (132) of these emerging pathogens are zoonotic. So, the vast majority of new disease threats for humans are from pathogens that we share with other animals. Interestingly, viruses and prions dominate this list (44 percent) and helminthes are relatively rare (6 percent) compared to the total human pathogens list.

In reality, the concept of emerging disease is simply the evolutionary consequences of us sharing pathogens with the rest of the biological world. Obviously, we have been accumulating pathogens from other species throughout our evolutionary history, and it is likely that this process will continue and perhaps even accelerate as the world becomes more crowded and the environment more degraded [2]. Influenza A viruses, of course, are typical zoonotic pathogens.

ZOONOTIC ORIGINS OF AVIAN INFLUENZA

A recent New England Journal of Medicine editorial [3] describes what the research priorities should be concerning avian influenza and the prospects for a pandemic. These research priorities include case management, development of vaccines and studies on vaccine efficacy, risk assessment, and the management of risk through intervention in the event of a human pandemic, and the role of animals. Unfortunately, this priority list is completely inverted. The role of animals should be the highest priority if it is not already too late to avert a pandemic from H5N1. The role of animals is of paramount importance in all zoonotic disease threats to humans, yet this aspect most often receives the least attention until it is too late to prevent an outbreak in humans [4].

As was mentioned in previous talks, all of the influenza A viruses originate from wild aquatic birds. Excellent reviews on the ecology and evolution of influenza A viruses are provided by Webster et al. [5, 6]. Phylogenetic evidence from sequence data suggests that all influenza A genotypes found in humans, pigs, horses, sea mammals, and a wide variety of bird species ultimately originate from migratory aquatic birds, mostly ducks and, to a lesser extent, shore birds. As with most zoonotic agents, reservoir hosts for influenza A viruses are usually asymptomatic but can transmit infection readily to other individuals or other susceptible species for varying periods of time, usually about two weeks.

Although influenza A viruses enjoy a global distribution, the different genotypes (H and N types) are not distributed evenly throughout the world as the major migratory bird flyways tend to maintain characteristic genotypes. Most of the viruses causing epidemics in humans originate in the East Asian flyway, but outbreaks in domestic birds (chickens and ducks) and rarely humans have occurred in other flyways as well. Epizootics in domestic birds create conditions conducive for transmission to humans, through increased contact and genetic adaptation, often through porcine (pig) intermediates [5, 6].

The potentially pandemic subtype H5N1 is directly infectious to humans from domestic birds, although cats are also susceptible and may be an additional route of exposure for humans [7]. However, the H5N1 subtype is also unusual in that it has recently caused epizootics in wild birds in Asia which may be contributing to its spread [8]. It appears to be lethal for wild birds, which should lessen their reservoir

potential; however, the risk to humans from infected wild birds remains to be determined.

INTERVENTION OPTIONS

All the discussion today has been focused on how we can prevent an H5N1 pandemic in humans and specifically on protecting humans from infection. This is the traditional approach of medical epidemiology in the face of a pending epidemic. But a more holistic approach to the problem would be to consider where these viruses are coming from in nature. What is the source of these viruses in the natural environment and what can we do about preventing them from becoming a threat to humans? Preemptive action at the level of wildlife reservoir would seem to be much more effective and far less costly than preparing for a human pandemic.

The usual options for intervention at the reservoir source for zoonotic diseases involve isolation or culling and possibly vaccination when domestic animals are reservoirs. Isolation and culling of domestic birds is currently being done in Southeast Asia and China, but it is extremely costly. Estimates of the economic loss from H5N1 to the domestic bird industry in Asia as of April 2005 are in the range of $10 billion to $15 billion [9]. The effectiveness of these measures in averting a human pandemic remains to be seen. A more cost-effective strategy might be to explore opportunities to intervene in the natural maintenance cycles of influenza A viruses occurring in wildlife.

Wholesale culling of wildlife should not be considered an option because its effectiveness in preventing a human pandemic is purely speculative, and the environmental consequences would be extremely severe. However, increased isolation between wildlife and domestic birds and humans that would decrease opportunities for cross-species transmission might be accomplished by creating or restoring natural wetlands in order to aggregate migratory aquatic birds in more remote areas. Likewise, domestic bird husbandry practices might be altered to minimize contact with wild waterfowl or discourage their presence. More research into these wildlife management strategies should be supported.

VACCINATION OF WILDLIFE RESERVOIRS

The focus of our discussion today has been on vaccination, albeit human vaccination, and I would like to introduce the concept of vaccinating wildlife instead of humans as an intervention strategy against zoonotic disease threats, such as influenza A viruses.

There are certain distinct advantages in vaccinating wildlife over humans. The obvious advantages are that you do not have to get informed consent or FDA approval, and the patients cannot sue. But also, there might not be as many individuals involved. Instead of trying to vaccinate billions of people to control a pandemic, it might be easier to try to vaccinate certain populations of key wildlife species that are responsible for the emergence of pandemic genotypes. There are certainly much fewer of these than there are people, but we must identify them and get them vaccinated. This would require a far greater knowledge of the evolutionary ecology of influenza A viruses than we currently have, but this knowledge probably can be obtained for a research investment of, say, $1 billion. This might be an intelligent investment considering the cost of vaccinating humans to prevent pandemics or, worse yet, not vaccinating humans during a pandemic. One billion dollars seems meager considering what is at stake (an estimated $800 billion in the first year of a human pandemic [10]).

Vaccination of wildlife reservoirs of human infections has been successfully demonstrated with another zoonotic virus

— rabies. In both Europe and North America, rabies in wild mammal reservoirs (fox and raccoon, respectively) has been dramatically reduced or eliminated over large geographic areas through oral vaccination resulting in a significant decrease or elimination of human disease risk [11, 12]. Although fatal, human rabies has always been a rare disease in modern times. Yet considerable effort has been made to understand its ecology and to manipulate the prevalence of this virus in nature. Vaccination of wildlife reservoirs of rabies has been much more effective than culling, which had been the previous practice to manage human risk of infection from reservoir animals [13].

Rabies is a relatively simple disease system, however, with one strain of virus usually being primarily maintained by a single reservoir host species [14]. Influenza A viruses are much more complex and maintained by many reservoir host species, which makes identifying an effective target a seemingly enormous challenge.

We have tried a wildlife vaccination approach with Lyme disease here in Connecticut. Like influenza A virus, the spirochetal agent of Lyme disease is genetically variable and is maintained by a large variety of vertebrate hosts, including both birds and mammals [15]. However, some reservoir species are much more important than others, and the white-footed mouse, perhaps the most common mammalian resident of northeastern forests, is thought to be the most important source of infection for humans. In the case of Lyme disease, ticks provide the means of transmission between reservoir host and humans, so direct contact with the reservoir host is not necessary. We reasoned that by vaccinating mice, there would be fewer infected ticks available in the environment to bite humans, and the risk of human disease would be reduced.

More than 1,000 mice were vaccinated over three years in a large field study near New Haven. Before developing an oral vaccine, we decided to do our preliminary study the old-fashioned way, by trapping and vaccinating individual mice with a syringe. The details can be found in our recently published paper [16], but the basic results were that we did find a reduction in the prevalence of infection in ticks occurring in the vaccination plots, vs. plots in which the mice were given a placebo injection. The reduction was not great, only 17 percent, but it did demonstrate that wildlife vaccination could reduce human disease risk for a vector-borne zoonotic disease. We may have overestimated the role of mice as a reservoir species, which demonstrates the need for understanding the ecology of zoonotic pathogens in their natural environment.

Ironically, the vaccine we used on mice was originally developed for humans. The human vaccine is no longer on the market because of liability issues [17]. Again, this was not an issue for the mice, and our mouse vaccination project did not require the approximate $100 million that was invested in clinical trials for the human Lyme disease vaccine [18].

ECOLOGY OF AVIAN INFLUENZA

What are the key ecological questions we need to ask in order to explore the potential for a wildlife intervention strategy against pandemic influenza A viruses? We need to know what is the distribution of the various subtypes among different avian species. It is not likely that all viral genotypes do equally well in all avian species. What is the pattern of the distribution of subtypes within a species? Presumably not all individuals are equally susceptible or equally infectious to others. What is the nature and outcome of interactions between viral subtypes within individual birds. Pathogens in nature do not usually occur in isolation, and competition and cross immunity are important in regulating interactions. And finally, what subtypes are most

likely to evolve into potentially pandemic genotypes for humans? A review of the literature reveals the fact that we do not know very much about the ecology of these viruses in nature. There are surprisingly few papers, and they are scattered among journals of many different disciplines, including wildlife diseases, ornithology, virology, and infectious diseases.

I have not conducted an exhaustive review of the literature, but I have found a few studies that begin to provide some answers to the key ecological questions posed. A recent study on influenza A viruses in migratory North American water birds [19] found that there are consistently more H subtypes in shore birds than in wild ducks. Also, the distribution of N types varies between shore birds and wild ducks, with some N types being dominant in ducks, while others are dominant in shore birds. So, there is some evidence that virus subtypes are not evenly distributed among all wildlife species, as would be expected.

Another study looking at co-infections of different subtypes within a single wild bird population is also revealing [18]. This study included several duck species, but the population was dominated by just three species (mallards, pintails, and blue-winged teals), and it was studied for several years. Mixed infections of two or more viral subtypes were observed in more than two out of three of the infected birds. The frequencies of mixed infections were compared with expected frequencies calculated from the product of single infections, which would indicate independence. This analysis revealed patterns of association among subtypes that were markedly different compared to an expected independent, or random, association. Co-infection with two particular subtypes (H6N1 and H6N4), for example, occurred 355 times more frequently than would be expected with random mixing. Another type of mixed infection occurred 4.8 times less frequently than random association. Several subtypes were never isolated alone and were always found in infections together with one or more of the other subtypes [19]. These deviations from expected random distributions reveal patterns in nature that beg explanations, especially when such deviations are so extreme (355X). A higher than expected frequency of positive association between two subtypes can be interpreted as having an advantage over single infections for both subtypes. Conversely, negative associations may indicate a disadvantage when two subtypes co-infect. In either case, random mixing of influenza A subtypes in nature appears not to be the norm. These observed patterns in nature are important in understanding the evolution of pandemic genotypes since reassortment of genes occurs during mixed infections [5, 6].

Understanding the evolution of potentially pandemic strains in nature will be a challenge, but investigations of influenza A viruses in wildlife have already provided some indication of the origin of H5N1 [20]. Phylogenetic analysis of NP gene sequences obtained from isolates of wild ducks in the Kobyaysky area of northern Siberia showed a sister-group relationship with H5N1 isolates from chickens and humans in Hong Kong in 1997. These findings indicate that the precursor genes of influenza viruses with pandemic potential are perpetuated in ducks nesting in Siberia, above the arctic circle, where avian influenza viruses are believed to survive through the winter in frozen lakes [21].

With the seemingly permanent threat of emergent influenza A pandemic strains evolving from natural avian reservoir cycles, intervention at the source clearly deserves more serious consideration. However, imposing changes on the natural evolutionary processes through vaccine intervention may have its own risks. Antigenic drift has already been observed to occur in response to poultry vaccination against H5N2 in Mexico [22], suggesting

that directly targeting high pathogenic genotypes may not be feasible. Also, forcing changes in the natural evolution of these viruses without a thorough understanding of the selection pressures and genetic responses operating in the natural wildlife reservoirs could result in a more adverse outcome.

However, given that all of the known genotypes of avian influenza viruses ultimately evolve from only ducks and shore birds, the targeted host range for a vaccine intervention strategy involving wildlife could involve relatively few species [5,6]. An in-depth understanding of viral evolution within and among a few key host species could reveal fundamental processes leading to the emergence of genotypes with pandemic potential early enough to provide a target that would contain those genotypes before they become prevalent and adapt to other species, domestic or human. Locating key reservoir species populations and developing an oral vaccine delivery system would be additional problems to address.

RESEARCH PRIORITIES REVISITED

Ecological studies of avian influenza viruses in nature are obviously essential to our understanding of the origin of potentially pandemic strains and would provide hope for an ability to predict the emergence of human pandemics. But why have there not been more ecological studies on avian influenza? The answer to this question can be revealed by examining the research priorities for responding to zoonotic disease threats here at home in the U.S. In 1999, there was a West Nile virus outbreak in New York City, just 50 miles from here (New Haven, Connecticut). West Nile virus is another zoonotic disease with an avian reservoir. If we look at the NIH funding priorities for West Nile virus for the current fiscal year, six years after the initial outbreak, we find that the priorities are focused upon vaccines, therapeutics and diagnostics (Figure 1). The basic research effort is presumably in the same area and so are the animal studies, which are murine (mouse) surrogates for studying infection in humans. There is some funding for vector-biology and control (less than 10 percent), but that seems to be the limit of field studies. The ecology of West Nile virus and the role of birds in maintaining the virus in nature seem not to be significant priorities. Just as with avian influenza, the research priorities for West Nile virus and other zoonotic disease threats, such as Lyme disease, should not focus primarily upon the defensive posture of protecting humans from infection. A much more aggressive, offensive effort should be made to reduce human risk of infection by intervention at the zoonotic level of wildlife reservoirs. Vaccination of wildlife reservoirs of human diseases is one possibility. Other strategies may be recognized through further ecological studies, but this will only happen with a serious re-evaluation of existing research priorities for addressing zoonotic disease threats for humans.

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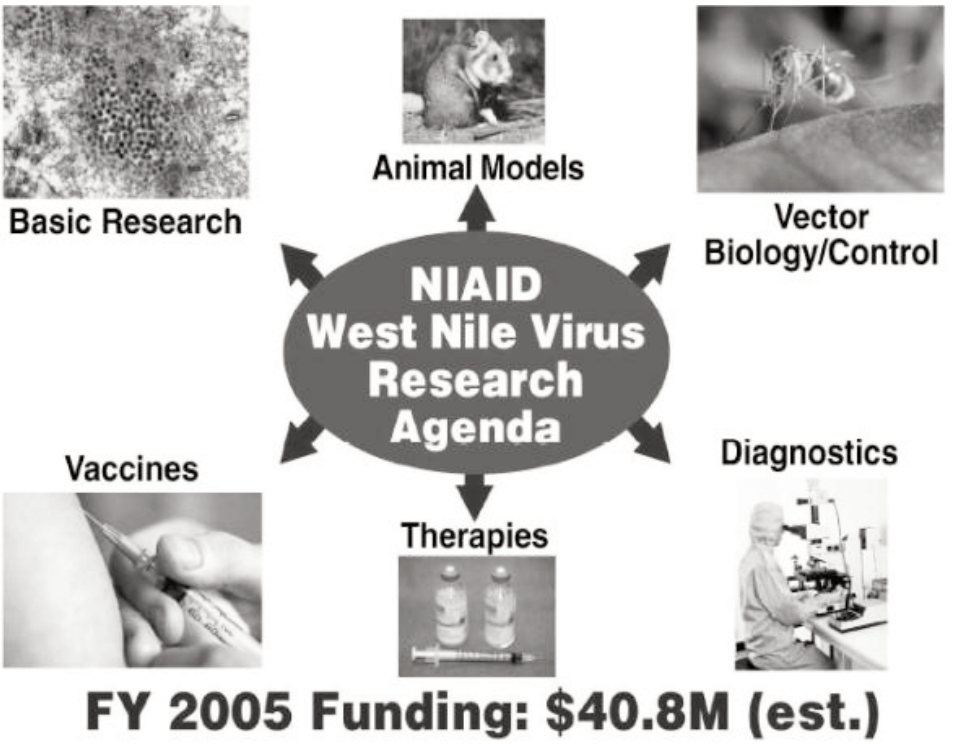

Figure 1. Research supported by NIH in response to the epidemic of West Nile virus, another zoonotic disease with an avian reservoir. Note the absence of research on birds which maintain West Nile virus in nature and are responsible for its spread throughout North America [22].

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