EMBO reports

Flu season

An interview with Jeffery K. Taubenberger, Chief of the Viral Pathogenesis and Evolution Section at the US National Institute of Allergy and Infectious Diseases

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EMBO reports (ER): In 2012, two publications by Ron Fouchier and Yoshihiro Kawaoka about increasing the transmissibility of highly pathogenic H5N1 avian flu virus created a public debate about flu research and biosecurity. Since Fouchier announced that he would repeat such gain-of-function experiments with the H7N9 bird flu virus, he was widely criticised for what some regard as conducting irresponsible high-risk experiments in the middle of Rotterdam. What are the potential benefits of these experiments, and do you think Fouchier should continue with his gain-of-function experiments on bird flu viruses?

Jeffery Taubenberger (JT): There are no simple answers, but it's worth thinking about this historically. People use the term gain-of-function as if this were a newly invented concept. But gain-of-function is what virologists have done for a hundred years. If you take an isolate of human influenza from a nasal or a throat swab and you grow it in a fertilized chicken egg, it can select for a clone that is able to grow in a chicken egg, which is not a native host. Mutations can rapidly accumulate in the virus, and you have now gained a function. It's the same thing with animal passage, so adapting a human or a chicken influenza virus to a ferret is something that people have done for eighty years.

The concern I think comes from several aspects. The H5N1 virus, which has been circulating since 1996, is very pathogenic for chickens and it has caused human infections with a theoretical case fatality rate of approaching 60%. The concern is about gain-of-function experiments that could lead to more mammalian transmissibility of these highly pathogenic viruses. We know that a zoonotically-derived animal influenza virus has to adapt to humans who are generally not susceptible to avian, equine, or canine influenza viruses. If we're ever actually going to be able to predict, prevent, or at least mitigate a pandemic, we need to understand some of the rules of how this happens. It just seems counter-intuitive to say that there's such a high risk of this happening in nature that we can't actually study it.

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I also think that there was some over-interpretation of the data. Ferret adaptation does not equal human adaptation. Ferrets can be infected with a number of avian, swine, and equine influenza viruses, or canine distemper viruses that can replicate, transmit and cause disease in ferrets, but that do not routinely cause disease in humans. The other thing that was initially lost in the Fouchier and Kawaoka experiments was the fact that these mutated viruses were not highly pathogenic in these animals, so this is another important point.

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ER: This seems to be similar to the Mexican flu strain, which seems to have lost its pathogenicity along transmission.

JT: The concern at least in the initial Mexico outbreak was that it had high morbidity and mortality in humans, but it's still a bit uncertain as to why that is. Evolutionary biologists have long hypothesized that influenza viruses or any virus that adapts to a human host would lose pathogenicity because it would be a selective advantage in the Darwinian sense to be able to cause disease and spread efficiently without killing or incapacitating the host.

ER: What was then special about the 1918 Spanish flu as most people actually died of secondary infections, and not of the virus itself?

JT: There are a lot of interesting things about the Spanish flu, and many unanswered questions. Since no viral isolates were available, one could only look at historical records. Having the sequence of the virus and being able to study it in animal models and *in vitro* systems, we've learned a lot. While we don't yet know completely how the pandemic

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virus formed—and we probably never will—it looks like this virus was an avian influenza-derived virus that may have adapted to mammals somewhere. It's been pathogenic in mice, in ferrets, in macaques without adaptation, so it does seem to have some kind of inherent pathogenicity.

The point that you mentioned about bacterial pneumonia is extremely important. Everyone who was treating patients or working in a laboratory in 1918 would have known that secondary bacterial pneumonias were extremely common and almost everyone in the pandemic died with bacterial pneumonia. And yet, somehow we seem to have forgotten this. I think that some people were so focused on the virulence of the virus, that they had a hard time allowing the idea that bacterial pneumonia was playing a role. But the way to look at it is exactly the opposite. A great measure of the virulence of an influenza virus and probably other respiratory viruses, is the ability to damage the respiratory epithelium so it is set up for a secondary bacterial pneumonia, which then kills you.

ER: Is this also the case for the avian H5N1 strain?

JT: Difficult to know. Unlike 1918, in which tens of thousands of autopsies or more were done, there have been extremely few autopsies performed for H5N1. I think it is still actually unresolved as to whether there would be evidence of secondary bacterial pneumonias. In the case of H5N1, one also wonders if there are not host factors that make certain people particularly susceptible to infection with this virus that otherwise can't replicate in humans.

ER: Maybe the high mortality comes from the fact that it's a self-selected population that becomes infected in the first place?

JT: Exactly, there are just a few hundred people over 18 years that have been exposed to this virus, that has been endemic in poultry populations all over Southeast Asia, Africa, the Middle East. And there have to have literally been millions or tens of millions of people who have had exposure to this virus and yet no evidence of infection; even serological evidence is lacking. It suggests to me that this virus is so poorly adapted, or so unable to infect humans, that exposure in most people does not even induce an antibody response. And yet maybe in some very tiny number of people there is some kind of genetic susceptibility, which causes a serious infection. I think that it would be very important to look at host factors. There's very little known about genetic susceptibility to infection in general, or specifically to influenza, but with the advent of deep sequencing and SNP analysis and HapMap sequencing, I think it would be quite interesting to do such studies.

People have constructed a set of arguments, that starts out with "H5N1 kills 60% of the people it infects, but it is not yet transmissible human-to-human" and "when it acquires mutations to become transmissible in humans, it will continue

to kill 60% of humans." That's the Armageddon scenario, but it seems unlikely. It is much more likely that either this virus cannot adapt to humans, or, if it were to adapt, it would behave as other pandemics have in the past.

ER: What was then so special about the 1918 pandemic? Was it the virus itself, or was it the environment after World War I that made the difference?

JT: That's a fascinating question, and we don't have all the answers. But I think that the 1918 virus was clearly more inherently pathogenic than the viruses that came after it, that is the 1957, 1968 or 2009 pandemic viruses. Another important feature is that the three pandemics that have occurred afterwards are all genetic descendants of the 1918 virus. David Morens and I have hypothesized about the idea of founder viruses, that is, the 1918 virus may have been a completely novel virus that somehow adapted to humans. And when such a host-switch event occurs, there is a strong selection pressure for a virus to adapt to a new host, otherwise this lineage goes extinct.

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But I don't think that inherent virulence, at least in influenza viruses, is selected for. Influenza is a blitzkrieg kind of virus: its peak replication is just a few days, by day five or six it may have damaged your lung to the extent that you are set up for a secondary bacterial pneumonia, but the virus is not even replicating anymore, it's off to someone else. I think that the 1918 virus accidentally had an inherent virulence in mammalian systems. Yoshi Kawaoka, Terry Tumpey and I all agree that the hemagglutinin, a major surface protein, of the 1918 virus seems to encode a virulence factor. If you take a non-pathogenic influenza virus, say a contemporary seasonal human virus that causes little or no disease in a mouse or a ferret, and if you construct a virus in the laboratory that has just the 1918 hemagglutinin on it, you can enhance the pathogenicity of that virus. It raises the possibility that the virulence of the 1918 virus, through its hemagglutinin, was just inherited without selection from an ancestral avian H1 virus that is not pathogenic in ducks but is pathogenic in mammals. A good example would be Ebola. Ebola is probably a bat virus, probably not pathogenic in the native host. But when it finds itself in a human, which is not something for which the virus is being selected, all sorts of terrible things happen. What we hope is that we can learn more about the biological basis of this and which avian influenza viruses have virulence factors. It would be nice to have a checklist of features or mutations that we think make viruses, if they adapt to humans, more dangerous.

ER: How do we translate this into better preparedness and public health measures to deal with a pandemic if one of these viruses emerges?

JT: We clearly need more surveillance, especially at the animal-human interface.

ER: Do you think that the global flu surveillance network generally works well?

JT: It's not uniform, obviously. Unfortunately for us many of the places that are experiencing some of these virus outbreaks are developing countries that don't necessarily have sufficient public health resources or infrastructure. But we also need more basic research. When I started the 1918 project, I thought naively that if we could work out the mutations that made 1918 adapt to humans, that this would be our checklist, and that you could then look at those mutations in H5 or some other virus. What I am now convinced of is that adaptation is a polygenic process that happens in the context of a particular virus with a particular set of mutations working in concert to achieve a physiological goal for the virus. There are two things a virus needs in a new host. It needs to be able to replicate and it has to be transmissible. Clearly there is major selection for those two events in a host-switch event. But there's clear evidence that these are individual processes.

ER: They're not linked with each other in terms of evolutionary advantage.

JT: Right. So our goal has been to look at evidence of parallel evolution within independent host-switch events. And we find it lacking. It comes down to the fact that we are still very naïve biologically. If one thinks of mutations as a linear coding sequence of a protein, and you say "Aha! In the polymerase B2 gene there's a mutation at amino acid 627. But another virus has a mutation at 590, so it's a different mutation." But maybe the virus is solving the same biological problem but just in a slightly different way. Until we can get to a structural understanding of how this works, how do host proteins interact with viral proteins and what are the consequences of these changes, we will have a hard time predicting. I think we will need to get up one more level in our basic science, where we achieve a structure-to-function relationship.

So we need more surveillance, we need more basic science. Of course then the two other things that are critically important are better vaccines and therapeutics. On the vaccine front, the response to the 2009 pandemic was obviously the best and most efficient response that has ever occurred. In most industrialized nations there was enough virus vaccine within 6 months of the emergence of the pandemic. But if it were a more pathogenic virus—and luckily for us 2009 was not—it would have had more of an impact. The peak of the pandemic, at least in the United States occurred around September–October and much of the vaccine wasn't distributed until after the peak, October–November.

ER: In some countries, there was also a strong public backlash against the vaccine and the vaccination campaign. Did you see something similar in the USA?

JT: There is a strong anti-vaccine movement in the US. I think that one of the consequences of this is a lack of historical perspective. You can just go back and you find that even in rich countries like Germany and the United States, in 1900, a large

number of children died of typhoid and diphtheria and measles. And because we've been so successful at eliminating infectious diseases through childhood vaccination, the only thing left to complain about are very rare vaccine-associated events. That's not to say that vaccines are completely safe, but this risk-benefit ratio at a public level, the risk of not having a measles vaccine, of not having a polio vaccine, of not having a diphtheria vaccine is so much greater, it's not even debatable. Measles is a virus that has no animal hosts, so like smallpox, could actually be eradicated, as could polio. There is no reason why they have not already been eradicated, other than the difficulties politically of doing that. But now outbreaks of measles occur in the United States, in Europe, and it's sad. Because this should not happen.

But back to influenza, our problem is that the vaccine certainly gives you decent protection in general if you're an immunocompetent person. But we could produce new generations of vaccines that would be better.

ER: Vaccines are based on the hemagglutinin and neuraminidase protein, and the most likely strains to come out for the flu season. If you take a step back and look at it from a higher level, what is the chance that we could develop a vaccine that provides a baseline protection against flu in general?

JT: We certainly can and that is another example of an advantage of sequencing the 1918 genome. One of the key directions for making a universal vaccine are to concentrate on conserved epitopes in the stalk, that is the bottom end of the hemagglutinin protein as it sticks up from the virus, not the outer surface, where most of the vaccines are directed. A number of monoclonal antibodies can protect animals in passive immunotherapy experiments which shows that immunity to these conserved antigens is actually important. The 1918 structure has been important in this effort: it turns out that antibodies raised against the stalk of the 1918 virus hemagglutinin might actually help us move toward a universal vaccine.

ER: Might this also help to close the time gap between outbreak and vaccination campaign?

JT: There are also independent efforts to decrease that time. Rather than producing vaccine in fertilized chicken eggs, you can do it in cell culture, you can do large vat cultures. I know that many companies in Europe have been doing this and made vaccines faster. But I think we can approach new technologies. People are doing all sorts of interesting things, making influenza proteins in tobacco plants or in other cell culture systems, DNA vaccines, vectored vaccines, there are all sorts of different approaches, and I don't know which one is the best.

ER: You are confident, that eventually it will succeed in coming up with a general vaccine?

JT: My view is that to produce a so-called universal vaccine that would work against any subtype of influenza such that you would get no viral replication at all, is not achievable. Actually, I don't think it's desirable. What one would want is protection from severe illness and death, but some viral replication might actually even be advantageous, in the sense that you boost your immune system by having an exposure. So people who are certainly at risk of influenza infection tend to be the elderly, the immunocompromised, people with chronic illnesses, respiratory or cardiac illnesses, diabetes, pregnant women. But for a vaccine, obviously what you want is community vaccination, you don't want to just vaccinate at-risk people, because you want to reduce spread in the community. This is the goal that we're trying to achieve: broad reactivity and broad protection but not necessarily sterilizing immunity.

We have a study just published looking at neuraminidase. While the current vaccine preparation contains neuraminidase, the vaccine often does not induce a neuraminidase immune response. The neuraminidase is a tetramer, it falls apart, and the vaccine is all quantitated by hemagglutinin. So neuraminidase is sort of the unloved stepsister of influenza vaccine. In a small pilot set of experiments we made a neuraminidase-only vaccine and were able to show that the neuraminidase from the 2009 pandemic could completely protect mice from a lethal infection with H5N1. We also have shown that it can protect against 1918 infection. Clearly neuraminidase immunity is important, and I think that if one could just add antigenic neuraminidase to the current vaccine, this would actually help a lot.

ER: You also mentioned antivirals and that we need new ones.

JT: Despite having good surveillance, despite having our biology, despite maybe having our universal vaccine, there are still people who are going to get sick with flu. The antivirals we have are not terribly effective. The neuraminidase inhibitors are fantastic drugs, an example of rational drug design. But the neuraminidase function, which is to help release finished virus particles from cells, is the very last stage of replication and so all the damage is already done. What we need are drugs that inhibit viral replication at an earlier stage, polymerase inhibitors and so on. We definitely need new classes of flu drugs, because you'll never be able to completely prevent pandemics. Influenza viruses live in hundreds of animal species and they can never be eliminated.

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ER: What about bacterial pneumonia? That is something that you can eventually treat with antibiotics.

JT: Well, we're running out of antibiotics too. In the 2009 pandemic for example, the majority of people we studied at autopsy died with bacterial pneumonias, but we saw community-acquired methicillin-resistant staph infections in a couple cases. This is becoming a bigger and bigger problem.

ER: Another aspect is control strategies on a local level. In Europe or the USA, as soon as avian H5N1 shows up anywhere in migrating birds, they start culling all birds in this

area. Is that very helpful? Or can we do better if we know more about the virus and how it evolves?

JT: Culling of domestic poultry, chickens, turkeys, quail certainly can be an effective form of local outbreak control. This term "highly pathogenic" is specifically focused on its ability to kill chickens really, it's not meant to apply to humans or other mammals. What I have heard from my veterinary and agricultural colleagues is that, in many places, the compensation to farmers is lacking and so there's a huge economic disincentive to want to do that. There are also people who have rare types of birds that they breed for show, or cock fighting, and some of these birds are very valuable and they hide them. Human behavior is a big factor. I think a lot of countries, and a lot of politicians like the idea that it's wild birds and nature, because that's not their problem, it's mother nature. But it looks like much or most of the spread of H5 has been through human activity: the spread of the virus actually comes across the silk road, that's not necessarily the migratory path of birds.

Moreover, now that people are wealthy enough to eat meat on a routine basis, the number of farm animals to provide this food is increasing exponentially. You have ten thousand chickens in this intense farming everywhere, and this is not just a problem with influenza, but also, for example, with Nipah virus in Malaysia, with pigs. Human activity, through our intense farming, through our crowding, through the fact that millions of people fly in airplanes across continents, is just going to increase the chances of not only zoonotic transfers of animal viruses to humans, but increase the spread of those viruses if they occur. SARS was on three continents in 10 days.

ER: As you say Spanish flu came basically out of nothing, so the risk of an unexpected pandemic is still there. SARS also came out of the blue.

JT: Take the 2009 pandemic, that's a great example. We have all this concern about H5N1, and what happens? A pig-derived H1N1 virus appears out of nowhere. The virus, genetically, was a reassortment event between two circulating swine strains, one commonly in North America and one most commonly in Europe and Asia. But, where did this reassortment event occur? In a pig? In a human? I don't know. When did it occur? I don't know. How long was it circulating in some animal population without being detected? And how long was it circulating in humans without being detected, until the initial outbreak in Mexico? I don't know. If you have circulation of viruses that cause limited disease in an animal, it's going to be hard to detect unless you're doing prospective surveillance, which is extremely expensive. Highly pathogenic viruses in chickens are easy to find. Every chicken in the farm dies in a couple days. But silent replication is not so easy to spot. Many pigs are infected with influenza viruses with limited symptoms, but they still shed virus. There's also no way that we could do much prospective surveillance in wild animal populations.

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My prediction would be that spillover infections in both directions, actually human viruses into pigs and animal viruses into humans, occur all the time at some very low level, in which there's little-to-no illness on either side and little-to-no spread. Unless one does very careful surveillance, you wouldn't see it. But this is the kind of work that needs to be done to see this level of genetic chatter among viruses, or whatever you want to call it. We need to realize that this probably happens continually.

ER: So, we're permanently at the edge of a new outbreak?

JT: Influenza has just evolved to want to, being anthropomorphic, do this. Some viruses have evolved to be very specific for their host. Every single rodent species probably has a specific hantavirus adapted to it, we have our set of herpes viruses, and every animal, all the way down to sea turtles, have their own herpes viruses, but they don't cross species barriers. Whereas influenza has taken this different lifestyle…

ER: Embracing the whole world…

JT: Let's be everywhere, yes. The problem with influenza is this unbelievable adaptability. We have numerous examples of our own patients here in the hospital, with the 2009 pandemic in which we do careful surveillance of the viral isolate from patients. We take a nasal swab every day. We sequence the genome of the virus, and now we're doing deep sequencing to look at quasispecies, and people who are treated with antiviral drugs. And you can see the development of antiviral resistance in just a couple of days. These viruses are very adaptable, and they're very good at surviving selection pressure. Drug selection pressure, vaccine immunity selection pressure, host-switch selection pressure, it's sort of these viruses' raison d'etre. That's also why it's so intellectually interesting to attack this problem.

ER: Coming back to Fouchier's work, some of the criticism that has been raised was the fact that it was done in a level 3 lab in the middle of Rotterdam. Some already argue that SARS should be a level 4 agent, and that flu should be up there. What do you think about these concerns regarding biosafety of flu research?

> *"We must keep in mind that it is the natural emergence of novel viral strains that continue to pose the largest public health risk."*

JT: Each country has its own regulations and I can only speak about the United States, but the US government has a set of criteria to evaluate pathogens, and they assign containment based on that. Influenza viruses are either BSL 2 or BSL 3 or BSL 3 with enhancement as the highest, and the reason that they haven't met the last criteria for BSL4 is that there are influenza antivirals and vaccines. I feel

confident that the combination of engineering in these containment facilities, personal protective equipment used, approved procedures, and biosecurity practices employed make this work safe for the scientists and the environment.

ER: The other angle is biosecurity. I guess some of the concern about the Kawaoka and Fouchier experiments was that someone with nefarious intent could abuse the information from their paper for criminal or terrorist purposes. What do you think in general is the risk that public knowledge about pathogenicity of dangerous viruses could be abused?

JT: One could never say that the risk is zero, but I think it is low. Viruses with pandemic potential, like influenza, are critically important to study because of the very real public health risk that they carry. Consequently, research conducted safely under approved protocols is essential if we are to prevent or mitigate a future pandemic. In a risk-benefit analysis, understanding the genetic basis of host switch events, designing better antiviral drugs and new generations of more broadly protective vaccines are all possible through continued research with viruses like 1918, H5N1, or H7N9. We must keep in mind that it is the natural emergence of novel viral strains that continue to pose the largest public health risk.

ER: Dr. Taubenberger, thank you for the interview.

The interview was conducted by Holger Breithaupt.