Pathogenesis and Transmissibility of North American Highly Pathogenic A(H5N1) Influenza A Virus in the Ferret Model

[Announcer] This program is presented by the Centers for Disease Control and Prevention.

[Sarah Gregory] Hello, I'm Sarah Gregory, and today I'm talking with Dr. Joanna Pulit-Penaloza, a biologist at the Centers for Disease Control and Prevention in Atlanta. We'll be discussing avian influenza A(H5N1) in a ferret model.

Welcome, Dr. Pulit-Penaloza. It's a pleasure to have you.

[Joanna Pulit-Penaloza] Thank you, Sarah. Thank you for inviting me.

[Sarah Gregory] Tell us about the HPAI H5N1 virus.

[Joanna Pulit-Penaloza] H5N1 virus is a type A influenza virus. The H5N1 stands for hemagglutinin 5 and neuraminidase 1. These are also referred to as HA and NA, and these are the glycoproteins that are present on the surface of the virus. The designation of highly pathogenic avian influenza (the HPAI), this refers to the virus' potential to kill domestic poultry. It has nothing to do with how infectious or high pathogenic this virus may be to people. The natural hosts for most influenza A viruses are wild, aquatic birds. And most of the avian strains that circulate in those birds are low pathogenic, and these strains cause asymptomatic infection or mild disease in birds. However, in poultry, occasionally these low pathogenic strains can mutate into highly pathogenic strains, and the highly pathogenic strains cause severe disease that affects multiple internal organs and frequently result in high mortality rates in poultry.

[Sarah Gregory] Is this influenza related to the avian flu outbreak of 2015?

[Joanna Pulit-Penaloza] Influenza viruses are constantly changing, but the hemagglutinin gene of the currently circulating H5N1 virus is related to the hemagglutinin gene of the viruses that caused the 2015 outbreaks in the US.

[Sarah Gregory] Do we know where it came from?

[Joanna Pulit-Penaloza] There's a long history. Going back in time, the ancestors of the highly pathogenic H5N1 bird flu first emerged (and this was in China) in 1996. The next year, in 1997, this virus caused large poultry outbreaks in Hong Kong and that resulted in the first documented infections in people. The outbreak was controlled at that time, but the virus re-emerged in 2003, and after which it spread widely throughout Asia, and then it went into Africa, Europe, and the Middle East. Over the years, the virus continued to evolve, leading to the emergence of new strains. And the first time related viruses were detected in North America was in 2014, and these were the viruses that caused the outbreaks in 2015.

So after the outbreaks in the US were controlled, this virus continued to circulate in other geographic locations, and it was evolving until the current H5N1 virus emerged. It was first identified in Europe during the fall of 2020. And after that, this H5N1 virus spread across Europe, into Africa, the Middle East, and Asia and it became the predominant subtype globally, and it displaced all the previously circulating H5 subtype avian viruses. And then, it was imported to North America by migratory birds in late 2021.

[Sarah Gregory] And what kind of birds has it affected?

[Joanna Pulit-Penaloza] The virus has been isolated from numerous species of birds, and that includes different species of ducks, but also geese, shorebirds, and gulls. And I've also seen reports of virus isolation from bald eagle, hawk, pelican, vulture, owl. And the infected wild birds then spread the virus to backyard and commercial poultry flocks.

[Sarah Gregory] It sounds like it's pretty contagious. How contagious is it?

[Joanna Pulit-Penaloza] Highly pathogenic avian influenza is very contagious in birds. It can spread between birds by direct bird-to-bird contact, but also it can spread by indirect contact with contaminated environment. And this is because infected birds shed the virus into the environment in their respiratory secretions and feces.

[Sarah Gregory] I know that in 2015, people were definitely affected. This strain is affecting people?

[Joanna Pulit-Penaloza] Since 1997, there have been over 800 confirmed cases of human infections with H5N1 viruses worldwide. But this current strain, there are very few infections that were reported for this strain. There was one case reported in the United States earlier this year, and the patient had a direct contact with birds that were presumably infected with this H5N1 strain. The patient complained of fatigue, but the symptom resolved, and the patient fully recovered. So far, the current H5N1 bird flu lacks mutations that in the past have been associated with viruses infecting people and causing severe illness.

[Sarah Gregory] So why is there so much concern about this flu? It's spreading quickly, apparently, but it's not affecting people. Is it killing poultry? Why the concern?

[Joanna Pulit-Penaloza] There are several reasons. So first of all, it is very contagious and outbreaks on commercial poultry farms result in significant agricultural losses. So anytime this kind of virus is detected, the flock needs to be depopulated to eradicate the virus from the farm. But also, contact with infected birds creates opportunities for transmission to people. The problem is that influenza viruses constantly undergo genetic changes and every time a bird flu infects a person, this creates opportunities for the virus to adapt to the new host. So if the H5N1 virus were to acquire mutations allowing it for efficient replication and transmission from person to person while it still maintains the capacity to cause severe disease, the consequences for public health could be very serious. And as I mentioned, although the current H5N1 virus has not been associated with many infections in people, over half of H5N1 infections that were reported since 1997 resulted in death. And to answer the second part of your question, it is spreading relatively quickly. It was imported into North America in late 2021 and it has really quickly (over a few months) spread into all four flyways of North America, and it has been detected in most states. Currently, I think 46 states reported this virus.

[Sarah Gregory] Is it unusual for a flu to spread so rapidly?

[Joanna Pulit-Penaloza] Because aquatic birds are the natural host of these viruses, global spread through migratory flyways is not unusual. And so, this is why surveillance in wild bird populations is so important. It helps detection of new strains, especially those that have enhanced transmission and pathogenesis in people.

[Sarah Gregory] Currently, it's already in 46 states. Which states are those and is it still spreading rapidly through Europe and Africa?

[Joanna Pulit-Penaloza] Yes. So the virus is currently in many different countries in Europe, Asia, Africa, and as I said, 46 states of the United States. And wild birds' migration patterns determine when and where the virus will spread next.

[Sarah Gregory] Why did you do this study?

[Joanna Pulit-Penaloza] One of the main reasons for this study was to provide information to help assess the pandemic risk potential of this virus, and this is done using Influenza Risk Assessment Tool. This tool was developed by both internal CDC and external experts, and it uses 10 risk elements, and the elements consider attributes of the human population, but also ecology and epidemiology of the virus as well as properties of the virus. And transmission in animal models is one of the elements, so this is why we used ferret model to evaluate this virus.

[Sarah Gregory] And where did the virus come from that you used in the study?

[Joanna Pulit-Penaloza] The isolate was obtained from the National Veterinary Services Laboratories, through the US Department of Agriculture. And also, the name of the virus provides several important pieces of information. So in the name, the 'A' stands for influenza A virus; then we have American wigeon, which is the species from which the virus was isolated; then we have 'SC', which is South Carolina (the state where the virus was isolated); followed by a number that is unique to this particular isolate; and then we have the year of isolation (and this was 2021).

[Sarah Gregory] You just mentioned you used ferrets as a model. Why are ferrets a good model for studying avian influenza?

[Joanna Pulit-Penaloza] The ferret's usefulness as a model for influenza became apparent in 1933, when influenza-induced rhinitis was first observed in a ferret. Since then, the model has been extensively used to study various influenza viruses, and it is because ferrets are naturally susceptible to influenza infection. Additionally, the ferret closely resembles humans with respect to clinical signs and pathogenesis. And also, influenza receptor distribution in the human and ferret respiratory tracks are similar. So for these reasons, ferret is the Gold-Standard model for studying pathogenesis and transmission of influenza viruses, and this includes avian influenza viruses.

[Sarah Gregory] So explain ferret transmission models to us—there's Direct Contact Transmission, Respiratory Droplet Transmission. What are these?

[Joanna Pulit-Penaloza] We used two different experimental setups to study transmission of a virus. The Direct Contact Transmission Model—here, we co-housed an inoculated ferret with a naïve ferret. In this set up we allow for the transmission to occur via contact, and this could be either direct contact or indirect contact through contaminated fomites—so for example, bedding or cage walls. But also in this setup, we allowed for the transmission to happen by inhalation of air that may carry virus-laden particles.

In the second model, we—the Respiratory Droplet Transmission Model—we house the inoculated and contact ferrets in separate cages. So we eliminated the contact transmission, but only allow for the transmission to occur through air.

[Sarah Gregory] What did you find from your experiments—clinical signs, tissue dissemination, transmission?

[Joanna Pulit-Penaloza] In this study, we inoculated six ferrets with a high dose of this H5N1 virus and we observed that the animals became productively infected, but the disease was generally mild; the ferrets exhibited less than five percent weight loss and it had very transient fever. We did not observe any signs of infection, such as sneezing or nasal discharge. We also had three additional infected ferrets that were humanely euthanized day three post inoculation so that we could do necropsy and determine the spread of the virus in organs. We observed that the virus was limited to the upper respiratory tract of ferrets, except for one animal which had low-level of virus in lungs. We did not detect any of the virus in blood, and also there was no virus detected in any extra pulmonary tissues, and these included intestines, olfactory bulb, brain, kidneys, spleen, and liver. And we also evaluated transmissibility of this virus using these two models I just described. To determine whenever the contact animals became productively infected, we collected nasal washes from these animals and then we looked for the presence of virus in the nasal washes. So what we observed is that there was no virus in any of the nasal washes from the contact animals, and that...it suggested that transmission did not occur between any of the ferret pairs. And we confirmed these results by collecting serum from all the ferrets, and this was about three weeks after inoculation or contact. And while all the infected animals (all the inoculated animals) had antibodies against the challenge virus, none of the contacts seroconverted. So that further demonstrated that this virus was not able to transmit between ferrets.

[Sarah Gregory] Tell us about in vitro (Calu-3 cells time course) experiments—what they are and what you found.

[Joanna Pulit-Penaloza] The Calu-3 cell line was derived from human bronchial epithelial cells. And when we grow these cells in the laboratory, they form tight monolayers, which share similarities with human airway epithelium, and the human airway epithelium is the primary site of influenza virus replication in people. So we wanted to determine whether this avian flu virus can replicate in human respiratory cells. But also, we wanted to see how well it replicated compared to human-adapted viruses. And also, we performed these experiments in two different temperatures—the 33 degrees Celsius temperature is representative of the temperature found in upper respiratory tract of people, and 37 degrees Celsius is temperature found in lower respiratory tract. And it is important to mention that avian influenza viruses are adapted to temperatures of avian enteric tract, and these are around 40 degrees.

So in this study, we used four human seasonal flu viruses for comparison. And overall, we noted that although we see strain-specific differences between all the viruses that we tested, the data showed that this highly pathogenic H5N1 virus can replicate efficiently in the types of cells that make up the human respiratory tract. There was one main difference, and this was observed in 33 degrees Celsius temperature. The avian virus showed a substantial delay in replication, and this kind of delay is characteristic of avian viruses, which are not adapted to replicate in mammalian respiratory tract.

[Sarah Gregory] What do you conclude from all of this?

[Joanna Pulit-Penaloza] Our data supports CDC's risk assessments. Currently, the assessment indicates that this highly pathogenic H5N1 virus does not pose a substantial risk to public health. However, because of the continued evolution in wild bird populations, there is need for close surveillance of circulating strains and continued assessment of new viruses so we can ensure that strains that might have enhanced mammalian fitness are quickly identified.

[Sarah Gregory] Were there challenges in doing this modeling?

[Joanna Pulit-Penaloza] Studying influenza virus in general is a challenge because every strain is unique. However, our protocols are well established and optimized to account for any potential variabilities. And our team has many years of experience, so we were capable to quickly respond and perform this analysis safely and efficiently.

[Sarah Gregory] Any surprises?

[Joanna Pulit-Penaloza] We knew that the viruses that were isolated in the US in 2014, they caused mild infection in ferrets and there was no transmission. So in that regard, the findings from our current study are in agreement with our previous work. However, because influenza viruses are unpredictable and there are differences between strains, it would not be surprising if other H5N1 isolates behaved differently in the ferret model.

[Sarah Gregory] With this rapid spread, what happens next?

[Joanna Pulit-Penaloza] There will be continued depopulation of commercial flocks where the virus is detected in efforts to eradicate the virus from poultry. Also, the virus will be monitored for any mutations that it might be acquiring in case a new strain evolves or there is an increase in infections in people, we might need to characterize another isolate, and risk assessment may need to be updated.

[Sarah Gregory] Is there a way to stop this? Is there a vaccine in the works for it?

[Joanna Pulit-Penaloza] There are few FDA approved H5N1 vaccines that were made for pandemic preparedness, and these are currently not recommended for wide general population use. But as the virus evolves and changes, candidate vaccine viruses have been and continue being developed. And these candidate vaccine viruses can be distributed to manufacturers to rapidly initiate vaccine production if a pandemic virus emerged in people.

[Sarah Gregory] These are vaccines for people then, not for poultry or wild birds?

[Joanna Pulit-Penaloza] Yes. These are vaccines developed for people.

[Sarah Gregory] Should people be concerned about wild birds in their yards? I know I have many.

[Joanna Pulit-Penaloza] This is a good question. So we need to keep in mind that people rarely get bird flu, and when they do, it is most often through direct contact with infected birds. And because birds can be infected with bird flu without appearing sick, as a general precaution people should avoid direct contact with wild birds and definitely avoid contact with birds that appear ill or have died.

[Sarah Gregory] Tell us about your job at CDC and how you became involved in this investigation.

[Joanna Pulit-Penaloza] I am a research scientist in the Influenza Division (the Immunology and Pathogenesis branch). There are 14 within that branch, and we are the Pathogenesis Laboratory Team. I have been routinely involved in similar risk assessments for a wide range of influenza viruses with pandemic potential. And besides avian influenza viruses, I have been also extensively working on swine viruses, especially those strains that were able to infect people. In addition, while we do these risk assessments in the ferret model, I'm also interested in incorporating aerosol-based measurements so we can improve our understanding of the adaptations that are necessary for airborne transmission of zoonotic viruses.

[Sarah Gregory] Well, thank you so much for talking with me today, Dr. Pulit-Penaloza.

[Joanna Pulit-Penaloza] Thank you so much.

[Sarah Gregory] And thanks for joining me out there. You can read the September 2022 article, Pathogenesis and Transmissibility of North American Highly Pathogenic Avian Influenza A(H5N1) Virus in Ferrets, online at cdc.gov/eid.

I'm Sarah Gregory for *Emerging Infectious Diseases*.

[Announcer] For the most accurate health information, visit cdc.gov or call 1-800-CDC-INFO.