Frequently asked questions and answers about avian influenza (bird flu)
Wageningen Bioveterinary Research (WBVR) - last update on 22 November 2016

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What types of avian influenza are there?

Avian influenza (bird flu) in poultry and other bird species is caused by influenza A viruses. Waterfowl (wild and domesticated) are the largest natural reservoir of these viruses and are the source of all known influenza A viruses (especially low-pathogenic avian influenza – LPAI). Influenza type A viruses are categorised based on two proteins on the surface of the viral envelope: hemagglutinin (H) and neuraminidase (N). These proteins are used to distinguish between avian influenza viruses. Currently, 16 hemagglutinin proteins (H1-H16) and 9 neuraminidase proteins (N1-N9) have been identified in birds, which could theoretically lead to 144 types of influenza A virus. Infection with LPAI viruses in wild birds and waterfowl almost always occurs without significant symptoms.
However, infection of domesticated poultry with influenza viruses can take many forms, ranging from no symptoms to moderate symptoms, but it can also lead to severe symptoms with up to 100% mortality. The mild variants of the avian influenza virus are referred to as low-pathogenic avian influenza (LPAI); the more severe variants are called highly pathogenic avian influenza (HPAI). All of the currently known HPAI versus are of the H5 of H7 subtypes, although not all viruses of these subtypes are HPAI.

Evolution of the highly pathogenic H5N8 virus

The HA segment of the H5N8 virus stems from the H5N1 virus (A/Goose/Guangdong/ 1/1996). Since 1996, the highly pathogenic H5N1 virus has been endemic in poultry populations in various Asiatic countries. In 2005-2006, the high-pathogenic H5N1 virus spread from Asia to Europe, the Middle East and Africa. A highly pathogenic H5N8 virus with genes from the H5N1 virus (A/Goose/Guangdong/1/1996) was first detected at a live bird market in China in 2010. The highly pathogenic H5N8 virus that was discovered in 2014 was a reassortant virus with the HA segment of the highly pathogenic H5N1 virus and other gene segments of several other AI viruses that circulate in China, and is categorised as a highly pathogenic H5 virus of clade 2.3.4.4. This clade is remarkably promiscuous and is found in combination with six different NA segments. These NA segments were obtained from low-pathogenic AI viruses, including N5 (from 2006-2010), N2 (from 2008-2012), N8 (since 2010) and more recently N6 (since 2013). In September 2016, highly pathogenic Influenza A virus subtype H5N8 virus was found in dead wild waterfowl on Ubsu-Nur lake, on the verge of South Siberia and Mongolia. This new, highly pathogenic H5N8 virus (2016) is not directly related to the highly pathogenic H5N8 virus from 2014; moreover it has once again acquired genetic material from other low-pathogenic AI viruses circulating in wild waterfowl.

Are other highly pathogenic viruses currently prevalent?

Highly pathogenic avian influenza was first described in 1894 in Italy, but the pathogenic organism was identified much later. Since 1959, the outbreaks have been tracked more effectively, and smaller and larger epidemics of both H5 and H7 HPAI viruses have been described. The impact of epidemics is much greater if they take place in areas with many poultry farms. The largest epidemic was caused by HPAI H5N1, which began in Asia. It appeared in the news around 2003/2004, but the epidemic probably started in 1996. The virus then moved from Asia to large areas in Africa and then to Europe. Epidemics are now more widely publicised than previously, which at least partly due to the fact that the H5N1 virus can infect humans, sometimes with fatal consequences. In the Netherlands, HPAI outbreaks have occurred only in 1926, 2003 and 2014.

What is WBVR doing to map out the spread of the highly pathogenic H5N8 virus (November 2016), and which factors is it investigating?

Various surveillance systems are being used to detect infections with the highly pathogenic H5N8 virus.

- First, there is surveillance of dead wild birds: If groups of dead wild birds are found by citizens, these are sent to the Netherlands Food and Consumer Product Safety Authority (NVWA) and then forwarded to Wageningen Bioveterinary Research (WBVR) to determine whether the virus can be detected in these dead birds.
- Second, there is mandatory notification of clinically suspicious situations on poultry farms: if increasing mortality or a rapid decline in egg laying or feed intake is observed by the poultry farmer or the veterinarian for the farm, this must be reported to the NVWA.
- In addition, if the disease history and autopsy findings from diseased or dead chickens that are sent for autopsy to the Animal Health Service Deventer (GD) and veterinarians cannot exclude avian influenza, tissue samples are forwarded to the WBVR for exclusionary diagnosis. Due to this surveillance system, WBVR receives samples to test for the presence of an H5N8 virus.

We monitor the results of the diagnostics for H5N8 virus infections in wild birds and poultry abroad, and we conduct DNA sequence analysis on the viruses, which we compare with existing sequences in an international database to determine where the virus originated, in which bird species and along which routes it has been transmitted.

What is the testing procedure to ascertain avian influenza in wild birds? How are tests conducted, and for which variants? And how many dead birds are tested?

If more than three dead waterfowl or more than 20 dead birds of a different species are found at a single location, this is reported to the Netherlands Food and Consumer Product Safety Authority (NVWA). The dead birds are then sent to Wageningen Bioveterinary Research (WBVR) to determine whether the virus can be detected. Samples from these birds are tested in the laboratory with a PCR test for influenza virus (all variants). If this test is positive, then the samples are first tested with PCRs to determine if H7 or H5 subtypes are present. All severe variants of avian influenza are from these subtypes. Other, more elaborate tests are used to determine whether a highly pathogenic variant is involved and to determine the N subtype.

Are waterfowl now more susceptible to avian influenza than several decades ago?

Wild waterfowl, especially ducks, are the source of influenza viruses. For maintaining the virus population, a balance between the virus and its hosts is required. In this sense, it cannot be expected that wild waterfowl are in general more susceptible now to avian influenza than they were several decades ago. In exceptional cases (such as infection with the Asian highly pathogenic H5N1 in 2005-2006 or the highly pathogenic H5N8 virus in 2014-2015 and 2016), wild birds and waterfowl can be infected with highly pathogenic avian influenza (HPAI) viruses due to the high infection pressure from poultry farming. These infections took place primarily in East and Southeast Asia. At these locations (for various reasons) HPAI virus infections on poultry farms could not be controlled in the same way as in the Western hemisphere. As a result, the viruses have continued to circulate. Because wild and domesticated birds and waterfowl are frequently in close contact with each other in Asian countries, wild birds and waterfowl sometimes become infected. Some wild bird and waterfowl species (such as some duck species) tolerate such an infection better than domesticated poultry (which usually die quickly after becoming infected). As a result, these infected wild birds can sometimes carry the virus with them on their annual migrations to other parts of the world. In case of overlapping migratory routes (such as breeding areas) or stopovers at foraging areas, the virus can be transmitted between various species of migratory birds, which can then carry the virus further. If the virus infection causes fewer disease symptoms and less mortality, it can maintain itself longer in these chains. A possible explanation of why the current H5N8 virus is now causing more disease and mortality than the H5N1 virus or the H5N8 virus did in 2014 is that it causes mortality only in certain species of waterfowl, such as loons, tufted ducks, swans, geese and seagulls. Another possible explanation is that infected waterfowl produce much more virus, causing more birds in the surroundings to become infected.

Is Dutch poultry now more susceptible to avian influenza than several decades previously? (Has genetic variation become lower within the entire group?)
There are no indications that Dutch poultry is now more susceptible to avian influenza than several decades previously. The first recorded highly pathogenic avian influenza outbreak was from 1926-1927. At that time it spread rapidly through the Veluwe, in the same region impacted by the severe avian influenza epidemic (type H7N7) in 2003.

How unusual is the current (as of 21 November 2016) geographical distribution of highly pathogenic H5N8 in wild birds the EU?
It is relatively unusual that highly pathogenic viruses circulate in wild waterfowl. Before 2014, this occurred only twice, for example with the Asiatic H5N1 virus in 2005-2006. Around 2009, it is likely that an H5N8 variant developed in China due to the exchange of genetic material from endemically circulating H5N1 with other low-pathogenic viruses in domestic poultry. The H5N8 virus was then transmitted to wild birds in which it can apparently maintain itself more effectively than the H5N1 virus, which was no longer detected in wild birds on a large scale in Europe after 2006. The H5N8 virus was probably carried by migrating waterfowl from China to breeding areas in the north (Siberia and Mongolia). From there, poultry farms in Korea and Japan were contaminated at the beginning of 2014 due to migrating birds, and later in 2014 in Western Europe, again in Japan and Korea, and the virus also appeared in North America (it was carried across the Bering Strait). In September 2016, highly pathogenic Influenza A virus subtype H5N8 virus was found in dead wild waterfowl on Ubsu-Nur lake, on the verge of South Siberia and Mongolia. This new H5N8 virus (2016) is not directly related to the H5N8 virus from 2014. Moreover, it once again acquired genetic material from other low-pathogenic AI viruses circulating in wild waterfowl. Based on genetic association, we know that this new H5N8 virus was again carried to Western Europe by migratory birds. It is possible that in overlapping foraging areas, the migratory birds infected other wild birds, which then migrated along various migration routes to the west and southwest. The current distribution of the new H5N8 virus in wild birds in the EU is therefore not unusual and corresponds to the spread of the H5N1 virus in 2005-2006.

Can WBVR say something about patterns that are visible in the distribution of the highly pathogenic H5N8 virus (November 2016)?
The dead wild birds found in European countries with confirmed H5N8 virus infections and in Israel are from areas crossed by known flight paths of migrating birds. Until now, H5N8 virus infections in Europe have been confirmed primarily in tufted ducks and loons, but also other species of ducks, swans and gulls, and more recently in some buzzards and crows in Germany. The latter species are scavengers and possibly ate dead wild birds that were infected. Until now, a limited number H5N8 outbreaks have been reported on poultry farms. These outbreaks occurred near locations where infected dead wild birds were found in these countries. This is also logical in an epidemiological sense, because large quantities of virus literally landed with the wild birds in these areas. Migrating wild waterfowl also contaminated the environment and very probably the local wild waterfowl as well. Consequently, the virus can continue to circulate for some time.

Can specific conclusions about this H5N8 virus be drawn from this current distribution pattern (November 2016)?
In comparison with 2014-2015, significantly more wild birds in the EU have become infected with the H5N8 virus. In 2014 we found indications for infection in migrating widgeon and teal which brought the virus to Europe, but that was not associated with mortality. It is still unknown why large numbers of wild birds are now dying as a result of avian influenza. There are indications that the current H5N8 virus is different from that from 2014. In addition, it is possible that many more migrating birds (and more species) have been infected, but also that the animals produce the virus in larger quantities. Consequently, local populations of wild birds can become infected more easily. In addition, mild weather conditions with temperatures above zero have ensured that migrating wild birds remain here longer and therefore had the opportunity to shed more virus, resulting in the infection of local wild birds. The virus was carried by more migrating wild birds to the south. As a result, there were indications the Rift Valley in the north of Israel that a poultry farm had become infected. The Rift Valley runs from the Beqaa Valley in Lebanon to Mozambique in Southeast Africa over a distance of 6000 km.

In the north of Israel, nearly 500 million wild birds migrate annually through the Rift Valley, which is narrow at that location. These birds are migrating from Europe to southern Africa. The possibly infected poultry farm is located in an area with aquaculture and wetland nature reserves, where migrating wild birds forage before they continue their difficult journey through desert regions to southern Africa.

How does the WBVR collaborate with other EU countries to understand the distribution of the highly pathogenic H5N8 virus (2016)?
The authorities of EU countries officially report outbreaks to the European Union (Animal Disease Notification System, ADNS) and to the World Organisation for Animal Health (OIE); these organisations publish this information on their websites. There are also other private initiatives that show the outbreaks on Google Maps. Research results are exchanged by the national reference laboratories, in part via OFFLU, an initiative of the Food and Agricultural Organisation of the UN (FAO) and the OIE. In addition, there is a "Global Consortium for H5N8 and related influenza viruses", a collaboration between laboratories in various countries to study the development of the virus.

What are the expectations based on the current distribution pattern on the highly pathogenic H5N8 virus (as of 21 November 2016)? Now that the virus appears to circulate so widely in wild birds; will it be possible to eliminate it, and if so, how?
The outbreaks on poultry farms Europe have been limited so far to farms near locations at which dead infected wild birds have been found. At the present time, the greatest risk for transmission of this avian influenza virus to poultry farms in the Netherlands appears to be near wetland areas.

It is unclear how long the virus will maintain itself in the wild birds in the Netherlands. In 2014/15, the virus was still found in a teal in January 2015. In 2005-2006 the epidemic began in October with outbreaks in Romania, and the last cases were reported in March 2006 in Germany. This was almost certainly due in part to the severe winter in 2006. Therefore, we should prepare for an infection threat from wild birds that will last for several months. Dead wild birds should be removed promptly because scavenging birds could spread the virus even further. To remain free from infection, therefore, poultry farms must maintain strict biosafety measures. This will require a great deal of discipline from poultry farmers; they must apply strict hygiene measures during a long period to prevent introduction of the virus. In addition, this requires a high level of alertness, and poultry farmers must not hesitate to notify the NVWA about a clinically suspicious situation in case of increasing mortality or a rapid decline in egg production or feed intake.

Does avian influenza occur frequently in the Netherlands?
In the Netherlands, the first report of a highly pathogenic avian influenza (HPAI) epidemic in domesticated poultry was from 1927. A subsequent HPAI epidemic did not occur for nearly 75 years, in 2003. In contrast, every year 35-50 introductions of low-pathogenic avian influenza virus are reported annually on poultry farms in the Netherlands, but in most cases these do not lead to significant clinical symptoms; approximately 10% of the cases involve a LPAI virus introduction of the H5 or H7 subtype. If an H5 or H7 virus is found on a poultry farm, all the birds on the farm are culled as a precaution. This is done to prevent the LPAI virus from mutating into an HPAI virus.

Is the risk of spreading avian flu virus now greater or smaller than several decades previously?
To answer this question, we must first distinguish two aspects: the risk of introducing the virus, followed by the risk of spreading the virus.

The risk of spreading the virus between farms depends initially on the density of the poultry farms and not as much on the density of animals per square kilometre. Since 2003, the number of poultry farms has declined. The risk of introducing the virus is not related to the number of farms, but is much more dependent on the type of farm. For example, the risk of introducing avian influenza on farms at which laying hens have access to outdoor runs is greater than the risk on farms at which the laying hens are kept indoors. Once the virus has infected a poultry farm, the risk of transmission to other farms is greater in areas with a high density of poultry farms.

In 2014, for example, the highly pathogenic H5N8 virus did not spread from infected poultry farms in Hekendorp and Ter Aar to other farms, which could be explained in part by the fact that the infected farms were located in an area with a low density of poultry farms.

Do current animal husbandry methods lead to a higher/lower risk of infection than the methods used several decades previously (and if so, why)?
Research conducted by WBVR in collaboration with the GD has shown that layer poultry farms with outdoor runs have an almost eight-fold higher risk of introduction of a low-pathogenic avian influenza virus than farms that keep their laying hens indoors. Because poultry are increasingly being kept outdoors, the risk of introduction of avian influenza viruses has increased, which is also expressed in the higher number of introductions of low-pathogenic avian influenza viruses during the last 10 years.

Are there any attempts to breed more resistant chickens?
In laboratory experiments, chickens have already been made more resistant to HPAI. This resistance is the result of genetic manipulation. In Europe, however, there is a moratorium on the use of genetically manipulated organisms, and it is unlikely that resistant chickens will become available in the short term through conventional breeding and selection procedures.

During the highly pathogenic H5N8 epidemic in 2014, how did you determine that separate outbreaks occurred in Hekendorp, Ter Aar and Kamperveen, but in Kamperveen one farm probably infected another farm?
WBVR isolated viruses from animals at the various poultry farms and mapped out the entire genome of these viruses. With this genomic data, WBVR compared the viruses and determined the most likely transmission routes. The analysis showed that there were only minimal differences between the viruses at the two farms in Kamperveen. Based on this finding, it appeared most likely that the virus had been introduced from one farm to the other, or that the poultry farms in Kamperveen were infected from the same event (for example, the virus was introduced to both farms by a single infected wild bird). Larger differences between the virus isolates (such as those found in Hekendorp, Ter Aar and Kamperveen I) make it more likely that the farms were infected due to unrelated introductions.

Based on outbreaks in the past, we know that once poultry farms are infected with a highly pathogenic avian flu virus, there is a risk of virus transmission between farms. This is probably what happened in Kamperveen.

In addition, it is possible that the virus was introduced to the Netherlands by migratory birds from their breeding grounds in Siberia. To cause infection, direct contact between these migratory birds and the poultry farms in Hekendorp, Ter Aar and Kamperveen was not required; other mechanisms were probably involved. The virus could have been present in the environment (e.g. bird faeces), and then have been introduced to the farms. It is also possible that migratory birds could have infected resident wild birds (which live all year in the Netherlands), and that faeces from these 'native' birds contaminated the environment.

Did the separate infections with the highly pathogenic H5N8 virus in 2014 have an impact on the current approach to avian influenza?
Two lessons were drawn from these separate infections. First, poultry farms with outdoor runs should keep their poultry indoors to prevent contact with wild waterfowl. Second, much attention is required for stringent biosafety measures. This is because the virus was still able to infect the farms in 2014 despite standard precautions.

Where did the highly pathogenic H5N8 virus (2014) originate?
The HPAI H5N8 virus originated in China. Due to a monitoring scheme, it was isolated there in 2009-2010 from apparently healthy ducks at a live poultry market. In 2014, the virus caused an outbreak on poultry farms in Japan and also spread rapidly in South Korea. The virus was identified in wild waterfowl in both Japan and South Korea. It is most likely that the virus was carried to Europe by migrating waterfowl from Asia, possibly with stopovers.

There are three reasons for this tentative conclusion: 1) the virus that was found in the Netherlands was very similar to the virus that had been previously found in Germany, South Korea and Japan, 2) no poultry had been imported from these parts of Asia to the Netherlands, and 3) this virus was not endemically present in the Netherlands on poultry farms.

What are the similarities and/or differences between the highly pathogenic H5N8 virus in the Netherlands, Germany and England in 2014, and the previous discoveries in Korea and Japan?

Genetically, the virus was virtually identical to the viruses found in Germany and England in 2014. In addition, all European isolates were closely related to those found in the summer of 2014 in Japan. They were also closely related to the viruses found in the first half of 2014 in Korea.

Does WBVR (or other institutes) also conduct virus research with rodents or other potential vectors of the avian flu virus? (If yes, which potential vectors?)

During the H5N8 epidemic in 2014, no rodents were submitted for testing. The biosafety schemes on poultry farms require continuous trapping of rodents (mice and rats). In 2003, no indications were found that people or rodents could transmit the virus because they had become infected. However, people can still introduce the virus to a farm, for example because they track in manure or other contaminated material.

Can the avian flu virus also enter a farm through the air (through the air intake)?

Dispersal of airborne influenza virus plays role primarily when many birds (for example on a large poultry farm) become infected and shed virus for a longer period. The virus produced in this way can become attached to dust or dry droppings and in this way become airborne. On poultry farms, exhaust fans blow air from inside the barns to the outside. It is therefore possible for the virus to be blown outside and enter a nearby farm through the air intake. However, the risk of this happening is currently very low: the isolated outbreaks at three locations in the Netherlands were quickly detected and virus shedding by sick animals on the infected farms was quickly eliminated.

Ultimately, is vaccination the only effective way to prevent contamination with avian influenza virus during an epidemic?

Even if an effective vaccine could be developed, which is certainly not impossible, this would not be a solution during an epidemic. Emergency vaccination is not feasible because highly pathogenic avian influenza viruses can move between and within farms so quickly that sufficient vaccination capacity cannot be attained. Moreover, the development of resistance to the virus (immunity) in vaccinated animals often requires more than a week, and that is much too slow to stop an outbreak. General preventive vaccination before an outbreak (general vaccination against H5 and/or H7) is difficult to implement due to current European legislation and the requirements of countries that import poultry products from the Netherlands. These importing countries frequently require that no vaccination takes place or that a vaccine is used for which the generated antibodies can be distinguished from the antibodies against the field virus. For the time being, a non-vaccination policy appears to be the best solution.

Is avian influenza a fact of life for the sector, or can poultry farmers take effective measures to counteract the disease?

It is clear that we are incapable of controlling avian influenza viruses in wild birds. However, farms can take many measures to reduce their risks. Strict application of biosafety measures should keep the virus outside of the farm; there many examples showing that this is possible. It is a question of establishing and strictly complying with these measures, which requires perseverance, dedication and discipline. Poultry farms that are located higher on the production pyramid (such as breeding farms) invest more in biosafety to keep pathogenic organisms outside. On these types of farms, infection with avian influenza is a rare exception. For poultry farms at which chickens have outdoor runs and can therefore come into contact with the environment, including wild birds/waterfowl and their droppings, the challenge of preventing infection is much greater.