Outbreaks of Highly Pathogenic Avian Influenza in Japan

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Abstract

In January 2004, an outbreak of highly pathogenic avian influenza, caused by the virus subtype H5N1, occurred at a farm in Yamaguchi Prefecture, Japan. This was Japan’s first outbreak since 1925, a span of 79 years. By March, there were additional outbreaks in Oita and Kyoto Prefectures at a total of four premises, resulting in the sacrifice of about 275,000 domestic fowl. The disease was successfully eradicated in Japan, and infection damage was kept to a minimum thanks to an intense eradication campaign. On the other hand, extensive outbreaks of the disease were confirmed at around the same time, mainly in Southeast Asian countries.

Three years later, in January 2007, there was another outbreak of highly pathogenic avian influenza caused by the H5N1 virus in Miyazaki Prefecture, Japan. Within less than two weeks, there were two more cases in Miyazaki Prefecture and another in Okayama Prefecture, for a total of four confirmed cases. At each of the four farms where cases occurred, the outbreak was quickly discovered and reported, and rapid epidemic prevention measures, such as culling, burial and incineration, were successful. The outbreaks were contained and no reoccurrences were reported.

However, the risk of H5N1 virus entering Japan again is not decreasing, because the virus is endemic in several parts of Asia. Therefore, it is still top priority for poultry producers to maintain biosecurity practices to prevent introduction of the virus into their flocks.

Key words: chicken, highly pathogenic avian influenza virus, Japan, wild waterfowl

1. Introduction

Highly pathogenic avian influenza is a peracute contagious disease of poultry that causes systemic infection (Easterday & Tumova, 1978). Since the mortality of the disease in poultry is as high as 100%, and it can spread rapidly, an outbreak can devastate the poultry industry. The main symptoms of highly pathogenic avian influenza in poultry are depression; loss of appetite; decreased or absent egg production; nervous behavioral signs; facial, comb or leg edema; blood spots or cyanosis; respiratory symptoms; and diarrhea (Fig. 1). In addition, symptoms and virus excretion vary depending on the bird species, their age and virus strain. Spread of this disease is mainly by contact with infected birds or with feces, saliva, nasal secretions, drinking water, feed, dust, insects, wild birds, human beings, small animals, equipment or vehicles contaminated with the virus.

In Japan, in cases where highly pathogenic avian influenza has been confirmed, appropriate measures such as eradication and movement restrictions on the affected holdings are implemented based on the Domestic Animal Infectious Diseases Control Law. In addition, although some countries use vaccination as a preventive measure, Japan in principle does not. Vaccination may suppress symptom onset and allow birds to shed the virus while remaining asymptomatic. Vaccination, therefore, may induce the habitual presence of the virus in the field. As special testing is required to distinguish the antibodies produced by vaccination from those produced by natural infection, Japan engages in prevention...
by using testing to detect infected poultry and cull them.

In this paper, an overview of highly pathogenic avian influenza outbreaks in Japan is presented and the possible sources of introduction of the virus are discussed.

2. Outbreaks in 2004

Japan had been free from highly pathogenic avian influenza (formerly known as “fowl plague”) until January and March 2004, when an outbreak of highly pathogenic avian influenza caused by the virus subtype H5N1 occurred on four premises in three prefectures (Fig. 2). This was the first report of highly pathogenic avian influenza in Japan for 79 years since the 1925 outbreak, which was caused by the virus subtype H7N7.

Case 1

The first case of highly pathogenic avian influenza occurred on a layer farm (35,000 birds) in Ato Town, Abu County, Yamaguchi Prefecture, in January 2004, after dead chickens were confirmed on December 28, 2003. Since the number of chicken deaths subsequently increased and spread to other houses, the egg farm requested the local Livestock Hygiene Service Center to conduct a disease diagnosis. On January 11, the suspected outbreak of avian influenza was reported to the Ministry of Agriculture, Forestry and Fisheries (MAFF).

Case 2

In February 2004, the second case of highly pathogenic avian influenza occurred at the home of a hobbyist bantam breeder (13 bantams and one domestic duck) in Kokonoe Town, Kusu County, Oita Prefecture. The sudden deaths of three bantams were discovered on February 14, 2004. Therefore, the raiser reported the cases to the local Livestock Hygiene Service Center. Receiving the report, the Service Center conducted an on-site inspection the same day and, taking two of the dead birds, started virus isolation tests. Furthermore, since four more bantams raised in the same shed died two days later (February 16), an on-site inspection was again conducted. In the evening of the same day, the virus suspected of being avian influenza was isolated from the specimens removed on the 14th, and the Service Center reported the fact to Oita Prefecture. At the same time, the six bantams and one duck still remaining at the home of the raiser were culled with the raiser’s agreement.

Case 3

The third case in Tanba Town, Kyoto Prefecture, on February 17, 2004, was particularly aggravated. The outbreak occurred in one poultry house (No. 8, capacity: approx. 30,000 fowl) of an egg production farm (225,000 fowl), and despite the deaths of many chickens, no report was made. Furthermore, infected chickens were shipped to meat processing plants in Hyogo and Aichi Prefectures. The infection spread to virtually all of the houses within a few days. The Service Center, which had received an anonymous telephone call reporting large-scale deaths at the farm, conducted an on-site inspection before dawn on February 27. Infection by the highly pathogenic avian influenza virus subtype H5N1 was confirmed on February 29.

Furthermore, avian influenza virus subtype H5N1 was also isolated from a total of nine crows between May 7 and April 9 in the restricted movement zone. No other infection of wild birds was confirmed.

Case 4

Since there was a rapid increase in the number of dead chickens on March 3 at a meat chicken farm
(15,000 birds) in Kyoto Prefecture, located about 4 km northeast of the infected farm of the third outbreak, the farm manager notified the Livestock Hygiene Service Center. The Service Center promptly conducted an on-site inspection of the farm and a simple test of the dead fowl at the farm. The virus was confirmed to be subtype H5N1 on March 8.

As an epidemic prevention response, necessary measures in accordance with the Domestic Animal Infectious Diseases Control Law were performed. All chickens on outbreak farms were slaughtered, disinfection was carried out, movement at nearby farms was restricted and epidemiological surveys were performed. This procedure prevented the spread of the virus to nearby farms and halted the outbreaks in all four cases.

Though all virus strains isolated in these outbreaks were from the same source, they were different virus strains that had mutated in a relatively short period. There is the possibility that the outbreaks in the three prefectures occurred independently due to different infection sources. The isolated virus strains indicate extremely strong pathogenicity to chickens. As ducks can survive infection (Kishida et al., 2005), they may be carriers of the virus. In experiments on infecting mice, the virus was not detected in the feces, and it is thought that proliferation through the feces does not readily occur (Isoda et al., 2004). Infection has not been established in hogs, suggesting that they also have resistance.

With respect to infection routes, an expert “Investigation Team on the Infection Route of Highly Pathogenic Avian Influenza” was formed (Fig. 3). The team conducted analysis and evaluation based on epidemiological surveys originating at each outbreak farm, as well as analysis of the characteristics of isolated viruses. An investigation of wild birds, including migratory waterfowl, was also conducted within a 10-km radius of each infected farm (Fig. 4). Although no highly pathogenic avian influenza viruses were isolated from free-living birds, the team reported that it was probable that migratory birds, such as ducks, brought the pathogenic virus to Japan from the Korean Peninsula, and that the feces of these ducks and other migratory birds were a source of infection that was carried by resident birds, rats, other animals and humans into poultry houses (http://www.maff.go.jp/tori/20040630e_report.pdf).

3. Outbreaks in 2005

On June 26, 2005, avian influenza virus subtype H5N2 was isolated in Mitsukaido, Ibaraki Prefecture. By December 25, there were 41 confirmed cases of infection (40 in Ibaraki Prefecture and 1 in Saitama Prefecture), including farms that tested positive for antibodies but had no mortalities and no virus detection. The virus was isolated in nine cases and each virus was closely related. No clinical abnormalities were found on the outbreak farms. Pathogenicity testing revealed that the virus belonged to the attenuated type, producing minimal symptoms and mortality. Although investigation of infection routes by the Investigation Team on the Infection Route of Highly Pathogenic Avian Influenza was unable to specify the virus origins or entry routes, in light of the virus characteristics and the localized outbreak area, it was unable to rule out illegal importation and use of an unauthorized vaccine derived from a Central and South American virus strain or of the virus itself. In addition, an epidemiological survey found that there was a high probability that the virus was transmitted among some of the farms by the movement of chickens between farms. In addition, the survey suggested that neighborhood transmission and the comings and goings of people and goods were major causes of transmission.

4. Outbreaks in 2007

In January and February 2007, there were four outbreaks: on a meat-chicken breeding farm, a meat-chicken farm and an egg farm in Miyazaki Prefecture, as well as an egg farm in Okayama Prefecture. In each case, the virus was an H5N1 subtype related to viruses isolated in China, Mongolia and South Korea.
Case 1
In early January, an increased number of deaths and facial puffiness were confirmed in a poultry house on a meat-chicken breeding farm (12,000 birds) in Kiyotake Town, Miyazaki Prefecture. An attending veterinarian was consulted, but simple testing results were negative. Since the number of dead birds subsequently increased, simple testing was repeated, and the result was positive. Therefore, the Livestock Hygiene Service Center for the area carried out virus isolation. Since it resulted in the isolation of a virus suspected of being a type A influenza virus on January 12, the National Institute of Animal Health carried out virus identification testing. On January 13, it confirmed that the virus in question was an H5 subtype. The dead chickens all came from one poultry house complex without separations, so it was not possible to specify the source of the outbreak. Initially, there were more dead males than females.

Case 2
On January 22, when a rapid increase in dead chickens in the central part of a poultry house on a meat-chicken farm (53,000 birds) in Hyuga City was confirmed, the attending veterinarian carried out simple testing. The results were all negative, but the attending veterinarian reported an overview to the Livestock Hygiene Service Center. On January 23, the Livestock Hygiene Service Center and the attending veterinarian visited the farm and assessed the illness. On January 23, the number of dead chickens increased by 326, and simple testing results were positive. That day, the farm was quarantined, and the farm and the area around the poultry house were disinfected and limed. On January 25, the National Institute of Animal Health confirmed infection with an H5 subtype, type A influenza virus.

Case 3
On January 22, two dead birds in one cage in a poultry house were discovered. On January 27, a further 15 birds were confirmed dead in locations near the first two deaths, so the Livestock Hygiene Service Center carried out a clinical examination and sampling. Symptomatic chickens were limited to the left rear corner of the poultry house as viewed from the entrance. Lethargy, lassitude and ruffled feathers were observed in several birds. Since the results of simple testing were positive, quarantine of the farm and voluntary restrictions on movement were requested. On the following day, January 28, disinfection and liming of the farm, the area around the poultry house, and the interior of the poultry house affected by the outbreak were performed. Furthermore, even before confirmation, poultry and egg shipping inspections were implemented for farms within a 10-km radius based on the recommendation of the Domestic Fowl Disease Committee. On January 29, the National Institute of Animal Health confirmed infection with an H5 subtype type A influenza virus. Samples were taken on January 30 as culling began. Antibody testing and virus isolation testing isolated the virus only from specimens taken from the left rear corner, which confirmed that the spread of the infection was limited.

Case 4
On an egg farm (93,000 birds) in Shintomi Town, Miyazaki Prefecture, no abnormalities were observed until January 30, when a number of dead chickens were found in the southwest corner of a poultry house. Extremely mild cyanosis in the combs of some dead chickens and lethargy in a number of live ones were confirmed. Simple testing by the Livestock Hygiene Service Center was positive. Virus isolation began that same day, and on February 1, the National Institute of Animal Health confirmed infection with an H5 subtype type A influenza virus.

During these outbreaks the farms reported promptly, and quick and effective disease-control response measures, such as culling, incineration, burial, and disinfection, were implemented. Furthermore, movement-restriction areas were set up in a 10-km radius from each outbreak farm. The movement restrictions were lifted on March 1, after all tests on poultry farms and pet chickens were negative, and no secondary cases were found.

The viruses isolated from each of the four farms were all H5N1 subtype, highly pathogenic, avian influenza viruses. Genetic analysis found at least 99% homology for each of eight gene segments. In addition, there was at least 99% homology with H5N1 subtype viruses isolated during previous outbreaks in Mongolia and South Korea in 2006. Phylogenetic tree analysis showed that all these viruses belonged to the same lineage (Fig. 5), which was the same group as the virus isolated from wild birds in Qinghai Province, China, in 2005. They differed from viruses isolated in 2004 in Yamaguchi, Oita and Kyoto Prefectures, and in Thailand, Vietnam and Indonesia.

On intravenous inoculation testing using an isolated virus, every inoculated chicken died within 26 hours of inoculation. Therefore, it was clear that the virus has high pathogenicity in chickens. Clinical presentation included comb cyanosis in some chickens, with grossly visible pathology, such as cardiac edema and hepatic petechiae. This pathology differed from that of the virus isolated in 2004, which caused sudden death without other notable symptoms.

In nasal inoculation testing of mice using an isolated virus, all 18 mice died within ten days after inoculation. Although no virus was recovered from the animals’ digestive tracts, the virus was recovered from their brains, lungs, spleens, livers and kidneys, indicating strong pathogenicity in mice. On the other hand, in rats tested under the same conditions, although antibodies were produced, no clinical symptoms were evident, and no virus growth within the rats’ bodies was found. These results indicate that even though they are both rodents, susceptibility differs between rats and mice.

On January 4, 2007, an emaciated mountain hawk-eagle (adult female) was captured in Sagaramura,
Kumagun, Kumamoto Prefecture, and it died immediately thereafter. Since the bird was emaciated despite having no external injuries, the Kyushu Regional Environment Office of Ministry of the Environment suspected lead poisoning and sent the carcass to the Ministry’s Kushiro-shitsugen Wildlife Center, which was engaged in a study of lead poisoning in raptors. Along with testing for lead poisoning, the Center used a simple kit to test for avian influenza. Since the simple kit test was positive, on February 10, the Center asked the Avian Zoonoses Research Center, Faculty of Agriculture, Tottori University, to carry out further testing. On March 18, isolation of the H5N1 subtype avian influenza virus was reported, and on March 23, it was determined to be a highly virulent form. The direct cause of death of the mountain hawk-eagle was unknown.

Mountain hawk-eagles are large raptors that inhabit mountainous areas from Hokkaido to Kyushu. They feed on a variety of birds, small mammals, reptiles, etc. They will feed on poultry or pets such as cats if they are left outside. They will also feed on carrion. Those that breed in Japan do not travel long distances, but remain in the area around their nests year round. Their range is said to be 5 to 10 km. Young birds may travel longer distances. In southwestern Japan, there may be individuals who have crossed over from the continent. In addition to Japan, they inhabit Sri Lanka, southern India, the Himalayas, southeastern China south of the Yangtze River, the Indochinese Peninsula, Taiwan, Hainan Island and the Korean Peninsula, and they have also been reported in northeastern China.

Analysis of the HA gene of the H5N1 highly pathogenic avian influenza virus isolated from the mountain hawk-eagle found the virus to have the G-E-R-R-R-K-K-R amino acid sequence in the HA cleavage region, which is typical of virulent types. As for homology with other H5 viruses, it is most homologous (at least 99.7%) with the virus isolated from wild birds in Mongolia in 2006. A phylogenetic tree for the HA gene was created in order to analyze the details of the relationships with other viruses (Fig. 5). It is related most closely to the A/chicken/Miyazaki/K11/07 strain isolated in Miyazaki, demonstrating that this virus is also a Qinghai Lake-type.

These results indicate that the Qinghai Lake-type H5N1 HPAI virus was newly introduced from the Asian continent into Japan and already existed in the natural environment of the Kyushu district at the beginning of January 2007. However, the mountain hawk-eagle is a resident bird in Japan and its range of inhabitation is within 5 to 20 km. Therefore, it would be unlikely that it carried the virus from the Asian continent to Japan. Thus, the mountain hawk-eagle was likely infected secondar-

![Evolutionary tree of the H5 HA gene for the Qinghai Lake-type virus group.](Fig. 5)
ily by preying on other infected wildlife.

The initial outbreaks in Cases 2 through 4 were in parts of the poultry houses that were away from the entrances and areas where people usually work. Each farm had curtains, bird nets, and wire mesh installed, but there were tears in the wire, gaps in exterior walls and areas where bird nets were not in place; thus, anti-bird measures were insufficient. Many wild birds and animals were confirmed to be living around the outbreak farms. Evidence of incursions of such animals into poultry houses included the presence of rat feces inside the Case 1 poultry house and the presence of carcasses of wild birds inside the Case 4 poultry house. In addition, at the Case 1 farm where males and females were kept together, the dead chickens were predominantly male, suggesting that males, which are aggressive during the breeding season, may have attacked infected wild animals that invaded the poultry house.

So, it appears that the viruses in these outbreaks entered the farms and poultry houses not through artificial means, such as the movement of people, feed, or materials, but through incursions by wild birds and/or animals (http://www.maff.go.jp/tori/20070906e_report.pdf).

5. Conclusion

Extensive epidemiological investigations were conducted on the affected farms to discover the source of the introduction of the virus into the flocks. However, except for the outbreaks in 2005, there was no evidence to indicate that the virus had been introduced through domestic or international movement/transportation of people, animals, vehicles or any other commodities. Therefore, though there is no direct evidence, such as isolation of virus from migratory birds, it can be surmised that such birds brought the viruses into Japan, and wild birds and/or animals brought them onto the farms. In order to carry out better investigation of infection routes from now on, ongoing monitoring of wild birds and on-site investigation before epidemic-control measures begin will be necessary.

Furthermore, the risk of H5N1 virus entering Japan again is not decreasing, because the virus is endemic in several parts of Asia. Therefore, it is still top priority for poultry producers to maintain biosecurity practices to prevent introduction of the virus into their flocks.

References

