Report of the Investigation Team on the Infection Route of Highly Pathogenic Avian Influenza

Infection Route of the 2007 Outbreak of Highly Pathogenic Avian Influenza in Japan

September 6, 2007

Investigation Team on the Infection Route
of Highly Pathogenic Avian Influenza

Food Safety and Consumer Affairs Bureau
Ministry of Agriculture, Forestry & Fisheries
TOKYO, JAPAN

Infection Routes of the 2007 Outbreaks of Highly Pathogenic Avian Influenza (Summary)

Investigation Team on the Infection Route of Highly Pathogenic Avian Influenza

1. Overview of the outbreak

• Outbreaks of the disease were confirmed on four farms (three in Miyazaki Prefecture and one in Okayama Prefecture) over roughly two weeks from January 13 through February 1. The styles of poultry raising, structure of poultry houses, and environments of the outbreak farms varied.
• No epidemiological relationships were found between outbreak farms or between the farms and overseas outbreak areas.
• A virus closely related to the strains isolated from the four farms was isolated from a mountain hawk-eagle captured on January 4 in Kumamoto Prefecture, indicating that the virus existed in Japan in early January.

2. Characteristics of isolated viruses

• The viruses isolated from the four farms and the mountain hawk-eagle were all closely related H5N1 subtype avian influenza viruses. They were highly homologous with viruses isolated in China, Mongolia, and South Korea, and differed from the virus isolated in Japan in 2004 and from viruses prevalent in Southeast Asia.
• Laboratory pathogenicity testing found high pathogenicity in chickens and mice and low pathogenicity in aigamo ducks and rats.
• Laboratory transmissibility testing found transmissibility from chickens to chickens and from aigamo ducks to chickens and aigamo ducks.

3. Entry routes of the virus into Japan

For the reasons listed below, although there is no direct evidence such as isolation of a virus from migratory birds, the bringing of the virus into Japan by such birds can be surmised in light of overseas cases. It is unlikely that an attenuated virus endemic in Japan mutated to a more virulent form, or that it was brought to Japan from overseas via human beings or imported poultry.
• The viruses isolated from the outbreak are highly homologous with 1) viruses isolated in China, Mongolia, and South Korea, 2) viruses isolated from wild waterfowl in South Korea late last year, and 3) the virus isolated from the wild mountain hawk-eagle in Kumamoto in January 2007.
• No attenuated Type A avian influenza H5N1 subtype viruses have been isolated in Japan in recent years.
• Due to quarantine measures in outbreak countries and the lack of any relationship between farm-related persons and outbreak countries, it is extremely unlikely that human beings brought in the virus.

4. Routes by which the virus invaded farms

For the reasons listed below, it is likely that wild birds and/or animals, rather than the movement of chickens, human beings, feed, or materials, brought in the virus.
• In Cases 1 and 2, there was no movement of chickens in or out of the farms. In Cases 3 and 4, although there were introductions of chicks, the outbreaks occurred in areas away from the new birds.

• In Cases 2 through 4, the first cases occurred in areas of the poultry houses away from entrances and areas where people usually work.
• No epidemiological relationships linking outbreak farms were found in terms of farm workers or visitors, or dealers in feed, medicines, and so on.
• At each outbreak farm, measures to keep wild birds and animals out of poultry houses were not necessarily adequate. Rat feces and bird carcasses were found inside poultry houses.

5. Conclusion

Although there is no direct evidence such as isolation of a virus from migratory birds, in light of overseas cases 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 are necessary.

During these outbreaks, early discovery and reporting enabled quick and effective disease-control. However, in order to steadily implement measures to prevent outbreaks at poultry farms from now on, the following steps are important in addition to knowledge of everyday animal hygiene management and its thorough implementation. Careful cleaning and disinfection, improved measures to keep out wild creatures, reexamination of animal hygiene management on farms, establishment of thorough high-security measures inside and outside poultry houses, and unified efforts on outbreak prevention measures by stakeholders such as prefectures and chicken producers are vital.

Contents

Introduction

In Miyazaki Prefecture on January 13, 2007, there was an outbreak of highly pathogenic avian influenza caused by the H5N1 virus. It was the first appearance of this virus subtype in Japan in three years, since the 2004 outbreak in Yamaguchi, Oita, and Kyoto Prefectures. 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.

Since 2003, this disease has repeatedly broken out in widespread outbreaks, mainly in East Asia, with no end currently in sight. In 2005, outbreaks spread to Europe and Africa as well, showing signs of becoming a worldwide epidemic. The primary transmission route for the disease in foreign countries was thought to be human movement of birds and goods, but in 2005 there were reports of outbreaks in various locations in Asia and Europe caused by wild birds such as geese and swans. There is thus concern about migratory birds as a disease vector. Furthermore, there have been cases of fatal infection with this virus through direct human-to-human transmission, mainly in Southeast Asia. The World Health Organization (WHO) is sounding the warning that the likelihood that a new strain of virus adapted to human beings will appear is increasing.

The current outbreak of this disease in Japan occurred just after highly pathogenic avian influenza was reported on a South Korean poultry farm in November, 2006, so vigilance in Japan was even higher than usual. 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 kept to a minimum, and no reoccurrences were confirmed.

An expert "Investigation Team on the Infection Route of Highly Pathogenic Avian Influenza" was quickly formed in order to elucidate the infection routes of the outbreaks. The team carried out on-site inspections including the outbreak farms and surrounding environments, interviews with relevant persons, and examination of characteristics of isolated viruses including genetic analysis. In April, the team published an interim report on elucidation of the infection routes of these domestic outbreaks. Subsequently, further epidemiological surveys and continued analysis of virus characteristics were carried out, along with the dispatch of a group of Japanese experts to South Korea, where the recent outbreak was similar to the Japanese one in terms of timing, virus genetic characteristics, and geographical conditions. Along with carrying out on-site inspections, the group exchanged opinions with the National Veterinary Research Quarantine Service of the South Korean Ministry of Agriculture and Forestry.

The investigation team has compiled this report based on the results of this analysis to elucidate infection routes. While the team was unable to obtain direct evidence of specific infection routes, obviously the purpose of an infection route investigation is not to uncover a criminal suspect, but rather to strengthen preventative measures against future outbreaks of the disease. This report therefore narrows down the possibilities and offers concrete proposals in response to each of them.

Finally, the investigation team wishes to thank those members who worked to create this report, everyone who joined in the response to the outbreak, and all who cooperated with the on-site surveys.

2007 September 6 Investigation Team on the Infection Route of Highly Pathogenic Avian Influenza

(Chair: Toshihiro Ito
Professor of Veterinary Public Health, Department of Pathology and Preventative Veterinary Medicine, School of Veterinary Medicine, Faculty of Agriculture, Tottori University)

List of members of the Investigation Team on the Infection Route of Highly Pathogenic Avian Influenza

Members

〇Toshihiro Ito	Professor of Veterinary Public Health, Department of Pathology and Preventative Veterinary Medicine, School of Veterinary Medicine, Faculty of Agriculture, Tottori University
Hiromi Okada	Senior Staff Member, Okayama Prefecture Takahashi Livestock Hygiene Service Center
Yutaka Kanai	Senior Research Biologist, Nature Conservation Division, Wild Bird Society of Japan
Toshiro Goto	Chief, Health Section, Miyazaki Prefecture Miyazaki Livestock Hygiene Service Center
Takehiko Saito	Senior Researcher, Research Team for Zoonotic Diseases, National Institute of Animal Health, National Agriculture and Food Research Organization
Kameo Shimura	Director, Center for Animal Disease Control and Prevention, National Institute of Animal Health, National Agriculture and Food Research Organization
Kumiko Yoneda	Senior Research Scientist, Japan Wildlife Research Center

Observers

Makoto Goto	Deputy Chief, Health Section Miyazaki Prefecture Nobeoka Livestock Hygiene Service Center
Akiko Nishiguchi	Senior Researcher, Epidemiology Research Team, National Institute of Animal Health, National agriculture and Food Research Organization

Note: ○ mark indicates Chair; other names are in Japanese alphabetical order
(current as of September 6, 2007)

Section 1 Overview of the outbreak of highly pathogenic avian influenza

1 Regarding highly pathogenic avian influenza

1.1 What is highly pathogenic avian influenza?

Highly pathogenic avian influenza is an acute infectious disease of chickens and other poultry that causes systemic symptoms. These symptoms vary, but because the disease has a high fatality rate and is highly transmissible, outbreaks can have a heavy impact on the poultry industry. The international livestock health organization World Organization for Animal Health (OIE) therefore designates the disease as one that should be reported internationally.

1.2 Definition of highly pathogenic avian influenza

In Japan, highly pathogenic avian influenza is defined as an illness of chickens, domestic ducks, quail, and turkeys (hereinafter "poultry") caused by infection with:

A) An influenza type A virus determined to be a highly pathogenic avian influenza virus according to the pathogenicity standards developed by the OIE (the "Manual of Standards for Diagnostic Tests and Vaccines") or

B) An H5 or H7 subtype influenza type A virus, not including A) above.

Article 2 of the Domestic Animal Infectious Diseases Control Law (law no. 166, May 31, 1951) designates highly pathogenic avian influenza as a "domestic animal infectious disease" and requires culling and other prevention mechanisms in the event of an outbreak.

Influenza type A infections in poultry that are not highly pathogenic avian influenza are not subject to culling and so on during outbreaks. Article 2 of the Enforcement Ordinance of the Domestic Animal Infectious Diseases Control Law (Ministry of Agriculture, Forestry and Fisheries Ordinance No. 35 of 1951) designates such infections as "reportable infectious disease" and requires only that outbreaks be reported to the prefectural governor.

1.3 Pathology (epidemiological characteristics)

The disease has diverse symptoms, but the primary ones include sudden death, respiratory symptoms, facial, comb, or leg edema, blood spots or cyanosis, decreased or absent egg production, neurological symptoms, and diarrhea or decreased feed and water intake.

In addition, symptoms and virus emission vary by type of bird and virus strain.

1.4 Infection routes, basic epidemic control measures, and treatment methods

This disease generally spreads through contact with infected birds or with excretions, feed, dust, water, flies, wild birds, human beings, equipment, or vehicles contaminated with the virus. There is no effective treatment method for this disease.

In Japan, based on the Domestic Animal Infectious Diseases Control Law and in accordance with the special guidelines for prevention of domestic animal infectious disease concerning highly pathogenic avian influenza (released by the Minister of Agriculture, Forestry and Fisheries on November 18, 2004), when an outbreak occurs, prevention measures such as the culling of infected poultry and restrictions on the movement of poultry and goods that may spread the disease are implemented in order to prevent and mitigate outbreaks.

In addition, although some countries use vaccination as a preventative measure, Japan in principle does not. While vaccination suppresses symptom onset, suppression of virus emission is more difficult. Vaccination therefore invites the habitual presence of the virus while not completely preventing new infections. Special testing is required to distinguish the antibodies produced by vaccination from those produced by infection with the wild virus. Rather than vaccinating, Japan therefore engages in prevention by using testing to detect infected poultry and cull them.

2 Outbreaks of highly pathogenic avian influenza in Japan

2.1 Outbreaks before January 2004

Japan had long been free of this disease. The 2004 outbreaks in Yamaguchi, Oita, and Kyoto Prefectures were Japan's first since 1925, a span of 79 years. According to records from 1925, outbreaks occurred in Nara, Chiba, and Tokyo Prefectures. Analysis years later of the strain isolated at that time found that a virulent form of the H7N7 subtype caused the outbreaks.

2.2 Outbreaks in 2004

In January through March 2004, there were four outbreaks in Japan, on an egg farm (35,000 birds) in Yamaguchi Prefecture, at the home of a hobbyist bantam breeder (13 bantams and 1 domestic duck) in Oita Prefecture, and on an egg farm (225,000 birds) and a meat chicken farm (15,000 birds) in Kyoto Prefecture. The causative virus was a virulent strain of the H5N1 subtype. It was typical highly pathogenic avian influenza, with infected chickens dying in succession in an acute course, so the disease was readily suspected by clinical presentation. Case 3 in Kyoto was very problematic. Despite the deaths of many chickens, no report was made, and infected chickens were shipped to meat processing plants in Hyogo and Aichi Prefectures. As an epidemic prevention response, necessary measures in accordance with the Domestic Animal Infectious Diseases Control Law and "Highly Pathogenic Avian Influenza Prevention Manual" (notification by the head of the Animal Health Division on September 17 2003) were performed. All chickens on outbreak farms were slaughtered, disinfection was carried out, movement on nearby farms was restricted, and epidemiological surveys were performed. This resulted in the prevention of spread to nearby farms, halting the outbreaks at four cases. Performance of these prevention measures, however, raised a number of issues. These issues included the problem of violations of the duty to report outbreaks of the disease, how to assist farmers cooperating with restrictions on movement, how to best slaughter and incinerate the poultry, the role of vaccination in epidemic prevention, the need to provide the public with correct information and to promote risk communication, and proper support systems to help affected farmers rebuild their businesses.

Based on this experience, the Domestic Animal Infectious Diseases Control Law was revised mainly to strengthen penalties for violating the duty to report and to systematize support for farmers cooperating with movement restrictions. Furthermore, special guidelines for prevention of domestic animal infectious disease concerning this disease (announced November 18, 2004, by the Minister of Agriculture, Forestry and Fisheries) were created and announced. In addition, necessary system improvements and work were carried out, including study of efficient slaughter methods, increased vaccine stores, risk communication, and creation of a mutual aid fund for livestock epidemics.

Regarding infection routes, an expert "Investigation Team on the Infection Route of Highly Pathogenic Avian Influenza" was formed. The team added analysis and evaluation based on epidemiological surveys originating at each outbreak farm, as well as analysis of the characteristics of isolated viruses. The team found 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, carried by resident birds, rats and other animals, and human beings into poultry houses (announced June 30, 2004).

2.3 Outbreaks in 2005

From June 26, 2005, when the influenza virus was isolated in Ibaraki Prefecture, through 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. From these, a virus was isolated in 9 cases. In each of these cases, the virus was an H5N2 subtype type A influenza virus. Genetically, each virus was extremely closely related. The Livestock Hygiene Service Centers with jurisdiction over the farms with confirmed infections and the surrounding farms carried out on-site inspections to confirm clinical presentations. No clinical abnormalities were found on the outbreak farms. Pathogenicity testing by the National Institute of Animal Health of the National Agriculture and Food Research Organization (hereinafter, the "National Institute of Animal Health"), which identified the virus; found it to be a weakly-toxic attenuated type.

Although investigation of infection routes by the Investigation Team on the Infection Route of Highly Pathogenic Avian Influenza was unable to specify virus origins or entry routes, in light of virus characteristics and the localized outbreak area, it was unable to rule out the illegal import 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 surmised that neighborhood transmission and the comings and goings of people and goods were major causes of transmission (released September 28, 2006.)

2.4 Outbreaks in 2007

2.4.1 Overview

In January and February 2007, there were four outbreaks, on a meat-chicken breeding farm (Kiyotakecho, 12,000 birds), a meat-chicken farm (Hyuga City, 53,000 birds), and an egg farm (Shintomicho, 93,000 birds) in Miyazaki Prefecture and an egg farm (Takahashi City, 12,000 birds) in Okayama Prefecture. On each farm, epidemic-control measures such as culling, incineration, burial, and disinfection were implemented. Furthermore, movement-restriction areas were set up in a 10-kilometer 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.

In each case, the virus was an H5N1 subtype related to viruses isolated in China, Mongolia, and South Korea.

2.4.2 Course of outbreaks

2.4.2.1 Case 1

In early January, a clinical presentation of increased deaths and facial puffiness was confirmed in a poultry house on a meat-chicken breeding farm (Kiyotakecho, 12,000 birds) in Kiyotakecho, Miyazaki Prefecture. An attending veterinarian was therefore consulted, but simple testing was negative. Because the number of dead birds subsequently increased, however, simple testing was repeated, and the result was positive. The Livestock Hygiene Service Center for the area therefore carried out virus isolation. Because this 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 a location for the outbreak. Initially, there were more dead males than females.

2.4.2.2 Case 2

On January 22, a rapid increase in dead chickens in the central part of a poultry house on a meat-chicken farm (Hyuga City, 53,000 birds) in Hyuga City was confirmed, so 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 was 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.

2.4.2.3 Case 3

January 22, 2 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 2, so the Livestock Hygiene Service Center carried out 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. Because 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 outbreak poultry house were performed. Furthermore, even before confirmation, poultry and eggs shipping inspections were implemented for farms within a 10-kilometer 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 confirming that spread of the infection was limited.

2.4.2.4 Case 4

On the egg farm (Shintomicho, 93,000 birds) in Shintomicho, 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 living 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.

[Course of 2004 outbreak]

- The first outbreak of virulent highly pathogenic influenza in Japan in 79 years occurred in Jan.–March 2004.
- Epidemic-prevention measures on the outbreak farms ended the outbreak in April.

[Response to 2004 outbreak]

- Revision of the Domestic Animal Infectious Diseases Control Law
- Compilation of "urgent comprehensive avian influenza measures"
- Setting of special guidelines for prevention of domestic animal infectious disease
- Creation of a mutual aid fund for livestock and facilitation of funding for continued operation
- Reserve vaccine for emergencies

[Course of the 2005 outbreak]

- Highly pathogenic avian influenza (attenuated type) broke out mainly in Ibaraki Prefecture beginning in June 2005.
- Based on the fact the infections were with an attenuated type, a farm monitoring program was implemented for farms meeting certain conditions.
- Culling ended in April 2006.

[Response to the 2005 outbreak]

- Release of report by the Investigation Team on the Infection Route of Highly Pathogenic Avian Influenza (Sep. 2006)
- Revision of epidemic control policy, adding measures for outbreaks of attenuated type virus (Dec. 2006)

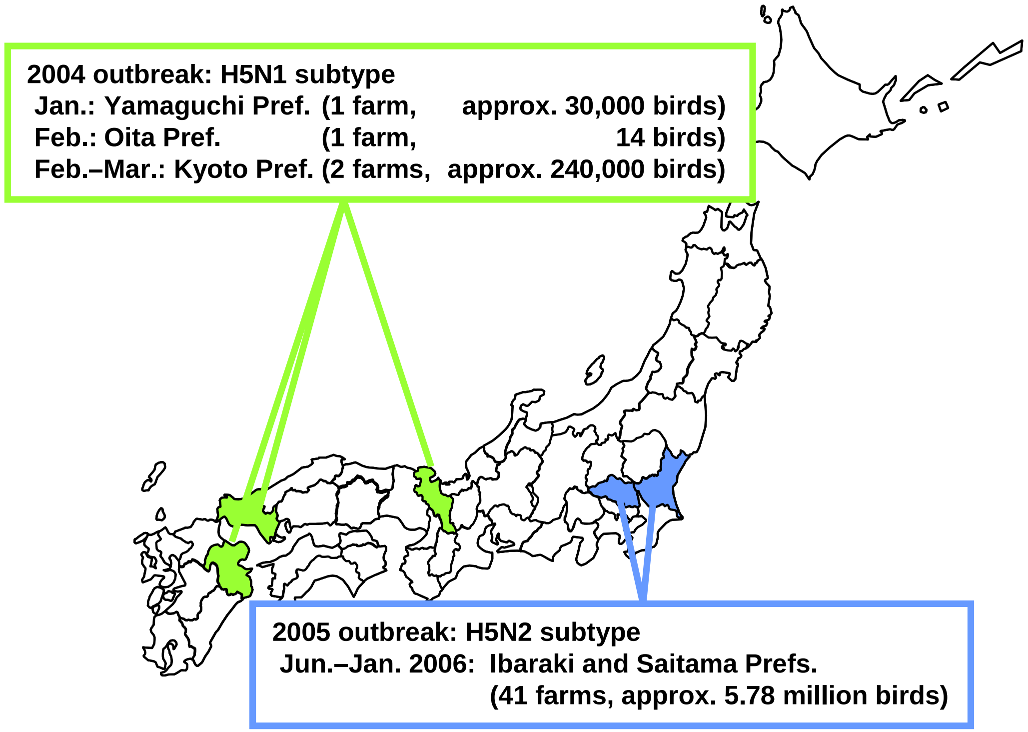


Figure 1. Outbreaks of highly pathogenic avian influenza in 2004 and 2005

1. Kiyotakecho Miyazaki Prefecture case

(Jan. 11: report of "suspected" case)

Jan.13: Highly pathogenic avian influenza confirmed, movement restriction on area farms implemented

16: Culling and other epidemic prevention measures completed on outbreak farm

Feb. 7: Movement and removal restrictions lifted

- Outbreak farm: Meat-chicken breeding farm (about 12,000 birds)
- Surrounding farms: 16 farms, about 194,000 birds

2. Hyuga City, Miyazaki Prefecture case

(Jan. 22, report of "suspected" case)

Jan.25: Highly pathogenic avian influenza confirmed, movement restriction on area farms implemented

30: Culling and other epidemic prevention measures completed on outbreak farm

Feb.21: Movement and removal restrictions lifted.

- Outbreak farm: Meat-chicken farm (about 53,000 birds)
- Surrounding farms: 21 farms, about 510,000 birds

3. Takahashi City, Okayama Prefecture case

(Jan. 27, report of "suspected" case)

Jan.29: Highly pathogenic avian influenza confirmed, movement restriction on area farms implemented

Feb. 7: Culling and other epidemic prevention measures completed on outbreak farm

Mar. 1: Movement and removal restrictions lifted.

- Farm: Egg farm (about 12,000 birds)
- Surrounding farms: 18 farms, about 950,000 birds

4. Shintomicho, Miyazaki Prefecture

(Jan. 30, report of "suspected" case)

Feb. 1: Highly pathogenic avian influenza confirmed, movement restriction on area farms implemented

7: Culling and other epidemic prevention measures completed on outbreak farm

Mar. 1: Movement and removal restrictions lifted.

- Farm: Egg farm (about 93,000 birds)
- Surrounding farms: 97 farms, about 2.88 million birds

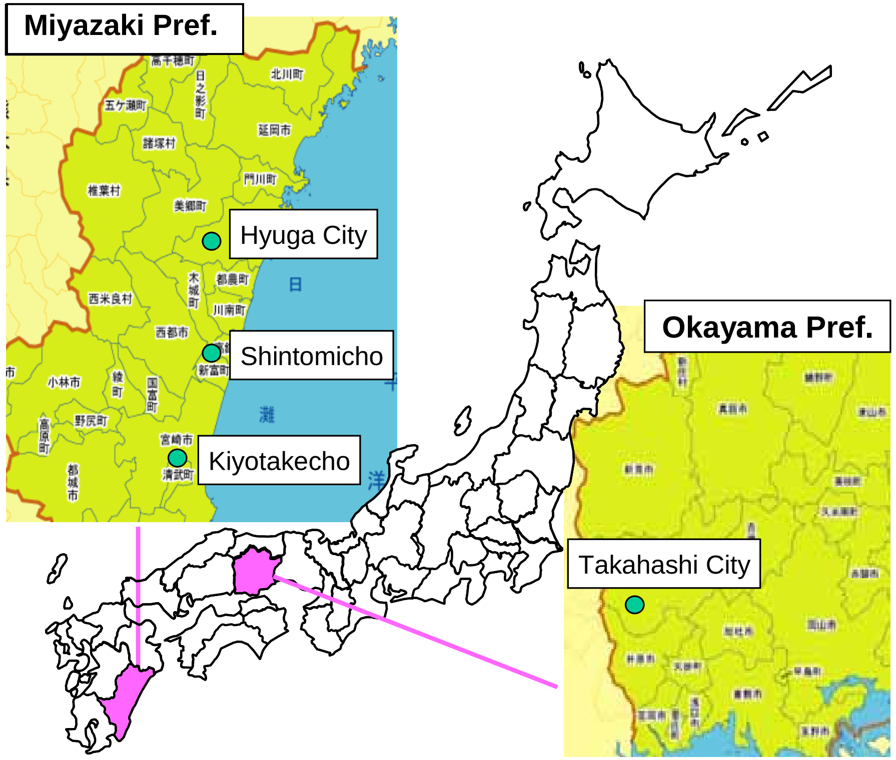


Figure 2. Outbreaks of highly pathogenic avian influenza in 2007

3 Outbreaks of highly pathogenic avian influenza overseas

3.1 Outbreaks in recent years

Avian influenza was confirmed in Asia at the end of 2003. In May 2005, avian influenza infection was confirmed in migratory birds in China's Qinghai Province. Further outbreaks were confirmed in Russia and Kazakhstan. Beginning in October, the disease spread to Turkey, elsewhere in Europe, and Africa. From February 2006 on, many cases of avian influenza infection were confirmed in Europe in swans and other migratory birds as well as in poultry.

In Asia in particular, avian influenza has been confirmed in many countries, such as China, Indonesia, Vietnam, South Korea, and Thailand. Cases of human infection have also been reported in Southeast Asia, including Indonesia and Vietnam, and in China.

In 2007 as well, outbreaks of highly pathogenic avian influenza have been reported in Europe, Africa, the Middle East, and Asia.

3.2 Import quarantine measures

In order to prevent the introduction of the disease into Japan through animals or livestock products, the Government of Japan has suspended imports of meat and live poultry from countries with outbreaks of highly pathogenic avian influenza in poultry until virus-free conditions can be confirmed. As of September 6, 2007, poultry imports from 49 countries and territories have been suspended. (In the case of the Netherlands, although there is no outbreak in poultry, Japan suspended imports from that country on March 16, 2006, because it uses an H5 subtype vaccine.) Regarding outbreaks of attenuated type highly pathogenic avian influenza, Japan applies a regionalist approach based on the veterinary administration system and surveillance in the outbreak country (USA, Mexico, UK, etc.).

As for poultry meat and so on from countries with outbreaks of highly pathogenic avian influenza, the only imports permitted are of products that have been heat treated at specified facilities based on animal hygiene conditions decided through bilateral agreement. As of August 2007, there are 56 such heat-treatment facilities for poultry meat in Thailand and 91 in China.

4 Domestic monitoring, etc.

4.1 Farm monitoring following South Korean outbreak

With the outbreak of highly pathogenic avian influenza in South Korea in November 2006, in order to strengthen domestic disease prevention, prefectural authorities visited or telephoned farms to encourage strict adherence to standards for managing animal hygiene as listed in part 1 of the guidelines for prevention of infectious disease, "Reporting abnormal poultry," such as prevention of the entry of wild birds into poultry houses, thorough disinfection upon farm entry and exit, and early reporting of any discovered abnormalities.

4.2 Farm monitoring following domestic outbreak

Because of the outbreak in Miyazaki Prefecture in January, prefectural authorities carried out thorough early detection and reporting measures regarding abnormal chickens. Emergency on-site inspections of farms were carried out to improve prefectural monitoring, while farms with at least 1,000 birds were required to report bird mortality weekly until Japan was free of the disease, rather than monthly as in the past.

(Reference) Survey of highly pathogenic avian influenza in South Korea

1. Purpose

In order to contribute to disease control measures in the event of future outbreaks, Japanese and Korean experts on highly pathogenic avian influenza met to exchange information.

2. Members

Head: Toshihiro Ito, Professor, Faculty of Agriculture, Tottori University (Infection Route Investigation Team Chair and member in charge of wild bird surveillance)

Members: Shigeo Yamaguchi, Research Manager, National Institute of Animal Health (in charge of virology)

Yutaka Kanai, Chief Researcher, Wild Bird Society of Japan (in charge of wild birds)

Akiko Nishiguchi, Senior Researcher, National Institute of Animal Health (in charge of epidemiological analysis)

Kazuhisa Hoshino, Animal Health Division, Food Safety and Consumer Affairs Bureau, Ministry of Agriculture, Forestry and Fisheries

3. Itinerary

June 18 (Mon.)–22 (Fri.), 2007, 5 days and 4 nights

4. Primary destinations

(1) Republic of Korea Ministry of Agriculture and Forestry headquarters

Animal Health Division, Livestock Policy Bureau, Republic of Korea Ministry of Agriculture and Forestry

(2) National Veterinary Research and Quarantine Service, Republic of Korea Ministry of Agriculture and Forestry

(a) Veterinary Epidemiology Department, Epidemiology Division

(b) Avian Influenza Laboratory, Poultry Disease Department, Disease Survey Division

5. Main information from South Korea

(1) Overview of outbreak

	Outbreak location	Date confirmed	Type	Notes
(1)	Iksan-city, Jeollabuk Province	2006 11/25	H5N1	Meat-chicken breeding farm 13,300 birds (of which about 6,500 died)
(2)	Iksan-city, Jeollabuk Province	2006 11/28	H5N1	Meat-chicken breeding farm 3 km from (1) 12,240 birds (of which about 600 died)
(3)	Gimje-city, Jeollabuk Province	2006 12/11	H5N1	Quail farm 18 km south of (2) 290,000 birds (of which about 3,720 died)
(4)	Asan-city, Chungcheongnam Province	2006 12/21	H5N1	Duck breeding farm 10,000 birds (of which 1 died)
(5)	Cheonan-city, Chungcheongnam Province	2007 1/20	H5N1	Egg farm 8 km from (4) 30,000 birds (of which 157 died)
(6)	Anseong-city, Gyeonggi Province	2007 2/10	H5N1	Egg farm 50–60 km from (4) and (5) 133,000 birds (of which 1,188 died)

(7)	Cheonan-city, Chungcheongnam Province	2007 3/8	H5N1	Duck breeding farm 24 km from (4) and 20 km from (5) 13,521 birds (of which 3 died)

(2) Basic epidemic-control measures

	500-m radius	3-km radius	10-km radius
Culling	Culling of all infected birds and susceptible animals	Preventative culling of poultry	—
Movement restrictions	Movement restrictions on poultry, eggs, and related matter		
Disinfection	• Disinfection of infected farms and farms in range • Disinfection of vehicles on main roads	Disinfection of vehicles on main roads	

Vaccination for highly pathogenic avian influenza is prohibited; disease control measures are implemented through testing and culling. Including preventative culling on outbreak farms, egg chickens, meat chickens, breeder chickens, domestic ducks, quail, and other livestock were culled during these outbreaks.

(3) Epidemiological survey

Epidemiological surveys were carried out through measures such as interviews with animal managers on outbreak farms, examination of records, confirmation of purchase sources and shipping destinations, serological and virological investigations, examination of nearby farms, and surveillance of wild birds. In addition, factors such as occurrences of highly pathogenic avian influenza in other countries, the role of wild birds in virus transmission, feed ingredients, hand-carried poultry products, and movement of people from outbreak countries were also examined.

Viruses isolated from the outbreaks were at least 99 percent homologous, with all belonging to the Qinghai Lake group.

(4) Surveillance

(1) Nationwide surveillance

On duck farms, serological surveillance and enhanced surveillance (cloacal swabs, laryngopharyngeal swabs, and fecal samples) were carried out. The results were all negative.

(2) Surveillance of wild birds

Testing of susceptible bird species known to have been infected in the past and of flocks on wetlands near outbreak farms was carried out. H5 antibodies were found in ducks.

(3) Universities and other institutions carried out wild bird surveillance. In late 2006, they isolated an H5N1 virus belonging to the Qinghai Lake group from the feces of migratory birds.

(5) Confirmation of virus-free status

The final disinfection of an outbreak farm was completed on March 17, 2007. With no further outbreaks, on June 18, 2007, the area once again is considered free of highly pathogenic avian influenza according to OIE code.

Section 2 Epidemiological surveys in outbreak areas

(Hiromi Okada, Toshiro Goto, Makoto Goto)

1 Livestock conditions in each outbreak area

1.1 Livestock conditions in Miyazaki Prefecture

Utilizing excellent resources such as a warm climate, diverse geography ranging from plains to mountains, and clean air and water, animal husbandry in Miyazaki Prefecture has developed on a scale that is among the largest in Japan. In particular, the prefecture ranks 1st in the nation in the number of meat chickens with 18,437,000 and 21st in egg chickens with 3,382,000, and it has one of the country's leading poultry processing plants. Poultry is a mainstay of the prefecture's economy.

Kiyotakecho is in a hilly area, surrounded by Miyazaki City on three sides, north, east, and south. In recent years, a number of housing complexes have been developed as the town has grown into a bedroom community for Miyazaki City. Rurbanization is advancing.

Hyuga City is located in northern Miyazaki Prefecture. Commerce is the key industry, but meat processing and other industries related to animal husbandry are also mainstays. Chicken farming accounts for 70 percent of agricultural output, with meat chicken production accounting for most of that. With 3,010,000 chickens, the city accounts for 17 percent of the prefecture's total.

Shintomicho calls itself "a town of animal husbandry and vegetables." Animal husbandry accounts for 50 percent of agricultural output, and egg chickens account for the highest agricultural output within animal husbandry. It is an important industry for the town.

1.2 Livestock conditions in Okayama Prefecture

Okayama Prefecture is among the leaders in Japan's poultry industry. With 7,900,000 egg chickens (152 farms), it produces 105,000 tonnes of eggs annually, ranking 7th nationally. It has 2,200,000 meat chickens (39 farms), and its annual shipping volume of about 40,600 tonnes ranks 11th.

In the outbreak area of Takahashi City, Okayama Prefecture, agriculture is a key industry. There are 24 chicken farms in the area (12 egg and 12 meat, as of March 31, 2007). Chicken farming accounts for about 49 percent of agricultural output, so it is an important industry.

2 Epidemiological information for each outbreak area

2.1 Epidemiological survey in Kiyotakecho, Miyazaki Prefecture

2.1.1 Overview of outbreak

2.1.1.1 Overview of epidemic control measures for the outbreak

Photograph 1. Panorama of the farm

The outbreak farm is located in a hilly area about 5 kilometers south-southeast from urban Miyazaki City. It is a meat-chicken breeding farm that keeps about 12,000 birds in three poultry houses. Beginning in early January, a clinical presentation including an increase in bird deaths in some poultry houses and facial puffiness was confirmed. On January 10, an attending veterinarian was therefore consulted, but simple testing was negative. Because the number of dead birds increased the next day as well, however, simple testing was repeated, and the result was positive. The Livestock Hygiene Service Center for the area therefore began virus isolation, and with animals suspected of infection under the Domestic Animal Infectious Diseases Control Law, requested quarantine of the farm and voluntary movement restriction on neighboring farms. On January 12, testing isolated a virus suspected of being a type A influenza virus. The National Institute of Animal Health therefore carried out virus identification testing. On January 13, it confirmed that the virus in question was an H5 subtype. That same day, a 10-kilometer radius around the farm was designated as a movement-restricted area. Culling of chickens on the outbreak farm was carried out on January 14. On January 15 and 16, the dead chickens on the outbreak farm were incinerated and the farm was disinfected, completing epidemic control measures. Subsequently, inspections to confirm virus-free status were carried out twice, including chicken farms and pet chickens inside the movement-restricted area. Because infection did not spread to neighboring areas, the movement restrictions implemented on January 13 were lifted at midnight on February 7.

2.1.2 Overview of outbreak site

2.1.2.1 Outbreak farm

There were 12,000 24-week old meat chickens (4,000 birds in each of three poultry houses). Males and females were kept together, with a ratio of 1 male to 10 females. To keep out unauthorized persons, there is a 1-meter-high fence around the farm, and at night a rope is stretched across the farm entrance and the poultry house doors are locked.

There are three steel-frame, cage-free, open-type poultry houses. Each poultry house has a single entrance and can only be entered through a service room (where egg collection and so on is carried out). The poultry house side walls are wire mesh only, without bird nets, but from the time chicks are introduced, they are closed off with curtains in order to control the amount of light inside the houses.

There are three feed tanks, one in each poultry house. Water is pumped up from the municipal water system and stored in tanks for supply to each of the three poultry houses. There are three full-time employees, including management. During busy periods, additional employees are brought in from related farms to help.

Workers change into dedicated work clothes and boots in the office before beginning work. Each poultry house has a step-in disinfection tank at its entrance along with boots for use only in that building.

The ordinary workflow is to check the status of the automatic feeder in the service room and then to enter the poultry house and examine the chickens for abnormalities and check the status of the automatic waterer. Finally, back in the service room, the worker gathers eggs and puts feed into the feeder. Workers are not assigned to a particular poultry house. Because the chickens are young at 24 weeks old, the eggs are not used or handled as hatching eggs.

Workers park north of Poultry House 1, while visitors park on the premises south of Poultry House 3. There is no vehicle disinfection equipment in either location.

Rodenticide is used as an anti-rat measure. The hygiene measure is disinfection of the poultry house interiors when they are empty (manure removal, cleaning, scattering of invert soap three times, and use of formalin, zole, iodine, external parasiticides, and lime swabbing). Outside the poultry houses, hydrated lime is scattered once every three to four months.

Vaccination program	
Age in days	Vaccine
1 day	ND, IB
8 days	IB
16 days	NB
22 days	IBD
25 days	FP
29 days	NB
32 days	MG • MS
46 days	NB
58 days	TRT
65 days	IB
69 days	AE
88 days	NB, IBD, TRT, CAV

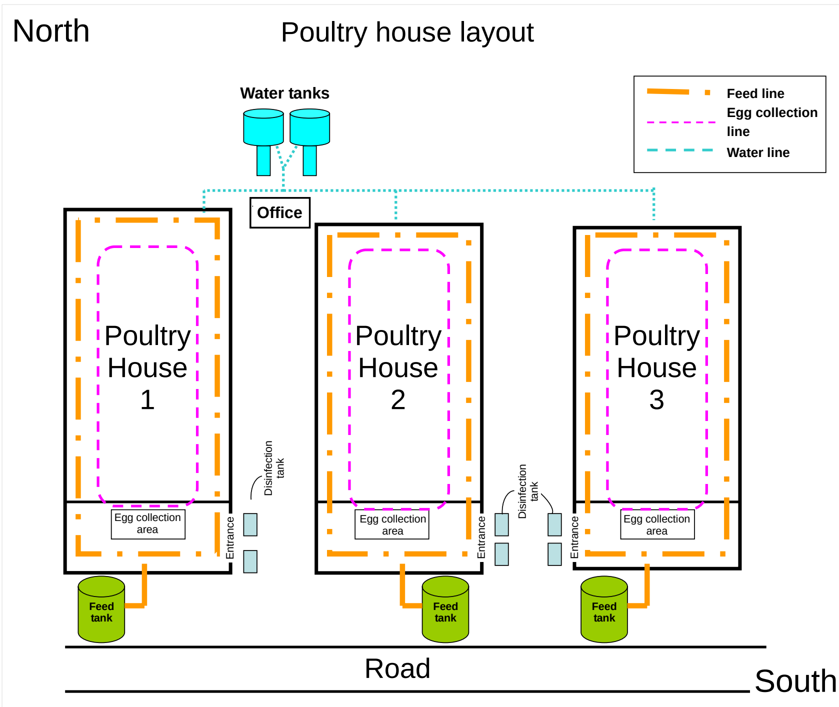


Figure 1. Vaccination program (left) and poultry house layout (right)

2.1.2.2 Surrounding environment of farm

The farm is located in a hilly area about 4 kilometers from the coast. It is surrounded by farmland, but with private houses dotted about 50–100 meters away. The Kiyotake River is 1 kilometer north of the farm, and there is an irrigation pond 500 meters from the farm. Migratory birds are common at that time of year.

The road that passes in front of the farm is a local residential road, so traffic is relatively heavy.

There are 16 farms with at least 1,000 birds within a 10-kilometer radius of the outbreak farm. Four of those farms are related to the outbreak farm.

2.1.3 Details of the outbreak

2.1.3.1 Course of the outbreak

In Poultry House 2, 4 birds died on January 7, 21 on January 8, and 18 on January 9. The dead chickens displayed facial puffiness, leading the farm manager to initially suspect swollen head syndrome (SHS). On the 9th, an attending veterinarian was called in. Because 246 birds died on the 10th, the attending veterinarian was called in again, but simple testing was negative (0/3 birds). On the 11th, 500 birds died. The attending veterinarian repeated simple testing, this time with positive results (15/20 birds). During this period, no particular abnormalities were observed in Poultry Houses 1 and 3.

The dead chickens in Poultry House 2 had free run of the interior, so there was no way to specify a particular location as the source of the outbreak. Initially, more males than females died.

2.1.4 Results of epidemiological survey

2.1.4.1 Survey of outbreak farm

2.1.4.1.1 Introduction of chicks

Chicks were introduced from farm M in Mie Prefecture on July 28, 2006.

2.1.4.1.2 Shipment of hatching eggs

Since the chickens had just begun laying, there were no shipments of hatching eggs.

2.1.4.1.3 Shipment of spent hens

There were no recent shipments of spent hens.

2.1.4.1.4 Disposal of dead birds

Workers remove dead chickens from inside the poultry house and dispose of them in a storage container for dead chickens located off the farm grounds. (This is shared by related farms.) A processor collects the dead chickens daily. The 500 birds that died on January 11 were stored on the farm.

2.1.4.1.5 Disposal of poultry manure

Manure is removed when all chickens are out of the houses (about every 18 months). There was no recent manure removal.

2.1.4.1.6 Bringing in of feed

Feed: Feed is brought in by vehicles belonging to a contracted transport company. Feed is brought in directly from the road to the feed tank, so the vehicles do not enter the farm. Feed is delivered once or twice per week. The most recent deliveries were January 5 and 11.

Water: Water is pumped up from the municipal water supply and sent to storage tanks on the farm. Water is supplied to the poultry houses after disinfection in the storage tanks.

Materials: In December and early January, workers brought materials necessary for egg production, such as nests, artificial grass, and egg collection belts that had been stored on the grounds into the poultry houses. When brought in, the materials were disinfected with invert soap, etc.

2.1.4.1.7 Dealers in veterinary medicines, etc.

Merchants deliver veterinary medicines and so on to the farm corporation headquarters. When necessary, workers retrieve them from headquarters. They are not delivered directly to the farm.

2.1.4.1.8 Wild animals

Some openings were found in the poultry houses. The farm manager had seen rats inside the poultry houses. The curtains were completely closed.

Birds such as crows, bulbuls, and wagtails often visit the fields on surrounding farms.

Following epidemic-control measures, large amounts of rat feces were found inside the poultry houses.

2.1.4.1.9 Vehicles

Feed transport vehicles: Feed is brought in by vehicles belonging to a contracted transport company. It is brought in directly from the road to the feed tank, so the vehicles do not enter the farm. Vehicle disinfection is not performed on the farm when feed is brought in. Before moving from one farm to another, however, the transport company voluntarily disinfects its vehicles.

2.1.4.1.10 Contact with human beings (people who enter poultry houses)

On January 5, a contractor entered Poultry House 3 in order to repair the feeder. The worker is a farm specialist and wore boots, etc. An attending veterinarian visited on January 9–11. No one else entered the poultry houses.

2.1.4.2 Epidemiological relationship with domestic outbreak farms

No relationships were found.

2.1.4.3 Connections with South Korea or other outbreak countries

Neither the farm manager, workers, nor family members had any recent history of foreign travel. There were no such connections.

2.1.4.4 Virus testing on the farm and in the surrounding area

After an outbreak of highly pathogenic avian influenza was confirmed, virus isolation testing was performed on chicken farms and pet birds inside the movement-restricted area as primary and secondary virus-free status confirmation testing. All tests were negative. Furthermore, because of the large number of bird hobbyists in the area around the outbreak farm, virus isolation testing was performed on all pet birds within 500 meters of the farm. All those tests were negative as well.

On the day of culling, a chicken presenting cyanosis of the comb and wattle was discovered in Poultry House 1. Virus isolation testing isolated an H5N1 virus.

In addition, virus isolation testing of poultry manure that had undergone fermentation disinfection was carried out on January 19 and 20 and February 13 and 16. All tests were negative.

2.2 Epidemiological survey in Hyuga City, Miyazaki Prefecture

2.2.1 Overview of outbreak

2.2.1.1 Overview of epidemic control measures for the outbreak

The outbreak farm is located in a mountainous area about 25 kilometers west of urban Hyuga City. The farm has about 53,000 meat chickens (broilers) farm in five poultry houses. On January 22, a sudden increase in dead chickens was noticed, and an attending veterinarian was contacted. The attending veterinarian visited the farm and appraised the illness. Simple testing was all negative, but the attending veterinarian reported a summary to the Livestock Hygiene Service Center. On January 23, Livestock Hygiene Service Center personnel and the attending veterinarian visited the farm to appraise the illness. That day, the number of dead chickens rose to 326, and simple testing was positive. With animals suspected of infection under the Domestic Animal Infectious Diseases Control Law, the Center implemented quarantine of the farm, carried out disinfection and liming of the farm and around the poultry houses, and requested voluntary movement restriction on neighboring farms. Infection with highly pathogenic avian influenza was confirmed on January 25, and the outbreak was reported that day. The area within a 10-kilometer radius was declared a movement-restricted area. Culling of the outbreak farm's chickens was carried out from January 26–28. On January 30, the carcasses, manure, feed, and other contaminated materials from the outbreak farm were transported to a burial site and the poultry houses were disinfected inside and out, completing epidemic control measures.

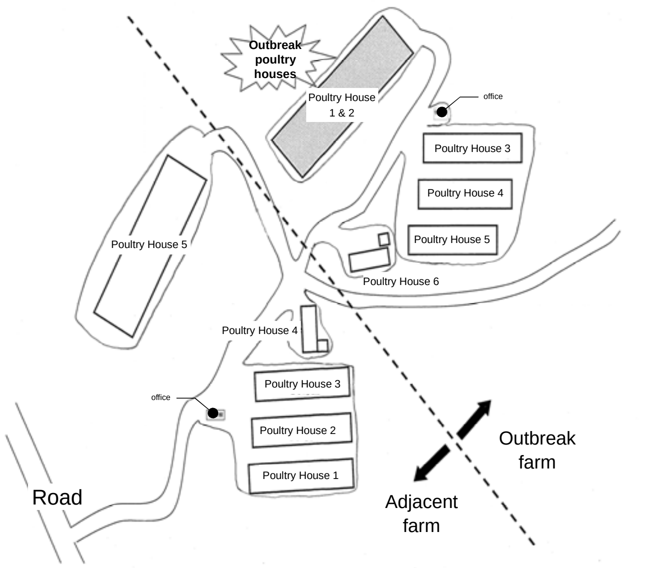


Figure 2. Diagram of outbreak farm area

Because there was a meat-chicken farm directly adjacent to the outbreak farm in this case, in order to contain the virus, the neighboring farm was considered part of the same grounds and its approximately 53,000 birds were considered suspected infections, so epidemic control measures were carried out there as well. Subsequently, testing to confirm virus-free status was carried out at all farms with chickens and at the homes of all bird fanciers within a 10-kilometer radius. Because infection did not spread in the area, the movement restrictions implemented on January 25 were lifted as of midnight on February 21.

2.2.2 Overview of outbreak site

2.2.2.1 Outbreak farm

Poultry House 1 & 2 (it is actually a single poultry house) is an open-type poultry house. On December 7, 22,420 chicks (equal numbers of males and females) were introduced. Poultry Houses 3, 4, 5, and 6 are semi-windowless poultry houses. On December 6, 10, 210, 9,710, 9,210, and 3,000 chicks were introduced into those respective houses. The total number of chicks was 54,550. (The poultry houses are numbered according to their feed tank numbers.) The outbreak poultry house (1 & 2) is a steel-frame building constructed six years ago. Vaccines received were neonatal Marek's disease/fowlpox injection and two doses of ND administered in drinking water after arrival at the farm. As illustrated in the diagram, the outbreak farm and another farm with different management are directly adjacent to one another. Both farms are affiliated with the same corporation. They operate jointly in terms of consideration of disease control measures in chick introduction and shipping.

The farm's water source is a nearby mountain stream, from which water is drawn, stored in tanks, and disinfected with chlorine. Drinking water for each poultry house is supplied by pickers, with water flowing one way, from the entrance (males by the door) to the rear (females). Feed for both farms are obtained from M Feed Company, which delivers six tonnes every three days by feed truck. Inside the poultry houses, an automatic feeder pipeline distributes feed into plastic trays, with flow in one direction, from the entrance to the rear. Furthermore, there is a large ventilation fan at the rear of each poultry house, so airflow is uniformly from the poultry house entrance (door) towards the rear.

Electric-meter readers visit the farm monthly; septic tank workers and corporate poultry managers visit irregularly. Sawdust is delivered to the farm; some is stored in a residence warehouse.

O Company performs chicken shipping and manure removal. Farm management practices gave due concern to disease control measures by disinfecting water, taking steps to control birds, periodically exterminating rats, and disinfecting equipment, including changing boots at each poultry house.

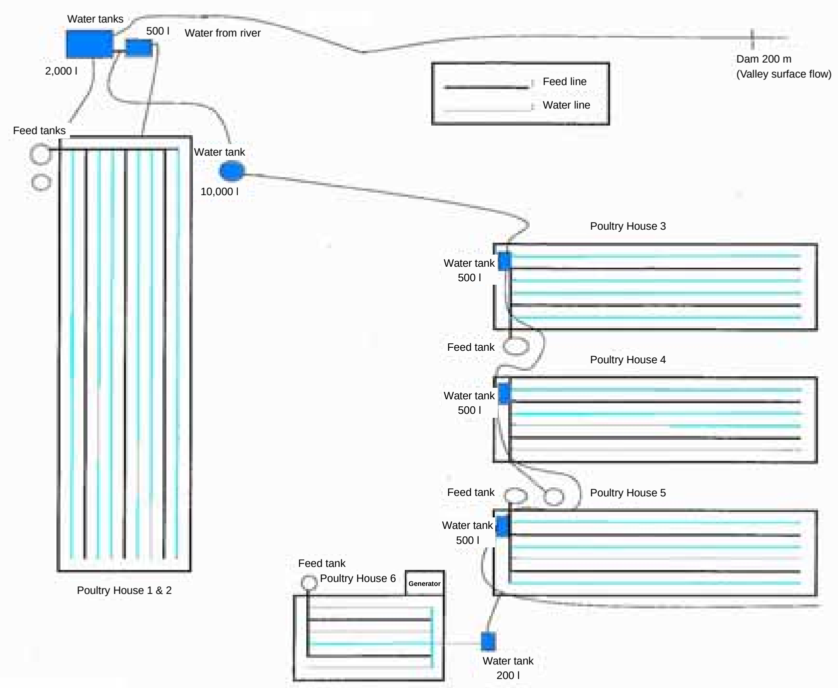


Figure 3. Diagram of outbreak farm

2.2.2.2 Surrounding environment of farm

The outbreak farm is located in a mountainous area at an elevation of 220–240 meters (sloping land), about 400 meters from a trunk road. The road from the trunk road to the farm has no private houses on it; the only homes are the two broiler farmhouses (the outbreak farm and the neighboring farm). Beyond the farms, it becomes a logging road. Therefore, vehicles and people involved with the farm seldom visit it. During the winter hunting season, an occasional hunter will pass through. The trunk road runs along the class-A Omaru River. Water birds such as wild ducks and great cormorants are occasionally seen on the river. The farm is surrounded by forest, so wild birds are seen. The farm and poultry houses have no fences or other devices to keep out wild animals from the forest. Wild animal tracks were found on the farm's grounds.

Photograph 2. Exterior of poultry house

2.2.3 Details of the outbreak

2.2.3.1 Course of the outbreak

The usual number of chicken deaths per day in the outbreak poultry house is 0–4, so the 12 deaths on January 21 were a large number. The 12 dead chickens were found along the 65-centimeter high net dividing males from females, as shown in the center of the Figure. The poultry house is open-type, but the curtains on both sides had not been open since the chicks arrived on December 3, so the environment was essentially the same as a windowless poultry house. On January 22, the number of dead chickens jumped to 243, so an attending veterinarian was contacted. Most of the dead chickens were concentrated on the male side of the net, with the deaths spreading towards the entrance from the locations on the 21st. Six or 7 of the dead chickens were on the female side. The farmer collected the carcasses and moved them outside the building. The attending veterinarian arrived at the farm at 4:00 p.m. and assessed the illness. Simple testing of 7 specimens was negative in each case. At 5:00 p.m., the attending veterinarian reported a summary of the dead chickens to the Livestock Hygiene Service Center.

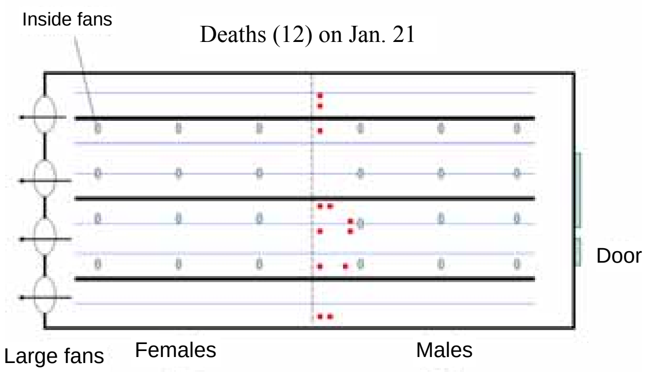


Figure 4. Outbreak farm (Poultry House 1 & 2)

The next day, January 23, the number of dead chickens increased to 326. Livestock Hygiene Service Center personnel and the attending veterinarian visited the farm and assessed the illness. The location of the dead chickens had spread further towards the door on the male side of the net. There were also 7 or 8 dead chickens on the female side; these were also collected and placed with the dead chickens near the door. The primary symptoms were lassitude and lethargy, with dull, ruffled feathers. The chickens were quiet, with no audible coughing, sneezing, or unusual calls. No cyanosis was found, and there was no clear edema or swelling of the face or legs. In addition, no diarrhea was observed, and necropsy found no remarkable pathology. Simple testing of 11 specimens found 1 clear positive reaction and 4 mild positives. Quarantine of the farm and disinfection and liming of the farm and around the poultry house were implemented that day. The number of deaths increased to 750 on the 24th and 1,850 on the 25th. There was little increase in deaths on the female side, however. In addition, cyanosis of wattles and legs that had not been observed early in the outbreak was now found, as was edema of the eyelids and face. On January 25, infection with highly pathogenic avian influenza was confirmed, and the outbreak was reported. A 10-kilometer radius was designated as a movement-restricted area.

Culling began the next day, January 26, and was completed for the outbreak poultry house that same day. The number of dead chickens on the 26th was 2,453, of which about 200–300 were on the female side. On the 28th, the carcasses, manure, feed, and other contaminants from the outbreak poultry house were buried in the forest about 500 meters from the farm, and the outbreak poultry house was disinfected inside and out and its interior swabbed with milk of lime. In addition, culling and manure removal for Poultry Houses 3, 4, 5, and 6, where infection with highly pathogenic avian influenza had not yet been confirmed, was carried out through the 30th, along with disinfection of poultry house interiors and exteriors and swabbing of the interiors with milk of lime.

2.2.4 Results of epidemiological survey

2.2.4.1 Survey of outbreak farm

2.2.4.1.1 Introduction of chicks

On December 7, 2006, chicks from C Hatchery were introduced into Poultry House 1 & 2. On December 6, chicks from C Hatchery were introduced into Poultry House 3, chicks from S Hatchery into Poultry House 4, chicks from M Hatchery into Poultry House 5, and a mixed batch of chicks from M, Y, and S Hatcheries into Poultry House 6. Each of these hatcheries is located within the prefecture, but none has any epidemiological relationship with the previous outbreak in Kiyotakecho.

2.2.4.1.2 Shipping of mature birds

Four to 10 handlers from a commercial bird handler (O Company) catch and ship the birds.

2.2.4.1.3 Disposal of dead birds

Dead chickens are stored at a stocking point about 3 kilometers from the farm and collected by a chicken carcass disposal company.

2.2.4.1.4 Disposal of poultry manure

Removal of poultry manure is handled by a specialist manure removal team from O Company. When poultry houses are empty, the farmer washes them with water and repeatedly disinfects them.

2.2.4.1.5 Bringing in of feed

Feed: The outbreak poultry house is supplied automatically from two feed tanks (numbers 1 and 2). Feed from the two tanks mixes inside the poultry house, so there is no difference in feed between males and females or by location inside the house. Feed carried from the feed tanks into the poultry house by pipeline is dispersed into feeding trays. Poultry Houses 3–6 each have one feed tank, but are otherwise supplied in an identical manner.

Water: Water is drawn from the mountain stream that flows next to the farm. Surface water from about 300 meters upstream is piped to a storage tank on the farm. Inside the tank, an appropriate amount of chlorine tablets is suspended in a net for disinfection, with additional tablets added while the previous tablets still last (every three days). Water is piped from this tank to each of the poultry houses.

2.2.4.1.6 Dealers in veterinary medicines, etc.

In January, pharmaceutical company employees made two deliveries of medicines and so on, but they were received at a home located about 2 kilometers from the farm.

2.2.4.1.7 Wild animals

Wild birds: There are many wild birds in the vicinity of the poultry house, including crows, kites, sparrows, bulbuls, pale thrushes, and flycatchers, but none were observed invading the poultry house. Water birds such as wild ducks and great cormorants are occasionally seen on the Omaru River near the farm. On January 7, two carcasses believed to be wild ducks were seen on the dry riverbed of the Omaru River about 700 meters from the farm.

Wild animals: Footprints of wild pigs and deer and deer feces are seen here and there around the poultry house, and raccoon dogs are seen as well. There were holes believed to be caused by rats in the curtain and wire netting in the center of the side wall of the outbreak poultry house (Poultry House 2). In addition, there was a hole (about 5 x 10 centimeters) in the wall of the poultry house where the feed pipeline enters. Rats have been seen inside the poultry house, but periodic efforts are made to exterminate them with poison and traps.

2.2.4.1.8 Vehicles

Vehicles entering and exiting the farm have their tires washed with liquid disinfectant at the farm entrance as a hygiene measure.

Feed transport vehicles: Feed transport vehicles enter the farm every three days, but take only about 20–30 minutes to transfer the feed. In January, Feed Tanks 1 & 2 at the outbreak poultry houses were each filled six times (on eight separate days) with a total of 36 tonnes of feed. Transport vehicles are prohibited from delivering to more than one farm on a single trip. Vehicle exteriors are automatically disinfected by the feed company's disinfection equipment before each delivery.

Sawdust trucks: For litter materials, sawdust made only in Japan (mainly in Kyushu) is added and spread regularly (every 7–10 days). On January 9 at about noon, a four-ton truck from a Hyuga City sawdust company unloaded one truckload of sawdust in front of Poultry House 4. The front of Poultry House 4 is paved with concrete. The unloaded sawdust was covered with a plastic sheet at about 5:00 p.m. the same day. On January 10, some was spread in Poultry House 1 & 2, and more was added to other poultry houses from the 11th through the 15th. The remaining half of the truckload was moved to a residence warehouse.

Electric-meter reader vehicles: On January 15, a meter reader came to check the meters on Poultry Houses 1 & 2 and 6. The reader subsequently checked the electric meters on the neighboring farm's Poultry Houses 4 and 5.

Live bird shipment trucks: A specialized team from O Company handles the work when chickens are shipped. No epidemiological relationship was found.

Manure removal trucks: A specialized team from O Company handles the work when manure is removed. No epidemiological relationship was found.

2.2.4.1.9 Contact with human beings (people who enter poultry houses)

A married couple handles the work of this farm. At 8:00 a.m., they inspect the inside of the poultry houses to observe the chickens and remove any dead ones. They complete this work at about 10:00. After that, they move any chicken carcasses to the stocking point. Usually, the husband inspects Poultry Houses 1 & 2 and 6, while the wife inspects Poultry Houses 3, 4, and 5. (This is so the inspections will end at about the same time.) Boots solely for wear in an individual poultry house are kept at each entrance. When inspecting poultry house interiors, those boots are put on and taken off each time. After noon until about 7:00 or 8:00 p.m., the interior of each poultry house is observed from the doorways.

In January, the only people to enter the poultry house were the farm couple and two helpers who helped them spread sawdust. (The helpers were the couple's sons, home for the New Year's holiday. Neither had any contact with another poultry farm.)

The poultry manager from the farm company did not visit during January. The attending veterinarian's first visit was on January 22. The four people who entered the poultry house have no history of foreign travel.

2.2.4.2 Epidemiological relationship with domestic outbreaks farms

Feed delivery: The feed company is completely unrelated to the one at the Kiyotakecho farm. No epidemiological relationship was found.

Removal of dead chickens: The companies that remove chicken carcasses are different, so their transport vehicles and collection equipment never intersect. The final disposal site, however, is the same for both companies. Disinfection and hygiene management at the disposal site for vehicles, equipment, and people is sufficient.

Drugs and disinfectants: The pharmaceutical companies the farms deal with are different. The epidemiological survey found no connection in the movement of people and so on.

2.2.4.3 Connections with South Korea or other outbreak countries

Misatocho's Nango District (formerly Nango Village), located 5 kilometers from the outbreak farm, and is also known as the “Kudara (Baekje) Village The area has had a relationship with Korea for over 1,300 years. It therefore works to draw tourists from South Korea by exhibiting cultural artifacts and so on based on Kudara (Korean) legends. In addition, Miyazaki Prefecture has many golf courses, sports facilities, and other tourist attractions, and strives to attract sightseers from South Korea to them. The prefecture therefore has many visitors.

2.2.4.4 Virus testing on the farm and in the surrounding area

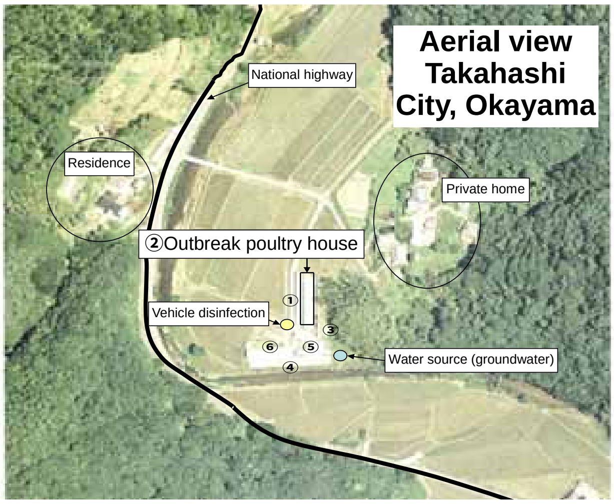
On January 25, the day the outbreak was confirmed, virus isolation and antibody testing were carried out on the outbreak farm using 10 birds from each of Poultry Houses 3, 4, 5, and 6 and 10 males and 10 females from the outbreak poultry house, for a total of 60 birds. On the 26th, 50 birds from the adjacent farm, 10 from each poultry house, underwent identical testing. No infection was found outside the outbreak poultry house. By February 11, the 10 chicken farms and homes with pet chickens within a 10-kilometer radius underwent virus isolation testing and antibody testing twice, but no infection was found. In addition, every bird owner within the movement-restricted areas was interviewed twice, but there were no abnormalities.

On March 7, virus isolation testing was performed on poultry house interiors, including roofs, walls, and floors, equipment, and soil around the poultry houses at the outbreak farm and the adjacent farm. No influenza virus was isolated.

2.3 Epidemiological survey in Takahashi City, Okayama Prefecture

2.3.1 Overview of outbreak

2.3.1.1 Overview of epidemic control measures for the outbreak



Photograph 3. Aerial view of farm

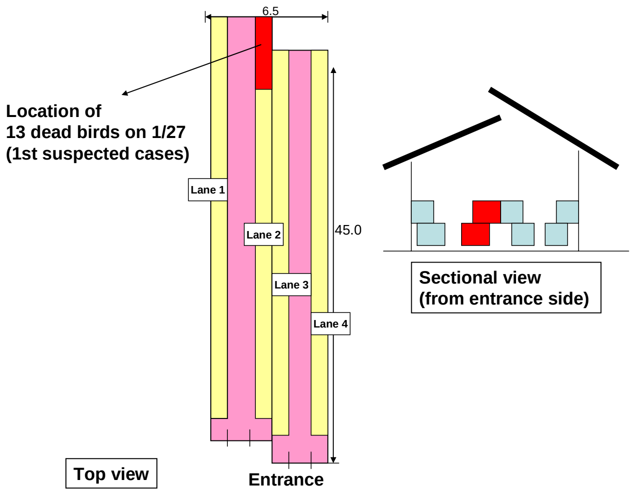


Figure 5. Diagram of Poultry House 2

The outbreak farm is about 15 kilometers southwest of central Takahashi City. Located next to a national highway and the river that parallels it, the egg farm has about 12,000 birds in six laying houses, three growing houses, and one brooding house. On January 22, two chickens in the same cage in one of the houses were found dead. Chicken deaths rose rapidly on January 27, and the farm contacted the Livestock Hygiene Service Center. Center personnel immediately visited the farm to appraise the illness. Two live and five dead chickens were brought back from the farm for simple testing, which was positive in each case. That same day, with animals suspected of infection under the Domestic Animal Infectious Diseases Control Law, the Center immediately implemented quarantine of the farm and requested voluntary movement restriction on neighboring farms. The next day, January 28, disinfection and liming of the farm and around the poultry houses was carried out and inspection of virus-free status (monitoring of poultry egg shipments) at farms within a 10-kilometer radius was performed. On January 29, highly pathogenic avian influenza was confirmed and the outbreak was reported. The area within a 10-kilometer radius of the farm was declared a movement-restricted area. Evaluation of chickens and contaminants was performed on January 29. Culling of the outbreak farm's chickens was carried out on January 30 and 31. The chicken carcasses were incinerated on February 1–4, and contaminants were buried by February 6. On February 7, the outbreak farm was disinfected and epidemic-control measures were completed. Subsequently, testing to confirm virus-free status, including chicken farmers and owners of pet chickens in the movement-restriction area, was carried out twice.

At the outbreak farm, disinfection of the poultry house was subsequently performed three times. When test results confirmed that the infection had not spread in the vicinity, movement restrictions were removed as of midnight on March 1.

2.3.2 Overview of outbreak site

2.3.2.1 Outbreak farm

All the poultry houses are wood construction, open-type, with low floors. Of the 10 poultry houses, Poultry Houses 1–6 are laying houses, Poultry House 7 is a brooding house, and Poultry Houses 8–10 are growing houses. At the time of the outbreak, Poultry Houses 7 and 8 were empty. The other houses, in order, held 1,240, 2,400, 480, 3,010, 580, 1,600, 970, and 1,020 chickens, for a total of about 11,300 birds. One of the poultry houses was old, having been built 37 years before. The outbreak poultry house (Poultry House 2) contained chickens of three different ages in days. Symptoms were seen only in some birds in one of these age groups. Vaccines received were NB, IBD, Pox, and quintuple mixed oil vaccine at appropriate ages in days. No problems ensued.

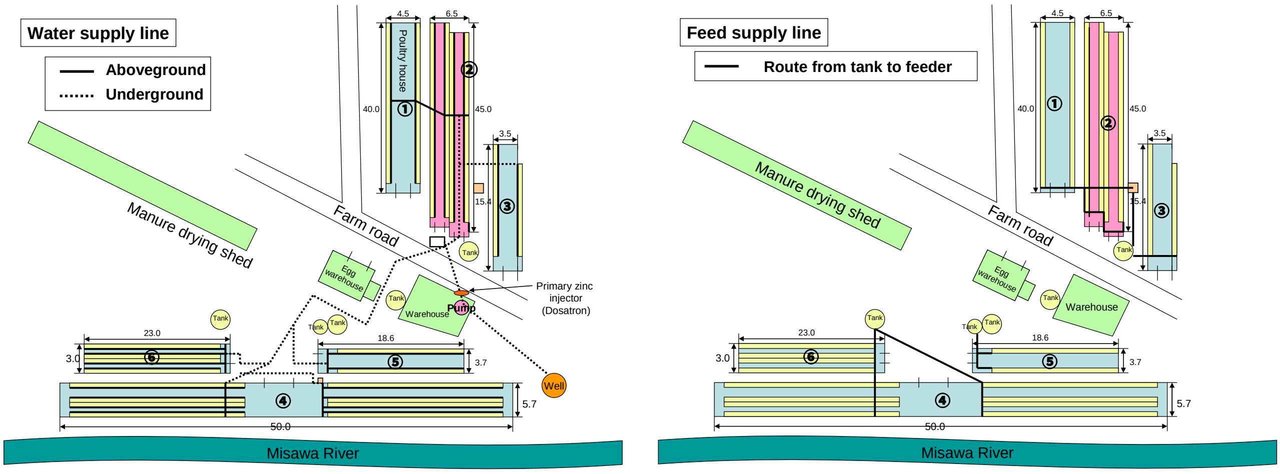


Figure 6. The farm's water (left) and feed (right) supply lines

The diagram above shows the farm's laying house layout and feed and water supply lines. The farm obtains its water from groundwater. During dry spells, it pumps water from the river. Water is supplied to the poultry houses after disinfections with sodium hypochlorite. Poultry house drinking water uses a picker for the laying houses and one growing house and tanks for the brooding house and two growing houses. (See 2.3.4.1.5) Poultry houses are cleaned and disinfected when chickens were all out. However, because a single poultry house would often have groups of chickens of varying ages in days, cleaning and disinfection of entire poultry houses is not performed often. Equipment is washed only.

2.3.2.2 Surrounding environment of farm

A farm road passes in front of the farm entrance. Although residents of the two private homes beyond the farm and, during the busy farming season, the owner of the rice field in front of the farm use the road, no one else but the farm couple and related businesses uses it. There is a national highway just to the north and south of the poultry house, and a river runs along the highway.

The farm is located at an elevation of 150–160 meters. Wild animals such as wild pigs and feral cats are known to inhabit the area. In addition, birds such as sparrows, crows, herons, and swallows are seen in the farm's vicinity in season.

There are 18 poultry farms (16 egg and 2 meat) with about 950,000 birds within 10 kilometers of the farm.

2.3.3 Details of the outbreak

2.3.3.1 Course of the outbreak

On January 22, the farmer found two dead chickens in one cage in the left rear of Poultry House 2 (about 2,300 birds) as viewed from the door. According to the farmer, it was the first time he had seen two dead birds in the same cage, so it made him very uneasy. He subsequently made that poultry house the last one visited for egg collection, disinfected with a simple spray device when entering or exiting the left rear, and ceased feed leveling. He also carefully sprayed his boots and clothes with disinfectant.

On January 27, the farmer found 15 dead chickens in Poultry House 2 and contacted the Livestock Hygiene Service Center at 9:00 a.m. The Center immediately sent two people to the farm, where they performed clinical inspection and took specimens.

The symptomatic chickens were limited to one corner at the left rear of Poultry House 2. Lethargy, lassitude, and ruffled feathers were found in several birds. Blood samples were taken from 10 symptomatic chickens and 10 apparently normal ones, and 13 dead birds and 2 symptomatic ones were placed in double plastic bags, which were carefully disinfected and sealed before transport to the Okayama Livestock Hygiene Service Center's Diagnostic Section.

Immediately after transport, samples were taken from the tracheas of seven birds (two living and five dead). Simple testing was positive in each case.

The Livestock Hygiene Service Center also carried out ND antibody testing, but found no abnormalities.

A quarantine of the farm and voluntary restrictions on movement of chickens and products were requested that same day. The next day, January 28, disinfection and liming of the farm, the area around the poultry houses, and the interior of the outbreak poultry house were performed. In addition, monitoring of poultry egg shipments for farms within a 10-kilometer radius was implemented in anticipation of a final diagnosis. Infection with highly pathogenic avian influenza was confirmed on January 29. That same day, the outbreak was reported and a 10-kilometer radius around the farm was declared a movement-restricted area.

Culling began on January 30 and was completed on the 31st. There was no dramatic increase in chicken deaths after the report on January 27. Only 63 chickens (including those tested on the 27th) had died before culling began. On January 30, when culling began, blood specimens and tracheal and cloacal swabs were taken from 10 birds in each poultry house (80 birds total) for the epidemiological survey, and antibody testing and virus isolation testing were performed. A virus was isolated from specimens taken from the left rear of Poultry House 2 (antibody testing was negative), confirming that infection had been confined to that area.

Incineration of the culled chickens began on February 1 at the municipal incinerator and was completed on February 4. During that period, the work of digging a hole for disposal and transporting contaminants such as eggs and manure was performed. Burial was completed on February 6. Disinfection of the farm was finished on February 7, completing epidemic-control measures.

2.3.4 Results of epidemiological survey

2.3.4.1 Survey of outbreak farm

2.3.4.1.1 Introduction of chicks

The most recent introduction of chicks was into Poultry House 7 (the brooding house) on November 18, 2006. Those chicks were later moved to Poultry Houses 9 and 10 (growing houses separate from the laying houses). No relationship to the outbreak was found.

2.3.4.1.2 Shipment of eggs

A specific dealer comes to the farm every day but Wednesday and Saturday to pick up eggs. The farm is the last destination after eggs are collected from other farms. Egg pick up continued through January 26.

2.3.4.1.3 Shipment of spent hens

Shipment of spent hens is handled as follows. A specific dealer visits the farm every few days. He takes spent hens that the farmer has temporarily placed in a cage belonging to the farm and puts them into his own cage for transport. The farmer washes the farm's cage after each use. Shipments within 10 days of the outbreak report were 82 birds on January 15, 52 on January 17, 40 on January 20, and 82 on January 22. The chickens shipped between January 15 and January 22 were from the right front of the outbreak poultry house, Poultry House 2, on a different lane from where the symptomatic chickens were found. Their date of first feeding was also different (520 days old on January 22). No abnormalities were noticed in any of the birds in any shipment. When the spent hens were shipped, the farmer placed them into the farm's cage in advance and then transferred them to the dealer's cage.

2.3.4.1.4 Disposal of dead birds

Dead chickens are collected daily during egg gathering and placed in a drying shed.

2.3.4.1.5 Disposal of poultry manure

Except during winter, manure is removed from the poultry houses and placed in a manure-drying shed for two weeks to dry. Every two weeks, a two-ton dump truck comes to the farm to pick up manure and transports it to a composting center. The last transports from the farm were on January 22 and 23, but the last transport of manure from the outbreak poultry house was in September 2006.

2.3.4.1.6 Bringing in of feed

Feed: The laying houses are fed automatically from feed tanks, with three round trips daily. When operating from the entrance side, feed is supplied to the underside of the second tier, and on the upper side when operating from rear to front. Leveling operates once per day. In the brooding and growing houses, the farmer feeds the birds manually twice per day.

Water: The laying houses are supplied with water via pipes from a well located north of Poultry House 4. When water is short, a pump brings water from to the farm from the nearby river. After storage in a tank, it is disinfected with sodium hypochlorite before being supplied to the poultry houses. The farmer carries water to the brooding and growing houses.

2.3.4.1.7 Dealers in veterinary medicines, etc.

A dealer delivers veterinary medicines and so on every three months, but had not been to the farm recently. They are usually received on the farm, but sometimes at the residence.

2.3.4.1.8 Wild animals

Wild birds: Sparrows and crows are seen around the farm year round, while in early spring swallows and herons are seen flying here and there. The area does not attract large numbers of water birds in winter. Bird netting was not complete. Inspection of Poultry Houses 2 and 4 found wire mesh attached, but the mesh was large. In addition, there were gaps between the roofs and walls of each poultry house.

Wild animals: Regarding rats, on May 29, 2006, the farm adopted the positive list system and stopped using rodenticides. Before stopping, rats had been seen on the farm, but none has been since. As for other wild animals, feral cats enter the farm, but the farmer is careful to prevent them from entering the poultry houses while he is working. Evidence of their presence in the egg warehouse has been found. After epidemic-control measures were completed, traces of their presence were briefly found, but none recently.

2.3.4.1.9 Vehicles

For outside vehicles, the farmer spreads hydrated lime west of the egg warehouse and has the vehicles drive through it.

Feed transport vehicles: Feed is delivered weekly by a pair of three-ton trucks from a specific transport company. The last delivery was January 23, when six tonnes of feed (two three-ton trucks) were brought. Drivers drive through the hydrated lime spread in front of the egg warehouse and carry their own simple spray devices filled with disinfectant to disinfect before and after entering and leaving the farm.

Egg pick up trucks: The drivers drive their trucks through the hydrated lime spread in front of the egg warehouse. Other merchants in the city come to the farm to buy eggs as needed. The last visit was in late December.

Manure transport vehicles: Dump trucks that transport manure are carefully washed after use. Drivers drive through the hydrated lime spread in front of the egg warehouse.

2.3.4.1.10 Contact with human beings (people who enter poultry houses)

Work is managed with the farm couple handling egg collection, with one part-time employee packing eggs in the egg warehouse during busy periods. The farmer begins taking care of the growing house at 7:00 a.m., and the couple collects eggs and checks for dead chickens in the laying houses beginning at about 9:30. They perform work as needed, without assignment to a particular poultry house. In 2:00 in the afternoon, they sort the eggs collected that day, continuing until egg pick up at 3:00 p.m. At 4:00, they begin the evening feeding, and continue the work of the farm until about 7:00 p.m. When entering poultry houses, they use a jet fog and step-in disinfection tank at the egg warehouse door, and spray when going in or out. The farm couple disinfects every time they go in or out of a poultry house. They do not have a regular veterinarian, but instead rely on the Livestock Hygiene Service Center for disease diagnosis, antibody testing, and so on.

The only people entering the poultry house in January were the farm couple and a

Livestock Hygiene Service Center employee on January 16. On January 24, a power company employee went to the farm to read the electric meter, but did not actually enter the farm. There were no other visitors.

2.3.4.2 Epidemiological relationship with domestic outbreak farms

No relationship with Miyazaki Prefecture was found in egg or compost shipping or in the farm couple or part-time employee's foreign travel.

2.3.4.3 Connections with South Korea and other outbreak countries

No connections were found.

2.3.4.4 Virus testing on the farm and in the surrounding area

The virus isolation testing performed on poultry farms within a 10-kilometer radius on January 28 was followed by two rounds of virus isolation testing and antibody testing at chicken farms and homes with pet chickens, on February 2–4 and February 17–18, but no infection was found. In addition, every bird owner within the movement-restricted areas was interviewed twice, but there were no abnormalities.

On May 14, virus isolation testing was performed on poultry house interiors, including roofs, walls, and floors, feed tanks, and soil around the poultry houses, and the burial site at the outbreak farm; no type A influenza virus was isolated.

2.4 Epidemiological survey in Shintomicho, Miyazaki Prefecture

2.4.1 Overview of outbreak

2.4.1.1 Overview of epidemic control measures for the outbreak

Photograph 4. Aerial view of farm

The outbreak farm is located about 2 kilometers west of urban Shintomicho. It is an egg farm with about 93,000 chickens in one poultry house (15 attached sections). On January 30, dead chickens concentrated in one lot in the southwest corner of the poultry house were discovered. Some of the dead chickens had very mild cyanosis of the comb, and lethargy was found in some living ones. That same day, with animals suspected of infection under the Domestic Animal Infectious Diseases Control Law, quarantine of the farm and voluntary movement restriction on neighboring farms were requested. At the Livestock Hygiene Service Center, diagnosis resulted in isolation of a virus, and on February 1, the National Institute of Animal Health confirmed that it was an H5 subtype type A influenza virus. That same day, a 10-kilometer radius from the farm was declared a movement-restricted area. Culling of the outbreak farm's chickens was carried out on February 2 and 3. Burial of the culled chickens was finished on February 5. Epidemic control measures were completed on February 7. In parallel with epidemic-control measures on the outbreak farm, primary testing to confirm virus-free status began on February 3, and secondary testing on February 16. Because there was no spread of the infection to surrounding areas, the movement restrictions implemented on February 1 were lifted as of midnight on March 1.

2.4.2 Overview of outbreak site

2.4.2.1 Outbreak farm

There were 93,000 laying chickens (in six lots: 549-day old, 465-day old, 398-day old, 320-day old, 172-day old, and 150-day old). Egg production was four tonnes per day.

The farm was surrounded by a 1.5-meter fence, but it was broken in places.

The farm has one entrance, which is closed off with a rope at night, and the poultry house door is locked at night as well. At the farm entrance, there is a power sprayer used to disinfect the vehicles of feed providers and so on.

The poultry house is steel frame construction, open-type, with a raised floor. It comprises 15 attached sections, with two rows with two tiers of cages on each side. The poultry house area is roughly 10,000 m^2 (100 m × 100 m).

The egg gathering room adjacent to the poultry house serves as its entrance, and the only stairs to the second floor are located in the rear of the egg gathering room. The first-floor of the poultry house is for manure accumulation.

The side walls of the poultry house are equipped with wire mesh and curtains, but there is no bird netting.

The curtains are constantly raised and lowered to control temperature inside the poultry house.

There are eight feed tanks to the east of the poultry house, one for each lot. Automatic feeders are used to supply the feed to the chickens.

Water is supplied by a well. No disinfection is used.

There are four-full time employees, including a manager.

Employees change into work clothes especially for the farm in the farm's office. A step-in disinfectant tank and spray disinfectors are provided at the poultry house entrance.

The ordinary workflow is to first go to the second floor and check the chickens for any abnormalities and to make sure the feeders and waterers are working. Egg collection is then performed in the egg gathering room. After egg gathering is complete, workers return to the second floor and remove any dead chickens.

Employee vehicles are disinfected at the farm entrance and then parked next to the case warehouse at the south end of the farm.

Feed dealers and so on who enter the farm are required to wear the farm's sanitary clothing and boots.

As a hygiene measure, whenever a lot is removed, the lot's aisles and cages are disinfected (manure removal, cleaning, and spreading of invert soap). After the Kiyotakecho outbreak, hydrated lime was spread outside the poultry house (mainly on the farm's roadways).

Containers and egg trays are disinfected with a power sprayer using an invert soap liquid immediately after being returned to the farm from the grading and packing center.

Vaccination program (carried out on raising farm)	
Age in days	Vaccine
1 day	IB
7 days	NB
21 days	POX, ILT, MG, IB
25 days	IBD
30 days	IB
50 days	NB
70 days	MB2AC
90 days	IB *Subsequently, IB every 3 months on the farm

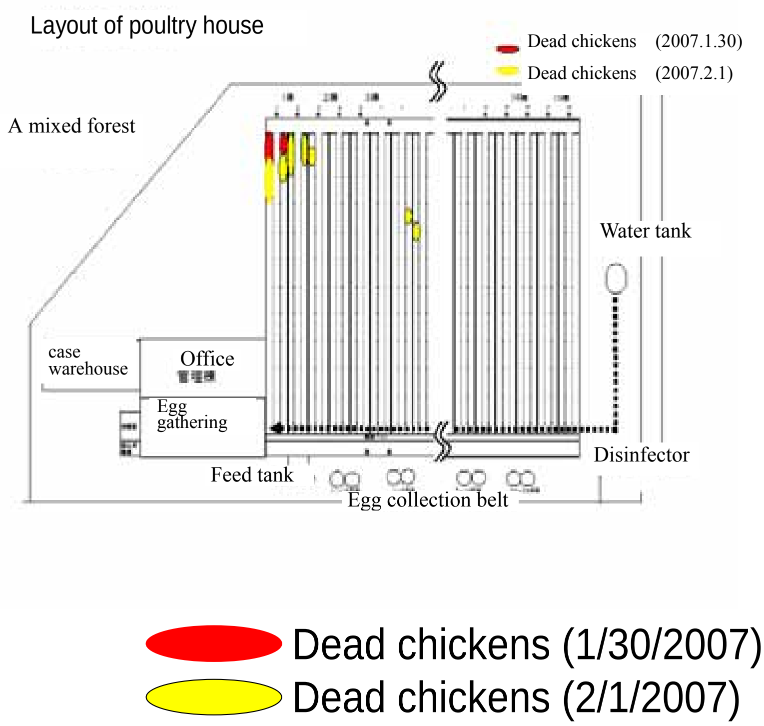


Figure 7. Vaccination program (left) and estimated spread of infection inside poultry house (right)

2.4.2.2 Surrounding environment of farm

There is a Japan Air Self-Defense Force base about 1 kilometer to the north; there are no residences nearby. The farm is surrounded on three sides by farmland. On the south side, a mixed forest comes right up to the poultry house.

Within a 10-kilometer radius of the farm, there are 97 farms with at least 1,000 chickens, totaling about 2.88 million birds. It is one of the prefecture's leading areas for egg production.

2.4.3 Details of the outbreak

2.4.3.1 Course of the outbreak

There were no abnormalities through the day before the outbreak. On January 30, an employee discovered 23 dead birds in a 549-day old lot concentrated in the southwest corner of the poultry house. Finding this abnormal, the farm manager reported it to the Livestock Hygiene Service Center.

Some of the dead chickens had very mild cyanosis of the comb, and lethargy was found in some living ones. Simple testing was positive (7 of 13 birds). Subsequently, chicken deaths slowly spread, mainly in the southwest corner of the poultry house. On February 1, 232 dead birds were found.

2.4.4 Results of epidemiological survey

2.4.4.1 Survey of outbreak farm

2.4.4.1.1 Introduction of chicks

The chicks were obtained from an affiliated growing farm in Miyazaki Prefecture. The most recent introduction was on January 16–19. The new chicks were placed in the northern end of the poultry house, farthest from the lot where the infection broke out.

2.4.4.1.2 Shipment of eggs

About every two days, good eggs are shipped to four grading and packing centers outside the prefecture, while broken eggs and so on are sent to a processing center inside the prefecture. Egg transport workers change into the farm's work clothes and boots at the farm entrance and spray with disinfectant at the entrance of the egg gathering room before packing containers into the transport vehicle.

2.4.4.1.3 Shipment of spent hens

The most recent shipments were on January 9 and 10. The birds went to a Miyazaki Prefecture dealer in spent hens. The dealer's containers for shipping the hens were disinfected at the farm entrance along with the transport vehicles. Employees from affiliated farms helped to catch the birds.

2.4.4.1.4 Disposal of dead birds

Farm employees remove dead chickens from the poultry house, place them in plastic bags, and put them into a storage container at the farm's entrance, where they are picked up by a disposal company. The company's employees and vehicles do not enter the farm's grounds.

2.4.4.1.5 Disposal of poultry manure

Manure has not been removed for five or six years.

2.4.4.1.6 Bringing in of feed

Feed: Feed is brought in daily. Feed transport vehicles go to all the way to the individual feed tanks outside the poultry house. An automatic feeder sends the feed from the feed tanks into the poultry house.

Water: Well water is pumped into a tank outside the poultry house and then distributed inside the building. No disinfection or other treatment takes place.

2.4.4.1.7 Dealers in veterinary medicines, etc.

Veterinary medicines and so on are received outside the farm's grounds. (There were no deliveries in January.)

2.4.4.1.8 Wild animals

Wild animals such as weasels, raccoon dogs, and foxes inhabit the area around the farm. Nearby, a relatively large irrigation pond attracts large numbers of migratory birds. There are also a large number of crows, sparrows, and other resident wild birds in the area. Although the poultry house has curtains, it has almost no anti-bird measures and has numerous tears and gaps. After epidemic-control measures were taken, sparrow carcasses were found inside the poultry house, and shrike carcasses were found on the south side along with what were apparently the bones and eggshells of wild birds.

Employees had noticed the incursion of sparrows and other wild birds into the poultry house.

2.4.4.1.9 Vehicles

Feed transport vehicles: Feed transport vehicles are disinfected at the farm's entrance before moving on to the feed tanks. Feed transport vehicles travel only between the farm and the feed factory, without mixing loads. They do not visit other farms on the way.

Egg transport vehicles: Egg transport vehicles are disinfected at the farm's entrance before moving on to the egg gathering room.

2.4.4.1.10 Contact with human beings (people who enter poultry houses)

On January 16–19, six employees of an affiliated growing farm entered to bring in new chicks. They performed spray disinfection of work clothes and step-in disinfection of boots.

2.4.4.2 Epidemiological relationship with domestic outbreaks farms

No relationship was found.

2.4.4.3 Connections with South Korea or other outbreak countries

Neither the farm manager, workers, nor family members had any recent history of foreign travel. There were no such connections.

2.4.4.4 Virus testing on the farm and in the surrounding area

After highly pathogenic avian influenza was confirmed, virus isolation testing was performed on chicken farms and homes with pet birds inside the movement-restricted area as primary and secondary virus-free status confirmation testing. All tests were negative.

On May 18, virus isolation testing of the manure inside the poultry house was carried out. The results were negative.

Section 3 Analysis of virus characteristics

(Takehiko Saito)

Viruses isolated from the four outbreaks of highly pathogenic avian influenza in Japan in January 2007 (Table 1) were examined for virological and molecular genetic characteristics and for pathogenicity in chickens, aigamo ducks, and rodents.

Table 1. Viruses causing outbreaks of highly pathogenic avian influenza

Strain name	Place specimens taken	Date specimens taken	Subtype
A/chicken/Miyazaki/K11/2007	Kiyotakecho, Miyazaki Prefecture	January 11	H5N1
A/chicken/Miyazaki/ H358/2007	Hyuga City, Miyazaki Prefecture	January 23	H5N1
A/chicken/Okayama/ T6/2007	Takahashi City, Okayama Prefecture	January 27	H5N1
A/chicken/Miyazaki/S749/2007	Shintomicho, Miyazaki Prefecture	January 30	H5N1

1 Molecular genetic characteristics

As shown in Table 1, the influenza viruses isolated from infected animals on three farms in Miyazaki Prefecture (Kiyotakecho, Hyuga City, and Shintomicho) and in Takahashi City, Okayama Prefecture, between January 11 and 30 were identified as H5N1 subtype highly pathogenic avian influenza (HPAI) viruses.

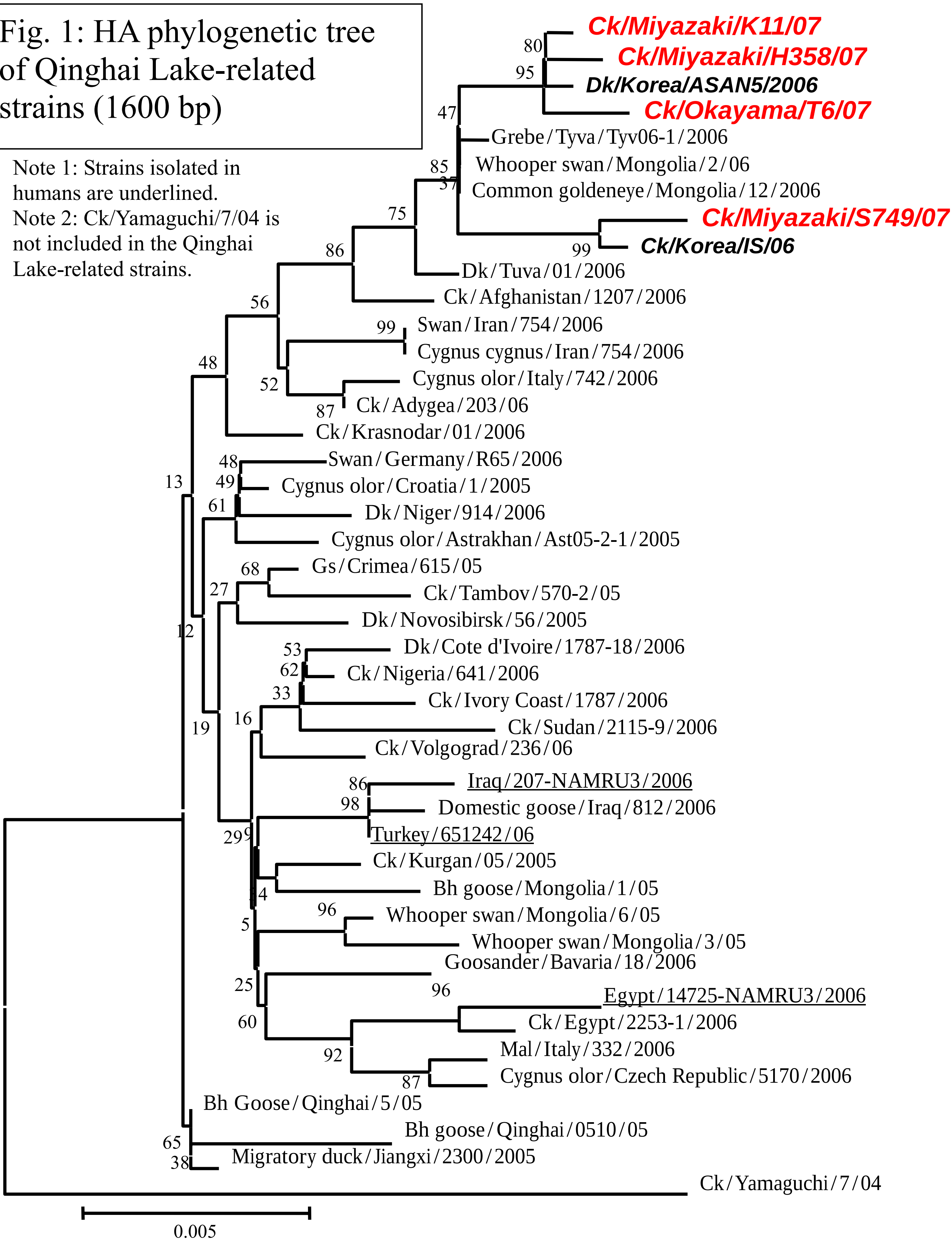
For the above four strains, base sequences for all gene segments (eight segments each) were determined, and base sequence homology with the A/chicken/Miyazaki/K11/2007 (K11 strain) virus from the initial case was examined. At the same time, the predicted amino acid sequence of the hemagglutinin (HA) protein cleavage region, which contributes to the virus's pathogenicity in chickens, was determined (Table 2). The results showed that the K11 strain had above 99 percent homology with the other three strains for all gene segments, suggesting that all four strains are closely related. In addition, homology with two strains from last year's outbreak of highly pathogenic avian influenza in South Korea was also above 99 percent, and homology with the A/whooper swan/Mongolia/2/2006 virus isolated from whooper swans in Mongolia was also above 99 percent for all segments, indicating that these viruses are closely related. On the other hand, the HPAI causative strain A/Ck/Yamaguchi/7/2004 that caused Japan's first outbreak in 79 years in 2004 is not highly homologous, indicating that the 2007 outbreaks were not a recurrence of the 2004 virus. The predicted amino acid sequence for the HA protein cleavage region for all four strains from the 2007 Japanese outbreaks was found to be the string of basic amino acids (GERRRKKR, R: arginine, K: lysine) characteristic of highly pathogenic strains.

Table 2. Base sequence homology of strains isolated in Japan, related viruses, and A/chicken/Miyazaki/K11/2007

Segment	Strains isolated in Japan in 2007			Strains isolated in South Korea in 2006		2006 Mongolian strain
	Ck/Miyazaki/ H358/2007	Ck/Okayama/ T6/2007	Ck/Miyazaki/S749/ 2007	Ck/Korea/ IS/2006	Ck/Korea/ ASAN/2006	A/whooper swan/Mongolia /2/2006
PB2	99.9	99.8	99.4	99.4	99.8	99.5
PB1	99.7	99.8	99.2	99.3	99.9	99.5
PA	99.9	99.8	99.6	99.6	99.9	99.5
HA	99.7	99.7	99.2	99.3	99.8	99.5
NP	99.8	99.9	99.6	99.5	99.9	99.5
NA	99.9	99.9	99.2	99.3	100	99.6
M	99.6	99.2	99.3	99.4	99.7	99.5
NS	99.6	99.6	99.2	99.1	99.4	99.4
Genetic lineage	Clade 2-2 (Qinghai Lake lineage)	Clade 2-2 (Qinghai Lake lineage)	Clade 2-2 (Qinghai Lake lineage)	Clade 2-2 (Qinghai Lake lineage)	Clade 2-2 (Qinghai Lake lineage)	Clade 2-2 (Qinghai Lake lineage)
HA cleavage region amino acids	GERRRKKR-GLF virulent type	GERRRKKR-GLF virulent type	GERRRKKR-GLF virulent type	GERRRKKR-GLF virulent type	GERRRKKR-GLF virulent type	GERRRKKR-GLF virulent type

Fig. 1: HA phylogenetic tree of Qinghai Lake-related strains (1600 bp)

Note 1: Strains isolated in humans are underlined.
Note 2: Ck/Yamaguchi/7/04 is not included in the Qinghai Lake-related strains.



In order to clarify the relationships of the viruses causing the 2007 Japanese outbreaks with H5N1 subtype viruses isolated in other countries, a phylogenetic tree was created using the neighbor-joining (NJ) method with HA gene base sequence as the criterion (Figure 1).

Starting with HPAI in 1996 in Guangdong Province, China, the H5N1 subtype HPAI HA genes that have spread worldwide but especially in Southeast Asia are currently subdivided as clade 1 (Thailand/Vietnam-related strains), clade 2-1 (Indonesia-related strains), clade 2-2 (Qinghai Lake-related strains), and clade 2-3 (Fujian Province-related strains). Phylogenetic tree analysis classifies the viruses in the recent Japanese outbreaks as clade 2-2 Qinghai Lake-related strains and shows that they are closely related to the HPAI-causative viruses from the 2006 South Korean outbreaks, as their genetic homology suggested. Clade 2-2 Qinghai Lake-related strains are viruses derived from the HPAI virus isolated in 2005 when large numbers of wild birds died at Qinghai Lake on the northeastern Qingzang Plateau in western China. Since it was identified in 2005, the area of confirmed infections in poultry and wild birds has spread as far as Europe and Africa in 2006, and in humans in Egypt, Turkey, and Iraq.

From amino acid sequences estimated from base sequences of other segments, it is clear that in all four strains the amino acid at position 627 of the PB2 gene is not glutamine, which is predominant in strains isolated from birds, but lysine, which is predominant in strains isolated from mammals. Among HPAI H5N1 strains, those isolated from human cases in 1997 and showing high virulence in mice and the strain isolated in Thailand from tigers are known to have lysine in position 627 of the PB2 gene. This suggests the possibility of a relationship with pathogenicity in mammals. Known mutations said to contribute to resistance to amantadine and neuraminidase inhibitors were not found.

2 Pathogenicity testing in chickens

2.1 Intravenous inoculation testing

In order to determine pathogenicity in chickens, the OIE pathogenicity testing method was followed. Eight 6- to 8-week-old SPF chickens were inoculated in the jugular vein with virus-infected chorioallantoic fluid diluted 10 times with sterilized PBS and their symptoms and survival were observed for 10 days (Table 3). No matter which virus was used, all inoculated chickens died within 26 hours (Photograph 1), confirming that these viruses are virulent types. Grossly visible findings in some chickens were wattle cyanosis (Photograph 2), conjunctival congestion, and hydropericardium.

Table 3. Results of intravenous inoculation pathogenicity testing

Strain name	Deaths/tested chickens	Age in weeks of tested chickens	Grossly visible pathology, etc.
Ck/Miyazaki/K11/2007	8/8 Within 26 hours of inoculation	NIAH SPF (P-1) 8 weeks old	Wattle cyanosis, conjunctival hemorrhage, hydropericardium
Ck/Miyazaki/H358/2007	8/8 Within 21 hours of inoculation	NIAH SPF (P-1) 6 weeks old	Hydropericardium
Ck/Okayama/T6/2007	8/8 Within 17 hours of inoculation	NIAH SPF (P-1) 7 weeks old	Hepatic petechiae
Ck/Miyazaki/S749/2007	8/8 Within 21 hours of inoculation	Nisseiken SPF (line M) 6 weeks old	Hydropericardium

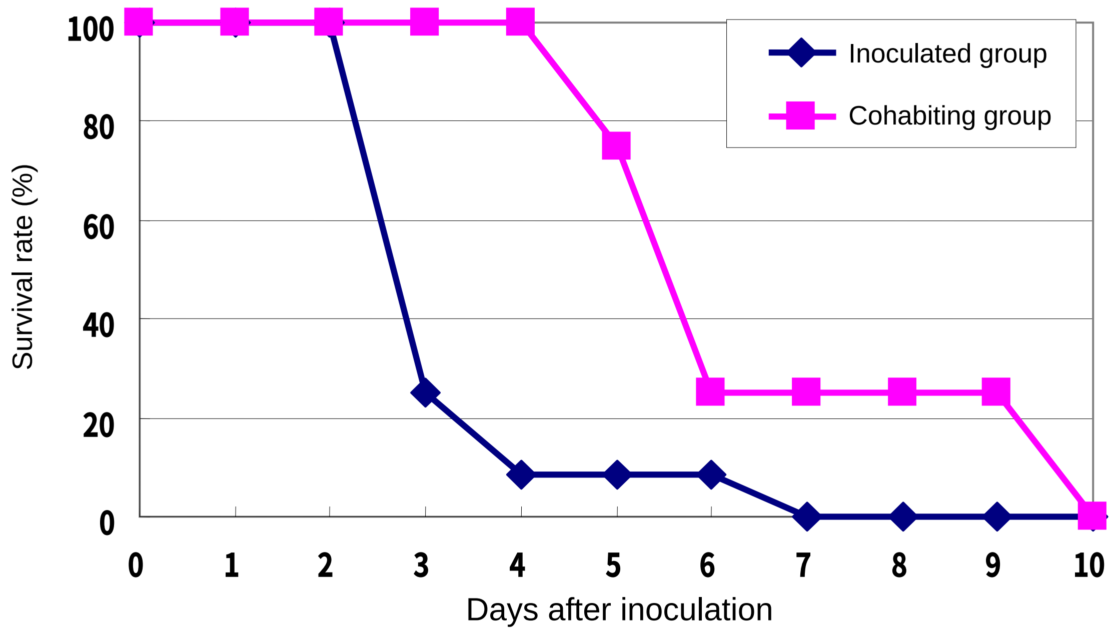
Photograph 1. Chickens that died after intravenous inoculation with the K11 strain
Photograph 2. Chicken with wattle cyanosis after intravenous inoculation with the K11 strain

2.2 Nasal inoculation and isolation testing of chickens

In order to calculate the death rate for nasal inoculation with the K11 strain, 16 6-week-old SPF chickens (Nisseiken Line-M) were inoculated nasally with 10^{6} EID_{50} virus. Furthermore, in order to study transmissibility to cohabiting chickens, uninfected chickens were placed with the inoculated birds five hours after virus inoculation. Two birds were euthanized on each of the first two days after nasal inoculation, and their major organs were removed and virus distribution in the body examined. Beginning on the third day after inoculation, dead chickens were examined in the same way for internal virus distribution. Organs examined were the brain, lungs, kidneys, spleen, liver, trachea, rectum, bursa of Fabricius, pancreas, leg muscles, and pectoral muscles. After harvesting, a 10-percent emulsion was created, and embryonated chicken eggs were used to measure the amount of virus contained.

All nasally inoculated chickens died three to seven days after inoculation. Nine chickens died 3 days after inoculation, two died 4 days after, and one died 7 days after. Death came after an average of 3.5 days. The four uninoculated chickens cohabiting with them all died as well, 5 to 10 days after joining the inoculated ones. One died after 5 days, two after 6 days, and one after 10 days. Death came after an average of 6.5 days.

Figure 2. Pathogenicity (and transmissibility) of nasal inoculation of chickens



Examination of virus distribution within the bodies of inoculated chickens (Table 4) did not recover any virus from the two chickens euthanized 1 day after inoculation. Of the two chickens euthanized 2 days after inoculation, the virus was detected in every organ of one of the chickens, with the exception of the pectoral muscles. The virus was detected in the trachea and heart at 10^4 EID_{50}/g, a higher virus titer than in other organs. The virus was also recovered from the rectum and the brain. In the two birds that died 3 days after inoculation, the virus was recovered at 10^4 EID_{50}/g or above from every organ examined except the pancreas. The virus was detected at 10^5 EID_{50}/g or above in the tracheas, lungs, and rectums of both birds.

Table 4. Distribution of virus in bodies of chickens inoculated with K11 strain

Organs examined	No. virus-positive birds/birds examined (virus titer: Log EID_{50}/g)		
	Days after inoculation		
	1	2	3
Brain	0/2	1/2 (3.5)	2/2 (4.0, 5.5)
Trachea	0/2	1/2 (4.0)	2/2 (5.5, 5.5)
Lung	0/2	1/2 (2.67)	2/2 (6.5, 6.5)
Heart	0/2	1/2 (4.0)	2/2 (3.5, 4.67)
Liver	0/2	1/2 (2.5)	2/2 (4.5, 5.5)
Kidney	0/2	1/2 (2.5)	2/2 (4.5, 5.5)
Spleen	0/2	1/2 (2.67)	2/2 (5.0, 4.0)
Pancreas	0/2	1/2 (2.67)	0/2
Bursa of Fabricius	0/2	1/2 (2.5)	2/2 (4.5, 4.5)
Rectum	0/2	1/2 (3.0)	2/2 (5.0, 7.0)
Pectoral muscle	0/2	0/2	2/2 (5.0. 4.67)
Leg muscle	0/2	1/2 (2.5)	2/2 (4.5, 4.5)

2.3 LD50 measurement for chickens

In order to calculate the median lethal dose by nasal inoculation for chickens (chicken lethal dose $_{50}$; CLD_{50}), 6-week-old SPF chickens (Nisseiken Line-M) were inoculated nasally with 0.1-ml virus solutions diluted by factors of 10 (10^1 EID_{50} to 10^5 EID_{50}). Four chickens were used with each dilution. They were kept in separate negative-pressure isolators and observed for 14 days.

The nasal inoculation dose lethal to 50 percent of chickens was $10^{2.5}$ EID_{50}. All chickens in the 10^5 EID_{50} inoculation group died 3–7 days after inoculation, all those in the 10^4 EID_{50} inoculation group died 3–10 days after inoculation, and all those in the $10^3 EID_{50}$ inoculation group died 3–11 days after inoculation. On the other hand, no chickens died in the 10^1 and 10^2 EID_{50} inoculation groups. Blood serum taken from surviving chickens 14 days after inoculation and examined for the presence of antibodies using hemagglutination inhibition testing with the inoculation virus as the antigen found antibody titers of less than 10 times in every case, so it appears that infection was not established.

2.4 Pathogenicity testing and LD50 measurement through nasal inoculation of aigamo ducks

In order to study pathogenicity in aigamo ducks and their median lethal dose, 4-week-old aigamo ducks were inoculated nasally with the K11 strain in dilutions of 10^6–10^1 EID_{50}/0.1 ml and observed for 14 days.

All of the aigamo ducks inoculated with the 10^4 EID_{50} inoculation survived, except one that died 5 days after inoculation. Clinical symptoms were as follows. One of the birds that received a 10^3 EID_{50} inoculation exhibited neurological symptoms such as torticollis and circling behavior. Those inoculated with 10^6–10^3 EID_{50} inoculations exhibited corneal opacity in all cases. In addition, white lesions on the pancreas and discoloration of the heart muscle were observed. Histologically, nonpyogenic encephalitis, honeycomb necrosis of the pancreas, myocardial necrosis, skeletal muscle myositis, keratitis, and necrosis of the skin on the wings and bill were seen along with virus antigens.

However, a correlation between dosage and death rate was not established, so a median lethal dose could not be determined.

Table 5. Pathogenicity testing and median lethal dose testing in aigamo ducks through nasal inoculation

Virus dosage (EID_{50})	No. of dead birds/birds	Clinical symptoms
10^6	0/8	Corneal opacity (8/8)
10^5	1/4	Corneal opacity (4/4)
10^4	0/4	Corneal opacity (4/4), neurological symptoms (1/4)
10^3	0/4	Corneal opacity (4/4), exophthalmos (1/4)
10^2	0/4	—
10^1	0/4	—

2.5 Transmission testing from infected aigamo ducks to chickens and aigamo

ducks

In order to model virus transmission among wild ducks and from wild ducks to poultry, aigamo ducks were inoculated nasally with the K11 strain (10^6 EID_{50}/0.1 ml/bird). After 24 hours, they were placed in isolators with SPF chickens (2 weeks old) or aigamo ducks (4 weeks old) to study whether cohabitation infection between aigamo ducks and chickens or between aigamo ducks and other aigamo ducks would occur.

All the uninoculated chickens cohabiting with aigamo ducks inoculated with the virus died, two after 5 days of cohabitation, one after 6 days, and two after 7 days. This proved that infection is transmissible from infected aigamo ducks to chickens. On the other hand, all the uninoculated aigamo ducks cohabiting with the inoculated aigamo ducks survived. All of them, however, exhibited corneal opacity, suggesting that they were infected.

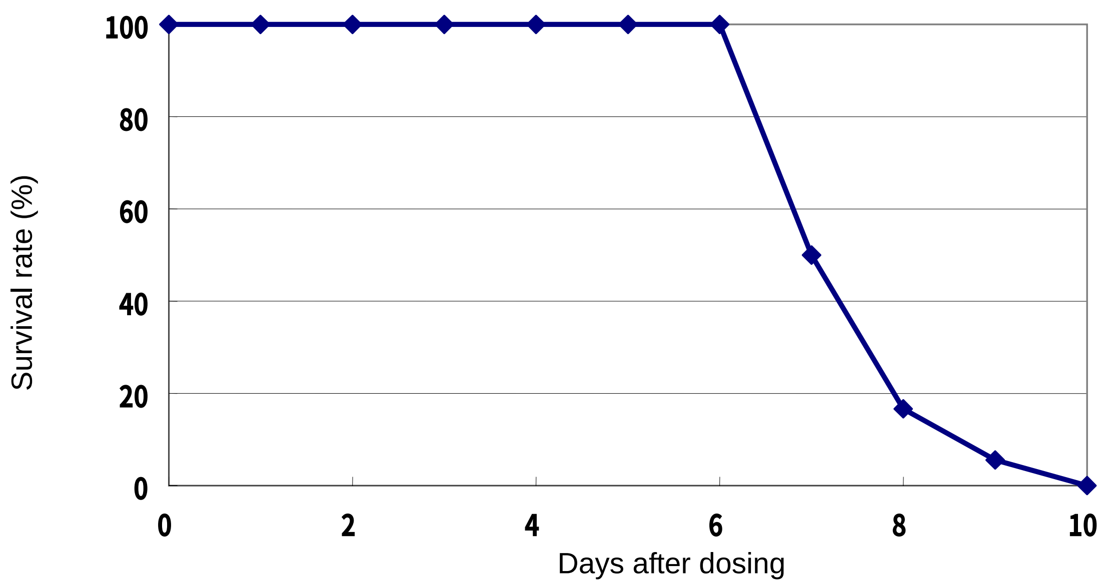
3 Pathogenicity testing using rodents

In order to estimate the infectiousness and pathogenicity of the K11 strain in rodents, mice and rats were used for infection testing as follows.

3.1 Mouse nasal inoculation testing

K11 strain adjusted to 10^6 EID_{50} was inoculated nasally, and the subsequent survival rate and average number of days to death were calculated (Figure 3). Furthermore, in order to study the distribution of the virus inside the body, three mice were euthanized on the 3rd day after nasal inoculation and three more on the 6th, and their brains, lungs, kidneys, spleens, livers, and digestive tracts were harvested. In order to measure virus titer in their organs, a 5–10-percent emulsion was created from the harvested organs and embryonated chicken eggs were used to measure the amount of virus contained. Virus titer in the organs of the mice that died 7 or more days after inoculation were examined in the same way.

Figure 3. Mouse nasal inoculation test



Nine of the mice inoculated nasally with the K11 strain died 7 days after inoculation, six after 8 days, two after 9 days, and one after 10 days, at which point all were dead. The average number of days to death was 7.7. Table 6 shows the distribution of the virus in the major organs of the mice euthanized after 3 and 6 days and that died after 7 days. Virus titer was high in the lungs and brain, but none was recovered from digestive tracts.

Table 6. Virus titer in major organs of mice inoculated with K11 strain

Organ	No. virus-positive/no. tested (virus titer ± S.D.: $\log_{10}$ EID_{50}/g)		
	3rd day after inoculation	6th day after inoculation	7th day after inoculation (death)
Brain	1/3 (2.3)	2/3 (4.1 ± 1.8)	3/3 (3.8 ± 0.9)
Lung	3/3 (7.0 ± 0.3)	3/3 (7.1 ± 0.2)	3/3 (6.0 ± 0.7)
Spleen	3/3 (3.7 ± 0.1)	3/3 (3.4 ± 0.2)	2/3 (1.8, 2.6)
Liver	1/3 (2.3)	1/3 (2.3)	0/3
Kidney	1/3 (2.3)	2/3 (4.5 ± 0.2)	0/3

3.2 Mouse LD50 measurement

The median lethal dose for mice (mouse lethal dose $_{50}$; MLD_{50}) was calculated by nasal inoculation of 6-week-old female BALB/c mice under Nembutal anesthesia with a 50-µl virus solution diluted in 10 strengths (from $0.5x10^{-1}$ to $0.5x10^{8}$ EID_{50}/50 µl). Four mice were used with each dilution, kept in negative-pressure isolators, and observed for 14 days.

The median lethal dose for nasal inoculation of mice was calculated to be 5 x 10^2 EID_{50}.

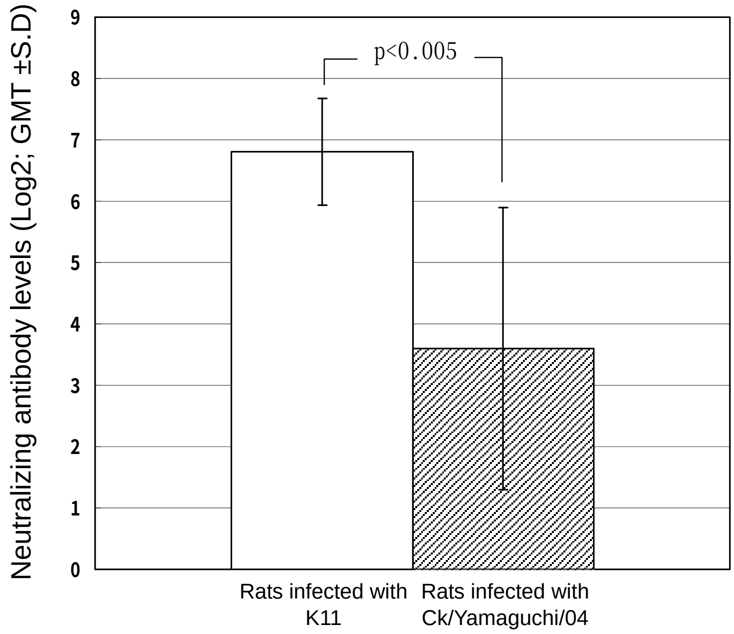
3.3 Rat pathogenicity testing

In order to estimate the virus susceptibility of rats (brown rats), 16 6-week-old female Sprague Dawley rats were inoculated nasally with the K11 strain at 10^6 EID_{50} while under Nembutal anesthesia and observed for two weeks. Furthermore, three mice were euthanized on the 3rd day after nasal inoculation and three more on the 6th, their major organs were harvested, and internal virus distribution was examined. The harvested organs were the brains, lungs, kidneys, spleens, and livers. A 10-percent emulsion was created, and embryonated chicken eggs were used to measure the amount of virus contained

The rats inoculated nasally presented no clinical symptoms, and none died. No virus was recovered from the major organs of the rats euthanized on the 3rd and 6th days after inoculation. As illustrated in Figure 4, the mean value (geometric mean; log 2 less than 4 times is calculated as 1) for K11-strain neutralizing antibody titer in blood serum taken after completion of observation was significantly higher than for the group infected with the Ck/Yamaguchi/04 strain. Furthermore, while the positive rate for the group infected with the K11 strain when neutralizing antibody titers of less than 4 times were taken as negative was 100 percent, for the group infected with the Ck/Yamaguchi/04 strain, it was 70 percent (7/10). These results suggest that although the virus multiplied to a certain extent inside the bodies of rats infected with the K11 strain infection, the growth was insufficient for excretion in feces or respiratory organs before the virus was eliminated from the body.

The above results make it clear that even through rats and mice are both rodents, the K11 strain varies in infectiousness, pathogenicity, and propagation within them.

Figure 4. Neutralizing antibody levels in infected rats



Section 4　Wild bird virus and antibody testing

(Toshihiro Ito)

1　Constant broadbased monitoring

1.1　Purpose

In 2003, the first outbreak of highly pathogenic avian influenza in Japan in 79 years was reported. Wild birds were suggested as a possible incursion route, the virus remains prevalent over a wide area centered on East Asia, and it is showing signs of becoming resident in Japan. The Ministry of the Environment has therefore carried out a series of studies of the status of the virus in wild birds in Japan, with the goal of monitoring the danger of incursion and infestation of this virus in Japan through migratory birds. During this fiscal year as well, monitoring was carried out a total of 13 times in 10 migratory-bird landing zones around Japan.

1.2　Testing methods

1.2.1　Sample collection

As shown in the table below, small terrestrial birds, shorebirds, and waders were captured at migratory bird stopovers, and pharyngeal and cloacal swabs and blood samples were taken. Locations, dates, bird species, and so on were recorded for each sample obtained. For blood samples, centrifuges were used in the field to separate serum, which was then refrigerated along with the other specimens and immediately sent to the Veterinary Public Health Science Laboratory, Faculty of Agriculture, Tottori University. For swans and wild ducks, fecal samples were also taken and sent in the same way.

1.2.2　Virus isolation and serological identification

Fecal samples were dissolved in phosphate buffered saline with added penicillin G potassium (10,000 units/ml; Banyu Pharmaceutical) and streptomycin sulfate (10,000 units/ml; Meiji Seika) to obtain 20–30 percent (w/w) and centrifuged at 2,000 rpm for 10 minutes. After that, 0.2 ml of the supernatant was injected into the chorioallantoic cavities of 10–11-day-old embryonated chicken eggs. The pharyngeal and cloacal swabs were dissolved in bouillon with the same antibiotics added and centrifuged at 2,000 rpm for 10 minutes. The supernatant was injected into embryonated chicken eggs in the same way. After incubation for 3 days at 35°C, the eggs were kept for one night at 4°C, and the chorioallantoic fluid was collected aseptically. Chicken hemagglutination (HA) testing was used to determine the presence of the virus in the chorioallantoic fluid. When no hemagglutination activity (HA activity) was found, the collected chorioallantoic fluid was injected again into new embryonated chicken eggs and the process was repeated. If HA activity was still not found, the sample was judged negative for the virus. Samples in which HA activity was detected were subjected to hemagglutination inhibition (HI) and neuraminidase inhibition (NI) tests using the microtiter method, and the serum subtype was identified.

1.3　Testing results

Testing results were as follows.

Test date	Location	Test subject	Virus isolation results	Antibody testing results
April	Okinawa City, Okinawa Prefecture	Waterfowl from Southeast Asia (Pacific golden plovers, sharp-tailed sandpiper, etc.: 68 birds)	Negative	Negative
April-May	Narashino City, Chiba Prefecture	Waterfowl from Southeast Asia (dunlins, grey plovers, etc.: 30 birds)	Negative	Negative
September	Wataricho, Miyagi Prefecture	Waterfowl from the Russian Far East (Terek sandpipers, grey-tailed tattlers, etc.: 56 birds)	Negative	Negative
	Narashino City, Chiba Prefecture	Waterfowl from the Russian Far East (Kentish plovers, grey-tailed tattlers, etc.: 4 birds)	Negative	Negative
October	Niigata City, Niigata Prefecture	Waterfowl from the Russian Far East (common teals, tundra swans, etc.: 38 birds)	Negative	NT
	Agano City, Niigata Prefecture	Waterfowl from the Russian Far East (northern pintails, etc.: 73 birds)	Negative	NT
November	Tsushima City, Nagasaki Prefecture	Terrestrial birds from China and Korea (yellow-throated buntings , rustic buntings, etc.: 110 birds)	Negative	Negative
January	Yamaguchi City, Yamaguchi Prefecture	Terrestrial birds from China and Korea (rooks, etc.: 37 birds)	Negative	Negative
	Narashino City, Chiba Prefecture	Waterfowl from the Russian Far East (dunlins, etc.: 46 birds)	Negative	Negative
	Arao City, Kumamoto Prefecture	Waterfowl from the Russian Far East (dunlins, etc.: 27 birds)	Negative	Negative
	Tamana City, Kumamoto Prefecture	Terrestrial birds from China and Korea (carrion crows, etc.: 12 birds)	Negative	Negative
	Ube City, Yamaguchi Prefecture	Waterfowl from the Russian Far East (black-headed gulls, etc.: 5 birds)	Negative	Negative
March	Okinawa City, Okinawa Prefecture	Waterfowl from Southeast Asia (common redshanks, Pacific golden plovers, etc.: 100 birds)	Negative	Negative
Total	13 times (10 locations)	606 birds	Negative	Negative

NT: Not tested

Virus isolation testing of feces and pharyngeal and cloacal swabs of these wild birds did not find the H5N1 highly pathogenic avian influenza virus in any specimen. Furthermore, HI testing of the sampled blood serum using the H5 subtype influenza virus A/whistling swan/Shimane/499/83(H5N3) strain as an antigen found no H5 antibodies in any specimen (serum diluted 1:4 or less).

2 Strengthened surveillance of South Korean and Japanese outbreaks

2.1 Purpose

In light of the confirmation in November 2006 of the highly pathogenic avian influenza virus (H5N1 subtype) in chickens in North Jeolla Province in southern South Korea, a five-month study of the status of the virus in wild birds in Japan began in December with the goal of enhancing wild bird surveillance.

2.2 Testing methods

2.2.1 Sample collection

As shown in the Table below, in addition to ordinary monitoring, fresh wild duck fecal samples were taken from major waterfowl habitats in western Japan. The specimens were individually labeled for location, date, species, and so on, refrigerated, and immediately sent to the Veterinary Public Health Science Laboratory, Faculty of Agriculture, Tottori University.

2.2.2 Virus isolation and serological identification

As in 1.2.2.

2.3 Testing results

Testing results were as follows.

December 2006

Location	No. of samples	Virus isolation results
Tottori City, Tottori Prefecture	320	Negative
Yamaguchi City, Yamaguchi Prefecture	15	Negative
Fukuoka City, Fukuoka Prefecture	10	Negative
Kashima City, Saga Prefecture	30	Negative
Ureshino City	10	Negative
Shiroishicho	10	Negative
Omura City, Nagasaki Prefecture	20	Negative
Tsushima City	18	Negative
Sasebo City	30	Negative
Kumamoto City, Kumamoto Prefecture	5	Negative
Tamana City	54	Negative
Izumi City, Kagoshima Prefecture	25	Negative
Higashikushiracho	134	Negative
Total	681	Negative

January 2007

Location	No. of samples	Virus isolation results
Tottori City, Tottori Prefecture	30	Negative
Yasugi City, Shimane Prefecture	48	Negative
Fukuoka City, Fukuoka Prefecture	41	Negative
Shingu-machi	30	Negative

Kashima City, Saga Prefecture	53	Negative
Ureshino City	25	Negative
Sasebo City, Nagasaki Prefecture	73	Negative
Omura City	15	Negative
Tsushima City	36	Negative
Higashisonogicho	50	Negative
Kumamoto City, Kumamoto Prefecture	53	Negative
Tamana City	90	Negative
Izumi City, Kagoshima Prefecture	50	Negative
Kagoshima City	50	Negative
Total	614	Negative

February 2007

Location	No. of samples	Virus isolation results
Kohokucho, Shiga Prefecture	50	Negative
Kameoka City, Kyoto Prefecture	50	Negative
Kamocho	50	Negative
Fukuchiyama City	12	Negative
Tondabayashi City, Osaka Prefecture	51	Negative
Kishiwada City	49	Negative
Sennan City	47	Negative
Kagogawa City, Hyogo Prefecture	50	Negative
Itami City	50	Negative
Yamatokoriyama City, Nara Prefecture	9	Negative
Nachikatsuuracho, Wakayama Prefecture	71	Negative
Shingu City	29	Negative
Yonago City, Tottori Prefecture	50	Negative
Hiezuson	50	Negative
Yasugi City, Shimane Prefecture	20	Negative
Izumo City	20	Negative
Okayama City, Okayama Prefecture	150	Negative
Hiroshima City, Hiroshima Prefecture	65	Negative
Hatsukaichi City	55	Negative
Yamaguchi City, Yamaguchi Prefecture	100	Negative
Ube City	50	Negative
Anan City, Tokushima Prefecture	110	Negative
Zentusji City, Kagawa Prefecture	50	Negative
Marugame City	65	Negative
Saijo City, Ehime Prefecture	50	Negative
Ozu City	50	Negative
Matsuyama City	50	Negative
Nankoku City, Kochi	50	Negative

Prefecture		
Susaki City	50	Negative
Kochi City	50	Negative
Kitakyushu City, Fukuoka Prefecture	50	Negative
Shingu-machi	50	Negative
Fukuoka City	35	Negative
Shiroishicho, Saga Prefecture	50	Negative
Imari City	100	Negative
Tsushima City, Nagasaki Prefecture	25	Negative
Sasebo City	100	Negative
Omura City	50	Negative
Kumamoto City, Kumamoto Prefecture	100	Negative
Usa City, Oita Prefecture	50	Negative
Kitsuki City	50	Negative
Oita City	50	Negative
Nichinan City, Miyazaki Prefecture	50	Negative
Nangocho	100	Negative
Kimotsukicho, Kagoshima Prefecture	50	Negative
Osakicho	50	Negative
Total	2563	Negative

2007 March

Location	No. of samples	Virus isolation results
Kohokucho, Shiga Prefecture	50	Negative
Takatsukicho	50	Negative
Kameoka City, Kyoto Prefecture	50	Negative
Tondabayashi City, Osaka Prefecture	50	Negative
Sennan City	30	Negative
Kagogawa City, Hyogo Prefecture	50	Negative
Itami City	50	Negative
Yamatokoriyama City, Nara Prefecture	50	Negative
Nachikatsuuracho, Wakayama Prefecture	52	Negative
Shingu City	6	Negative
Yonago City, Tottori Prefecture	50	Negative
Hiezuson	50	Negative
Yasugi City, Shimane Prefecture	50	Negative
Izumo City	50	Negative
Okayama City, Okayama Prefecture	100	Negative
Hiroshima City, Hiroshima Prefecture	50	Negative
Hatsukaichi City	27	Negative
Yamaguchi City, Yamaguchi Prefecture	100	Negative
Anan City, Tokushima	60	Negative

Prefecture		
Zentusji City, Kagawa Prefecture	50	Negative
Marugame City	50	Negative
Saijo City, Ehime Prefecture	50	Negative
Ozu City	50	Negative
Nankoku City, Kochi Prefecture	50	Negative
Susaki City	50	Negative
Kitakyushu City, Fukuoka Prefecture	50	Negative
Shingu-machi	2	Negative
Shiroishicho, Saga Prefecture	50	Negative
Imari City	50	Negative
Tsushima City, Nagasaki Prefecture	25	Negative
Sasebo City	100	Negative
Kumamoto City, Kumamoto Prefecture	50	Negative
Tamana City	50	Negative
Usa City, Oita Prefecture	50	Negative
Kitsuki City	50	Negative
Nichinan City, Miyazaki Prefecture	21	Negative
Kimotsukicho, Kagoshima Prefecture	23	Negative
Osakicho	50	Negative
Total	1846	Negative

April 2007

Location	No. of samples	Virus isolation results
Nagahama City, Shiga Prefecture	30	Negative
Kameoka City, Kyoto Prefecture	30	Negative
Kishiwada City, Osaka Prefecture	29	Negative
Kagogawa City, Hyogo Prefecture	30	Negative
Nachikatsuuracho, Wakayama Prefecture	30	Negative
Yonago City, Tottori Prefecture	30	Negative
Matsue City, Shimane Prefecture	30	Negative
Okayama City, Okayama Prefecture	30	Negative
Hiroshima City, Hiroshima Prefecture	31	Negative
Anan City, Tokushima Prefecture	30	Negative
Marugame City, Kagawa Prefecture	20	Negative
Matsuyama City, Ehime Prefecture	30	Negative
Susaki City, Kochi Prefecture	23	Negative
Kitakyushu City, Fukuoka	30	Negative

Prefecture		
Shiroishicho, Saga Prefecture	30	Negative
Imari City, Saga Prefecture	30	Negative
Kumamoto City, Kumamoto Prefecture	30	Negative
Kitsuki City, Oita Prefecture	30	Negative
Miyazaki City, Miyazaki Prefecture	30	Negative
Kimotsukicho, Kagoshima Prefecture	30	Negative
Total	583	Negative

Virus isolation testing of these wild waterfowl feces found no H5N1 highly pathogenic avian influenza virus in any specimen.

3 Testing for the virus in wild birds in the vicinity of outbreak farms following confirmation of outbreaks

3.1 Purpose

In light of the outbreaks of highly pathogenic avian influenza in Miyazaki and Okayama Prefectures, wild birds near the outbreak farms were examined for viruses and antibodies in order to contribute to elucidation of wild bird infection status and infection routes.

3.2 Testing methods

3.2.1 Sample collection

A survey of wild waterfowl inhabitation within 10-kilometer radiuses of the outbreak farms in Kiyotakecho, Hyuga City, and Shintomicho, Miyazaki Prefecture, and Takahashi City, Okayama Prefecture, was carried out. At the same time, areas where sampling is possible were examined. Based on that research, fresh wild duck fecal samples were obtained. The specimens were individually labeled for location, date, species, and so on, refrigerated, and immediately sent to the Veterinary Public Health Science Laboratory, Faculty of Agriculture, Tottori University. Furthermore, a study of other wild birds (terrestrial migratory birds) within 10-kilometer radiuses of the outbreak farms was carried out in the same way, with a survey of their inhabitation and examination of possible sampling sites. Based on that research, mist nets and so on were used to capture wild birds, and pharyngeal and cloacal swabs and blood samples were taken. The specimens were individually labeled for location, date, species, and so on, and centrifuges were used to separate serum from the blood samples. The specimens were refrigerated and immediately sent to the Veterinary Public Health Science Laboratory, Faculty of Agriculture, Tottori University.

3.2.2 Virus isolation and serological identification

As in 1.2.2.

3.3 Testing results

Testing results are as follows.

Examination of wild duck feces in areas around outbreak farms

Test date	Location	No. of samples	Virus isolation testing results	Antibody presence test results
January 24-27	Kiyotakecho, Miyazaki Prefecture	150	Negative	Negative
February 1-3	Hyuga City, Miyazaki Prefecture	100	Negative	Negative
February 6-8	Takahashi City, Okayama Prefecture	104	Negative	Negative
February 6-8	Shintomicho, Miyazaki Prefecture	109	Negative	Negative
Total		463	Negative	Negative

Examination of small terrestrial birds captured in areas around outbreak farms

Test date	Location	No. of samples	Virus isolation results	Antibody testing results
January 24-27	Kiyotakecho, Miyazaki Prefecture	102 *1	Negative	Negative
February 1-3	Hyuga City, Miyazaki Prefecture	102 *2	Negative	Negative
February 6-8	Takahashi City, Okayama Prefecture	105 *3	Negative	Negative
February 6-8	Shintomicho, Miyazaki Prefecture	104 *4	Negative	Negative
Total		413 birds	Negative	Negative

*1 Migratory birds (red-flanked bluetails 1, Daurian redstarts 6, pale thrushes 7, thrushes 1, black-faced buntings 24)
Resident birds (white wagtail 1, Japanese wagtails 2, bulbuls 2, shrike 1, bush warbler 1, fantail warblers 8, meadow bunting 1, grey-headed bunting 1, Oriental greenfinches 44)

*2 Migratory birds (red-flanked bluetails 3, Daurian redstarts 4, pale thrushes 6, black-faced buntings 10, yellow-throated buntings 23, olive-backed pipit 1)
Resident birds (bulbuls 2, shrikes 2, bush warblers 5, Japanese white-eyes 8, meadow buntings 2, varied tits 4, long-tailed tits 23, great tits 7, red-billed leiothrixes 2)

*3 Migratory birds (red-flanked bluetails 10, Daurian redstart 1, pale thrushes 10, black-faced buntings 40, yellow-throated bunting 1, olive-backed pipit 1, Eurasian siskin 1, grey bunting 1)
Resident birds (Japanese wagtail 1, shrikes 2, bush warblers 5, Japanese white-eyes 3, meadow buntings 11, Oriental greenfinches 1, varied tits 1, long-tailed tits 12, great tits 2, pygmy woodpecker 1, common kingfisher 1)

*4 Migratory birds (red-flanked bluetails 1, Daurian redstarts 3, pale thrushes 15, black-faced buntings 45, yellow-throated buntings 5, bramblings 2, Siberian rubythroats 1)
Resident birds (bulbuls 2, shrikes 1, bush warblers 8, Japanese white-eyes 15, meadow buntings 3, varied tits 1, great tits 2)

Virus isolation testing of these wild bird feces and pharyngeal and cloacal swabs found no H5N1 highly pathogenic avian influenza virus in any specimen. Furthermore, HI testing of the sampled blood serum using the H5 subtype influenza virus A/whistling swan/Shimane/499/83(H5N3) strain as an antigen found no H5 antibodies in any specimen (serum diluted 1:4 or less).

4 Isolation of the virus from mountain hawk-eagles in Kumamoto Prefecture

4.1 Particulars (excerpted from Ministry of the Environment press release)

On January 4, 2007, an emaciated mountain hawk-eagle (adult female) was collected in Sagaramura, Kumagun, Kumamoto Prefecture, and died immediately after. Because the bird was emaciated despite having no external injuries, the Kyushu Regional Environment Office of the 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. Regardless of whether lead poisoning was involved, because 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 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 is unknown. (For the general ecology of the mountain hawk-eagle, see Section 5 4.2, "The general ecology of the mountain hawk-eagle.")

4.2 Research methods

4.2.1 Virus isolation and serological identification

Pharyngeal and cloacal swabs were taken from the carcass of the mountain hawk-eagle, dissolved in bouillon with antibiotics added, and centrifuged at 2,000 rpm for 10 minutes. After that, the supernatant was injected into 10-day-old embryonated chicken eggs. After incubation for 2 days at 35°C, the eggs were kept for one night at 4°C, and the chorioallantoic fluid was collected aseptically. Chicken hemagglutination (HA) testing was used to determine the presence of the virus in the chorioallantoic fluid. Specimens in which HA activity was detected were subjected to hemagglutination inhibition (HI) and neuraminidase inhibition (NI) tests using the microtiter method, and the serum subtype was identified.

4.2.2 HA gene sequencing

The HA gene from the virus isolated from the mountain hawk-eagle virus was amplified using the primers below, and the base sequence was determined.

pol.ISW499-HA(+)(CACACACGTCTCCGGGAGCAAAAGCAGGGGTCTRATCTAYYAAAAT)

H5HA360 (TCAACGACTATGAAGAGCTG)

H5H665 (TATGTGTCTGTAGGAACATCAACGC)

H5HR441 (CCTGATGAGGCATCGTGGTTGGACC)

H5HR1021 (CCATACCAACCGTCTACCATTCC)

H5HR782 (TTGGCTTTAAGATTGT CCAG)

H5HA920 (ATGGGTGCAATAAACTCCAG)

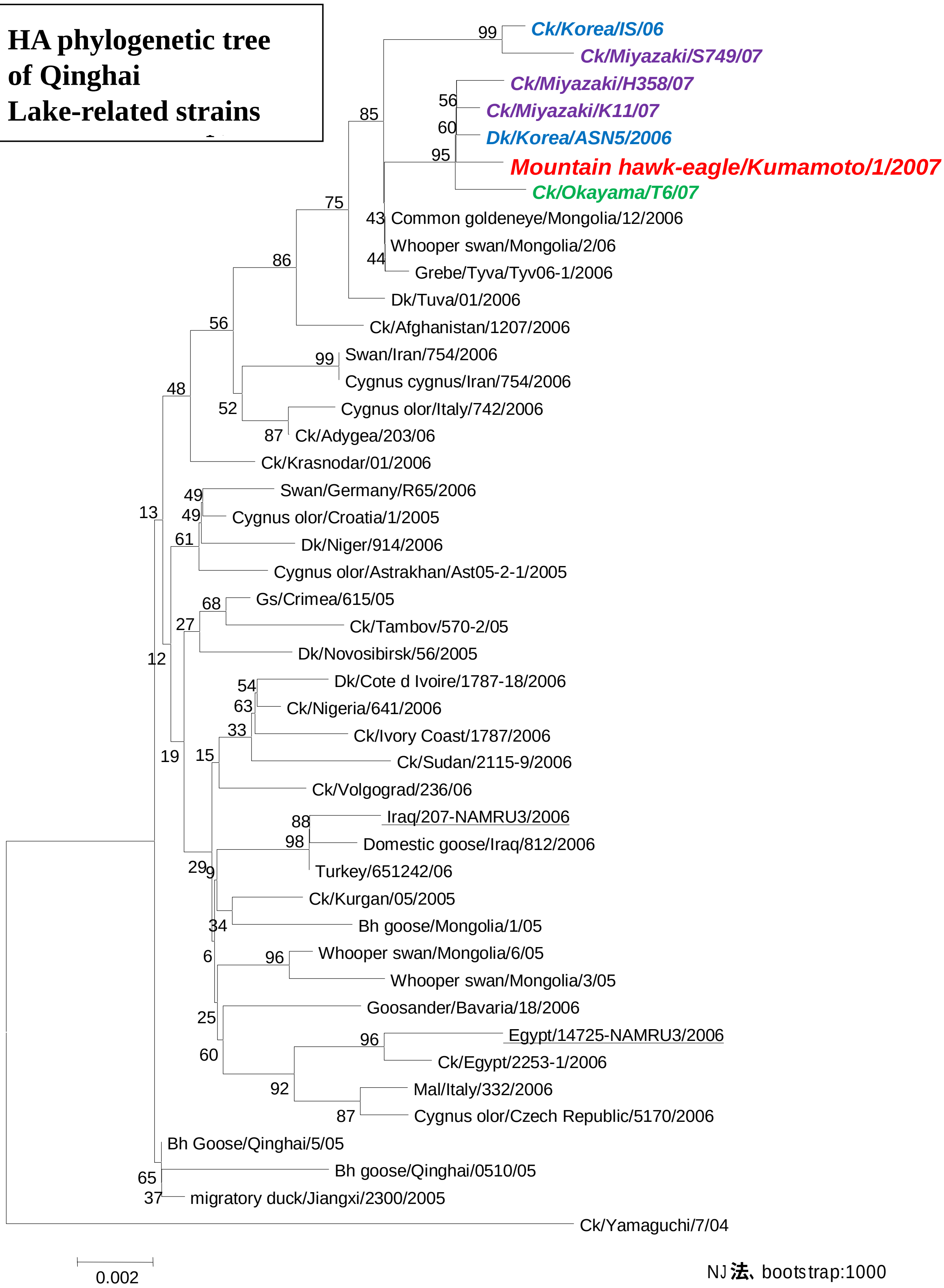
4.2.3 Mapping an evolutionary tree

A phylogenetic tree was created with the neighbor-joining method using HA gene HA1 region base sequence data for 38 isolated wild H5 subtypes from the DNA Data Bank (DDBJ) as well as data on the five strains isolated from chickens and the mountain hawk-eagle.

4.3 Research results

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 typical of virulent types. As for homology with other H5 viruses, it is most homologous (at least 99.7 percent) 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 relationships with other viruses (see attached figure). It is closest to the A/chicken/Miyazaki/K11/07 strain isolated in Miyazaki, demonstrating that this virus is also a Qinghai Lake-type.

HA phylogenetic tree of Qinghai Lake-related strains



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Section 5 Information on wild birds

(Yutaka Kanai)

Wild birds may be infected with the avian influenza virus at any time. Wild ducks in particular require caution, because even when infected they rarely show any symptoms. Because Japan's outbreaks occurred in winter, this section summarizes information on the relationships between wintering bird migrations and habitats and outbreaks.

1 Wintering bird migrations and habitats

1.1 Wintering bird species

Because Japan is located in the middle latitudes, the birds that inhabit it vary by season. According to the Ornithological Society of Japan's *Check-List of Japanese Birds (6th ed.)*, about 550 species of birds have been confirmed to live in Japan. Of these, about 130 species of residential birds are present year round, while about 100 species of wintering birds fly in for the wintering. Even among the residential bird species present in Japan all year, some individuals may have flown in for the winter.

Waterfowl such as geese, swans, and ducks are well known as wintering migratory birds, but they also include many small forest birds such as thrushes and meadow buntings.

1.2 Migration routes of wintering birds

The breeding grounds of Japan's wintering birds are mainly in Alaska, the Russian Far East, eastern Siberia, eastern Mongolia, and northeastern China. Banding research on wild ducks has shown that they fly to Japan from those regions (Yamashina Institute for Ornithology 2002). The main migration routes to Japan are (1) Kamchatka Peninsula to Hokkaido, (2) the Russian Far East to Sakhalin and Hokkaido, (3) the Russian Far East and northeastern China to Honshu across the Sea of Japan, and (4) from northeastern China to the Korean Peninsula to southwestern Japan.

Figure 2 summarizes the results of past satellite tracking (Photograph 1) of waterfowl that winter in Japan. Wild geese are known to use the Kamchatka Peninsula route (Ozaki, et al., 1999), Tundra Swans (Higuchi, et al., 1991) and Whooper Swans (Kanai, et al., 1997) the Sakhalin route, Tundra Swans the Sea of Japan route (Kamiya and Ozaki 1999), and Hooded Cranes, White-naped Cranes (Higuchi, et al., 1992), and Black-faced Spoonbills (Wild Bird Society of Japan and Ministry of the Environment) the Korean Peninsula route. The Ministry of the Environment commissioned Professor Hiroyoshi Higuchi of the Laboratory of Biodiversity Science, University of Tokyo, to carry out a "project to elucidate the flight paths of migratory birds" by satellite tracking of wild ducks. In addition, as a research area within the coordinated policies on new and reemergent infectious diseases of the Council for Science and Technology Policy, Cabinet Office of Japan, research on "ecological elucidation and genome analysis of viruses from wild birds" led by Dr. Akio Yamada of the National Institute of Infectious Diseases began in FY 2005. Professor Hiroyoshi Higuchi led research tracking the migration of wild ducks by satellite. These projects also found that birds fly to Japan via Kamchatka and Sakhalin, and from the Russian Far East across the Sea of Japan (Ministry of the Environment 2007, Higuchi 2007).

Although little is known about the migration routes of birds that winter in forests, it is likely that many of these forest birds follow the same routes.

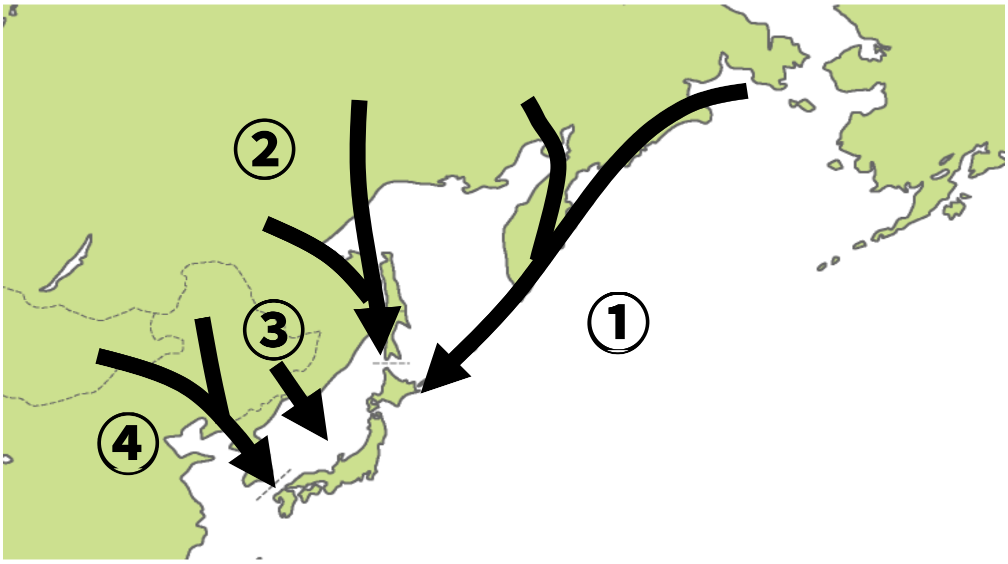


Figure 1. Main migration routes of wintering birds to Japan
(1) Kamchatka Peninsula route, (2) Sakhalin route, (3) Sea of Japan route, (4) Korean Peninsula route

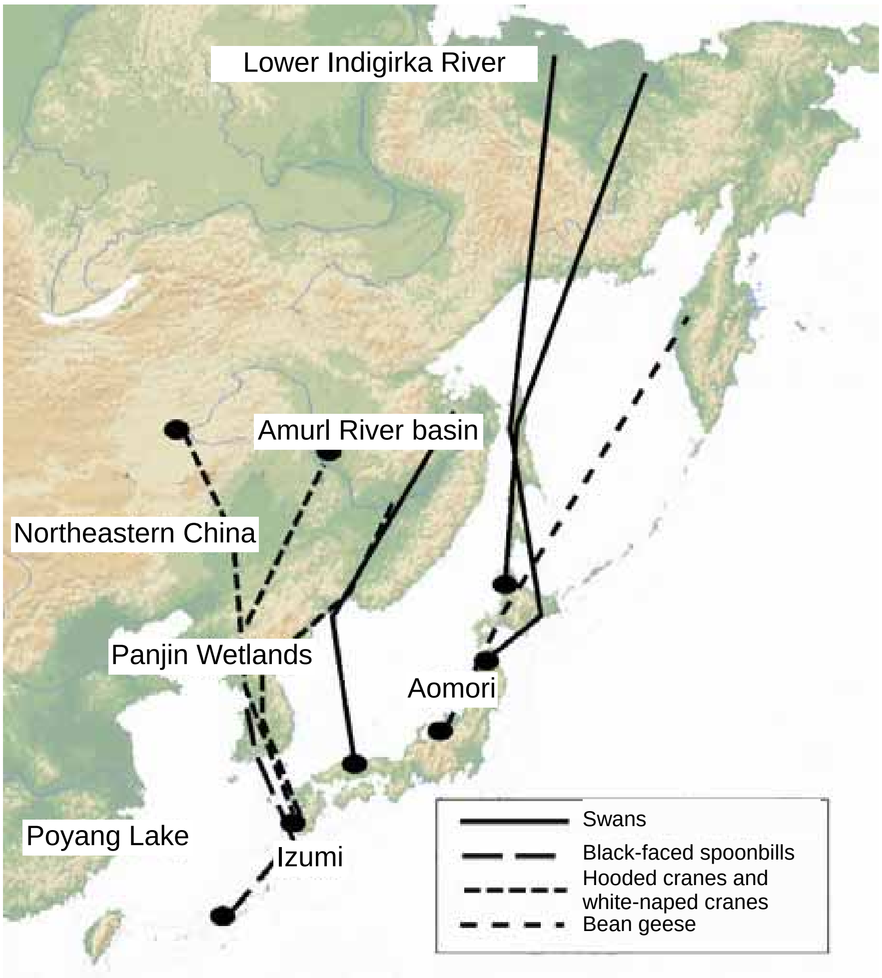


Figure 2. Migration routes of wintering waterfowl to Japan as found by satellite tracking
The results of satellite tracking from 1989 through 2005 are summarized.

Photograph 1. Whooper swan wearing a transmitter
(Kominato, Aomori Prefecture)

1.3 Migration periods

For both wintering waterfowl and terrestrial birds, winter bird migration peaks from October into November and is mostly complete in early December. From mid-December, some individuals will move south as cold climate bring snow accumulation and freezing of surface water. Because recent winters have been warmer with less snow, wintering grounds have been moving north as more waterfowl remain in northern Tohoku and Hokkaido.

Figure 3 depicts daily changes in the number of terrestrial wintering birds caught and released each day during the autumn migration period at the Otayama Banding Station in Fukui Prefecture (Ministry of the Environment and Yamashina Institute for Ornithology 1997). The data were accumulated over 25 years, from 1973 through 1997. The Figure depicts the average number of birds caught and released on each day. The migration begins in early October, peaks in late October through early November, and is almost over by mid-November.

Figure 4 shows changes in the number of wild geese, swans, and ducks inhabiting Katanokamoike in Kaga City, Ishikawa Prefecture, from autumn to spring in 2004–2005 and 2005–2006 (Wild Bird Society of Japan and Katanokamoike Ecosystem Management Council 2006). In both years, the number of inhabitants began increasing in October, with more increasing in during November through early December, and little change after that. The migration thus ends in early December.

Forest-dwelling migratory birds probably live in mountainous areas immediately after arriving in Japan, but move to lower and warmer areas as ripe fruits, nuts, and berries decrease, snow increases, and temperatures fall. In fact, however, little is known about their movements. With warmer winters and less snowfall, the wintering grounds of wild geese, swans, and ducks have been moving northwards. More of these birds are wintering in northern Tohoku and Hokkaido, which used to be only stopovers, although they do move south when snow is heavy. Like waterfowl, forest-dwelling birds probably shift their wintering grounds in relation to climate during the wintering period.

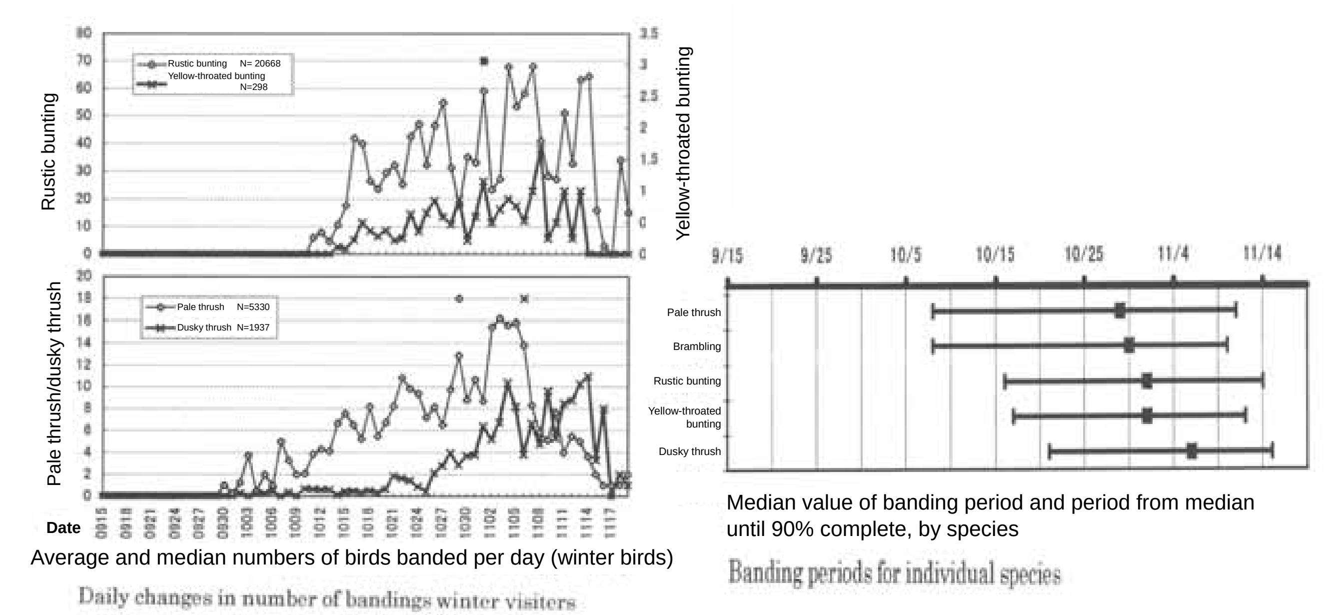
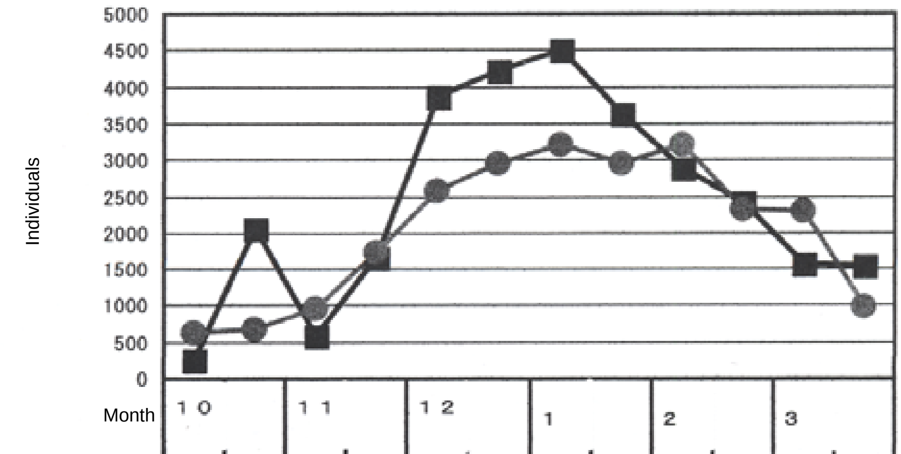


Figure 3. Changes over time in the number of birds banded at the Otayama Banding Station in Fukui Prefecture

Terrestrial wintering birds arrive in Japan mainly from early October through mid-November. (Ministry of the Environment and Yamashina Institute for Ornithology 1997 FY 1996 Report on Banding Research)



Month	10		11		12		1		2		3	
FY 2005	247	2057	590	1662	3877	4227	4513	3642	2885	2417	1573	1540
FY 2004	642	683	971	1748	2575	2962	3227	2961	3226	2331	2290	985

Figure 4. Changes in the number of wild ducks inhabiting Katanokamoike, Kaga City, Ishikawa Prefecture

Increases in the number of inhabitants due to the arrival of migratory birds are mainly in November through early December.

1.4 Distribution of major waterfowl habitats

Figure 5 shows large-scale waterfowl habitats in Japan that meet the registration criteria of the Ramsar Convention. Geese, swans, and ducks rest in wetlands around ponds, marshes, and rivers, and feed nearby. Because geese and ducks feed on water organisms such as fallen grains of rice in rice paddies and seeds and shoots along watersides, lowlands with many rice paddies and bodies of water meet their habitat needs. The major waterfowl habitats in Japan are therefore in lowlands in Hokkaido, Tohoku, Kanto, Hokuriku, Tokai, Kinki, northern Kyushu, and western Kyushu.

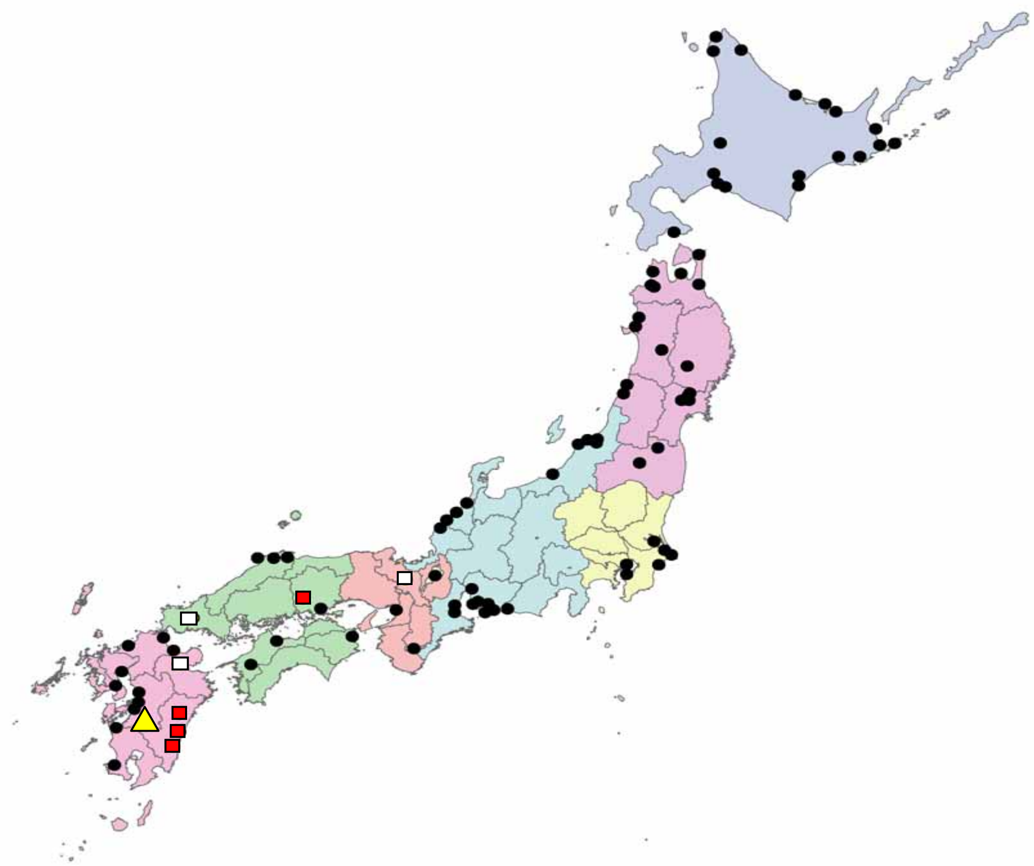


Figure 5. Locations of major waterfowl habitats and infection outbreak areas (and collection location)

Large-scale waterfowl habitats that meet the criteria for Ramsar Convention registration are shown as black circles. The white squares represent the 2004 outbreaks, the red squares the 2007 infection outbreak areas, and the yellow triangle the location in Kumamoto Prefecture where the mountain hawk-eagle was found.

1.5 Relationship between Japan and South Korea

South Korea on the southern Korean Peninsula and the Tohoku region in Honshu lie at about the same latitude. Like Japan, South Korea is a wintering ground for birds in East Asia, and the winter bird species and their migration periods are about the same. Swan and geese migrations end on the southern Korean Peninsula, so those that winter in Japan must use migration routes other than the Korean Peninsula route. As in Japan, waterfowl habitats in South Korea are in lowlands, along the western coast. Large-scale draining in recent years has increased the area of rice paddies, resulting in improved feeding conditions for geese and ducks. Warmer winters have ended the freezing of bodies of water, increasing the number of individual cranes and ducks that winter on the Korean Peninsula rather than moving on to Japan. Among forest-dwelling birds, pale thrushes, Daurian redstarts, and yellow-throated buntings are resident birds in South Korea but wintering birds in Japan.

Very little is known about whether birds journey between Japan and Korea during the wintering period from December through February. When feeding becomes difficult in South

Korea because there is heavy snowfall or severe cold waves causing bodies of water to freeze, birds might move to Japan. There appears not to have been any such widespread snow accumulation or freezing from December 2006 through February 2007.

2 Periods of infection outbreaks and migration periods

Table 1 summarizes infection outbreaks in Japan and Korea according to a temporal axis. In South Korea, outbreaks occur beginning in the late November autumn migration period and continue through the wintering period until March. An H5N1 subtype virus was detected in the feces of wild ducks at a river near an outbreak area on December 21 during the wintering period.

Infection outbreaks in Japan are at least a month later than those in South Korea, occurring in early through late January 2007, after the end of the major migration period. This outbreak pattern is extremely similar to the pattern for the Japanese and Korean outbreaks during 2003–2004.

Month		Migration period	Infection-related information
November	1	Autumn migration	
	5		
	10		
	15		
	20		(1) Iksan-city, Jeollabuk Province 19 birds dead
	25		
			(2) Iksan-city, Jeollabuk Province 2-case outbreak announced
	30		
December	5		
			(3) Gimje-city, Jeollabuk Province 20 quail dead
	10		(4) Asan-city, Chungcheongnam Province Duck egg production reduced
	15		
	20		Collection of duck feces at Pungse Stream River and Miho River H5N1 virus later confirmed
	25		
	30		
January			Infected mountain hawk-eagle captured for protection in Youra, Saguramura, Kumagun, Kumamoto Prefecture Dies
	5		(1) Kiyotakecho, Miyazaki Prefecture 21 birds dead
	10		
	15		
	20		(5) Cheonan-city, Chungcheongnam Province 157 birds dead
			(2) Togocho, Hyuga City, Miyazaki Prefecture 243 birds dead
	25		(3) Kawakamicho, Takahashi City, Okayama Prefecture Deaths
	30		(4) Shintomicho, Miyazaki Prefecture 23 birds dead
February	5		(6) Anseong-city, Gyeonggi Province Infection
	10		
	15		
	20	Spring migration	
	25		
March	5		
			(7) Cheonan-city, Duck farm Infection
	10		

Table 1. Japanese and Korean infection outbreak periods and major migration periods
Areas in rectangles in the Table represent outbreaks in Japan.

3 Infection outbreak locations and wild bird habitats

3.1 Infection outbreak areas and waterfowl habitats

Infection outbreak areas in Japan in both 2004 and 2007 were in southwestern Japan. They are scattered in mountainous areas far from concentrations of large-scale waterfowl habitats with enough birds to meet the criteria for Ramsar Convention registration.

Miyazaki Prefecture has less level ground than other parts of Kyushu, so the number of wild ducks that winter there is relatively small. The major wintering ground in the prefecture is in Miyazaki City between the Oyodo River and the Omaru River, an area where there are many rice paddies and irrigation ponds. The Case 4 outbreak area is in this location.

Okayama Prefecture has large-scale goose and duck habitats near Okayama City at Kojima Bay and the Yoshii River estuary, and in the Kasaoka reclaimed land area and other lowlands facing the Seto Inland Sea. Overall, however, the prefecture is rather mountainous, and waterfowl habitats are limited to coastal areas. The infection outbreak area and large-scale waterfowl habitats have little relevance to one another.

The South Korean outbreaks are concentrated in lowlands along that country's west coast, where there are many large-scale goose and duck habitats (Li and Mundkur 2007; see Figure 6). There are rice paddies and medium-sized rivers next to the farms, conditions that invite the arrival of ducks. Some of the 2006–2007 outbreak areas are notably close to the 2003–2004 outbreak areas. Natural environmental conditions differ from those in the Japanese outbreaks.

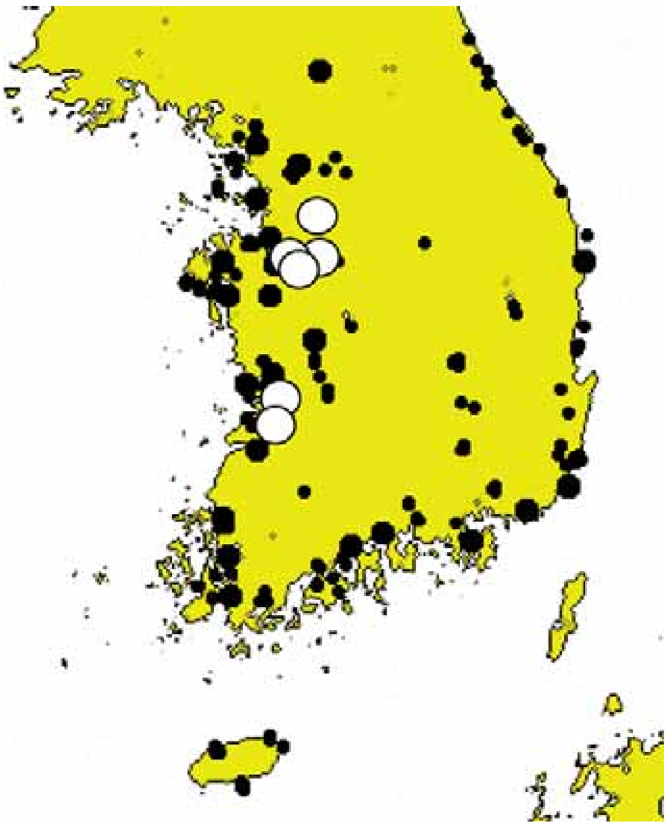


Figure 6. Distributions of South Korean waterfowl wintering grounds and 2006-2007 outbreaks

The black dots represent waterfowl habitats, while the white circles represent infection outbreak areas. The outbreaks in South Korea occurred in low-lying rice paddy districts near the coast, close to major waterfowl wintering grounds. Waterfowl habitats are taken from the *Asian Waterbird Census 2002-2004* (Li and Mundkur

2007).

3.2 Natural environments in infection outbreak areas and wild bird habitats

3.2.1 Kiyotakecho, Miyazaki Prefecture

On a plateau near the Kiyotake River, the area is surrounded by housing and farmland. Large-scale cultivation takes place in the plain along the Kiyotake River, but there are few rice paddies where waterfowl can glean fallen grains in winter. It is not a favorable habitat for wild ducks. Interviewees reported that ducks fly to farmland next to the infection outbreak area, but in fact these are rare cases. The area is home to Oriental greenfinches, white wagtails, and other birds that inhabit farmland, but there are few forest-dwelling birds. Swallows fly there.

No wild birds were found inside the poultry houses. No infections were found in pet birds in the area, which are likely to have more contact with sparrows and other wild birds that live around human habitations.

Photograph 2. Location of the outbreak area in Kiyotakecho, Miyazaki Prefecture

It is a plateau surrounded by farmland and housing, without the wetlands that waterfowl prefer. The farms grow daikon radishes, cabbages, and wheat. In winter, the fields are left bare or as grassland.

3.2.2 Hyuga City, Miyazaki Prefecture

It is a steeply sloping mountainous area. The adjacent river is narrow, and there is little grassland. There is little in the way of plains along the river, and few wild ducks are likely to inhabit the region. The outbreak farm is in a branch valley on steeply sloping land. Waterfowl do not come there. The area is surrounded by secondary forest, so there are many mountain-forest birds. Reportedly, there were no incursions of wild birds into the poultry houses.

Photograph 3. Location of the outbreak area in Hyuga City, Miyazaki Prefecture

The entrance to the valley where the farm is located (left) and the area around the farm (right)

Photograph 4. Environment surrounding poultry houses

The poultry houses are in mountain valley. Waterfowl are unlikely to come there.

3.2.3 Takahashi City, Okayama Prefecture

It is similar to the Hyuga City outbreak area in its sloping mountainous land. The adjacent river is narrow, with little grassland. There is little in the way of plains along the river, and few wild ducks are likely to inhabit the region. The outbreak farm is next to some riverside rice paddies, but the paddies do not cover a large area. Widgeons and mallards inhabit an area about 5 kilometers away. There is grassland along the riverbanks there, and because ducks can find food in the grass and the river, they are unlikely to move very far away. The mountain slopes in the area are covered with secondary forest, so there are many mountain-forest birds in the area.

There were reportedly no incursions of wild birds into the poultry houses. Japanese wagtails are seen on the land next to the farm, and feces thought to belong to that species were found on poultry house walls. Japanese wagtails do not travel far and spend the entire year in one area.

Photograph 5. Environment of the outbreak area in Takahashi City, Okayama Prefecture

While there are rice paddies and a river, they are not large, and waterfowl are unlikely to come there (left). The poultry houses are next to grassland and mountain forest, so many terrestrial birds are likely to visit (right).

3.2.4 Shintomicho, Miyazaki Prefecture

Located on a plateau along the Hitotsuse River, the poultry house is next to farmland and mixed forest. Eggshells and chickens feathers were scattered in the mixed forest by the poultry house, and bird and weasel feces were found. Because there are many rice paddies and irrigation ponds along the Hitotsuse River, many wild ducks inhabit the area. In particular, the Kotaike across the Hitotsuse River from the outbreak area is one of the prefecture's leading wild duck wintering grounds. During the 2000 wintering period, over 2,000 ducks were recorded there.

Because the farm is surrounded by fields and mixed forest, wild ducks probably do not come directly to the adjacent area. There are many terrestrial birds in the area's mixed forests and on its farmland. There were reportedly incursions of sparrows and other small birds into the poultry house.

Photograph 6. Environment in the outbreak area in Shintomicho, Miyazaki Prefecture (left) and rice paddies near the outbreak area (right)

4 Infection in Mountain Hawk-eagles

4.1 Infection outbreaks in Mountain Hawk-eagles

On January 4, 2007, an emaciated Mountain Hawk-eagle (adult female) was collected from a forest road in Sagaramura, Kumagun, Kumamoto Prefecture and died immediately afterwards. On March 18, Tottori University reported isolation of an H5N1 subtype avian influenza virus. On March 23, its high virulence was confirmed. The direct cause of the bird's death is unknown. (For details, see Section 4 4.1 "Particulars.")

The geography where the bird was found is steeply sloping, with a riverbed at an elevation of 200 meters and the adjacent ridges at over 700 meters. The area is covered with mixed evergreen coniferous and deciduous broadleaf trees, but the trees are not very tall. Three hundred meters upstream is the planned site of a river dam, and 500 meters upstream, there is an open area that is under construction. Although there is a deepwater settlement about 700 meters downstream, overall there are few dwellings in the area.

4.2 The general ecology of the Mountain Hawk-eagle

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 kilometers. Young birds may travel long 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 have been reported in Northeastern China as well.

4.3 Discussion of source of infection

It is unclear whether this Mountain Hawk-eagle was from the area where it was picked up or had moved there from another area. It is thus unclear where it was infected. However, it is more likely to have been infected by consuming food with the virus somewhere in Kyushu than to have been infected in Korea and then flown across to Japan. There were no infections of poultry or abnormal wild bird deaths in the area where the mountain hawk-eagle was picked up. Because of the species' varied diet, it is difficult to surmise the source of the infection.

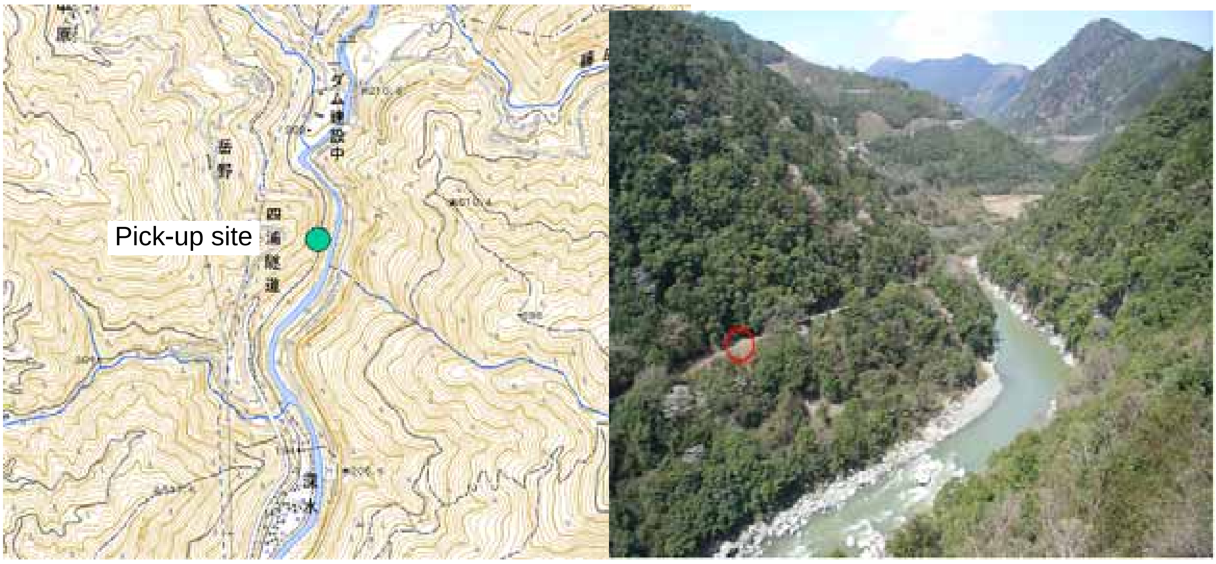


Figure 7. Map (left) and photograph (right) of the location where the infected Mountain Hawk-eagle was picked up in Sagaramura, Kumamoto Prefecture.
The circle is where the bird was found.

5 Wild birds and conveyance of the virus

5.1 Summary on introduction of the virus into Japan by wild birds

Because the outbreaks of infection with highly pathogenic avian influenza in Japan in 2007 occurred during the wintering season for wild birds whose migration routes include the Korean Peninsula, where there had been outbreaks, it was considered possible that the virus was brought to Japan by migratory birds. Furthermore, there was an infection in a wild Mountain Hawk-eagle, suggesting that the virus exists in the wild.

However, examination of current knowledge of wild birds, as will be discussed below, provides no evidence or reasons to conclude that wild bird migration brought the virus from far away.

Current knowledge of wild birds can be summarized as follows.

(1) The infection outbreaks in Japan occurred after the October and November migration periods of both waterfowl and terrestrial birds had ended.
(2) Research sponsored by the Ministry of the Environment on the presence of the virus in wild birds in major wintering grounds and infection outbreak areas did not find the virus.
(3) Most of the infection outbreak areas are distant from major habitats of the waterfowl considered likely to have the virus, and few of the farms are in environments that attract waterfowl.
(4) Migratory terrestrial birds inhabit the area around the outbreak poultry farms. Although terrestrial birds can be infected, they are unlikely to carry the virus for long periods if infected.
(5) The infection in a Mountain Hawk-eagle suggests that the virus exists in the wild, but it is unknown whether wild birds were the source of that infection.

5.2 Conditions that attract wild birds to poultry houses

Animal husbandry buildings are attractive to wild birds in numerous ways. Food is the primary factor that attracts them. This section examines factors attracting wild birds at the outbreak poultry farms, especially food sources.

(1) Feed

Chicken feed is a major attraction for wild birds. When feed is spilled on a farm, it attracts grain-eaters such as sparrows, Oriental Turtledoves, and Meadow Buntings. There was no remarkable feed spillage on any of the outbreak farms.

(2) Insects

Flies and other insects generated by poultry houses are an attractive food source for wild birds. This attraction is especially powerful in winter, when there are few insects outdoors. When wild birds defecate in places where there are flies, the birds' feces can be carried on or inside the flies' bodies.

Flies and other insects were observed to inhabit the infection outbreak poultry farms, so there is a strong possibility that they attracted wagtails, thrushes, and other birds that feed heavily on insects.

(3) Plants around poultry houses

The fruits and seeds of plants are an important food source for wild birds. When birds eat fruit, seeds may remain in their feces, and rodents and other small seed-eating animals may consume the feces along with the seeds. The Hyuga City outbreak poultry farm is surrounded by a forest, where there are probably many food sources, but the Japanese wax trees next to the poultry house were fruiting. In order to avoid attracting wild birds, fruit-bearing trees should not be planted on chicken farms. Furthermore, a 1-meter wide area around poultry houses should be paved so that bushes cannot grow and attract wild birds to the vicinity of the poultry houses.

In areas where trees are scarce, they will attract birds as places to hide or rest. At the Shintomicho poultry farm, there are relatively large trees near a forest. Some branches touched the poultry house or its roof. In such cases, the branches become pathways for weasels and other small mammals as well as birds.

Photograph 7. Japanese wax trees planted next to a poultry house in Hyuga City

The infection began inside the poultry house approximately where the person is standing.

Photograph 8. Trees in contact with the poultry house in Shintomicho

A bamboo-grass thicket extends all the way from a neighboring forest to the adjacent trees. Tree branches touch the roof of the poultry house. Not only do wild birds use them for rest or refuge, small animals use them as pathways.

5.3 Introduction of the virus into poultry houses by wild birds

The question of wild birds introducing the virus from the wild into poultry houses can be addressed as follows.

5.3.1 Vents and windows (placement of bird netting)

In order to prevent incursions by wild birds, windows and vents should be covered with netting with a mesh too small for them to pass through. In order to prevent the incursion of sparrows, a mesh with an outer diameter of no more than 3 centimeters is necessary. At the poultry farms in Kiyotakecho and Hyuga City, curtains were lowered, but they had gaps and tears. At the Takahashi City poultry farm, there were thick spider webs, with no apparent gaps for wild birds to enter. At the Shintomicho poultry farm, the bird netting was torn near the area where the infection began.

5.3.2 Building construction

If there are gaps between a building's roof, walls, beams, and so on, sparrows and other hole-dwelling wild birds will enter the building. The structure of the Shintomicho poultry house created multiple gaps that enabled the incursion of wild birds. At the other poultry farms, however, poultry house structure did not permit constant incursions by wild birds.

For the above reasons, the possibility that wild birds brought the virus into the poultry houses from the wild is low, except in the case of the Shintomicho poultry farm. Even in the case of the Shintomicho poultry farm, however, incursions by wild animals other than birds were possible. Careful examination of the possibility that wild animals other than birds brought the virus into the outbreak poultry houses is therefore necessary. Because the possibility always exists that wild birds carry the virus, however, disease control measures require that chicken farms and poultry house take steps against wild birds.

5.4 Measures required from now on

When considering the contribution of wild birds to outbreaks of highly pathogenic avian influenza in Japan, there are numerous points where knowledge is insufficient. In order to improve the epidemic prevention system, the following measures should be implemented.

5.4.1 Understanding the movement of birds

There is little knowledge concerning migration and movement during wintering periods in Japan or abroad. The research described below should be enhanced in order to improve understanding of the movement of birds.

• Expansion of research using satellite tracking

Satellite tracking is the best method for understanding the movement of birds. During the tracking period, daily movements can be grasped.

• Improved banding and color marking

Because satellite tracking is expensive, only a few birds can be tracked. Furthermore, the birds must be large enough to carry transmitters. Banding with leg bands and so on is essential because it allows the movements of small birds such as those that dwell in forests to be tracked. Color marking research that allows records to be collected by observing colored leg bands is an extremely effective tracking method in areas where there are large populations of birders. In Asia, not only in Japan but also in Taiwan, South Korea, and China, the bird watching population is increasing, enhancing the efficiency of tracking with color marking.

• Radar tracking

Large flocks of birds can be tracked using ship, aircraft, and weather radar. In Japan, there are few examples of movement research using radar. Expanding this research should add to understanding of bird movement.

5.4.2 Understanding the habitation of wild geese, ducks, and swans

A detailed understanding of the habitation of wild geese, ducks, and swans, which have a high possibility of carrying the avian influenza virus, should be obtained.

• Grasping changes in the numbers inhabiting each area

The status of goose, duck, and swan migrations will become clearer with an understanding of periodic changes in populations. Because visual population surveys are simple to perform, sites should be designated for regular surveys. The Ministry of the Environment began periodic population surveys in national wildlife refuges in February 2007.

• Improved study of feeding and other behavior

Geese, ducks, and swans often use bodies of water for resting, and feed in nearby rice paddies and wetlands. It is necessary to improve studies on the extent of feeding areas and their environmental conditions in order to determine points of contact with poultry farms and human beings. Furthermore, residents and tourists feed geese, ducks, and swans at various locations. The behaviors of waterfowl and humans at these feeding sits should also be studied, and care taken that tourists do not step in waterfowl feces or otherwise have excessive contact with them.

5.4.3 Understanding the habitation of birds in the vicinity of poultry farms

The kinds of birds that visit poultry farms, their behavior (feeding, resting, and breeding), and the conditions that attract them should be understood.

5.4.4 Establishment of a system for international information exchange

Because highly pathogenic avian influenza virus enters Japan from outside the country, information on wild birds is as necessary as information regarding outbreak areas. A system to share information with other countries regarding natural environments in foreign outbreak areas and bird inhabitation, migration, and other movement is necessary.

Countries throughout Asia participate in the Asian Waterbird Census (AWC) regarding waterfowl habitation. As for wild birds and outbreaks of highly pathogenic avian influenza, the Secretariat of the Convention on the Conservation of Migratory Species of Wild Animals (CMS; also known as the Bonn Convention) has a working group on highly pathogenic avian influenza and wild birds so that signatory countries can exchange information. However, most Asian countries, including Japan, do not belong to the Bonn Convention. Japan has treaties and agreements with the USA, Russia, China, and Australia on the conservation of migratory birds, and it regularly exchanges information about migratory birds with those countries and with South Korea. Furthermore, Partnership for the East Asian-Australasian Flyway (EAAF) among Asian countries began in November 2006 for the conservation of migratory waterfowl. Systems for exchanging information about the relationship between highly pathogenic avian influenza and wild birds should be created within these frameworks for international conservation of wild birds.

Works cited

Kiyoaki Ozaki, Takahiro Komatsu, Y. Gerashimov, Shigemoto Komeda, and Toshio Ikeuchi, 1999. "Discovery of bean goose breeding grounds by satellite tracking," Ornithological Society of Japan FY 1999 Conference Address Abstracts 49

Yutaka Kanai, Fumio Sato, Mutsuyuki Ueta, Jason Minton, Hiroyoshi Higuchi, Masaki Soma, Nagahisa Mita, and Shigeru Matsui, 1997. "The migration routes and important restsites of Whooper Swans satellite-tracked from northern Japan," *Strix* 15: 1-13.

Kaname Kamiya and Kiyoaki Ozaki, 1999. "Migration survey of tundra swans that fly to the Yonago waterfowl park," Ornithological Society of Japan FY 1999 Conference Address Abstracts 48

Ministry of the Environment, 2007. "Migration routes of ducks to Japan," http://www.env.go.jp/nature/dobutsu/bird_flu/migratory/dabbler.html

Ministry of the Environment, 2006. November 16, 2006, press release materials on East Asian-Australasian Flyway Partnership (cooperative project on migratory waterfowl conservation)

Wild Bird Society of Japan and Ministry of the Environment, 2005. "Satellite tracking of black-faced spoonbills," http://www.sizenken.biodic.go.jp/flyway/

Wild Bird Society of Japan and Katanokamoike Ecosystem Management Council, 2006. FY 2005 Annual report of the Kaga City Kamoike Observation Center

Hiroyoshi Higuchi, Fumio Sato, Shigeru Matsui, Masaki Soma, and Noboru Kanmuri, 1991 "Satellite tracking of the migration routes of Whistling Swans Cygnus *columbianus*," *Journal of the Yamashina Institute for Ornithology* 23:6–12.

Hiroyoshi Higuchi, Kiyoaki Ozaki, Tsuyoshi Fujita, Masaki Soma, Noboru Kanmuri, and Mutsuyuki Ueta, 1992. "Satellite tracking of the migration routes of cranes from southern Japan," *Strix* 11: 1-20

Hiroyoshi Higuchi, 2007. Movement routes of migratory birds in East Asia—The state of long-distance movement and domestic movement—Coordinated Science and Technology Policies: Symposium on new and emerging infectious diseases, "Elucidation of transmission of highly pathogenic avian influenza virus and research on measures against new types of influenza," Abstracts: 13-29

Yamashina Institute for Ornithology, 2002. *Bird Atlas*, Yamashina Institute for Ornithology

Li, Z. W. D. and Mundkur, T. 2007. Numbers and distribution of waterbirds and wetlands in the Asia-Pacific region. Results of the Asian Waterbird Census: 2002-2004. Wetlands International, Kuala Lumpur, Malaysia.

Section 6 Information on wild animals

(Kumiko Yoneda)

1 Wild animals and avian influenza virus infection

In 2004, Japan saw its first outbreak of highly pathogenic avian influenza (HPAI) in 79 years. In Kyoto and Osaka, nine cases of jungle crows with H5N1 subtype influenza virus infection, possibly from chickens, were confirmed (Ministry of Agriculture, Forestry and Fisheries press release 2004; Ministry of the Environment press release 2004). In, South Korea, which had outbreaks shortly before the Japanese ones, infection was confirmed in magpies (Japan Wildlife Research Center 2004; Kwan et al. 2005). Subsequently, the virus spread from China to Russia, Europe and Africa, with many reported cases of infection in wild birds.

In order to compile information only on cases where infection with the H5N1 subtype influenza virus was confirmed, the author extracted cases from "Weekly Disease Information," which shows the information officially reported by relevant government agencies in each country to the World Organization for Animal Health (OIE). A summary of infections from 2004 to the present follows below. Where the details of reports to the OIE were unclear, the author also considered information from government announcements and cases registered with gene banks. Cases of infections in captive birds in zoos or similar facilities are not included.

1.1 Southeast Asia

The H5N1 subtype virus that has infected poultry and people in Thailand, Vietnam, Cambodia, and Lao PDR belongs to a different group than the virus that caused outbreaks in China and other regions, and the virus in Indonesia also belongs to a different group than the Indochinese one (Chen et al. 2006b). Southeast Asia has attracted attention for its human infections, but it looks like little research has been done on infections in wild birds and other animals. Poultry infections in the region are characterized by deaths of domestic ducks, and there may be significant impact on wild birds as well. Details are difficult to find for this region and the extent of infections in wild birds, in terms of both area and the number of species, may be greater than reported.

Thailand conducts survey on infection in wild birds and there are several news reports regarding the government announcement on confirmed cases of infection. Infection in mammals such as cats and tigers, probably stemming from poultry, was reported. Vietnam has not carried out large-scale survey on wild birds because of its policy that wild bird surveys should be performed after poultry outbreaks end because it is impossible to discern whether wild or domestic birds are the source during a poultry outbreak (personal communication). The author was unable to obtain information regarding infection in wild birds in Indonesia. In the Philippines, survey watching for incursions by the virus is carried out, but to date no infections have been reported.

There have been no official reports of infection in wild birds in Southeast Asia since 2005, but there have been repeated outbreaks of infection in poultry and humans. An H5N1 subtype virus was isolated from herons in Indonesia in 2006, and there is a report that pigeons were infected and died in Thailand in January 2007. In addition, there is a report that during a poultry outbreak in Pakistan in 2007, infection with an H5N1 subtype virus was confirmed in the carcasses of 4 crows.

< Mass outbreaks in wild birds >

- January 2004: There were 59 deaths in a Cambodian wildlife rescue center. (An H5N1 subtype virus was confirmed in 1 heron, 4 carnivorous birds - crows and raptors, and 1 parakeet.) The same center reports that tigers, leopards, and other cats were also infected

(FAO AIDE news No. 16).

- February 2004: In Thailand, in addition to confirmed infection in crows in a zoo in Bangkok (Bangkok Post 2004/02/07), there was a mass die-off of 200 Asian openbills in a wetlands in a Bangkok suburb, with H5N1 subtype influenza A virus infection confirmed in at least 2 of them (Bangkok Post 2004/02/17).
- December 2004: In a wild bird survey in central Thailand, infection was confirmed in 2 Asian openbills and 1 little cormorant, and in 6 non-migratory small birds including pigeons (ProMED 20041214.3303).

1.2 Hong Kong

In Hong Kong, about 150 waterfowl died in December 2002 (Ellis et al. 2004). A strict monitoring system was subsequently established, and examination of several thousand to more than ten thousand wild bird carcasses is carried out annually and the results publicized. Although there have been no mass die-offs since then, around January every year, isolation of H5N1 subtype viruses from the carcasses of wild birds is announced. In 2006, the virus began to be found in small birds (species often kept as pets), and the number of cases has been increasing. In 2007, it has been detected in terrestrial birds even after May (Government of Hong Kong press release). It is also reported that the virus's characteristics have changed since the 2002 outbreak (ProMED 20070405.1148).

< Outbreaks in wild birds >

- January 2004: 1 peregrine falcon
- November 2004–January 2005: 2 grey herons, 1 pond heron
- January–February 2006: Detected in 14 scattered cases (1 little egrets, 7 crows, 6 non-migratory small birds), and in 2 (feral?) chicken carcasses
- December 2006–March 2007: detected in 20 scattered cases (13 non-migratory small birds, 7 carnivorous birds - long-tailed shrikes, crows, and raptors)

1.3 China, Mongolia, and eastern Russia

The origin of the H5N1 subtype influenza virus now epidemic is said to be a virus that was isolated in geese in China's Guangdong Province in 1996. China's poultry, especially its domestic ducks, harbor diverse viruses, which are considered to be still changing (Chen et al. 2004).

In May 2005, there was an outbreak of mass deaths at Qinghai Lake in central China, and a subsequent outbreak of deaths among Anseriformes such as swans, and grebes at lakes on the border between Mongolia and Russia and in wetlands farther north. There was a recurrence in April–June 2006, with deaths in the thousands. In 2007, news of the outbreaks was not released until June.

< Mass outbreaks in wild birds >

- Around January through March 2004, during an outbreak in poultry in China's Hubei Province, there were many deaths of Eurasian wigeons at a lake. An extremely virulent H5N1 subtype virus was isolated from carcasses (Yu et al. 2007).
- In 2004 in China's Henan Province, an H5N1 subtype HPAI virus was detected in 4 out of 38 captured sparrows. It was suggested that the poultry virus might have changed genetically and might be widely infecting sparrows (Kou et al. 2005).
- In May 2005 at Qinghai Lake, there was an outbreak with over 6,000 waterfowl deaths, mainly among bar-headed geese (Chen et al. 2006).
- In July 2005 in Novosibirsk, Russia, there was a poultry outbreak. During the same period, deaths among grebes and wild ducks were reported (L'vov et al. 2006a).
- In August 2005, the deaths of about 100 ducks and geese and swans at Erhel Lake and Khunt Lake in northern Mongolia were reported.

- In October 2005 and April 2006 in China's Liaoning Province, there were outbreaks with multiple dead birds including magpies. During October 2005, there was also an outbreak among poultry, but there was no poultry outbreak in April 2006. (It was possible that vaccinations prevented infections from becoming evident.)
- In April through June 2006, there was another outbreak with about 1,000 deaths, mainly bar-headed geese, in Qinghai Province. In the Tibet Autonomous Region to the southwest of Qinghai Province, there was an outbreak that killed 2,600 birds by the end of July.
- In May and June 2006, the deaths of 13 birds including whooper swans, gulls, and ducks and geese at a lake in northern Mongolia were reported.
- In June 2006, about 1,600 great crested grebes, great cormorants, coots, common terns, and geese died at Uvs Nuur Lake on the border between Mongolia and Russia. The virus was detected in individuals without symptoms as well (L'vov et al. 2006b).

1.4 Europe

Following the July 2005 outbreak in Novosibirsk, Russia, poultry outbreaks gradually spread westward. In Europe, deaths of wild birds, especially mute swans, were seen in areas without poultry outbreaks. They continued sporadically until June 2006. Mass outbreaks among wild birds included swans in the Caspian Sea coastal region, as well as deaths of ducks and geese and hawks in Germany and France. The virus responsible for these outbreaks came from the same lineage as the Qinghai Lake virus.

< Mass outbreaks in wild birds >

- In October 2005 in Romania's Black Sea coastal region, there were 137 mute swan deaths simultaneous with a poultry outbreak. Scattered carcasses were found after that, and in December over 700 swans died in Russia. In the Caspian Sea and Black Sea coastal regions through March 2006, swans, ducks, crows, and so on died in outbreaks in Ukraine, Bulgaria, Azerbaijan, Turkey, Georgia, Iran, and Kazakhstan. Poultry outbreaks in Romania continued until June 2006, and a small number of deaths of geese and coots were confirmed in February and March.
- In October 2005 in Croatia, 15 mute swans died twice. The virus was detected in 30 black-headed gulls without symptoms captured for survey at that time. In January 2006, mute swans died in Greece, and deaths of mute swans and other wild birds broke out in Egypt, Italy, Bosnia and Herzegovina, Slovenia, Hungary, Slovakia, Serbia and Montenegro, France, Switzerland, Austria, Sweden, Germany, the UK, Poland, Czech, Denmark, and Spain.
- Outbreaks were especially numerous in France and Germany. In France, about 100 birds including mute swan, tufted duck, common pochard, barnacle goose, herons, and common buzzard died during February and March. In Germany, there were many outbreaks on the Baltic Sea island of Rügen and in wetlands in Bavaria in the southern part of the country. Over 300 birds of swans, geese, ducks, gulls, cormorants, hawks, and crows died in February through May.

1.5 Africa

In Africa, there were not many reports of infection in wild birds. Most of the cases were raptors in areas with poultry outbreaks, so secondary infection from poultry was suspected.

< Outbreaks in wild birds >

- In February through June 2006, the deaths of 3 hooded vultures in Burkina Faso (Ducatez, et al. 2007), 1 vulture in Nigeria, and 1 sparrow hawk and 1 wild bird (species unknown) in Cote d'Ivoire were reported.

1.6 Infection of mammals

Cases of natural infection with an H5N1 subtype influenza virus in carnivorous mammals have been found because of emaciation or deaths. Both in the wild and in captivity, the infections are thought to have come from eating infected birds.

The case in the wild was the discovery of an emaciated stone marten in March 2006 on Germany's Rügen Island (WHO/EPR/Disease Outbreak News, 9 March 2006). Other cases of natural infections are not wild animals, and Thailand (Songserm, et al. 2006a; ProMED 20040617.1614), Austria (Leschnik, et al. 2007), Germany (WHO/EPR/Disease Outbreak News, 9 March 2006), Iraq (Yingst, et al. 2006), and Indonesia (ProMED 20060620.1700) confirmed infection in dead cats and Thailand in a dead dog (Songserm et al. 2006b).

Cases in captivity were the deaths of a clouded leopard (ProMED 20040213.0480), leopards and tigers (Keawcharoen, et al. 2004; Thanawongnuwech, et al. 2005; ProMED 2004/02/21.0560) in Thailand in 2003–2004, and an Owston palm civet in Vietnam in 2005 (ProMED 20050826.2527).

In addition, there is a low rate of subclinical infection in pigs and it was reported during a poultry outbreak in Vietnam (Choi, et al. 2005), and in China beginning in 2001 (ProMED 20040822.2330). In the experimental infection with the 1997 Hong Kong strain and the 2004 Vietnam strain, the virus replicated in pigs but did not spread to the contact pigs (Shortridge, et al. 1998; Choi, et al. 2005).

In the experimental infection, virus infection and replication were confirmed in ferrets (Zitzow, et al. 2002), crab-eating macaques (Kuiken, et al. 2003), rhesus macaques (Chen, et al. 2006a), rats (Shortridge 1998), and mice (Chen, et al. 2006, etc.). Ferrets are reported to be especially susceptible.

Literature cited

OIE Weekly Disease Information
http://www.oie.int/wahid-prod/public.php?page=weekly_report_index&admin=0

Ministry of Agriculture, Forestry and Fisheries press release 2004
http://www.maff.go.jp/www/press/cont/20040324press_7.htm

Ministry of the Environment press release 2004
http://www.env.go.jp/press/press.php?serial=4898

Government of Hong Kong press release 2007
http://www.afcd.gov.hk/english/whatsnew/what_qua/files/common/h5n1_2007.pdf

Chen, et al. (2004) The evolution of H5N1 influenza viruses in ducks in southern China. Proceedings of the National Academy of Science USA 101(28): 10452-10457.

Chen, et al. (2006a) Properties and dissemination of H5N1 viruses isolated during an influenza outbreak in migratory waterfowl in western China. Journal of Virology 80(12): 5976-5983.

Chen, et al. (2006b) Establishment of multiple sublineages of H5N1 influenza virus in Asia: Implications for pandemic control. Proceedings of the National Academy of Science USA 103(8): 2845-2850.

Choi, et al. (2005) Studies of H5N1 influenza virus infection of pigs by using viruses isolated in Vietnam and Thailand in 2004. Journal of Virology 79(16): 10821-10825.

Ducatez, et al. (2007) Genetic characterization of HPAI (H5N1) viruses from poultry and wild vultures, Burkina Faso. Emerging Infectious Diseases 13(4): 611-613.

Ellis, et al. (2004) Investigation of outbreaks of highly pathogenic H5N1 avian influenza in waterfowl and wild birds in Hong Kong in late 2002. Avian Pathology 33(5): 492-505.

Keawcharoen et al. (2004) Avian influenza H5N1 in tigers and leopards. Emerging Infectious Diseases 10(12): 2189-2191.

Kou, et al. (2005) New genotype of avian influenza H5N1 viruses isolated from tree sparrows in China. Journal of Virology 79(24): 15460-15466.

Kuiken, et al. (2003) Pathology of human influenza A (H5N1) virus infection in Cynomolgus macaques (*Macaca fascicularis*). Veterinary Pathology 40: 304-310.

Kwan, et al. (2005) Highly pathogenic avian influenza in magpies (*Pica pica sericea*) in South Korea. J. Wildl. Dis. 41(3): 618-623.

Leschnik, et al. (2007) Subclinical infection with avian influenza A (H5N1) virus in cats. Emerging Infectious Diseases 13(2): 243-247.

L'vov, et al. (2006a) Isolation of influenza A/H5N1 virus strains from poultry and wild birds in West Siberia during epizooty (July 2005) and their depositing to the state collection of viruses (August 8, 2005). Vopr Virusol. 51(1): 11-14. (Russian, English abstract)

L'vov, et al. (2006b) Isolation of highly pathogenic avian influenza (HPAI) A/5H5N1 strains from wild birds in the epizootic outbreak on the Ubsu-Nur Lake (June 2006) and their incorporation to the Russian Federation State Collection of viruses (July 3, 2006). Vopr Virusol. 51(6): 14-18. (Russian, English abstract)

Shortridge, et al. (1998) Characterization of avian H5N1 influenza viruses from poultry in Hong Kong. Virology 252: 331-342.

Japan Wildlife Research Center (2004) Report on FY 2003 Commissioned Urgent Research Project on South Korean Migratory Birds

Songserm, et al. (2006a) Avian influenza H5N1 in naturally infected domestic cat. Emerging Infectious Diseases 12(4): 681-683.

Songserm, et al. (2006b) Fatal avian influenza A H5N1 in a dog. Emerging Infectious Diseases 12(11): 1744-1747.

Thanawongnuwech, et al. (2005) Probable Tiger-to-Tiger Transmission of Avian Influenza H5N1. Emerging Infectious Diseases 11(5): 699-701.

Yingst, et al. (2006) Qinghai-like H5N1 from domestic cats, Northern Iraq. Emerging Infectious Diseases 12(8): 1295-1297.

Yu, et al. (2007) Avian influenza (H5N1) virus in waterfowl and chickens, central China. Emerging Infectious Diseases 13(5): 772-775.

Zitzow, et al. (2002) Pathogenesis of avian influenza A (H5N1) viruses in ferrets. Journal of Virology 76(9): 4420-4429.

2 Wild animals living in the vicinity of outbreak areas

2.1 Wild animals and infection routes

Of the four locations with highly pathogenic avian influenza outbreaks in Japan in 2007, Kiyotakecho and Shintomicho are farmlands in a plain, while Hyuga City and Takahashi City are semi-mountainous areas. The presence of rats and mice in the outbreak poultry houses in Kiyotakecho and Hyuga City before the outbreaks was confirmed. Previously, rats and mice were relatively common in the Takahashi City outbreak poultry house, but this year there were fewer. Rats or mice were not confirmed in the Shintomicho outbreak poultry house, but wild birds did enter it. It was therefore necessary to examine the role that rats or mice and wild birds may have played in carrying the virus into the poultry houses in this outbreak.

The rats that entered the poultry houses were likely residential rodents such as roof rats or house mice. Interviews also found that in addition to dogs and cats, other animals that lived in the vicinity of the outbreak poultry houses included wild boar, sika deer, raccoon dogs, red foxes, weasels, and bats. Tracks and feces were found near the poultry houses. Is there any possibility that these animals contributed to the outbreaks of highly pathogenic avian influenza?

Feces from chickens infected with the H5N1 subtype influenza virus epidemic in Hong Kong in 1997 that were kept at 4°C without drying reportedly remained infective for more than 40 days (Shortridge, et al. 1998). Viruses are sensitive to ultraviolet rays, so they will not necessarily remain infective for that long outdoors. But these recent outbreaks occurred in winter, when temperatures are low, so it is possible that the virus could have remained infective in the environment. Such a virus could become attached to the bodies of animals or insects and be physically carried. Furthermore, it is conceivable that such a virus could have been incorporated into the bodies of wild animals along with soil or other organisms. Alternatively, birds with the virus might have died, and the virus might have entered the bodies of animals that ate their carcasses. Because, as discussed above, pigs (domesticated wild boars), ferrets (a family of weasel), cats, rats, and mice can be infected with the avian influenza virus, it is possible that the virus could enter the bodies of wild boars, weasels, cats, rats and mice, proliferate there, and be carried for long distances. (However, because pigs are not known to excrete the H5N1 subtype influenza virus even when infected, wild boars might not become virus carriers.)

For example, it is conceivable that a bird infected with the virus may have flown to Japan and then died. A cat may have eaten its carcass and then excreted the virus near one of the poultry houses. A rat may then have carried the virus into the poultry house. However, this would transport only a small amount of the virus, and then these coincidences would have to be repeated three times for the other outbreaks. The probability is extremely low.

If the virus volume is high, weasels, cats, rats or mice will become sick and display neurological symptoms and/or die. No one reported observing such abnormalities in cats or wild animals in the vicinity of the outbreak areas. Furthermore, no virus was detected in the rat and mouse feces taken as samples after disinfection of the poultry houses. In short, there is absolutely no evidence of mediacy by wild animals in these outbreaks.

2.2 Wild animals in the vicinity of outbreak areas

An overview of the ecology of major wild animals follows. These animals are likely to come near poultry houses in search of chicken feed or the insects that live in poultry manure. Carnivorous animals may seek the chickens themselves, and if eggs and other waste are disposed of near poultry houses, naturally that will attract animals too.

Roof rat (*Rattus rattus*): These are large (about the size of lab rats) house rodents that inhabit warm, dry places such as attics. They can clime agilely and run on small ducts or electric wires. They are primarily nocturnal and eat grains and seeds, but about 10 percent of their food comes from animal matter.

Norway rat (*Rattus norvegicus*): Somewhat larger than black rats, in addition to buildings, they inhabit relatively wet ground such as sewers, rivers, and beaches. Primarily nocturnal, they eat more animal matter than black rats.

House mouse (*Mus musculus*): These are small (about the size of lab mice) house rodents. In addition to buildings, they inhabit grassland, farm fields, rice paddies, wasteland, and so on. They are nocturnal and feed on grains, seeds, and vegetables.

Wild boar (*Sus scrofa*): Ranging from forests to farmland, they are omnivores that dig up the ground to feed on plant and animal matter. Females live in small herds, while males are solitary. On farmland, they usually come out at night.

Siberian weasel (*Mustela sibirica*): A non-native Eurasian species introduced around 1949, they are somewhat larger than Japanese weasels (*M. itatsi*). In western Japan, they range from farmland to the feet of mountains. Japanese weasels inhabit more mountainous areas than Siberian weasels. In addition to rats, mice, birds, crustaceans, and fish, they also eat fruit and farm crops.

Raccoon dog: (*Nyctereutes procyonoides*) They range widely throughout Japan, from city suburbs to mountains. They feed on rats, mice, birds, insects, fruit, and so on, but also on garbage. They are nocturnal and live in family groups.

Red fox (*Vulpes vulpes)*: They range widely throughout Japan, from city suburbs to mountains, but except in Hokkaido they are outnumbered by raccoon dogs. The feed on rats, mice, birds, large insects, and so on, but will also eat fruit. After kits are born in early spring, foxes live in family groups through summer.

A detailed ecology of the relationships between these wild animals and poultry houses has not been studied, and it was not possible to determine whether they play a role in transmitting avian influenza virus or other viruses.

Reference

Hisashi Abe ed. in chief (2005) *A Guide to Mammals in Japan* (revised ed.) Tokai University Press

Section 7 Comprehensive discussion

(Toshihiro Ito and Kameo Shimura)

1 Characteristics of outbreaks

1.1 Characteristics of outbreak farms

The characteristics of the four outbreak farms in two prefectures are varied. Case 1 was on a meat-chicken breeding farm with 12,000 birds in open-type, cage-free poultry houses with a mixed female-male population. Case 2 was on a commercial meat-chicken farm with 53,000 birds in open-type and semi-windowless poultry houses. Case 3 was on an egg farm with 12,000 birds in open-type poultry houses with low floors. Case 4 was on an egg farm with 93,000 birds in an open-type poultry house with a raised floor. Cases 1 and 4 were on corporate-run farms, while Cases 2 and 3 were on family farms.

1.2 Characteristics of outbreak regions

The three farms in Miyazaki Prefecture were about 30 kilometers away from each other, while Case 3 in Okayama Prefecture was about 380 kilometers away. The surrounding environments of the outbreak farms were all different. On is in a city suburb with a mix of farmland and housing, one is in a ravine in steep mountains, one is next to rice paddies next to a mountain river, and one is in an agricultural area on a level plateau.

1.3 Epidemiological relationship

Epidemiological surveys of chickens, people, vehicles, goods, and so on were unable to find any epidemiological relationships among outbreak farms. Furthermore, there were no epidemiological relationships between any of the outbreak farms and surrounding farms in terms of people or goods coming and going.

1.4 Characteristics of outbreak periods

In 2006, an H5N1 subtype virus related to the virus isolated during the mass waterfowl die-off in Qinghai Province, China, in 2005 was isolated from poultry and wild birds throughout almost all of Eurasia and part of Africa. Near Japan, there was an outbreak of the same virus subtype on poultry farms in South Korea in November 2006. Through March 2007, outbreaks at seven chicken and duck farms were reported.

At the Japanese outbreak farms, abnormalities were noted, in order from Case 1, on January 7, 21, 22, and 30, 2007. In addition, a virus related to those isolated in the outbreaks was isolated from a mountain hawk-eagle. That bird was captured on January 4, three days before Case 1 began, so it is likely that the virus was in the area around the initial site from early January and remained in the vicinity of outbreak areas through the latter half of the month.

2 Characteristics of isolated virus

2.1 Characteristics of isolated avian influenza

The viruses isolated from each of the four farms in Miyazaki Prefecture (Kiyotakecho, Hyuga City, and Shintomicho) and Okayama Prefecture (Takahashi City) were all H5N1 subtype highly pathogenic avian influenza viruses. Genetic analysis found at least 99-percent homology for each of eight gene segments. In addition, there was at least 99-percent homology with H5N1 subtype viruses isolated during previous outbreaks in Mongolia and South Korea in 2006. Phylogenetic tree analysis showed that all these viruses belong to the same lineage. The viruses belong to the same group as the virus isolated from wild birds in

Qinghai Province, China, in 2005. They differ from viruses isolated in 2004 in Yamaguchi, Oita, and Kyoto Prefectures and in Thailand, Vietnam, and Indonesia.

2.2 Pathogenicity of the isolated virus and susceptibility of hosts

2.2.1 Chickens

In intravenous inoculation testing using an isolated virus, every inoculated chicken died within 26 hours of inoculation, and in nasal inoculation testing, every inoculated chicken died after 3–7 days. It is therefore 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 is different from the virus isolated in 2004, which caused sudden death without other notable symptoms.

2.2.2 Birds other than chickens

In nasal inoculation testing of aigamo ducks using an isolated virus, although corneal opacity was evident among birds inoculated with medium to large doses of virus, only one presented neurological symptoms and one died. Pathogenicity among aigamo ducks therefore appears to be low.

In addition, because a related virus was isolated from an emaciated mountain hawk-eagle found in Kumagun, Kumamoto Prefecture, on January 4, 2007, the virus is also infective in those wild birds.

2.2.3 Mammals

In nasal inoculation testing of mice using an isolated virus, all 18 mice died within 10 days after inoculation. Although no virus was recovered from digestive tracts, virus was recovered from 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.

2.2.4 Transmissibility of the virus

Chickens cohabiting with other chickens inoculated nasally with isolated virus all died 5–10 days after cohabitation began. In addition, at the Case 1 and Case 2 farms, the number of dead birds rapidly increased. These results suggest that the virus is highly transmissible among chickens. In addition, all chickens cohabiting with aigamo ducks inoculated nasally with the isolated virus died, while other aigamo ducks cohabiting with inoculated ones evidenced corneal opacity. These results confirm transmission of the isolated virus from aigamo ducks to chickens and aigamo ducks.

3 Entry routes of the virus into Japan

3.1 The possibility that a domestic virus mutated from a less virulent strain

Since the 2004 outbreaks of highly pathogenic avian influenza, Japan has carried out nationwide surveillance, confirming the absence of H5N1 subtype virus in Japanese poultry. Furthermore, the virus isolated during the recent outbreaks is extremely closely related to viruses isolated in nearby South Korea, Mongolia, and China. This suggests that it is highly unlikely that a virus endemic in Japan mutated to a more virulent form and caused the outbreaks, and that the virus was probably a new incursion from overseas.

3.2 The possibility that wild (migratory) birds introduced the virus

An extremely closely related virus was isolated from the emaciated mountain hawk-eagle (a resident bird) that died after capture on January 4, 2007, in Kumamoto Prefecture. This was before Case 1 broke out about 70 kilometers away on January 13, 2007. In addition, there were outbreaks with related viruses in South Korea, and virus and antibodies were isolated from wild waterfowl in the areas around those outbreaks. These facts suggest that wild (migratory) birds may have brought the virus to Japan from China or the Korean Peninsula. There is no direct evidence such as isolation of a virus from migratory birds, however, so it cannot be positively stated that wild birds brought the virus to Japan from overseas.

3.3 The possibility that the virus was introduced through imported poultry or meat

Imports of birds, meat, and so on from countries with outbreaks of highly pathogenic avian influenza are suspended, so this incursion route is extremely unlikely.

3.4 The possibility that the virus was introduced through human beings

In recent years, the Kyushu region has had flourishing contacts with South Korea, drawing many tourists from that country. The outbreak farms, however, are far from tourist paths, so it is unlikely that tourists from South Korea could have brought the virus directly. Furthermore, epidemiological surveys of the farms found that workers and their families have no history of travel to or contacts with countries with outbreaks of highly pathogenic avian influenza. These facts make it unlikely that the virus was introduced by human beings.

Thus, infection routes cannot be definitely determined, but the bringing of the virus to Japan by wild (migratory) birds can be surmised in light of overseas cases and the isolation of viruses from wild birds in Japan and abroad.

4 Routes by which the virus invaded farms

4.1 The possibility that the virus was introduced by the movement of chickens

In Cases 1 and 2, there was no movement of chickens in or out of the outbreak farms during the period when the virus is estimated to have arrived there. In Cases 3 and 4, there were shipments of spent hens and introduction of chicks on the farms shortly before the outbreaks. In both cases, however, the outbreak occurred in an area away from the new birds. It is unlikely that the virus was introduced by the movement of chickens at any of the farms.

4.2 The possibility that the virus was introduced by the entry of people or the bringing in of goods

No epidemiological relationships linking outbreak farms were found in terms of farm workers or visitors, or dealers in feed, medicines, and so on. There were also gaps among the outbreaks in time and distance. These facts make transmission of the virus among the four farms or its introduction through such means unlikely.

In Cases 2 through 4, the first cases occurred in specific areas of the poultry houses, away from entrances and areas where people usually work. This suggests that human beings did not bring the virus into the poultry houses.

At the Case 1 farm, materials such as nests and artificial grass were brought into the poultry house before the outbreak, but disinfection was carried out beforehand, so it is unlikely that the virus entered along with those materials.

4.3 The possibility that the virus entered through wild animals

4.3.1 Virus contamination in the vicinity of outbreak farms

Before any abnormality was noted at the first outbreak farm, an extremely closely related virus was isolated from a mountain hawk-eagle captured about 70 kilometers from that farm. Surveys of wild birds around the outbreak farms, however, were unable to find any virus infections, so if there were any viruses near the outbreak farms, they were probably at a low level.

4.3.2 Incursion routes into poultry houses

The initial outbreaks in Cases 2 through 4 were in parts of the poultry houses 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, so anti-bird measures were not complete.

Many wild birds and animals were confirmed to be living around the outbreak farms. Confirming incursions of such animals into poultry houses were rat feces inside the Case 1 poultry house and the 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 heavily male, suggesting that males, which are aggressive during the breeding season, may have attacked infected wild animals that invaded the poultry house.

In light of the above, 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.

5 Conclusion and proposals

During these outbreaks, the farms reported promptly, and quick and effective disease-control response prevented spread to nearby farms and successfully prevented an epidemic.

Each virus isolated from an outbreak farm was closely related to viruses recently isolated in China, Mongolia, and South Korea, and to viruses isolated late last year from wild birds in South Korea and from the wild mountain hawk-eagle (a resident bird) captured in Kumamoto Prefecture in January. Although because there is no direct evidence such as isolation of a virus from migratory birds it cannot be positively stated that wild birds brought the virus to Japan from overseas migratory birds, the bringing of the virus to Japan by migratory birds can be surmised in light of overseas cases.

Furthermore, because measures to prevent the incursion of wild birds and animals at the outbreak farms were incomplete, and because no relationship could be established with human workflows or the introduction of outside chicks and the location of the first dead chickens, the bringing of the virus onto the farms by wild birds and/or animals is likely.

In light of the outbreaks, the following outbreak prevention measures and infection route investigations are necessary. In particular, in order to steadily implement measures to prevent outbreaks at poultry farms, the following steps are important in addition to knowledge of everyday animal hygiene management and its thorough implementation. From the perspective of (1) and (2) below, stakeholders such as prefectures and chicken producers need to work together to establish thorough high-security measures inside and outside poultry houses and further reexamine the status of animal hygiene management.

(1) In order to prevent wild birds and animals from approaching poultry houses, keep farms clean and orderly, remove trees and bushes from around poultry houses, regularly clean

and disinfect, work to prevent the growth of sanitary insects, and avoid polluting the area around farms.

(2) Close up gaps that allow wild birds and animals to enter poultry houses. To prevent wild bird incursions, install bird nets with an external mesh diameter of 2 centimeters or less, smooth protrusions and indentations in exterior walls of poultry houses, and remove electric poles and other structures from around poultry houses.

(3) In order to detect incursions of virus into Japan early on, maintain and enhance monitoring of wild birds, including wild bird deaths.

(4) Compared with past outbreaks, this time an Infection Route Investigation Team was able to conduct on-site research and gather information at an early stage. In the future, however, an Infection Route Investigation Team should be formed and begin gathering information on-site even before epidemic-control measures begin. Although there will be some overlap with epidemic-control work, the earliest possible implementation of this measure should be examined.

Appendices

Imports of Birds (Reference Material 1)

Chickens (newborn chicks)

Country	2005	2006
United States of America	141,385	109,604
United Kingdom	298,547	161,510
Netherlands	98,910	26,967
Canada	57,231	78,187
Germany	132,377	122,743
Hungary	40,715	
France	251,491	207,568
Australia		3,058

Geese

Country	2005	2006
France	30	

Total	2005	2006
Total geese	30	
Total chickens (newborn chicks)	1,020,656	709,637
Total other poultry (newborn chicks)	2,383	9,245
Total poultry	1,023,069	718,882

Other poultry (newborn chicks)

Country	2005	2006
UK	2,383	6,344
France		2,901

Unspecified birds

Country	2005	2006	Country	2005	2006
United States of America	3,518	930	Togo	70	
Argentina	5	49	Turkey	6	
United Kingdom	236	503	New Zealand	385	45
Israel	2		Panama	1	
Iran	8		Hawaii	320	
India	1		Philippines	321	379
Indonesia	5		Brazil	115	
Ukraine	127		France	4,245	1,922
Uzbekistan	438		Bulgaria	102	
Australia	609		Peru	62	39
Austria	1	9	Belgium	3,969	9,266
Netherlands	4,993	1,247	Malaysia	6	
Ghana	359		Mexico		312
Guyana	63	523	Mongolia	2	
Canada	5	40	Jordan	130	
Guinea	5,170		Lebanon	50	
Cuba		6	Russia	1,710	
Singapore	2,932	3,624	Hong Kong	3	
Switzerland	1		Cote d'Ivoire	63	
Spain	381	165	Taiwan	61,794	83,084
Surinam	81		Republic of Korea	11,964	7,563
Thailand	2		People's Republic of China	4	
Tanzania	5,141	25	South Africa	3	
Czech	584	20	Unknown	5	
Germany	118	70			
			Total unspecified birds	110,110	109,821

* "Other poultry" include ducks, turkeys, quail, geese, and seagulls.
* "Unspecified birds" are birds not included in items for quarantine as designated by Article 45 Section 1 of the Domestic Animal Infectious Diseases Control Law Enforcement Regulations and are subject to import and export since 2006 under Article 56 Section 2 of the Law Concerning the Prevention of Infectious

Diseases and Medical Care for Patients of Infectious Diseases. (They are handled by Ministry of Health, Labor and Welfare quarantine stations.).

* Figures for 2006 are preliminary.

Imports of poultry, etc. (Reference Material 2)

(Units: items, tonnes)

Country		2005				2006			
		Meat/organs	Eggs	Other	Total	Meat/organs	Eggs	Other	Total
Iceland	Items			13	13			22	22
	Amount			1	1			2	2
USA	Items	2,022	723	216	2,961	1,709	340	233	2,282
	Amount	31,220	17,696	393	49,309	30,433	5,972	850	37,255
Argentina	Items	61	1	1	63	57			57
	Amount	1,134	0	0	1,134	1,181			1,181
UK	Items	13	4	25	42	5	2	12	19
	Amount	0	0	0	1	0	0	0	0
Israel	Items	259			259	66			66
	Amount	45			45	9			9
India	Items	3	1	2	6	2		1	3
	Amount	0	0	0	0	0		0	0
Indonesia	Items	1			1				
	Amount	0			0				
Ukraine	Items			2	2			1	1
	Amount			6	6			7	7
Australia	Items	39		1	40	80	1	8	89
	Amount	29		0	29	143	0	0	144
Austria	Items			12	12			7	7
	Amount			6	6			2	2
Netherlands	Items		123	2	125		30	1	31
	Amount		4,290	0	4,290		922	0	922
Ghana	Items	1			1				
	Amount	0			0				
Canada	Items	41	46	26	113	27	63	11	101
	Amount	168	860	1	1,029	171	984	0	1,155
Guam	Items					1			1
	Amount					0			0
Kenya	Items			2	2				
	Amount			0	0				
Costa Rica	Items	3			3				
	Amount	1			1				
Saipan	Items	3			3	5			5
	Amount	0			0	0			0
Saudi Arabia	Items						1		1
	Amount						0		0
Switzerland	Items							1	1
	Amount							0	0
Spain	Items	56	6	1	63	67	10		77
	Amount	33	105	0	138	25	210		235
Sri Lanka	Items	1			1	1			1
	Amount	0			0	0			0
Serbia and Montenegro	Items			1	1				
	Amount			0	0				
Thailand	Items	12,664		57	12,721	12,704	1	3	12,708
	Amount	147,217		275	147,492	149,813	0	3	149,815
Czech	Items			5	5			1	1
	Amount			4	4			1	1
Chile	Items	309		9	318	115		5	120
	Amount	6,060		28	6,089	1,904		11	1,914
Denmark	Items	20	18	3	41	10	8	1	19
	Amount	316	388	0	703	134	207	0	341
Germany	Items	9	239	34	282	2	230	40	272
	Amount	1	0	190	190	0	0	99	99
New Caledonia (French, including Chester)	Items	2			2			1	1
	Amount	0			0			0	0
New Zealand	Items			35	35			28	28
	Amount			18	18			12	12

Country		2005 Meat/organs	2005 Eggs	2005 Other	2005 Total	2006 Meat/organs	2006 Eggs	2006 Other	2006 Total
Pakistan	Items			1	1				
	Amount			0	0				
Panama	Items	1			1				
	Amount	0			0				
Hawaii	Items	20			20	24			24
	Amount	0			0	0			0
Hungary	Items	1,453		41	1,494	1,142		99	1,241
	Amount	555		115	670	489		273	762
Philippines	Items	250		11	261	81		4	85
	Amount	2,466		12	2,478	412		3	415
Bhutan	Items		1	1	2				
	Amount		0	0	0				
Brazil	Items	14,814	644	581	16,039	12,742	168	625	13,535
	Amount	387,274	12,979	3,174	403,427	339,774	3,320	5,048	348,142
France	Items	5,208	6	33	5,247	3,484		45	3,529
	Amount	2,148	0	29	2,177	1,644		73	1,717
Bulgaria	Items	2			2				
	Amount	44			44				
Vietnam	Items			9	9			9	9
	Amount			83	83			77	77
Peru	Items	2			2	7		1	8
	Amount	23			23	148		0	148
Belgium	Items	14	2		16	9			9
	Amount	32	0		32	0			0
Poland	Items	76		133	209	55		133	188
	Amount	986		258	1,243	1,077		317	1,394
Madagascar	Items							1	1
	Amount							0	0
Malaysia	Items			2	2			2	2
	Amount			18	18			15	15
Mexico	Items	15	99	1	115		44		44
	Amount	233	0	0	233		20		20
Russia	Items			1	1				
	Amount			3	3				
Hong Kong	Items			3	3			3	3
	Amount			4	4			5	5
Taiwan	Items	389	15	478	882	374	10	502	886
	Amount	4,951	13	1,208	6,172	5,355	14	1,452	6,820
Republic of Korea	Items	186	2	2	190	123	1	1	125
	Amount	1,027	2	2	1,031	779	1	0	779
People's Republic of China	Items	25,690		1,328	27,018	27,753		598	28,351
	Amount	192,963		4,528	197,491	204,216		2,607	206,823
South Africa	Items			11	11			42	42
	Amount			1	1			3	3
Total	Items	63,627	1,930	3,083	68,640	60,645	909	2,441	63,995
	Amount	778,926	36,332	10,356	825,615	737,708	11,649	10,860	760,217

Note: Since the outbreak of highly pathogenic avian influenza, only heat-treated imports of poultry meat and organs are allowed from the People's Republic of China and Thailand.

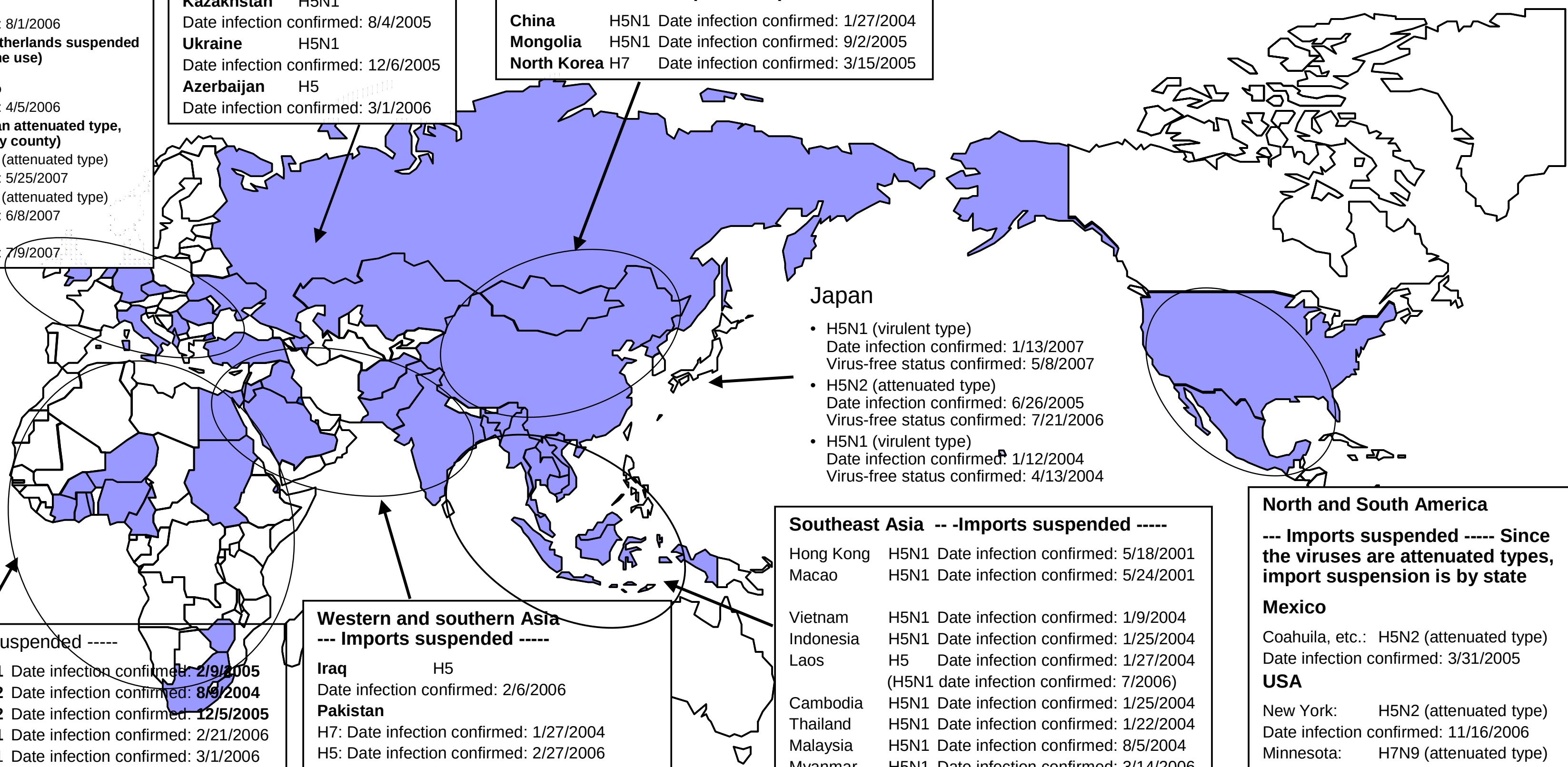
- "Other" includes bones, skin, feathers, blood, serum, and eggs for laboratory use. (Blood, serum, and eggs for laboratory use are not included in "Amount.")
- Amounts of 0 mean less than 1 tonne.

* 2006 figures are preliminary.

Outbreaks of highly pathogenic avian influenza in poultry
(Reference Material 3)

[= Imports suspended (49 countries and territories)]

As of September 6, 2007



Europe

------- Imports suspended ----------

Italy H7N3
Date infection confirmed: 10/23/2002
Romania H5N1
Date infection confirmed: 10/11/2005
Turkey H5N1
Date infection confirmed: 10/11/2005
Albania H5N1
Date infection confirmed: 3/9/2006
Czech H5N1
Date infection confirmed: 6/22/2007
Netherlands H7N7
Date infection confirmed: 8/1/2006
(Note: Imports from Netherlands suspended 3/16/2006 due to vaccine use)

Serbia and Montenegro
Date infection confirmed: 4/5/2006
UK (Since the virus is an attenuated type, import suspension is by county)
Conwy H7N2 (attenuated type)
Date infection confirmed: 5/25/2007
Merseyside H7N2 (attenuated type)
Date infection confirmed: 6/8/2007
Germany H7N7
Date infection confirmed: 7/9/2007

--- Imports suspended ------

Russia H5N1
Date infection confirmed: 7/22/2005
Kazakhstan H5N1
Date infection confirmed: 8/4/2005
Ukraine H5N1
Date infection confirmed: 12/6/2005
Azerbaijan H5
Date infection confirmed: 3/1/2006

Northeast Asia --- Imports suspended -----

China H5N1 Date infection confirmed: 1/27/2004
Mongolia H5N1 Date infection confirmed: 9/2/2005
North Korea H7 Date infection confirmed: 3/15/2005

Japan

- H5N1 (virulent type)
  Date infection confirmed: 1/13/2007
  Virus-free status confirmed: 5/8/2007
- H5N2 (attenuated type)
  Date infection confirmed: 6/26/2005
  Virus-free status confirmed: 7/21/2006
- H5N1 (virulent type)
  Date infection confirmed: 1/12/2004
  Virus-free status confirmed: 4/13/2004

Southeast Asia -- -Imports suspended -----

Hong Kong H5N1 Date infection confirmed: 5/18/2001
Macao H5N1 Date infection confirmed: 5/24/2001

Vietnam H5N1 Date infection confirmed: 1/9/2004
Indonesia H5N1 Date infection confirmed: 1/25/2004
Laos H5 Date infection confirmed: 1/27/2004
(H5N1 date infection confirmed: 7/2006)
Cambodia H5N1 Date infection confirmed: 1/25/2004
Thailand H5N1 Date infection confirmed: 1/22/2004
Malaysia H5N1 Date infection confirmed: 8/5/2004
Myanmar H5N1 Date infection confirmed: 3/14/2006
Bangladesh H5N1 Date infection confirmed: 3/27/2007

North and South America

--- Imports suspended ----- Since the viruses are attenuated types, import suspension is by state

Mexico

Coahuila, etc.: H5N2 (attenuated type)
Date infection confirmed: 3/31/2005

USA

New York: H5N2 (attenuated type)
Date infection confirmed: 11/16/2006
Minnesota: H7N9 (attenuated type)
Date infection confirmed: 5/10/2007
South Dakota: H5 (attenuated type)
Date infection confirmed: 6/26/2007
Nebraska: H7N9 (attenuated type)
Date infection confirmed: 7/2/2007
Virginia: H5N1 (attenuated type)
Date infection confirmed: 7/10/2007

Africa --Imports suspended -----

Nigeria	**H5N1**	Date infection confirmed: **2/9/2005**
South Africa	**H5N2**	Date infection confirmed: **8/9/2004**
Zimbabwe	**H5N2**	Date infection confirmed: **12/5/2005**
Egypt	**H5N1**	Date infection confirmed: 2/21/2006
Niger	H5N1	Date infection confirmed: 3/1/2006
Cameroon	H5N1	Date infection confirmed: 3/14/2006
Sudan	H5N1	Date infection confirmed: 4/21/2006
Cote d'Ivoire	**H5N1**	Date infection confirmed: **4/27/2006**
Burkina Faso	H5N1	Date infection confirmed: 5/31/2006
Djibouti	H5N1	Date infection confirmed: 5/31/2006
Ghana	H5N1	Date infection confirmed: 5/7/2007
Togo	H5N1	Date infection confirmed: 6/26/2007

Western and southern Asia
--- Imports suspended -----

Iraq H5
Date infection confirmed: 2/6/2006
Pakistan
H7: Date infection confirmed: 1/27/2004
H5: Date infection confirmed: 2/27/2006
India H5N1
Date infection confirmed: 2/21/2006
Afghanistan H5N1
Date infection confirmed: 3/17/2006
Israel H5N1
Date infection confirmed: 3/20/2006
Jordan H5N1
Date infection confirmed: 3/27/2006
Palestinian Autonomous Territories H5N1
Date infection confirmed: 4/18/2006

Kuwait H5N1
Date infection confirmed: 3/1/2007
Saudi Arabia H5N1
Date infection confirmed: 3/27/2007