Report of the Highly Pathogenic Avian Influenza Infection Route Elucidation Team

Routes of infection of highly pathogenic avian influenza in Japan

June 30, 2004

Highly Pathogenic Avian Influenza Infection Route Elucidation Team
Food Safety and Consumer Bureau
Ministry of Agriculture , Forestry & Fisheries
TOKYO , JAPAN

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Report Summary

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1. Routes of infection

(1) Characteristics of outbreaks

* There are considerable differences between the scale of poultry operations and raising method at the four locations of outbreaks in three prefectures and no mutual relationship can be recognized. In addition, though they have in common that fact that the locations of outbreak are near mountainous terrain, the surrounding environment is different.

(2) Characteristics of the isolated avian influenza virus

* Though all virus strains isolated in Japan are from the same source, they are different virus strains that had mutated relatively recently and there is the possibility that the outbreaks in the three prefectures occurred independently due to different infection sources.
* The isolated virus strains indicate extremely strong pathogenicity to chickens.
* Though the susceptibility of crows is not particularly strong, there is the possibility that, even if infected, there are many that are able to endure the infection.
* Though ducks are infected by the disease, they endure without dying. They may, however, be carriers of the virus.
* In infection tests using mice, the virus was not detected in the feces and it is thought that proliferation through the feces does not readily occur.
* Infection has not been established in hogs, suggesting a resistance.
* As characteristics of proliferation, rather than rapid spread due to the movement of humans or animals, it is thought that the spread to new farms does not readily occur unless the chickens are exposed a given volume of the virus.

(3) Possibility of mutation from low pathogenicity to high pathogenicity virus strains

* It would be difficult to consider that, in the recent outbreaks in Japan, low pathogenic avian influenza virus changed to highly pathogenic virus.

(4) Possibility of incursion from overseas and the route of incursion

* It is unlikely that virus brought to the farms through the medium of humans or objects from overseas led to the outbreaks but there is a possibility that it was brought in by migratory birds from Korean Peninsula or elsewhere.

(5) Route of incursion to farms and chicken poultry houses

* There is the possibility that the feces of ducks and other migratory birds were the source of infection and that the virus was brought into the poultry houses by resident birds, mice or other animals inhabiting the vicinity or humans, etc.
* In the outbreak in Oita Prefecture, only bantams raised in a specific poultry house were infected and the possibility cannot be denied that water supplied only to that poultry house was contaminated by feces of birds that were coincidentally infected.

* Even if virus is not excreted in the feces of mice, there is the possibility that the virus was attached to the bodies of mice and that they brought the virus into the poultry houses from the surrounding area. In addition, it also cannot be denied that flies and other insects from the surrounding area into the poultry houses with the virus attached to their bodies.

(6) Proliferation between farms

The route of incursion to the poultry house involved in the fourth outbreak may have been through humans, vehicles, birds or wild animals from the third outbreak before measures were adopted to prevent the spread of the virus. In addition, the possibility that the virus was brought into the vicinity of the farm by news media people also cannot be denied. Though identifying the direct route of incursion into the poultry houses is difficult, it could have occurred through sparrows, mice or other wild animals, insects, etc.

2. Recommendations for outbreak and proliferation preventive measures

(1) Epidemiological surveys

1) Epidemiological surveys should be initiated by launching epidemiological survey teams at the time of an outbreak, collecting data as promptly as possible, gathering materials for the preparation of required scientific data, etc., with the assumption that an outbreak of infectious disease could occur at any time.
2) The epidemiological survey teams should include not only veterinary science specialists but also experts in wild birds, wild animals, harmful pests and other fields.
3) It is necessary for the epidemiological survey teams to go to the site and give instructions relating for the epidemiological surveys in cooperation with the local Livestock Hygiene Service Center

(2) International collaboration and cooperation

If it were possible to prevent outbreaks between countries through information exchanges, technology cooperation and other means with the other countries of Asia as well as countries throughout the world, that would ultimately lead to the prevention of outbreaks in Japan as well and international collaboration and cooperation is therefore essential.

(3) Promotion of research

It will be necessary hereafter to pursue the further promotion of research relating to highly pathogenic avian influenza.

Joint initiatives by industry, academia and the government aiming in particular for the development of new vaccines have begun and there are hopes for research results.

(4) Preventive measures for the future

1) Since the disease is an infectious disease with incursion across national boundaries, it is necessary to pursue close exchanges of information with other countries and contribute to the prevention of outbreaks of the disease in all countries through joint research, technological cooperation and other activities with China, Korea, Vietnam, Thailand and other countries of Southeast Asia.
2) It is necessary to strengthen wild bird measures at poultry farms. It is especially necessary to give the utmost attention to range-fed poultry, which have many opportunities for contact with wild birds. In addition, in the case of open-type poultry houses, bird nets are to be installed over the windows to prevent entry into the house by sparrows, crows and other wild birds, while making every effort to assure full control of feed in the houses and reinforcing measures to prevent the incursion to chicken feces,

compost yards, etc.

3) The importation of birds, including pet birds, should be prohibited from countries where outbreaks of the disease have occurred.
4) There is also a need for measures to deal with wild animals. Measures to deal with mice and weasels as well as flies, cockroaches and other harmful pests should be strengthened.
5) Only water suitable for drinking water or that has been disinfected should be used for the water supply. At least, unboiled water that is accessible by wild birds or animals should not be given directly to poultry.
6) Boot disinfection tanks should be installed permanently at the entrances to poultry houses and internal entranceways and, while thoroughly disinfecting vehicles, tools, employees, etc., access to the houses by outsiders should be severely restricted.
7) Employees who work at poultry farms should be fully trained in hygiene management, the workflow in poultry farms should be improved and work records thoroughly promoted. The most recent information should constantly be provided relating to the conditions of disease outbreaks and caution aroused.
8) It is necessary to be fully attentive to the prevention of crime at poultry farms.
9) It is necessary to promote the early detection of the disease by carrying out effective monitoring and health observation from the early stage.

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Introduction

In January 2004, highly pathogenic avian influenza was discovered in Yamaguchi Prefecture, the first time in Japan in 79 years. By March, there were additional outbreaks in Oita and Kyoto Prefectures at a total of four poultry raising facilities entailing the sacrifice of about 275,000 domestic fowl. Large-scale outbreaks of the disease were also confirmed at about the same time centered in East Asia and in some countries, besides fowl, even humans were exposed to infection that proved to be fatal and, therefore, those outbreaks not only involved livestock hygiene but also became an issue of nationwide interest as a public health problem. It was fortunately possible to keep infection damage to a minimum in Japan thanks to earnest epidemic prevention measures introduced through the unified efforts of those involved in the outbreak areas.

Nevertheless, in the case of the outbreak in Kyoto Prefecture, after the outbreak expanded in the poultry farm, not only did the outbreak go unreported by those involved but there were also shipments of infected poultry, spreading the infection to facilities where the shipments were delivered, arousing wide-spread apprehension among the general public. In spite of the fact that there have been no reported cases anywhere in the world of human infection of the disease through broiler or eggs, consumption dropped broadly as the result of rumors and, besides the areas of outbreak, the domestic commercial poultry industry as a whole was severely impacted.

Within this context, the relevant government ministries and agencies worked in close cooperation to devise measures to cope comprehensively both in terms of preventing the spread of the disease and other livestock hygiene problems and protecting the health of the people including assurance of food safety and security and the prevention of human infection. A meeting of relevant ministries was thus held on March 16 to consider avian influenza measures, resulting in a compilation of urgent comprehensive avian influenza measures. Among them, the decision was made to establish a team of experts in the Ministry of Agriculture, Forestry and Fisheries (MAFF) to elucidate the infection route and to move ahead promptly with its operations.

Prompted by this, the Highly Pathogenic Avian Influenza Infection Route Elucidation Team was set up in the MAFF on March 29 with membership consisting of experts and persons involved in epidemic prevention in the prefectures where outbreaks had occurred with the aim of comprehensively verifying infection sources and routes in Japan with the cooperation of relevant ministries and organizations. Individual study group meetings were held on May 7, June 11 and June 30 and a wrap-up was promoted. In the examination process, on-site surveys were also conducted by the study group members in early June at each of the outbreak sites.

Since it was thought necessary to move ahead promptly with a reinforcement of measures for the prevention of disease proliferation taking the results of the elucidation of infection routes into account, a final report was completed within the short term of about three months. In addition, since initial response measures for epidemic prevention are given priority at the time of an outbreak, it became clear that the inability to collect sufficient information that would lead to the elucidation of the infection route remains as an issue for future resolution. As always with the elucidation of the route of infection of infectious diseases, there are difficulties that arise in identifying specific infection sources and routes and there are also aspects that must necessarily depend on inference since retrospective surveys are conducted later; however, we hope that this report will contribute to the reinforcement of measures for the prevention of outbreaks in the future.

Finally, I would like to express my appreciation to the team members and to all those involved in the response to the infection at the time of the outbreaks for your many efforts in the preparation of this report.

June 30, 2004

Nobuyuki Terakado
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Highly Pathogenic Avian Influenza Infection Route Elucidation Team

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In order of Japanese syllabary
(as of June 30, 2004)

Section 1 Summary of the Outbreaks of Highly Pathogenic Avian Influenza

1. Highly Pathogenic Avian Influenza

1.1. What is highly pathogenic avian influenza?

Highly pathogenic avian influenza is an acute infectious disease that causes systemic symptoms in chickens and other domestic fowl. Though the symptoms are varied, the fatality rate is high and the disease is highly transmittable. Therefore, given the serious impact that it has on the poultry industry when it occurs, it has been included on List A among the most serious diseases by the World Organization for Animal Health (OIE), an international organization dealing with animal health.

1.2. Definition of highly pathogenic avian influenza

The definition of highly pathogenic avian influenza in Japan is

a) Type A influenza virus diagnosed to be highly pathogenic avian influenza based on the Manual of Standards for Diagnostic Tests and Vaccines 2000, diagnostic standards for pathogenicity strength, etc., prepared by the OIE

- or -

b) Diseases of chickens, ducks, quail or turkeys ("domestic fowl") considered to be caused by subtype H5 or H7 of type A avian influenza (with the exception of a) above). Since it is necessary to adopt measures such as culling as provided for by law at the time of the occurrence of highly pathogenic avian influenza, it is a designated infectious livestock disease under Article 2 of the Domestic Animal Infectious Diseases Control Law (law #166, 1951).

Domestic fowl infected by type A influenza other than highly pathogenic avian influenza are not subject to culling or other measures at the time of occurrence and it is designated as an infectious disease requiring notification (notifiable infectious livestock disease) under Article 2 of the Domestic Animal Infectious Diseases Control Law Enforcement Regulations (MAFF ordinance #35, 1951).

1.3. Symptoms (epidemiological characteristics)

The symptoms are varied, though major symptoms are sudden death, respiratory symptoms, edema of the face, comb or legs, blood spots or cyanosis, reduced or suspended egg-laying, neurological symptoms, diarrhea or reduced intake of feed or water. In addition, the symptoms and amount of virus discharge differ depending on the type of fowl and isolated virus strain.

1.4. Infection route, basic prevention measures and treatment methods

Infection with this disease is caused through contact with feces, feed, fine particles, water, flies, wild birds, humans or equipment or vehicles required for farm management. Since there are no treatment methods for this disease, it is necessary to promote its eradication and prevent its permanent establishment by adopting prevention measures such as culling infected fowl and restricting the movement of fowl, objects, etc., that may spread the disease.

Moreover, in some countries, vaccinations are adopted as a preventive measure and, even though clinical symptoms can be inhibited with vaccines, due to the fact that it is not possible to prevent infection itself and that it is not possible to distinguish between antibodies produced as the result of infection and as a result of vaccine injection, vaccines in principle are not used in Japan and epidemic prevention is implemented by identifying infected fowl through tests and culling.

2. State of outbreaks in Japan

2.1. Outbreaks prior to January 2004

There have been no outbreaks of the disease in Japan since 1925, which means that the outbreak in January 2004 was the first in 79 years. Based on records from that time, outbreaks are evident in Nara, Chiba and Tokyo Prefectures and the strain isolated at that time (Chiba strain) was revealed through analysis in later years to be H7N7.

Outbreaks have been seen in many countries in recent years including Chile, Hong Kong and Italy in 2002, the Netherlands, Belgium, Germany, Hong Kong and the Republic of Korea ("Korea") in 2003. In the Netherlands, in particular, almost thirty million fowl were culled by the conclusion of the outbreak, inducing enormous damage.

Taking these conditions into account, the MAFF prepared the Highly Pathogenic Avian Influenza Prevention Manual in September 2003 (Food Safety and Consumer Affairs Bureau, Animal Health and Animal Products Safety Division, Notification 15 Shouan #1736, dated September 17, 2003; "(Epidemic Prevention Manual") in order to prepare preventive measures for an outbreak of the disease.

Moreover, since an outbreak of the disease was recognized in Korea on December 12, 2003, a notification was released to the prefectures on December 24 for exhaustive observation of poultry health, prevention of incursion of wild birds into poultry houses and thoroughgoing dissemination of education to poultry raisers, veterinarians and others.

2.2. Outbreak in Yamaguchi Prefecture (first case)

2.2.1. Course of events leading to the outbreak

Dead chickens were confirmed at an egg production farm (34,640 chickens) in Ato-Cho, Abu-Gun, Yamaguchi Prefecture on December 28, 2003. Since the number of chicken deaths subsequently increased and spread to other houses, the egg farm requested the local Livestock Hygiene Service Center having jurisdiction to conduct a disease diagnosis. The Service Center conducted another on-site survey on January 9, collected additional test samples and, upon subjecting the samples to a virus isolation test, recognized a virus suspected of being avian influenza. A report to that effect was submitted to the Yamaguchi Prefecture Agriculture and Forestry Department, Livestock Division, on January 11, which in turn reported the suspected outbreak of avian influenza to the MAFF.

On the same day, Yamaguchi Prefecture sent test samples to the National Institute of Animal Health, which conducted disease diagnosis, confirming infection by type A subtype H5 avian influenza virus on January 12. The fowl were thus found to be suffering from highly pathogenic avian influenza infection.

When the National Institute of Animal Health subsequently conducted disease diagnosis of dead fowl, the detected virus was confirmed to be highly pathogenic avian influenza of serum subtype H5N1 on January 13.

2.2.2. State of response to epidemic prevention

2.2.2.1. Initial epidemic prevention measures

As initial epidemic prevention measures, Yamaguchi Prefecture carried out various measures including restricted entry to the farm where the outbreak occurred by outsiders, constraints on egg shipments and disinfection of poultry houses.

After confirmation of the outbreak, Yamaguchi Prefecture devised the necessary epidemic prevention measures including the culling of all fowl at the farm, disinfection, restrictions on movement at other farms in the vicinity and implementation of epidemiological surveys in line with the Domestic Animal Infectious Diseases Control Law and the epidemic prevention manual in cooperation with the Prefectural Public Health Bureau. Of these, the epidemic prevention measures at the infected farm were completed by January 21.

Restrictions on movement: The movement of chickens and other domestic fowl as well as objects that could potentially cause the spread of the virus is prohibited within a zone with a radius of 30 kilometers centered in the infected farm as a way of preventing expansion of the infection until virus-free conditions are confirmed at farms in the vicinity.

2.2.2.2. Expert Committee (Domestic Fowl Disease Committee)

The second meeting of the Domestic Fowl Disease Committee, Livestock Hygiene Department, Consumption and Safety Subcommittee, Investigative Council on the Basic Problems Concerning Food, Agriculture and Rural Areas ("Domestic Fowl Disease Committee"), was held at the MAFF on January 15 to hear the opinions of experts regarding specific measures taking the conditions of the outbreak into account and the following views were expressed.

(1) Prevention measures, etc.
  a) Exhaustive measures are to be adopted for the time being to prevent the spread of the disease in line with the epidemic prevention manual.
  b) Confirmation of virus-free conditions should be implemented based mainly on the existence of clinical symptoms
  c) Though the use of vaccines is not appropriate under current conditions, preparations should be examined in the event of a further spread of the disease.

(2) Epidemiological surveys of farms, etc., should continue to be promoted until the route of infection is elucidated.

In addition, given the fact that the series of epidemic preventive measures at the infected farm had been completed by January 21, the third meeting of the Domestic Fowl Disease Committee was convened on February 3 and the following views were expressed.

(1) In regard to the confirmation of virus-free conditions, on-site inspections based on the epidemic prevention manual, antibody tests and virus isolation tests should be promoted.
(2) Releasing restrictions on movement should be examined while obtaining the advice of the Committee taking the results of tests for the confirmation of virus-free conditions into account.
(3) In regard to the emergency vaccine stores, use at the present time is not appropriate and, if it cannot be avoided for the prevention of the further spread of the disease, vaccines should be used methodically and systematically in line with instructions by the Minister of the MAFF and prefectural governors based on the Domestic Animal Infectious Diseases Control Law and the views of the Committee should be heard regarding the specific methods.

2.2.2.3. Release of restrictions on movement

No other infections were confirmed in any of the flocks in virus-free confirmation tests conducted by Yamaguchi Prefecture on February 14 to confirm that the poultry farms, etc., within the zone of restricted movement were free of virus and it was also confirmed that all of the samples tested negative in the antibody and virus isolation tests.

Taking these circumstances into account, it was confirmed at the meeting of the Highly Pathogenic Avian Influenza Infection Countermeasures Headquarters held at the MAFF on February 18 that the restrictions on movement would be lifted as of 12:01am, February 19, and the MAFF notified Yamaguchi Prefecture to that effect. Prompted by the notification, Yamaguchi Prefecture released all movement restrictions effective 12:01am, February 19.

2.3. Outbreak in Oita Prefecture (second case)

2.3.1. Course of events leading to the outbreak

Three bantams died at the home of a fancier of pet bantams (13 bantams, 1 duck) in Kokonoe-machi, Kusu-gun, Oita Prefecture, on February 14, 2004. The raiser therefore reported the event to the local Livestock Hygiene Service Center through the local administrative office. Receiving the report, the Service Center conducted an on-site inspection the same day and, taking two of the dead birds, started an autopsy and virus isolation tests. Furthermore, since a report was received from the raiser on the morning of February 16 that an additional four bantams had died, an on-site inspection was conducted again. Since virus suspected of being avian influenza was isolated from the specimens removed on the 14^{th} on the evening of the same day, the Service Center reported that fact to the Oita Prefecture. At the same time, the six bantams and one duck still remaining at the home of the raiser were culled with the agreement of the raiser.

On the 17^{th}, Oita Prefecture sent test samples to the National Institute of Animal Health, which conducted a disease diagnosis on the dead fowl and, since it was recognized that they had been infected with type A subtype H5 avian influenza virus, the fowl were confirmed to be infected with highly pathogenic avian influenza. The virus was later confirmed to be type H5N1.

2.3.2. State of response to epidemic prevention

2.3.2.1. Initial epidemic prevention measures

All of the fowl had either died or had been culled by the time that the diagnosis of avian influenza was finalized on February 17. As initial epidemic prevention measure, entry into the infected location by outsiders was restricted and the poultry house was disinfected, etc. At the same time, a zone with a radius of 30 kilometers was designated centered in the infected location.

After confirmation of the outbreak, measures required for epidemic prevention were devised including disinfection of the infected location and the implementation of epidemiological surveys in cooperation with the Prefectural Public Health Bureau. Of these, the epidemic prevention measures at the infected location were completed by February 18.

2.3.2.2. Expert Committee (Domestic Fowl Disease Committee)

The fourth meeting of the Domestic Fowl Disease Committee was held at the MAFF on February 23 and the following views were expressed.

(1) Efforts should be made at the time of outbreak to collect appropriate samples and take all possible measures to prevent infection of the people who are involved in the measures to contribute to epidemiological examinations later.
(2) Areas where no other infections were confirmed through primary tests to confirm virus-free conditions (virus isolation tests and serum antibody tests) taking the stipulations of the epidemic prevention manual into consideration should be gradually changed from movement restriction zones to limited shipment zones and, furthermore, if other infections are not confirmed through secondary virus-free tests, it would be appropriate to reduce the size of the limited shipment zones.
(3) Taking into account the second outbreak, a reinforcement of the nationwide monitoring infrastructure and outbreak prevention measures should be promoted.
(4) A revised draft of the epidemic prevention manual should be prepared by the ministry for the specific management of future movement restrictions, monitoring methods and so forth taking into account experiences in responding to epidemic prevention obtained through the first outbreak and the views of the committee members should be heard.

2.3.2.3. Release of restrictions on movement

Since no other infections were confirmed as the result of the primary virus-free confirmation tests (virus isolation tests and serum antibody tests) conducted at poultry farms within the restricted movement zone on February 27 taking the views of experts into account, after discussions between Oita Prefecture and the MAFF, it was decided to reduce the size of the restricted movement zone effective 12:01am, February 28, and to re-designate the zone spanning from 5km to 30km from the location of infection to a limited shipment zone.

In addition, since no other infections were recognized in the results of the secondary virus-free confirmation tests conducted by March 3, shipment limitations were lifted effective 12:01am, March 4, leaving only the movement restricted zone within a radius of 5km from the location of infection.

Moreover, since no other infections were discovered in the results of the tertiary virus-free confirmation tests, restrictions within the movement restricted zone with radius of 5km from the location of infection were lifted effective 12:01am March 11.

2.4. Outbreak in Kyoto Prefecture (third case)

2.4.1. Course of events leading to the outbreak

There was an increase in dead fowl in the Poultry House No. 8 (capacity: approx. 30,000 fowl) of an egg production facility (225,000 fowl) in Tanba-cho, Kyoto Prefecture, on February 17, 2004, and the infection spread to virtually all of the houses within a few days. The raiser did not report this to the Livestock Hygiene Service Center while this was occurring. The Service Center, which had received an anonymous telephone call reporting large-scale deaths at the farm, conducted an on-site inspection before dawn on February 27. Since the large-scale deaths of poultry were confirmed as a result and positive results were obtained in a simple test, the Kyoto Prefecture Livestock Department contacted the MAFF with the report that avian influenza was suspected. Kyoto Prefecture later sent test samples to the National Institute of Animal Health, which conducted disease diagnosis of the test samples, whereupon infection by type A subtype H5 avian influenza virus was confirmed on February 29 and it was thus revealed that the fowl were infected with highly pathogenic avian influenza virus. The virus was confirmed to be subtype H5N1 on March 1.

2.4.2. State of response to epidemic prevention

2.4.2.1. Initial epidemic prevention measures

Initial epidemic prevention measures included restricted entry to the farm where the outbreak occurred by outsiders, constraints on egg shipments and disinfection of poultry houses. In addition, there were also movement restrictions within a zone with a radius of 30km centered in the infected farm.

Furthermore, after confirmation of the outbreak, the necessary epidemic prevention measures were devised including the culling of the fowl at the farm, disinfection, restrictions on movement at other farms in the vicinity and implementation of epidemiological surveys in cooperation with the Prefectural Public Health Bureau. Of these, the epidemic prevention measures at the infected farm were completed by March 22.

In later surveys, in spite of the deaths of large numbers of fowl at the farm beginning February 17, no report was submitted to Kyoto Prefecture and it became clear meanwhile that poultry were shipped to chicken processing plants in Hyogo and Aichi Prefectures on February 25 and 26.

2.4.2.2. Treatment to the poultry shipped to the poultry processing plant in Hyogo Prefecture

Of the poultry shipped from the farm to poultry processing plants in Hyogo Prefecture, a simple test of conducted of those still unprocessed, which tested positive on February 28, and, once the virus was isolated, they were considered to be diseased fowl. In addition, poultry were also delivered to the chicken processing plants from Okayama and Hiroshima Prefectures on February 26 and 27 together with the poultry from the infected farm and, when unprocessed poultry were subjected to simple tests, it was confirmed that those shipped from Okayama Prefecture also tested positive the same day. The virus isolated from these was confirmed to be subtype H5N1 on March 3.

Disinfection and restrictions on entry by outsiders into the processing plants in Hyogo Prefecture were implemented on February 28 and constraints on movement were demanded of farms within a radius of 30km. Since the relevant farms and other facilities were identified on the 29th, however, following discussions with the MAFF, the scope of voluntary constraints on movement was reduced to a radius of 5km and virus-free confirmation tests were conducted at farms within that zone. Since, as a result, no other infections were confirmed that would suspect avian influenza, the voluntary constraints on movement were lifted as of 12:01 am on March 17.

In addition, the poultry residue discharged from the chicken processing plant were treated at a rendering plant in Kagawa Prefecture, a test of the poultry residue prior to rendering was conducted at the rendering plant on March 1 and influenza virus was confirmed (confirmed to be subtype H5N1 on March 5). Even so, when the feather meal and chicken meal was likewise tested after rendering of the poultry residue, influenza virus was not confirmed and it was thus confirmed that the virus had been inactivated by the rendering process. For the sake of safety, Kagawa Prefecture had the rendering plant disinfected and it resumed operations again on March 5.

2.4.2.3. Treatment of the poultry shipped to the poultry processing plant in Aichi Prefecture

All of the poultry shipped from the farm to the poultry processing plant in Aichi Prefecture were processed and it was confirmed that a portion for other than human consumption use had been shipped, which was returned to the plant and incinerated.

In addition, poultry residue discharged from the plant was processed at a rendering plant in Aichi Prefecture. The rendering plant incinerated the meal after rendering along with beef residue. For the sake of safety, Aichi Prefecture had the plant disinfected.

2.4.2.4. Expert Committee (Domestic Fowl Disease Committee)

The fifth meeting of the Domestic Fowl Disease Committee was held at the MAFF on March 3 and the following views were expressed.

(1) Also taking into account the delay in confirmation of the outbreak, the restricted movement zone with a radius of 30km should be maintained for the time being and an examination of future handling should be undertaken at the time when the primary virus-free confirmation tests have been concluded.
(2) Since there was thought to be virus incursion from Kyoto to the poultry processing plants, etc., to which the farm had shipped poultry, epidemiological surveys of relevant farms, etc., should be conducted exhaustively without setting movement restrictions.
(3) Given the time required for confirmation of the outbreak, the prefectures and relevant organizations should be urged to reinforce outbreak prevention measures and monitoring activities

2.4.2.5. Release of restrictions on movement

Since no other infections were confirmed as a result of the primary virus-free confirmation tests conducted in the restricted movement zone by Kyoto Prefecture on March 31, after discussions with the MAFF, the prefecture decided to reduce the size of the zone as of 12:01am, April 1, and switched to a restricted shipment zone in the area from 5km to 30km from the infected farm.

In addition, the following views were expressed when sixth meeting of the Domestic Fowl Disease Committee was held on April 7.

(1) In regard to epidemic prevention measures relating to the outbreak in Kyoto Prefecture, if no other infections are confirmed through the secondary virus-free confirmation tests currently being conducted, shipment restrictions should be lifted and, if no further reports are received, there would be no problem with the cancellation of all movement restrictions as of 12:01am, April 13, 21 days after the conclusion of the final epidemic prevention measures.
(2) In principle, there is no need from the perspective of animal hygiene to recover poultry eggs and meat from the infected farm generally processed at poultry processing plants, GP centers, etc., for human consumption.
(3) In regard to the epidemic prevention manual, monitoring by the prefectures should be reinforced (expansion of monitoring targets from 1 farm per prefecture to 1 farm per Livestock Hygiene Service Center), epidemiological test methods and methods for virus-free confirmation tests within movement restriction zones should be specifically defined and other reviews should be promoted.

Since no other infections were recognized as the result of secondary virus-free confirmation tests conducted by Kyoto Prefecture by April 10, shipment restrictions were lifted as of 12:01am on April 11 leaving only a movement restriction zone with a radius of 5km from the infected farm.

Since no other infections were subsequently recognized, the 5km movement restriction zone was cancelled as of 12:01am April 13.

2.4.3. Confirmation of infection in crows (*Corvus macrorhynchos*)

Type A serum subtype H5N1 avian influenza virus was isolated from a total of nine jungle crows between May 7 and April 9 in the restricted movement zone in the third outbreak. A summary is given below. No other infection of wild birds was confirmed.

	Discovery date	Date of H5 subtype determination	Data of H5N1 subtype determination
Kyoto Prefecture (infected farm: 1, Sonobe-cho: 1)	3/4, 5	3/8	3/9
Osaka Prefecture (Ibaraki City: 1)	3/5	3/11	3/15
Kyoto Prefecture (Tanba-cho: 2)	3/4, 5	3/12	3/13
Kyoto Prefecture (Tanba-cho: 1)	3/10	3/15	3/16
Kyoto Prefecture (Kameoka City: 1)	3/14	3/18	3/20
Osaka Prefecture (Ibaraki City: 1)	3/17	3/22	3/24
Kyoto Prefecture (Kameoka City: 1)	4/2	4/9	4/9

2.5. Outbreak in Kyoto Prefecture (fourth case)

2.5.1. Course of events leading to the outbreak

Since there was a rapid increase in dead fowl on March 3 at a broiler farm (15,000 fowl) in Kyoto Prefecture located about 4km northeast of the infected farm of the third outbreak, the farm manager notified the Livestock Hygiene Service Center. The Service Center promptly conducted an on-site inspection of the farm and a simple test of the dead fowl at the farm. Since they tested positive as a result, the Kyoto Prefecture Livestock Department notified the MAFF of the suspicion of avian influenza virus infection. Kyoto Prefecture sent samples of the dead fowl to the National Institute of Animal Health on March 5 and disease diagnosis was conducted. Since it was confirmed as a result that the isolated virus was type A subtype H5 avian influenza virus, it was determined that the fowl were infected with highly pathogenic avian influenza. In addition, the virus was confirmed to be subtype H5N1 on March 8.

2.5.2. State of response to epidemic prevention

As initial epidemic prevention measures, various measures were implemented including restricted entry to the farm where the outbreak occurred by outsiders and disinfection of poultry houses beginning March 3.

Since the outbreak of dead fowl continued, Kyoto Prefecture, without waiting for the results of confirmation tests, ordered the culling of fowl at the farm on March 4 since their infection was suspected. The culling of the fowl was completed on March 5 and all epidemic prevention tasks were completed by March 11.

Since this outbreak was related to the third case in the restricted movement zone, a response was implemented concomitant with the third outbreak without setting a new restricted movement zone.

Section 2 Epidemiological Surveys in the Outbreak Locations

1. Epidemiological Surveys in Yamaguchi Prefecture

(Takayuki Mizuhara)

1.1. Summary of the outbreak

1.1.1. Summary of the outbreak and epidemic prevention measures

Abnormal deaths on December 28 were noted by the farm manager on December 29 and the veterinarian having jurisdiction contacted the Livestock Hygiene Service Center on the same day. On the 30th, the Service Center conducted an on-site inspection and disease diagnosis. Along with subsequent increased deaths and transmission to other poultry houses, disease diagnosis was conducted again on January 9, whereupon highly pathogenic avian influenza infection was confirmed on January 12 and the outbreak was reported the same day. A movement restriction zone with a radius of 30km was designated. Culling of fowl at the infected farm was carried out from January 13 to 15 and epidemic prevention measures, including the burying of the contaminated material and disinfection, were completed by January 21. On-site inspections of all poultry farms in the prefecture were completed by January 17 and it was confirmed that there were no other infections. The virus-free conditions of poultry farms within a 30km range and small-scale operations were confirmed beginning February 5. Since there was not spread of the infection to surrounding areas, the movement restriction measures were lifted effective 12:01am on February 19.

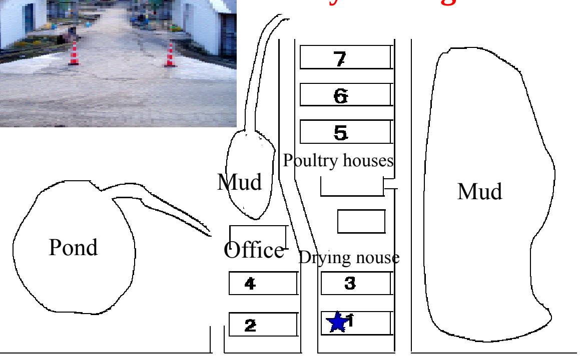
1.1.2. Summary of the outbreak location

1.1.2.1. Farm where the outbreak occurred

Egg production and open-type poultry houses, house no. 6 of the seven houses was unused, there was a total of 34,640 fowl.

1.1.2.2. Surrounding conditions

Poultry house layout diagram



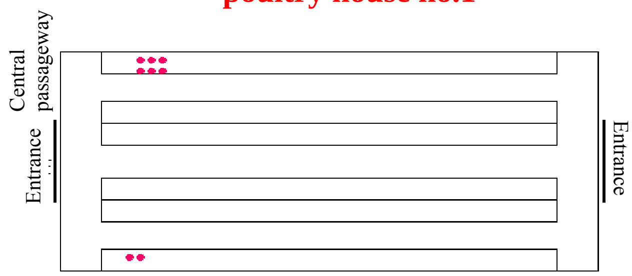
The farm is in a hilly area at an elevation of 280m about 1.5km from an arterial highway. The access road is only wide enough for a single vehicle and it provides access to only two homes before reaching a cul-de-sac (there is very little vehicle or foot traffic). There is a pond about 50m in circumference located about 50m to the northeast and ducks can be seen coming to the pond. There are three egg production farms about 4km to the east-southeast over the ridge. There is a river and two dams that are frequented by many wild birds within a radius of about 6km and there is vacant land near the site were wild boars are known to appear.

1.1.3. Detailed description of outbreak conditions

1.1.3.1. Course of events leading to the outbreak

On December 28, eight dead chickens were confirmed at two locations near windows of the entrance from the central passageway in poultry house no.1 (about 6,000 fowl). Of the four rows of cages, the dead chickens were found at two locations near the outer walls on each side. Prior to this, about 3 chickens at most had died a day. The manager and two employees noted the green-colored feces, sudden death and lethargy and, following that, deaths increased concentrically from the two sides where the dead chickens had been found. The number of dead chickens in poultry house no.1 gradually increased, reaching 221 by January 5. Later, when the number peaked on January 11, the number of deaths exceeded 800 per day. By the 12th, the day prior to the start of culling, nearly 70% of the fowl in poultry house no.1 had died.

Location of the initial outbreak in poultry house no.1



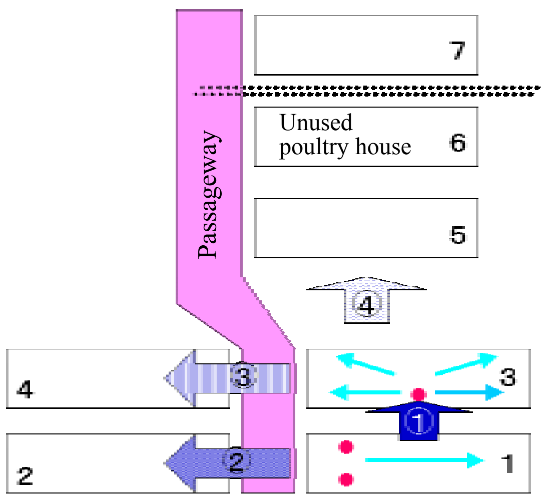
On January 5, eight days after the abnormality was first noted, the deaths of 19 chickens were confirmed in poultry house no.3 (about 5,000 fowl). Deaths increased concentrically from near the center of the window side of poultry house no.1 and, by January 13, the number of deaths had exceeded 1,300. Deaths were confirmed in poultry houses nos. 4 and 2 next after house no.3, followed by nos. 5 and 7 in succession.

Since the virus isolated from samples used in disease diagnoses conducted on January 9 had hemagglutinating properties, a report was sent to the MAFF on January 11 to the effect that an outbreak of avian influenza was suspected. Type A subtype H5 avian influenza infection was confirmed at the Livestock Hygiene Service Center on January 12. The fowl were determined to be infected with highly pathogenic avian influenza.

1.1.3.2. Conditions of work tasks at the farm

A total of six employees, including the manager, manage the fowl at the farm. Though they wore long rubber boots and work garments exclusively for use at the site, there were no boot disinfection tanks at the entrances to the poultry houses nor were there any boots or work garments specifically for individual poultry houses. Though the personnel in charge were generally assigned to feeding, management, repair, egg collection, feces removal and other tasks, those in charge of these tasks by poultry house was not decided and essentially all of the employees carried out work activities in all of the houses. Egg collection was carried out in houses nos.1, 2, 4, 3 and 5, in that order, and three would enter house no.1 through the entrance leading from the central passageway for egg collection and the other four would collect eggs from their rows.

Transitions in outbreaks in poultry houses



In house no.1, egg collection started at near the location where the outbreak occurred. The manager was primarily in charge of feces removal (houses nos.1 and 2 only, it was not implemented in the other houses) and transfer to the drying house. Dead chickens were buried on vacant ground in back of the farm office.

1.2. Epidemiological survey results

1.2.1. Survey of the infected farm

1.2.1.1. Introduction of chicks

Pullets were delivered to house no.7 from December 24 through 26; however, the farm supplying the chicks located in Fukuoka Prefecture tested negative and the house in which they were introduced was not the first to be infected (it was the last). The trucks delivering the chicks (headquarters) did not stop by any other farms along the way. Furthermore, introduction of chicks in house no.1, where the outbreak occurred first, had been in April.

1.2.1.2. Human contact (persons entering the poultry houses)

Employees: Three of them lived in a house across from the poultry houses. Two lived in town and the last one lived in a nearby city. Contact with waterfowl and other wild birds was not confirmed at all. None of them had traveled overseas during the previous two month period.

Veterinarian: After a visit on December 16 for notification of test results, the veterinarian visited one other farm and disinfected his automobile upon return. There was a total of 18 visits between the visit on December 29 and the time of the definite diagnosis. However, whenever entering and leaving the farm, he disinfected his hands, clothing, boots, etc., and, when returning, disinfected his boots and automobile. There were no outbreaks at the farms that he visited.

Construction workers: An acquaintance in the town came to the farm from December 15 through the 18 for curtain construction work in poultry house no.7 and did no disinfect when leaving. It has not been determined at all whether there was any contact with waterfowl or other wild birds. The person had not traveled overseas during the most recent two-month period.

1.2.1.3. Vehicles

Feed delivery trucks: Deliveries were made essentially every day (4 vehicles of a specific company). The vehicles were disinfected at the entrance to the farm and were disinfected again upon return to the company. These trucks only traveled back and forth between the farm and company and did not make any stops at other farms.

Egg shipment trucks: The trucks came every day (8 vehicles of a specific company). The trucks were not disinfected at the time of arrival at the farm but were disinfected upon arrival at the GP Center. The trucks only traveled between the farm and GP Center and did not make any stops at other farms.

1.2.1.4. Animal incursions

Wild birds: The employees saw sparrows, crows and pigeons coming into the poultry houses. Ducks can be seen at the pond located at a distance of 50m and their cries can be heard at night. The poultry house entrances were often left open during the day. No dead birds had been seen in the vicinity.

Mice and other wild animals: Poison bait type rodent poison was used during the summer and was applied continuously, replenished when required.

1.2.1.5. Contact with objects

Feed: In the poultry house where the outbreak occurred first, feed was automatically supplied from the feed blend tank and was supplied manually in the other houses using a supply cart. The poultry was not provided with any feed other than the feed blend.

Water: Water was supplied to each of the poultry houses by pipe from a well located near poultry house no.7. Poultry house no.1 alone was not special.

Rack containers: The driver of the farm truck delivered six containers to house no.6 on December 14 prior to the introduction of chicks.

Calendars: B, an employee of a product company, and C, an employee of a construction company, and D, an employee of an electrical contracting company, brought calendars to the farm on December 3, December 8 and at the end of the year (specific time unknown), respectively. In addition, veterinarian E brought a calendar on December 16 at the time of seasonal greetings. None of them, except the employee of the electrical contracting company, were disinfected. Contact with waterfowl or other wild birds has not been confirmed at all and none of them had traveled overseas during the previous two months.

1.2.1.6. Other visitors

Pharmaceutical company employee F: Came for greetings on December 18 and was disinfected upon arrival. A total of seven poultry farms were visited between December 3 and 8. Contact with waterfowl or other wild birds has not been confirmed at all and the employee had not traveled overseas during the previous two months.

Pharmaceutical company employee G: Delivered vaccine on December 25 and was disinfected upon arrival. A total of 19 farms were visited between December 1 and 22. Contact with waterfowl or other wild birds has not been confirmed at all; however, the employee did visit a pet shop on the same day (no contact with birds). The employee had not traveled overseas during the previous two months.

Neighbor H: Visited the farm on December 9 and 11 and was not disinfected upon arrival. H was raising 5 ducks and 70 chickens. None of the chickens had died since May 2002 and infection has not been confirmed.

1.2.2. Relationship to the outbreaks in Oita and Kyoto Prefectures

Through interviews, etc., with the six employees and the 37 people who had entered the farm, no response was received indicating any relationship to the outbreaks in Oita and Kyoto Prefectures through the movement of people, objects, etc.

The eggs were delivered to the GP Center in a nearby city and culled chickens were disposed of on the farm premises. Compost was at the accumulation stage on the farm premises since the farm had started operations in April 2003 and none had been delivered outside of the farm. In this respect as well, no relationship to Oita or Kyoto Prefecture was discernable.

1.2.3. Relationship between the outbreak location and Korea or other countries with outbreaks

There is a ferry in Yamaguchi Prefecture linking Shimonoseki, at the western tip of

Yamaguchi Prefecture, and Pusan (Korea), which began operations in 1970, that covers the distance of 228 kilometers each way in about 13 hours. Though there are about 80,000 passengers a year, the high speed ferry (3 hours each way) in neighboring Fukuoka Prefecture has more than 300,000 passengers a year, and it is not a main distribution route with Korea. Tourists also tend to go to the hot springs and theme parks in northern Kyushu rather than to Yamaguchi Prefecture.

Ato-cho, the outbreak site, has virtually no exchanges with countries where outbreaks of highly pathogenic avian influenza have occurred other than Korea and there are few tourists and foreign workers. Nothing notable can be cited in terms of a relationship with other countries where outbreaks have occurred.

1.2.4. Virus tests on the farm and in the vicinity

Virus isolation were conducted by February 19, when movement restrictions were lifted, of 10 samples of feces thought to originate in wild birds collected on the farm premises, 5 samples of water from the pond at a distance of 50m and 72 samples of water at points where wild birds (mallards, mandarin ducks, etc.) that had been observed and 13 samples of feces thought to have originated in wild birds within a distance of about 2km, all of which tested negative. In addition, virus was also not isolated from two rats caught in the poultry houses on February 11 and 26.

2. Epidemiological Surveys in Oita Prefecture

(Makoto Yoshitake)

2.1. Summary of the outbreak

2.1.1. Summary of the outbreak and epidemic prevention measures

Three long-tailed bantams from among 13 bantams and 1 duck being raised in a home garden died on February 14, 2004. An additional four died on February 16. The Livestock Hygiene Service Center conducted an on-site inspection and disease diagnosis the same day. Since type A influenza was identified using a simple test kit, the remaining 6 bantams and 1 duck were culled. It was recognized to be subtype H5 highly pathogenic avian influenza on February 17.

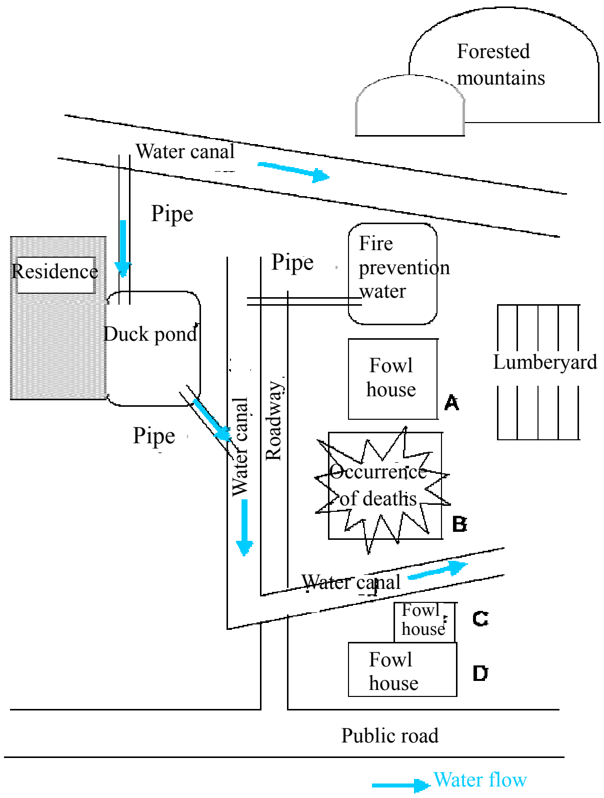
A movement restriction zone with a radius of 30km centered in the fowl house was therefore designated on the 17^{th}. The culling of the remaining fowl was completed on the 16^{th} and burial of the house and other contaminated material was completed on the 18^{th} (completion of initial epidemic prevention measures). Subsequently, poultry farms and small-scale poultry operations within the movement restricted zone were inspected to confirm virus-free conditions and, since the infection had not spread to the surrounding area, movement restrictions were cancelled as of 12:01am on March 11.

2.1.2. Summary of the outbreak location

2.1.2.1. Outbreak location

The location of the garden of a private residence located about 150m from a station on the local JR train line, where 13 long-tailed bantams and 1 duck were being raised as pets. There were four fowl houses in the garden. Five bantams (female) were being raised in house A, 7 bantams (female) in house B, 1 bantam (male) in house C and the duck in house D There is a carp pond with a length of about 15m around the outside of the garden and the duck was free to move around the premises.

A lumber mill is operated at the home where the outbreak occurred and the houses are located in the garden linking to the lumberyard and there is a mountainous area on the north side of the site.



2.1.2.2. Surrounding conditions

The fowl houses were located in the Kusu Basin at a elevation of 450m. The area is dotted with forestland and, across the JR line track, there is a mountain with an elevation of 850m located 2km to the northeast, a mountain with an elevation of 600m 2km to the north, a mountain with an elevation of 1,140m 4.5km to the west and a mountain with an elevation of 770m 1km to the south. The Kusu River, which flows along the railway track, and its tributaries flow through the area. In addition, many people, especially students, pass through the area everyday. Wild birds have been observed in the garden pond. Within 5km of the location of outbreak, there are no poultry farms raising 1,000 or more fowl and there are about 230 homes where about 10 chickens are being raised for personal use or as pets with a total of about 1,300 fowl.

2.1.3. Detailed description of outbreak conditions

2.1.3.1. Course of events leading to the outbreak

During the forenoon of February 14, one bantam of the seven being raised in house B died suddenly without indicating any clinical symptoms and was buried on vacant ground on the residence premises. No particularly unusual conditions were recognized through the previous night. Since an additional two bantams in house B died suddenly during the afternoon that day, the raiser contacted the local Livestock Hygiene Service Center the same day through the local administrative office and the Service Center conducted an autopsy of the two dead bantams and virus tests (embryonated egg culture) that day. A report was received from the raiser during the morning of February 16 that an additional four bantams had died and, as the results of additional disease diagnosis, it was revealed to be type A influenza using the simple test kit by evening that day. The remaining six bantams and one duck were therefore culled with the approval of the raiser. Four of the bantams that had died demonstrated reddening of the face. Antibody tests were also conducted of the coresiding bantams and duck but they all tested negative.

Autopsy Findings

Autopsy findings	No.	External appearance			Internal appearance						
		Face	Legs	Cyanosis	Cervical subcutaneous	Esophagus	Trachea	Appendix	Ovarian duct	Ovaries	Diarrhea
Autopsy 2/14	1	Eyelid edema	-	-	-	-	-	Dark red at end	General petechia	Bleeding	-
Autopsy 2/14	2	Eyelid edema	-	-	-	-	-	Dark red at end	General petechia	Bleeding	-
Autopsy 2/16	1	-	-	Slight	Edema	Hyperemia & bleeding	-	Dark red all over	-	-	-
Autopsy 2/16	2	-	-	Slight	Edema	-	-	-	-	-	-
Autopsy 2/16	3	-	-	Slight	Edema	-	Bleeding	-	-	-	-
Autopsy 2/16	4	Eyelid edema	-	Slight	Edema	-	Slight bleeding	-	-	-	Green diarrhea

2.1.3.2. Conditions of work tasks at the home

The fowl were given food and water everyday (by the raiser). The feed was a feed blend obtained at the farmer's co-operative. They were occasionally given vegetable residue and other vegetable matter. Drinking water was well water using a simple water pipe supplied daily in wash basins placed in each of the houses. Since the water in house B had frozen on the evening prior to the initial deaths (February 13), water was provided after first melting the ice using water draining from the pond through a drain pipe.   Well water was provided to the other houses as usual.

Though the bantams were kept in the houses without being allowed out, the duck was allowed to move freely and was able to move around the premises at will.

2.2. Epidemiological survey results

2.2.1. Surveys of the location of outbreak

2.2.1.1. Origin of delivered chicks, etc.

Bantams: The raiser obtained bantam eggs from Oguni-machi, Kumamoto Prefecture, 3 years previously (2001) and had incubated them himself.

Duck: The raiser had found the duck, which had come down the Kusu River from upstream while out on a walk in July 2003.   He took it home and continued to raise it.

2.2.1.2. Human contact (persons entering the poultry houses)

Family members: Only the raiser gave the fowl feed and water.   Though he operates a lumber mill, it is impossible to go into the mountains during the winter because of the cold.

Besides family members, when grandchildren come to play, they have at times approached the fowl houses.   The employees work in the lumberyard adjacent to the residence premises where the fowl houses are located but have never approached closely to them.

Livestock related persons: There are no visitors other than the farmer's co-operative, which delivers the feed.

Forestry related persons: Since the raiser operates a lumber mill, there are visitors in the lumber-related business but they have not approached the fowl houses.

2.2.1.3. Vehicles

Feed delivery trucks: There were no feed deliveries after January 27. When delivering, farmer's co-operative staff use a light truck.

Lumber-related trucks: The lumber mill only handles domestically produced lumber.

2.2.1.4. Animal incursions

Wild birds: Since the fowl houses were strongly built using wood and metal wire mesh, it was not possible for sparrows or other wild bird to enter. Sparrows often come to the pond adjoining the fowl houses and blue herons have also come but none were observed prior to the outbreak.

Photo: External appearance of one of the fowl houses

Wild birds not ordinarily observed arrived in quite large numbers (several 1,000) in mid-January this year and about 30-40 of them died and fell at a location nearby.   The bird was about the size of a swallow with a long bill, black back and white on the side of the feathers and webbed feet.   Besides the raiser, two other people in the vicinity also observed these birds but were unable to identify the species.

Mice: Unconfirmed by the raiser.

Other: Weasels, martens, foxes and raccoon dogs have approached, though none were witnessed prior to the outbreak.

2.2.1.5. Contact with objects

Feed: The feed blend (Power Layer 16Y) was purchased at JA Kusu Kokonoe (January 27, 2004) and fed to the fowl. Besides that, they are also fed vegetable residue, rice screenings, etc., but are not given table leftovers.

Photo: Pond where the duck was raised (drainage ditch on the upper right)

Water: They were normally given well water but, on the evening of the day prior to the outbreak, the fowl in house B only were given pond water (water canal from the water intake of No.2 Machida Power Plant on the Naruko River) (since the water in the water container had frozen, the ice in the container was melted with water taken from the pond drain pipe and they were given water directly from the pond).

Lumber: All of the lumber handled is domestic lumber and no imported lumber is handled.

2.2.2. Relationship to the outbreaks in Yamaguchi and Kyoto Prefectures

The raiser had not traveled to either Kyoto Prefecture or Yamaguchi Prefecture prior to the outbreak.

2.2.3. Relationship between the outbreak location and Korea or other countries with outbreaks

The raiser had not traveled to Korea or other country where outbreaks have occurred or to Kyoto or Yamaguchi Prefecture.

3. Epidemiological Surveys in Kyoto Prefecture

(Takashi Nakanishi)

3.1. Summary of the farm involved in the third outbreak

3.1.1. Summary of the outbreak

3.1.1.1. Summary of epidemic prevention measures

The farm where the outbreak occurred is one of the largest scale egg production farms in the prefecture with a combination of 10 poultry houses and GP center and about 225,000 fowl. On about February 17, there was an increase in dead fowl in poultry house no.8 (about 30,000 chickens). Next there was an increase in dead fowl in the neighboring poultry house no.9 about five days later. By the 26th, about nine days after the initial occurrence, there was an increase in dead chickens in all of the poultry houses. There was no report by the farm owner to the local Livestock Hygiene Service Center and an on-site inspection was conducted before dawn on the 27th after an anonymous telephone call was received.

Photo: Interior of poultry house no.9 (from the entrance)

It was determined to be highly pathogenic avian influenza on the 29th. All epidemic prevention measures, including the burial of contaminated material and disinfection, were concluded by March 22.

Virus-free confirmation surveys were conducted at poultry farms and small-scale operations within a range of 30km and, since the infection had not spread into the surrounding area, all movement restrictions, which had been established at infected farm, were released effective 12:01am on April 13.

3.1.1.2. Summary of the infected farm

3.1.1.2.1. Infected farm

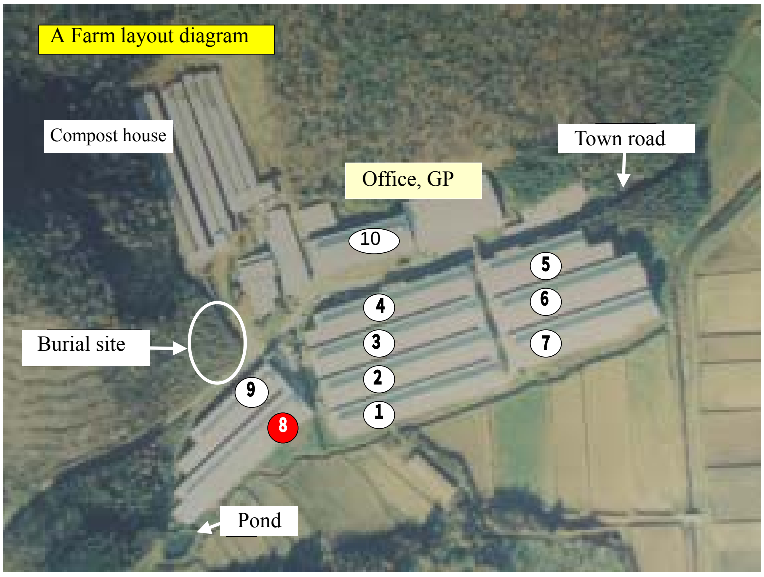
The farm consists of 10 poultry houses with open-type elevated-floor chick 4-stage double type (2 fowl) for egg layers with a total of about 225,000 fowl. There are 28 employees. Besides the infected farm, the company also directly operates four other farms (Hyogo and Okayama Prefectures) and one affiliated farm (Okayama Prefecture).

The 10 poultry houses at the farm are segmented into numbers 1-4, 5-7, 8-9 and 10 with a structure that links all by corridors.

3.1.1.2.2. Surrounding conditions

Although a town road passes through the farm, it is unpaved, narrow and has very light traffic. Nine of the poultry houses are located south of the road and one house, compost yard and GP center are located on the north side. There is a pond with a circumference of about 30m near the initially infected house (no.8). The pond holds water from a stream about 1m in width and water that wells up in the pond and is the water source for houses 8 and 9. There is no bird net covering the pond and it is visited by wild birds including waterfowl.

The farm is at an elevation of about 230m located in a valley between mountains about 170m higher than the farm in elevation on the south side and mountains about 140m higher on the north side and sightings of wild boar, foxes, raccoon dogs and other wild animals have been confirmed. In addition, many crows frequent the area around the compost yard.



A poultry farm about 4km away is the site of the second confirmed outbreak in the prefecture.

3.1.1.3. Detailed description of outbreak conditions

3.1.1.3.1. Course of events leading to the outbreak

A death of a group of fowl was confirmed by an employee near the center of poultry house no.8 (30,000 fowl) on February 17. At first, the dead fowl were scattered among the stages of the 4-stage cages. Dead fowl later increased along rows of cages where deaths were confirmed and, from the 20th, it had spread throughout the entire house with more than 1,000 deaths a day. Forced molting was carried out on the 18th and 19th in house no.8 and feeding was resumed from the 20th.

There was an increase in deaths near the entrance of adjacent house no.9 about 3m distant from house no.8 from about February 22 and in house no.3, located across a passageway from no.8, from February 23. The infection spread later to houses 1, 2 and 4 in the same cluster.

From about February 24, an increase in deaths was confirmed in house no.6, across a passageway from house no.3, later spreading to houses 5 and 7 in the same cluster and to house no.10 across the town road. By the 27th, there was an increase in deaths in all of the houses.

3.1.1.3.2. Conditions of work tasks at the farm

The 28 employees of the infected farm were divided into three groups, poultry houses, compost and GP center, and, other than the removal of culled chickens and other times when manual work was especially required, none of them other than the three who are in charge of the poultry houses ever entered the houses. In addition, those in charge of the houses were assigned to specific houses (buildings) (1-4, 5-7, 8-10) and normally the employees with access to the houses (buildings) were specified.

Manure was periodically moved to the primary fermentation facility across the town road and was later moved to the secondary fermentation facility for processing.

Dead fowl were discarded in the manure in the primary fermentation facility.

3.1.2. Epidemiological survey results

3.1.2.1. Survey of the infected farm

3.1.2.1.1. Introduction of chicks

31,752 chicks from one of the company's directly operated farms (Okayama Prefecture) were delivered by company truck to poultry house no.6 on February 6-8 but the infection did not occur first in house no.6.

3.1.2.1.2. Human contact

Entry into the poultry houses by persons other than employees after the beginning of February has not been confirmed. Boot disinfection tanks were installed before the outbreak at the entrance to the poultry houses (buildings) and in the service rooms and the employees who worked in the poultry houses wore special-purpose nylon poultry house capes as they worked. It has not been confirmed whether or not any of the employees had traveled overseas prior to the outbreak or raised fowl themselves at home.

There is no particular management veterinarian and, if something unusual occurred, the feed company's veterinarian was consulted by telephone, etc. The feed company's veterinarian had not visited the farm since the beginning of this year.

Late at night on February 26, a media reporter came to the farm to gather information and, by the time the police arrived at about 10:00am the next morning and set up barricades, it has been confirmed that a number of reporters had gathered in front of the farm office to interview the president.

3.1.2.1.3. Vehicles

Disinfection at the farm: Lime had been spread randomly in the poultry houses and in front of the GP center 1-2 months prior to the outbreak.

Feed trucks: The trucks of a contract supplier (contract with 1 supplier) delivered feed virtually every day. At the time of feed delivery, the feed was delivered to the feed tanks along the town road and the trucks did not enter the farm site itself. In addition, at the time of delivery, the trucks did not go to any other farms, etc.

Egg shipments: The trucks of contract companies (contracts with 2 companies) came to the farm every day and delivered the eggs primarily to an affiliated center in Himeji City. Occasionally, stores in the neighborhood would come to purchase eggs and there were transactions in February on the 10th, 13th, 20th and 25th.

Manure shipments: The entire volume of bagged fermented poultry manure was removed virtually every day by company trucks and the trucks of a contract company (contract with 1 company) and delivered to home centers, etc., in the Kansai area. They did not stop by other farms at the time of delivery. Farms in the area occasionally came to pick up manure.

Culled fowl shipments: 15,532 culled fowl from house no.3 were shipped to broiler processing plants in Hyogo and Aichi Prefectures on February 25-26. Adult chickens were simply placed in cages that were secured in place with rope without any sheets or other coverings.

3.1.2.1.4. Animal incursions

Various wild birds, including crows, had been observed entering the poultry houses. The wire mesh used on the ventilation ports on the roofs of the poultry houses was large enough to permit the entry of small birds the size of sparrows and, in addition, entry was also possible through ventilation fan ports installed in the center of the houses or through holes in the wire mesh. A wild bird somewhat smaller than a pigeon was found flying around inside house no.8 on about February 10 and an employee observed a crow entering house no.8 sometime after the beginning of February, though the specific time is unknown.

There were many mice and a nest was found under the ceiling of house no.8, where the initial infection occurred, and scraps of insulation that had fallen from the ceiling was scattered around in the vicinity of the cages where the first group of fowl deaths was confirmed. Stray cats had also settled in the houses.

Since the dead fowl were taken to the compost yard, it was confirmed that thousands of crows gathered at the site to eat the dead birds.

3.1.2.1.5. Contact with objects

Feed: All of the houses were automatically supplied with feed blend.

Water: Water pumped up from underground was supplied to houses 1-7. It was possible to switch between underground water and pond water for supplies to houses 8 and 9 and water was supplied without disinfection. Pond water was being used at the time of the outbreak. House no.10 was supplied with underground water and valley water.

A nipple system (a device that supplies water only when chickens peck a protruding nipple) was used for the water supply

Photo: Water pond in back of poultry house no.8 (there was no net at the time of the outbreak)

Medication: Medication was purchased from the Himeji branch of company A. It was regularly delivered to the farm through scheduled transport with the headquarters. There is no record of purchases from January onward.

3.1.2.1.6. Other

The egg conveyor belt in house no.8 broke on about February 7-8 and fell down on the chicken manure on the first floor. The location was on the side across the interior passageway across from the row of cages were the first group of deaths was confirmed.

3.1.2.2. Relationship to the outbreaks in Oita and Yamaguchi Prefectures

No connections with Oita Prefecture or Yamaguchi Prefecture through shipments of eggs, compost, etc., or travel by employees have been confirmed

3.1.2.3. Relationship between the outbreak location and Korea or other countries with outbreaks

Nothing in particular has been confirmed.

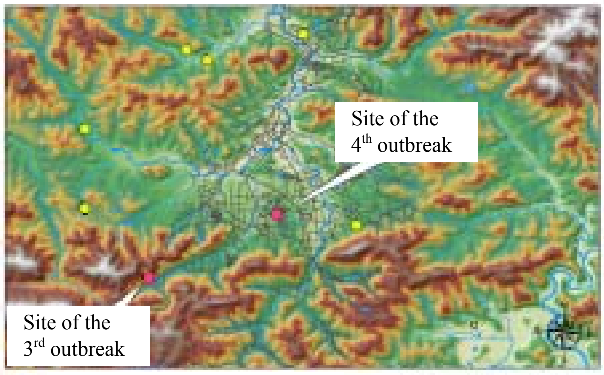
3.2. Summary of the farm involved in the fourth outbreak

3.2.1. Summary of the outbreak

3.2.1.1. Summary epidemic prevention measures

On March 3, 21 fowl died in poultry house no.10, where about 3,000 fowl were being raised, at a broiler farm (about 15,000 fowl) located some 4km northeast of the farm involved in the third outbreak. The raiser reported this to the Livestock Hygiene Service Center the same day and an on-site inspection and disease diagnosis were conducted. It was confirmed to be highly pathogenic avian influenza on March 5.

Map of the Tanba-cho, Kyoto Prefecture, area



Poultry farms with more than 1,000 fowl

The culling of the fowl started on March 4 prior to the definite diagnosis and all of the epidemic prevention measures, including burial of contaminated material and disinfection, were completed by March 11.

Given the short distance from the farm where the third outbreak occurred, new movement restrictions for the outbreak at this farm were not put in place.

3.2.1.2. Summary of the infected farm

3.2.1.2.1. Infected farm

The poultry farm, which is affiliated with a large breeding hen farm, is operated as a (joint stock) corporation by four members of the same family and the eldest son is a veterinarian. Twelve single-story poultry houses (combination of open-type and semi-windowless houses; the outbreak occurred in an open-type house) are located on the same site as the family residence. At the time of the outbreak, there were 15,000 meat poultry and five of the houses were in use.

3.2.1.2.2. Surrounding conditions

The line connecting the first farm to be infected and this farm is a valley crowded between mountains. The Sone River and a town road run along the belt-like zone traversing from southwest to northeast. The farm infected first is located on the upstream side.

Photo: Left-side exterior wall of poultry house no.10 (roadway beyond in the direction of the arrow)

The local administrative office and residences are located nearby and the public roadway that passes by the poultry houses has rather heavy traffic. Poultry house no.10, the first to be infected, is located near the road at a distance of about 30m. It can be seen from the road through a wooded area and is not enclosed in a fence or other enclosure. There is a pond in the athletic park to the northwest of the poultry houses that is frequented by many ducks and other wild birds and there is also a pond with a circumference of about 800m located 200m to the southeast.

3.2.1.3. Detailed description of outbreak conditions

3.2.1.3.1. Course of events leading to the outbreak

Although the normal death rate in each poultry house had been 0-4 fowl per day, there were 21 deaths in house no.10 on March 3. The Livestock Hygiene Service Center was notified the same day and a disease diagnosis was conducted. Culling started the next day since infection was suspected.

3.2.1.3.2. Conditions of work tasks at the farm

The labor force consists of four people, the father, mother, eldest son (veterinarian) and second son. The sequence of work tasks was not especially specified. Prompted by the outbreak in Yamaguchi Prefecture, they had purchased a power sprayer in late January and had furthermore installed barricades at the front entrance to prevent entry. They reinforced control of virus incursion from the outside by completely eliminating entry by objects and humans. Disinfection was implemented by installing boot disinfection tanks at the entrance to the poultry houses.

3.2.2. Epidemiological survey results

3.2.2.1. Survey of the infected farm

3.2. 2.1.1. Introduction of chicks

Chicks were brought into poultry house no.2 on February 27 but that was not the house where the outbreak occurred first.

3.2.2.1.2. Human contact

People other than the family members who entered the poultry houses after the beginning of February were limited to an electrical technician who came to replace the feed supply equipment timer in house no.10, where the outbreak first occurred, on February 22 and an employee of the Livestock Hygiene Service Center who stopped by while out making the rounds on the 28th. None of these people had recently traveled overseas or were acquainted with anyone at the site of the third outbreak.

Other than these, the only confirmed entries onto the farm premises within one or two weeks prior to the outbreak were five or six reporters who came onto the farm premises during the morning of the 27th by climbing over the barricades.

3.2.2.1.3. Vehicles

Feed delivery trucks: Transport company trucks came to the farm on February 23 and 25 and March 1. At the time of delivery, the trucks were disinfected at the entrance and the feed was input into feed tanks installed at each house. Only feed for this farm was loaded (5 tons) in the trucks, which made simple round trips between the feed plant and the farm without stopping by any other farms.

Broiler shipments: Trucks from a contract company (one company) came a total of 8 times between February 5 and 20. Approximately 3,400 poultry at an age of about 90 days per shipment were shipped by special trucks from a contract company (1 company) and by their own farm trucks. Company trucks were disinfected when shipping and farm trucks were disinfected only upon arrival back at the farm.

Chicken manure shipments: Chicken manure was shipped to local farms using their own farm trucks. The trucks were disinfected upon arrival back at the farm.

3.2.2.1.4. Animal incursions

The doors of the poultry houses are left open constantly. The raiser had not witnessed any incursions by wild birds into the infected poultry house. The raiser has also stated that they hardly ever saw crows in the vicinity of the poultry houses.

3.2.2.1.5. Contact with objects

Feed: Feed blend is supplied automatically from feed tanks to all poultry houses.

Water: Water drawn from a well is supplied to each house by pipe. The well has a diameter of 1m and does not have a lid and, until March 1, water was supplied without disinfection.

Photo: Well for water supply

Medications: Medications are received from the Kyoto branch of company A. Orders are placed through an affiliated company and deliveries are made by courier service, etc. The most recent delivery was on March 3 and there were no deliveries during February.

3.2.2.2. Relationship to the outbreaks in Oita and Yamaguchi Prefectures and the first outbreak in Kyoto Prefecture

Based on the travel records, etc., of people who accessed the poultry houses or the farm, there is no confirmed relationship to Oita or Yamaguchi Prefecture. In regard to the relationship to the site of the third outbreak, there are differences in the type of operations (egg vs. broiler production) and no commonalities have been confirmed between persons related to feed or product shipments with access to the two farms. In addition, though medication is supplied by the same company, the branch is different and the transport route is completely different.

3.2.2.3. Relationship between the outbreak location and Korea or other countries with outbreaks

Nothing special has been confirmed.

Section 3 Analysis of Virus Properties

(Kenji Tsukamoto)

1. Molecular epidemiological analysis

It is possible to clarify the mutual correlation between the outbreaks of highly pathogenic avian influenza that occurred in western Japan through a detailed comparison of the properties of the isolated viruses. In addition, outbreaks of the disease also occurred in seven other countries in East Asia at around the same time and, in order to conjecture the route of incursion of the virus into Japan, it is necessary to make molecular epidemiological comparisons with the virus isolated in those countries.

Genetic data relating to the strains isolated in Japan and those isolated in the other countries have been clarified by the Urgent Study of the Epidemiology of Highly Pathogenic Avian Influenza made possible by special coordination funds for supporting science and technology (SCF) of the Ministry of Education, Culture, Sports, Science and Technology (MEXT). Elucidation of the infection routes was estimated based on the results.

1.1. Sample viruses

The viruses isolated from the four outbreaks of highly pathogenic avian influenza are summarized in Table 1 below. In addition, temporal relationships are given in Fig. 1.

The first case occurred at an egg production farm in Ato-cho, Yamaguchi Prefecture. It was on December 28, 2003, when dead fowl were first found and confirmation of the virus was carried out on January 12, 2004.

The second case involved infected bantams being raised by a fancier in Kokonoe-machi, Oita Prefecture. Deaths occurred on February 14 and 16 and the virus was confirmed on February 17. The outbreak occurred in a location with no contacts with poultry farms in terms of the movement of chickens, objects, humans, etc.

The third case occurred at an egg production farm in Tanba-cho, Kyoto Prefecture. Fowl deaths were confirmed from about February 17 and the virus was confirmed on February 28. In this outbreak, prior to the establishment of movement restrictions, infected chickens and carcasses were shipped to broiler processing plants or chemical rendering plants and, therefore, viruses were also isolated from those locations and genetic analysis was conducted.

The fourth case occurred at a broiler farm located at a distance of about 4km from the infected farm of the third case and chicken deaths were confirmed on March 3, when epidemic prevention measures were put in place for the third outbreak. Confirmation of the virus was carried out on March 5. The geographical and time relationships to the third outbreak suggest the possibility of a spread of infection to the fourth outbreak.

Table 1 Isolated strains used on the analyses

No.	Isolated strain	Abbreviation	Collection/ determination	Outbreak location	Origin
1	A/Chicken/Yamaguchi/7/2004	Yamaguchi strain	04/1/9	Ato-cho, Yamaguchi Prefecture (case 1)	Chickens
2	A/Chicken/Oita/8/2004	Oita strain	04/2/17	Kokonoe-machi, Oita Prefecture (case 2)	Bantams
3	A/Chicken/Kyoto/3/2004	Kyoto 3rd strain	04/2/28	Tanba-cho, Kyoto Prefecture (case 3)	Chickens
4		Hyogo broiler Kyoto strain	04/3/3	Hyogo Prefecture broiler processing plant (originating in Kyoto)	Chickens
5		Hyogo broiler Okayama strain	04/3/3	Same (originating in Okayama)	Chickens
6		Kagawa chemical plant strain	04/3/2	Kagawa Prefecture chemical treatment plant (originating in Kyoto)	Chicken feathers
7	A/Chicken/Kyoto/4/2004	Kyoto 4th strain	04/3/5	Tanba-cho, Kyoto Prefecture (case 4)	Chickens
8	A/Crow/Kyoto/53/2004	Kyoto crow – strain 1	04/3/5	Tanba-cho, Kyoto prefecture (case 3)	Jungle crow
9	A/Crow/Kyoto/70/2004	Kyoto crow – strain 2	04/3/4	Sonobe-cho, Kyoto Prefecture	Jungle crow
10	A/Crow/Osaka/102/2004	Osaka crow – strain 1	04/3/5	Ibaraki City, Osaka Prefecture (private home)	Jungle crow
11	A/Crow/Osaka/1660/2004	Osaka crow – strain 2	04/3/17	Ibaraki City, Osaka Prefecture (private home)	Jungle crow
12	A/Chicken/Korea/ES/2003	Korea strain	03/12/10	Korea (1st case in Korea)	Chickens
13	A/Chicken/Supanburi/2/2004	Thailand chicken strain	2004	Thailand	Chickens
14	A/Quail/Angthong/72/2004	Thailand quail strain	2004	Thailand	Quails
15	A/Duck/Angthong/71/2004	Thailand duck strain	2004	Thailand	Ducks
16	A/Vietnam/1196/2004	Vietnam human strain	2004	Vietnam	Humans
17	A/Duck/Korea/ES/2003	Korea chicken strain	03/12/10	Korea	Chickens
18	A/Duck/China/E319-2/2003	Taiwan smuggled strain	03/12	Taiwan	Ducks
19	A/Duck/Anyang/AVL-1/2001	Korea import duck strain	2001	From Shanghai	Ducks
20	A/Chiken/HK/YU562/2001	Hong Kong chicken 01 strain	2001	Hong Kong	Chickens
21	A/HK/156/1997	Hong Kong 97 strain	1997	Hong Kong	Humans

In addition, in the third outbreak, dead crows were found inside of the 30km movement restriction zone while epidemic prevention measures were being implemented. Virus was detected in seven crows in Kyoto and two in Osaka and genetic analysis is also being conducted.

Furthermore, multiple simultaneous outbreaks of avian influenza also occurred in countries in East Asia. In order to examine the relationship between the outbreaks in Japan and those in the other East Asian countries, genetic similarity is being investigated between the chicken strain in the first outbreak in Korea (1 strain), the Thailand chicken, quail and duck strains (1 each), the Vietnamese human strain registered in the gene bank, the Taiwan strain from duck smuggled from China and the Hong Kong chicken strains (1997 & 2001) with the Yamaguchi Prefecture strain.

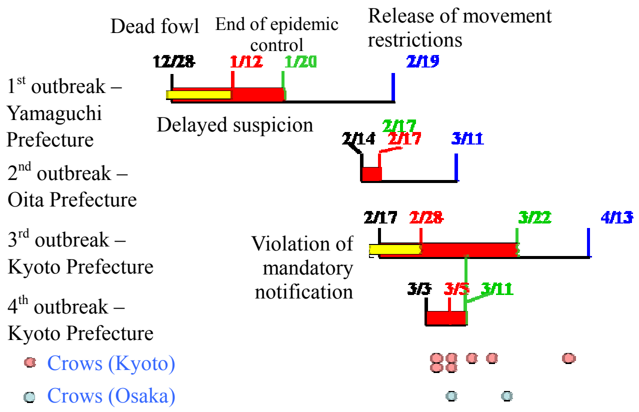


Fig. 1 Time relationships between the outbreaks

1.2. Analysis method

The avian influenza virus has eight different gene segments in the particles (PB1, PB2, PA, HA, NA, M, NS). Molecular epidemiological analyses of the virus are basically conducted over the entire area of all eight segments and, if partial genetic data of the eight segments could be adequately determined, partial areas are compared.

Of these, the HA segment is a major segment that prescribes the pathogenicity of the virus and it has a faster mutation rate than the other segments. Furthermore, since it is a segment that is suitable for ascertaining differences between strains and mutations, it is the most widely used of the eight segments in epidemiological analyses. We focused in particular on the HA segment.

1.3. Mutual similarity of viruses isolated in Japan

The route of infection of the viruses isolated in Japan was conjectured based on genetic analysis, time of infection, records of the movement of materials and geographical distance.

They are thought to be independent outbreaks due to the fact that the time of occurrence of the outbreaks in Yamaguchi, Oita and Kyoto Prefectures differs, that they are geographically separated from one another, that there is no definite correlation between the fowl and records of movement of materials and that there are differences, though slight, between the base sequence homology of the isolated viruses (Table 2, Fig. 2). Strictly speaking, there is a strong possibility that these outbreaks are the result of virus being brought to the farms from different sources along separate routes.

Table 2 Genetic comparison of virus strains isolated in Japan

Gene segment	Comparative base count	Homology with Yamaguchi strain (%)				Homology with Oita strain (%)			Homology with Kyoto strain (%)	
	(full code area)	Oita strain	Kyoto strain	Osaka crow -strain 1	Osaka crow-strain 2	Kyoto strain	Osaka crow -strain 1	Osaka crow – strain 2	Osaka crow - strain 1	Osaka crow - strain 2
PB2	2280	99.7	99.7	99.6	99.8	99.8	99.7	99.9	99.7	99.7
PB1	2274	99.7	100	99.9	99.8	99.7	99.7	99.6	100	99.8
PA	2151	99.8	99.7	99.7	99.9	99.7	99.7	99.9	99.5	99.7
HA	1704	99.5	99.6	99.7	99.4	99.5	99.6	99.4	99.8	99.6
NP	1497	99.9	99.9	99.9	99.9	100	99.9	100	99.9	99.9
NA	1350	99.5	99.4	99.3	99.6	99.6	99.3	99.6	99.5	99.4
M	982	100	99.9	99.9	99.9	99.9	99.9	99.9	100	100
NS	823	99.6	99.6	99.6	99.4	99.8	99.8	99.5	99.8	99.5

Table 3 Genetic comparison of three strains from the third outbreak in Kyoto and epidemiologically-related isolated strains

Gene segment	Comparative base count	Homology with Kyoto 3rd outbreak strain (%)									
		Hyogo broiler Kyoto strain	Hyogo broiler Okayama strain	Kagawa chemical plant strain	Kyoto 4th outbreak strain	Kyoto crow -1 (Sonobe)	Kyoto crow -2 (3rd outbreak)	Kyoto crow - 3 (Tanba)	Kyoto crow -4 (Kameoka)	Osaka crow -1 (Ibaraki)	Osaka crow - 2 (Ibaraki)
PB2	590	100	100	100	100	100	100	100	100	99.7	99.7
PB1	679	100	100	100	100	100	100	100	100	100	99.8
PA	903	100	100	100	100	100	100	100	100	99.5	99.7
HA	1704	99.9	99.6	99.9	99.8	99.9	99.9	99.8	99.8	99.8	99.5
NP	977	100	100	100	100	100	100	100	100	99.9	99.9
NA	576	100	100	100	100	99.8	100	100	100	99.5	99.4
M	651	100	100	100	100	100	100	100	100	100	100
NS	436	100	100	100	100	99.8	100	100	100	99.8	99.5

Given the fact that the outbreaks involving the Hyogo broiler plant Kyoto strain, Hyogo broiler Okayama strain, Kagawa chemical rendering plant strain and the Kyoto 4th outbreak strain all occurred at about the same time, that the record of movement of the fowl is clearly known and that base homology is essentially identical, the 3rd outbreak is considered to be the source of the infection (Table 3, Fig. 2)

In addition, since the time of discovery of Kyoto crow strains 1 and 2 coincides with the 3rd outbreak and base homology is essentially identical, the 3rd outbreak is thought to the be source of the crow infection (Table 3, Fig. 2).

Meanwhile, the fact that Osaka crow strains 1 and 2 differ somewhat in base homology and are distantly separated from the farm infected in the 3rd outbreak, there is thought to be possibility that that farm may not have been the direct source of infection.(Tables 2 & 3),

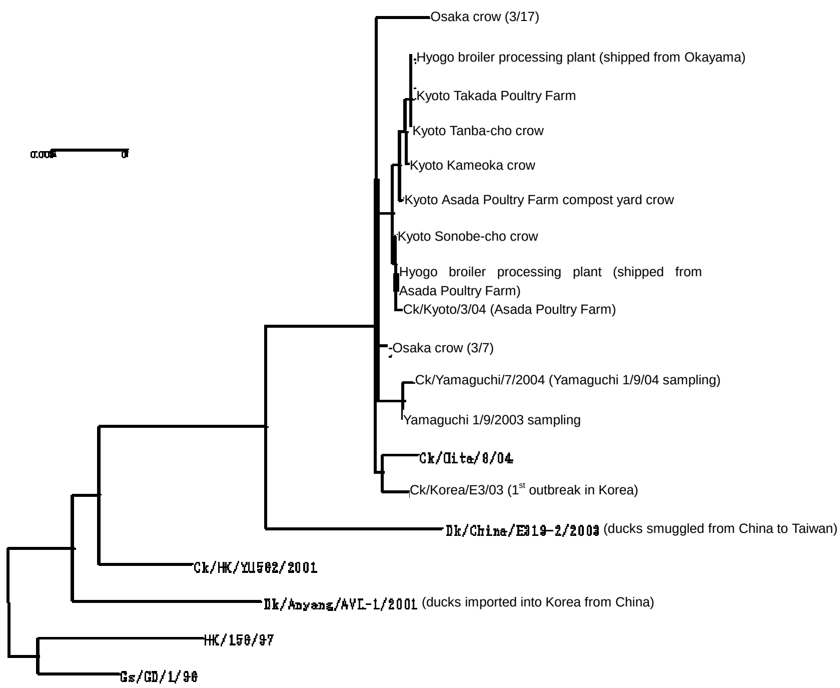


Fig. 2 Molecular phylogenetic tree of the HA segment of virus isolated in Japan

1.4. Similarity to viruses isolated in other East Asian countries

The Yamaguchi strain is thought to have greater affinity with the Korean strain since the base homology of all eight segments with the Korea strain is at the high level of 99% or more while the base homology of all segments with the three strains isolated in China, Hong Kong, Vietnam and Thailand is low at 92-99% (Tables 4 & 5). That is also evident from the molecular phylogenetic tree of the HA segment (omitted). The Oita strain, Kyoto 3rd outbreak strain (Kyoto 4th outbreak strain, Kyoto crow strains) and the Osaka crow strain are all thought to have the same degree of affinity to the Korean strain (Fig. 5). In addition, the similarity of the three strains isolated in China in 2004 and the strains isolated in Japan was examined but the homology of most segments was in the range of 93-98% and they are considered to be somewhat different viruses (data not shown).

Table 4 Genetic comparison of Korean and Japanese strains

Gene segment	Comparative base count (all coding regions)	Base homology with Korea strain (%)				
		Yamaguchi strain	Oita strain	Kyoto 3rd outbreak strain	Osaka crow - 1	Osaka crow - 2
PB2	2280	99.7	99.7	99.7	99.6	99.8
PB1	2274	99.8	99.6	99.9	99.9	99.7
PA	2151	99.7	99.6	99.5	99.6	99.8
HA	1704	99.5	99.6	99.6	99.7	99.4
NP	1497	99.7	99.8	99.8	99.7	99.8
NA	1350	99.6	99.8	99.6	99.6	99.7
M	982	99.9	99.9	99.8	99.8	99.8
NS	823	99.4	99.5	99.5	99.5	99.3

Table 5 Comparison of strains from Japan and from overseas

Gene segment	Base homology with strain isolated in Yamaguchi Prefecture (%)										
	Korea	China	China	.Thailand	Thailand	Thailand	Thailand	Vietnam	Hong Kong	Hong Kong	Hong Kong
	Chicken	Duck	Duck	Human*	Chicken	Duck	Quail	Human*	Human	Chicken	Human*
	(Ck/Korea/ES/03)	(Dk/China/E319-2/03)	D(k/Anyang/AVL-1/01)	(Thailand/)(KAN-1/04)	(Ck/Supanburi//2/04)	(Dk/Angthong/71/04)	(Qa/Angthong/72/04)	(Vietnam/1/)96/04	(HK/213/03)	(Ck/HK/YU562/01)	(HK/156/97)
PB2	99	98	94	NT	98	98	98	98	98	97	86
PB1	99	98	92	NT	98	99	98	98	99	98	91
PA	99	93	91	NT	93	93	93	92	93	93	89
HA	99	97	96	96	96	96	96	97	97	97	95
NP	99	99	92	NT	98	98	98	98	99	91	91
NA	99	97	97	96	97	97	97	97	89	89	88
M	99	98	96	NT	98	98	98	98	98	97	91
NS	99	98	93	NT	98	97	98	98	98	97	90

NT: Not examined (base sequence was not available)
*: Comparison of partial region

1.5. Discussion

Since the viruses isolated from chickens, bantams and crows in Japan in 2004 had equally high pathogenicity and had base homology of 99% or more, they are thought to have high mutual similarity. However, the mutual base homology of the Yamaguchi strain, Oita strain, Kyoto 3rd outbreak strain and Osaka crow strains is 99.4 – 99.8% and, since there is no base homology that approached 100%, as is apparent between the Kyoto 3rd outbreak strain and epidemiologically related strains, the four virus strains are thought to have already mutated before their incursion into Japan and there is thought to be the possibility that they may have been brought into Japan by different routes.

Meanwhile, in a comparison of virus strains isolated in East Asian countries, among the current virus strain samples, there was greater similarity with the strain isolated from chickens in Korea than the strain isolated from humans in Vietnam, the strain isolated from chickens in Thailand, the strain isolated from smuggled duck in Taiwan, the strain isolated in China and other strains (Tables 4 & 5). Since the outbreak in Korea started in early December 2003, it is thought possible that the virus came to Japan from the Korean Peninsula. However, since the strains currently obtainable are strains from limited areas of specific countries, there is also the possibility that the virus entered both Korea and Japan at essentially the same time from another area.

Multiple simultaneous outbreaks of this disease have occurred in wide-reaching regions of East Asia and there are no other examples anywhere else in the world. It is possible to conjecture that the outbreaks in Japan also occurred at essentially the same time in three or four geographically separate locations due to viruses that differ to a degree as the source. It would be difficult to imagine that there was frequent movement of materials, etc., between those countries with outbreaks and Japan and it is necessary to consider the involvement of wild birds.

If the source of the highly pathogenic avian influenza virus that has been isolated is conjectured based on HA genes, it would be possible to trace it back to the subtype H5N1 virus that was detected at a goose farm in Guangdong Province in 1996 (Fig. 3). The descendents of this virus were later detected repeatedly in Hong Kong (1997, 1999, 2000, 2001-03) and were also detected in ducks from Shanghai (2001) and duck meat originating in Shandong Province (2003). Based on this, subtype H5N1 virus has gradually proliferated from southern China toward northern China and is thought to have spread to eight countries in East Asia in 2003-04. The outbreaks in East Asia have calmed compared to earlier times, though, compared to a year ago, the area of contamination has undeniably expanded. If migratory birds were involved in the virus infestation, it is probably necessary to be constantly vigilant as long as the virus contamination continues in East Asia.

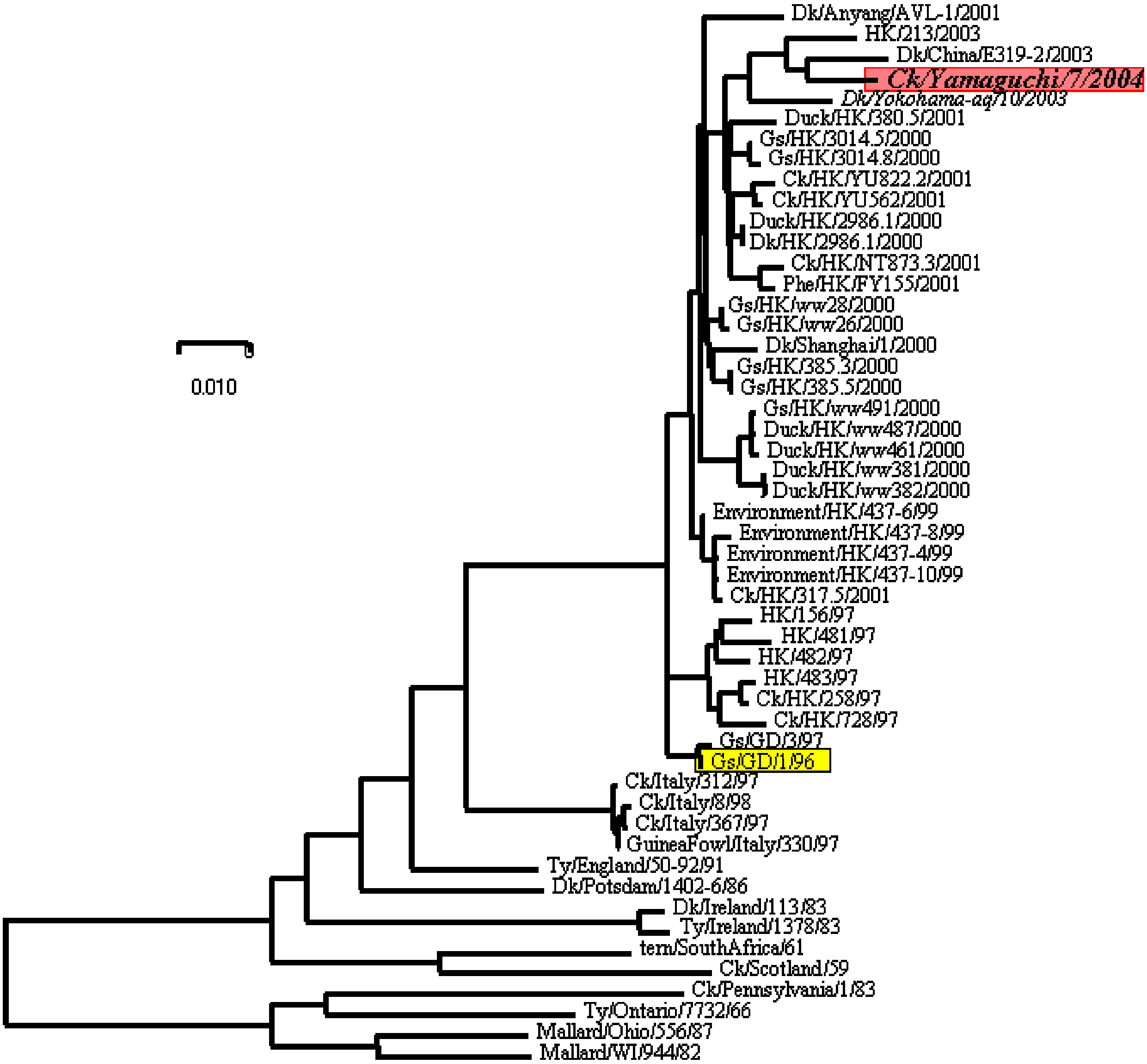


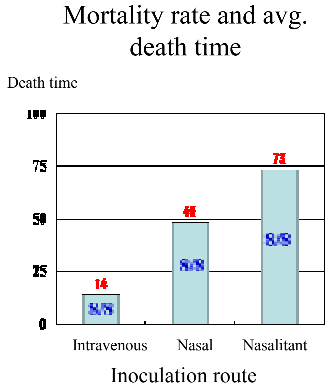
Fig. 3 Molecular phylogenetic tree of the HA segment

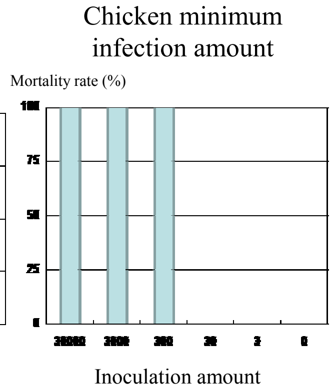
2. Susceptibility by birds and mammals

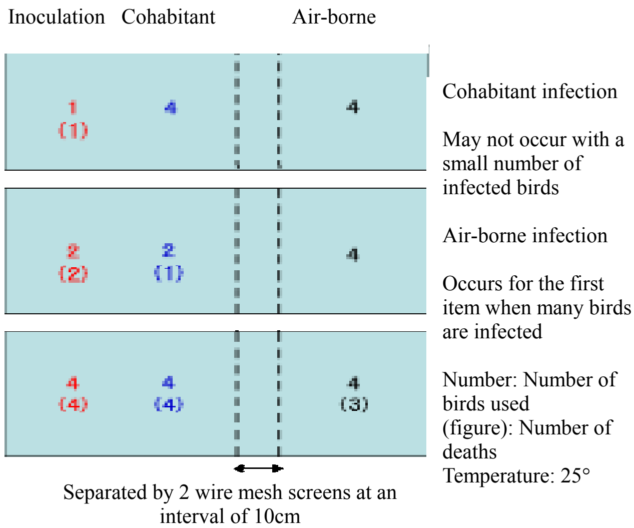
In regard to the Yamaguchi strain isolated in Japan, the susceptibility of chickens, mallards, budgerigars, gray starlings, jungle crows, sparrows, mice and hogs was examined in the Urgent Study of the Epidemiology of Highly Pathogenic Avian Influenza made possible by special coordination funds for supporting science and technology (SCF) of the MEXT. The results are introduced below together with a discussion of possible virus carriers.

2.1. Susceptibility of chickens

When the pathogenicity of the isolated viruses (Yamaguchi, Oita and Kyoto strains) to chickens was examined by intravenously inoculating chickens by methods based on international standards, it was found that all inoculated chickens died within 24 hours. The time of death due to the Kyoto strain was an average of 14 hours. It was confirmed based on this that the isolated strains were all highly pathogenic. In addition, when 8 chickens were given nasal inoculations of the Yamaguchi strain, all died within 3 days (avg. 40 hours) and 8 cohabiting chickens also all died within 4 days (avg. 73 hours) (National Institute of Animal Health). In addition, in the case of nasal inoculation, it was found that chickens were infected and died with a dose of about 300 virus particles.







However, it was confirmed in infection tests that the spread to cohabiting chickens and air-borne transmission may not readily occur if the number of early infected fowl is small. The results of transmission tests may make it possible to explain the conditions of the outbreak in Yamaguchi Prefecture, in which the infection spread gradually in the early stages. On the other hand, the infection in the third case in Kyoto Prefecture spread rapidly throughout all of the poultry houses. It has been pointed out that, since they are houses with elevated floors enclosed in plastic curtains, much virus-contaminated dust floated in the air, thereby possibly effectively causing air-borne transmission in those chicken houses.

2.2. Susceptibility of ducks

When mallards were given nasal inoculations, virus was recovered from all internal organs on the 3rd day; however, it is reported that a high level of antibodies were detected on the 14th day with no occurrence of illness or death (Hokkaido University). Based on this, it is possible that mallards were the carrier that brought the virus from Korea or other countries to Japan.

2.3. Susceptibility of budgerigars

High titers of virus were isolated from the entire bodies of budgerigars (*Melopsittacus undulatus*) given nasal inoculations of Yamaguchi strain virus and, after exhibiting severe neurological symptoms, all died by the 5th day (Hokkaido University). Budgerigars are considered to be highly susceptible to this virus.

2.4. Susceptibility of gray starlings

High titers of virus were isolated from the entire bodies of gray starlings given nasal inoculations of the Yamaguchi strain virus. It was reported, however, that it is unknown whether or not the infection was lethal since there were also deaths in the control group during the test period (Hokkaido University).

2.5. Susceptibility of crows

Subtype H5N1 virus was isolated from 9 dead jungle crows found in Kyoto and Osaka Prefectures over a period of about a month and virus antigens were detected in the tissues of 3 crows. Based on this, the possibility has been pointed out of strong susceptibility of crows to this virus. Thereupon, 4 jungle crows were given oral inoculations of virus and, though antibodies were confirmed on the 14th day, all but one remained healthy during the observation period and there were no deaths (National Institute of Animal Health). This would indicate that crows may not be particularly susceptible to H5N1 virus.

2.6. Susceptibility of sparrows

The susceptibility of sparrows to the Yamaguchi strain was examined since they are one possible species of wild bird that may inhabit the area around poultry houses. No symptoms were observed in sparrows given nasal inoculations; however, more than 90% died within 2-7 days and high titers of virus were detected throughout the body (National Institute of Animal Health). Based on this, it is clear that sparrows are highly susceptible to the virus.

2.7. Susceptibility of mice

The susceptibility of mice to the virus was examined using nasal inoculation. As a result, it was found that a dose of 1 million virus particles is required to cause death in mice and, after death, virus was detected in the lungs and brain. However, it is clear that it was not detected in feces (National Institute of Animal Health). As a result, it was found that, though mice do become infected, it would difficult to consider it to be an animal that would carry the virus into poultry houses other than mechanical infestation. The susceptibility of rats was not examined.

2.8. Susceptibility of hogs

The susceptibility of miniature hogs was examined using nasal inoculation of the Yamaguchi strain and, as a result, the virus was not detected from the internal organs or nasal cavity swabs and serum antibodies also tested negative on the 14th day. It was therefore reported that miniature hogs were not infected by the virus (Hokkaido University).

2.9. Discussion

Based on the test results obtained from the mallards, it is possible that the virus was brought from Korea or other countries to Japan by mallards or other migrating wild birds and that their feces later contaminated ponds or other bodies of water.

Contaminated water can be considered the most dangerous route of infection. When 1g of feces (10 million virus particles) was dissolved in a tub (100L), the virus density was 100 particles/ml of water; actually, however, uric acid in the feces has the effect of weakening this. However, even water that is contaminated with virus of very low density, the lethal dose for the establishment of infection in chickens is about 300 particles and, if about 200ml of water per day is ingested, there is the possibility the chickens could become infected if they continued to drink water during a single day. It is therefore probably important to conduct surveys of ponds located near poultry farms that are visited by waterfowl during the winter.

Meanwhile, if crows, sparrows, gray starlings and other resident birds are infected with virus in the natural environment, there is the possibility that areas surrounding poultry houses become contaminated and, though there is the possibility that viruses are carried directly into the poultry houses, it is not known how resident birds are infected in the natural environment. The amount of virus particles required to infect resident birds is unknown and, since it is thought that the amount of water intake at one time is small, if resident birds become infected in the natural environment, it may only occur due to the intake of water with a high virus concentration or the intake of virus in droppings. There is probably a need to further examine the route of infection of resident birds.

Moreover, since dead wild birds would probably serve as definite indicators of contamination, it would be desirable to continue the surveys. Subtype H5N1 virus was isolated from ducks, geese, swans, egrets, blue herons, eagles, owls, crows, magpies, quail, pheasants, parakeets, cranes, flamingoes, openbills and pigeons in East Asia between 2002 and 2004 (J. Virol. 78:4892-4901, 2004).

Section 4 Survey of the conditions of virus and antibody retention in wild birds

(Toshiro Ito)

1. Purpose

In this survey, we took samples from wild birds (terrestrial and water birds) in the vicinity of the outbreak sites in Yamaguchi, Oita and Kyoto Prefectures and studied the conditions of virus and serum antibody retention with the aim of helping specify the sources and infection routes of the highly pathogenic avian influenza outbreaks that occurred in Japan. We conjectured the routes of incursion of the virus into Japan and examined the possibility of the involvement of wild birds as the virus carrier based on the results obtained and information regarding flights of wild birds to Japan.

2. Research method

2.1. Isolation of virus from waterfowl in the vicinity of outbreak sites

We conducted a survey of wild waterfowl habitats within a minimum radius of 10km from the outbreak sites in Ato-cho, Yamaguchi Prefecture, Kokonoe-machi, Oita Prefecture, and Tanba-cho, Kyoto Prefecture and, at the same time, surveyed areas where it would be possible to collect samples. Based on the results of the surveys, we collected fresh feces samples from wild waterfowl. We recorded the collection location, time, bird species and other data of the samples obtained and promptly sent them in a frozen state to the Veterinary Public Health Science Laboratory at Tottori National University, Faculty of Agriculture.

2.2. Isolation of virus from the wild (terrestrial) birds in the vicinity of the outbreak sites and seroepidemiological surveys

We conducted a survey of wild (terrestrial migratory) bird habitats within a minimum radius of 10km from the outbreak sites and, at the same time, surveyed areas where it would be possible to collect samples. Based on the results of the surveys, we captured birds using mist nets, etc., and collected feces, throat swab, cloaca swab and blood samples. We recorded the collection location, time, bird species and other data of the samples obtained, carried out serum separation of the blood on-site using a centrifuge and promptly sent the remaining samples in a frozen state to the Veterinary Public Health Science Laboratory at Tottori National University, Faculty of Agriculture.

2.3. Virus isolation and serological identification

The feces samples were dissolved in a phosphate buffered saline solution with the addition of penicillin G potassium (10,000 units/ml; Banyu Pharmaceutical Co., Ltd.) and streptomycin sulfate (10,000 units/ml; Meiji Seika Kaisha, Ltd.) to obtain 20-30% (w/w) and, after centrifuging for 10 minutes at 2,000rpm, the supernatant was inoculated into the chorioallantoic cavity of 10-11 day old embryonated hen's eggs. The throat and cloaca swab fluid was dissolved in bouillon with the same antibiotic substances added and, after centrifuging for 10 minutes at 2,000rpm, the supernatant was likewise inoculated in embryonated hen's eggs. It was incubated at 35°C for 3 days and left for 1 night at 4°C and then the chorioallantoic fluid was aseptically collected. The existence of virus in the chorioallantoic fluid was determined by the chicken hemagglutination (HA) test. If hemagglutinating (HA) activity was not recognized, the recovered chorioallantoic fluid was be inoculated again in embryonated hen's eggs and the same procedure was repeated. If HA activity was not later recognized, it was judged to be negative for the virus. Samples in which virus was detected were subjected to hemagglutination inhibition (HI) and neuraminidase inhibition (NI) tests according to the microtiter method and the serum subtype was identified.

2.4. Determination of genetic base sequences of the HA gene

The HA genes of A/teal/Tottori/150/02 (H5N3; “strain 150”) isolated from teal fece s collected at Nikko Pond in the suburbs of Ketaka-cho, Tottori Prefecture, in January 2 002, and A/whistling swan/Shimane/580/02 (H5N3; “strain 580”) isolated from whistling swan feces collected from a rice paddy field in the suburbs of Yasugi City, Shimane Pr efecture, in December 2002 was amplified using the primer described below and the bas e sequences were determined. [; pol. ISW499-HA (+) (CACACACGTCTCCGGGAGCA AAAGCAGGGGTCTRATCTAYYAAAAT) ; H5HA360 (TCAACGACTATGAAGAGCTG) ; H5H665 (TATGTGTCTGTAGGAACATCAACGC) ; H5HR441 (CCTGATGAGGCATC GTGGTTGGACC) ; H5HR1021 (CCATACCAACCGTCTACCATTCC) ; H5HR782 (TTG GCTTTAAGATTGTCCAG) ; H5HA920 (ATGGGTGCAATAAACTCCAG)

2.5. Production of a phylogenetic tree

A phylogenetic tree was produced consisting of a total of 23 strains, including 21 strains of field isolation subtype H5 with base sequences obtained from the DNA Data Bank (DDBJ) and the above low pathogenic H5 virus strains 150 and 580, by the neighbor-joining (NJ) method using base sequence data for the HAI portion of the HA gene of each.

3. Study results

3.1. Survey of virus isolation from wild waterfowl in the vicinity of the infected farm in Yamaguchi Prefecture

We conducted a survey of wild waterfowl habitats within a radius of about 10km centered in the farm infected with highly pathogenic avian influenza and collected waterfowl feces based on the results of the survey. The collection data is given below.

Table 1 Feces samples of wild waterfowl collected from the vicinity of the infected farm in Yamaguchi Prefecture

Collection date	Collection location	Bird species	No. of samples
2/17/04	Abu River Dam	Mandarin duck	65
	Ikumo Dam	Mandarin duck, Eurasian wigeon (Anas Penelope)	26
	Sasanami Dam	mandarin duck, Baikal teal	48
	Ato-cho	jungle crow (Corvus macrorhynchos)	50
Total			189

The collected samples were sent to Tottori University and, as the result of virus isolation tests, all of the samples tested negative.

3.2. Isolation of virus from the wild (terrestrial) birds in the vicinity of the infected farm in Yamaguchi Prefecture and seroepidemiological surveys

We conducted a survey of wild (terrestrial) bird habitats within a radius of about 10km centered in the farm infected with highly pathogenic avian influenza and selected areas where samples collection would be possible. When making the selection, we were careful to select locations as close as possible to the site of the highly pathogenic avian influenza outbreak.

Based on the results of the survey, we captured birds with mist nets and collected feces, respiratory swab and blood samples. The collection locations, captured bird species and the number of samples are given below.

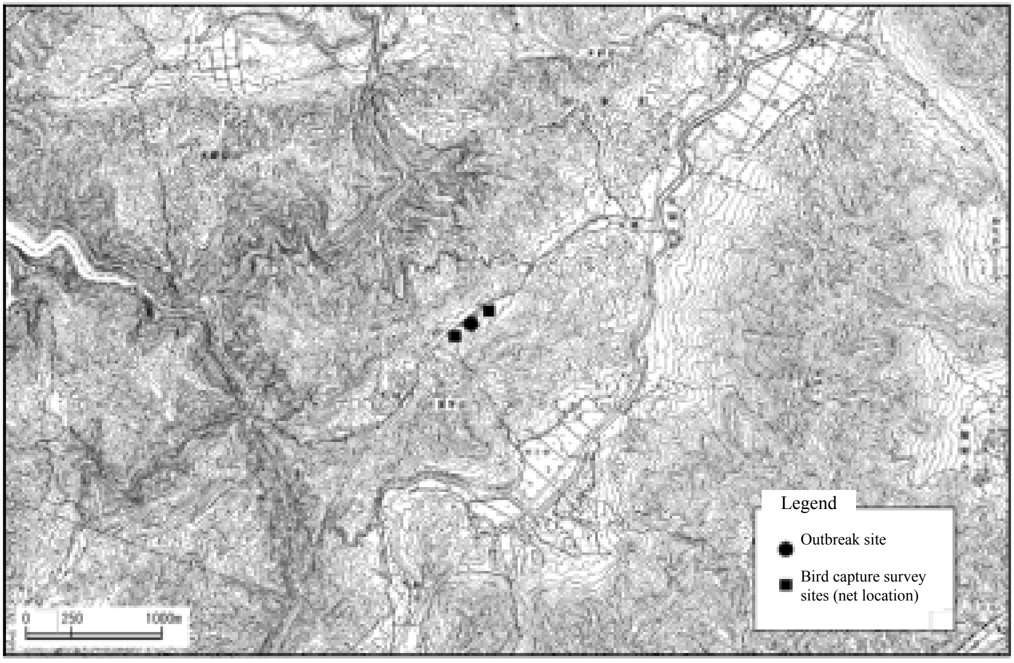


Fig. 1 Site of the outbreak of highly pathogenic avian influenza in Ato-cho, Yamaguchi Prefecture, and bird capture survey locations

Table 2 Terrestrial birds captured in the vicinity of the infected farm in Yamaguchi Prefecture

Order	Family	Species	No. of birds
Charadriiformes	Scolopacidae	Gallinago solitaria	1
Passeriformes	Pycnonotidae	Hypsipetes amaurotis	1
	Laniidae	Lanius bucephalus (bull-headed shrike)	3
	Troglodytidae	Troglodytes troglodytes	1
	Turdidae	Erithacus cyanurus	3
		Phoenicurus auroreus	4
		Turdus pallidus	9
		Turdus naumanni	16
		Cettia diphone	12
	Aegithalidae	Aegithalos caudatus	4
	Paridae	Parus major	2
	Emberizidae	Emberiza elegans	4
		Emberiza spodocephala	8
	Fringillidae	Carduelis cinica	1
		Carduelis spinus	1
		Uragus sibiricus	9
Total	9 families	17 species	91

Feces, throat and cloaca swab and blood samples were collected from these captured birds and were sent to Tottori University and, as the result of virus isolation tests, all of the samples tested negative. In addition, using the collected blood samples, HI tests were conducted with the subtype H5 influenza virus A/whistling swan/Shimane/499/83 (H5N3) strain as the antigen and, as a result, all of the samples tested negative for H5 antigens (serum dilution: 1:4 or less).

3.3. Survey of virus isolation from wild waterfowl in the vicinity of the infected site in Oita Prefecture

We conducted a survey of wild waterfowl habitats within a radius of about 10km centered in the site infected with highly pathogenic avian influenza and collected waterfowl feces based on the results of the survey. The collection data is given below.

Table 3 Feces samples of wild waterfowl collected from the vicinity of the infected site in Oita Prefecture

Collection date	Collection location	Bird species	No. of samples
3/3/04	Osumi, Kusu-cho	Carrion crow (Corvus corone)	50
3/4/04	Nakazumari, Kusu-cho	Anas crecca and other waterfowl	41
	Tsukawaki, Kusu-cho	Corvus frugilegus	50
3/5/04	Amagaya Reservoir, Kokonoe-machi	Ducks	21
3/7/04	Amagaya Reservoir, Kokonoe-machi	Ducks	20
3/8/04	Yamashita Pond, Kokonoe-machi	Waterfowl	17
	Yamashita Pond, Kokonoe-machi	Anas crecca or Eurasian wigeon	24
	Tanosencho-muta	Corvus frugilegus	8
Total			231

The collected samples were sent to Tottori University and, as the result of virus isolation tests, all of the samples tested negative.

3.4. Isolation of virus from the wild (terrestrial) birds in the vicinity of the infected site in Oita Prefecture and seroepidemiological surveys

We conducted a survey of wild (terrestrial) bird habitats within a radius of about 10km centered in the site infected with highly pathogenic avian influenza and selected areas where samples collection would be possible. When making the selection, we were careful to select locations as close as possible to the site of the highly pathogenic avian influenza outbreak.

Based on the results of the survey, we captured birds with mist nets and collected feces, respiratory swab and blood samples. The collection locations, captured bird species and the number of samples are given below.

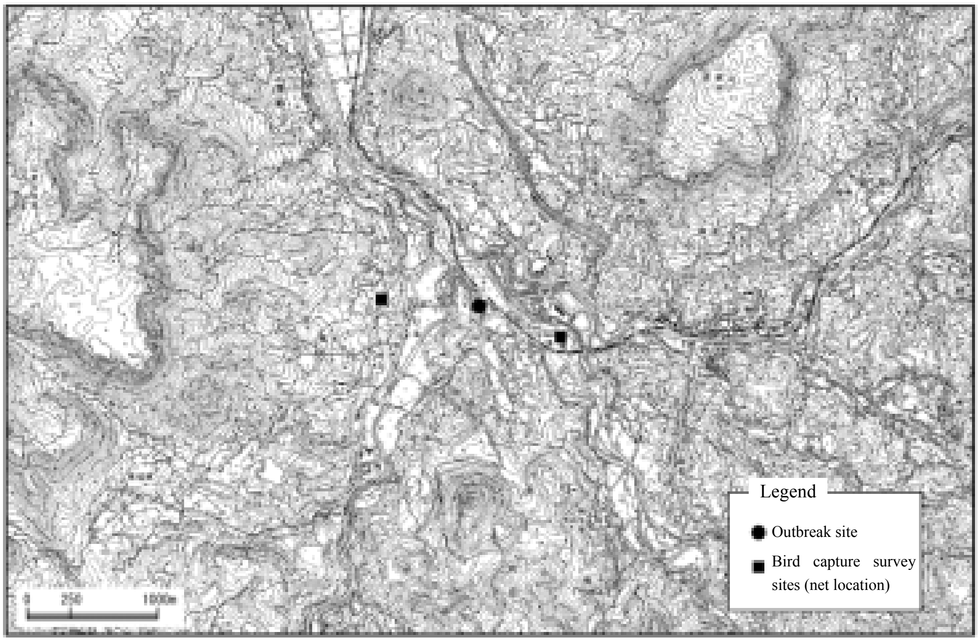


Fig. 2 Site of the outbreak of highly pathogenic avian influenza in Kokonoe-machi, Oita Prefecture, and bird capture survey location

Table 4 Terrestrial birds captured in the vicinity of the infected site in Oita Prefecture

Order	Family	Species	No. of birds
Passeriformes	Laniidae	Lanius bucephalus	2
	Muscicapidae	Phoenicurus auroreus	1
		Turdus naumanni	7
		Cettia diphone	2
	Aegithalidae	Aegithalos caudatus	3
	Emberizidae	Emberiza cioides	76
		Emberiza rustica	4
		Emberiza elegans	1
		Emberiza spodocephala	3
Total	4 familes	9 species	99

Feces, respiratory swab and blood samples were collected from these captured birds and were sent to Tottori University and, as the result of virus isolation tests, all of the samples tested negative. In addition, using the collected blood samples, HI tests were conducted and, as a result, all of the samples tested negative for H5 antigens.

3.5. Survey of virus isolation from wild waterfowl in the vicinity of the infected farm in Kyoto Prefecture

We conducted a survey of wild waterfowl habitats within a radius of about 10km centered in the infected farm and collected waterfowl feces based on the results of the survey. The collection data is given below.

Table 5 Feces samples of wild waterfowl and crows collected from the vicinity of the infected farm in Kyoto Prefecture

Collection date	Collection location	Bird species	No. of samples
3/10/04	Kuritani Pond, Kami-Miyata, Tanba-cho	Mallards	50
3/9-11/04 – 3/11	Innai, Tanba-cho	Jungle crow (Corvus macrorhynchos) Carrion crow (Corvus corone)	45
3/11/04	Mori, Tanba-cho	Large-billed & carrion crows	2
3/15/04 – 3/18	Vicinity of outbreak site, Tanba-cho	large-billed & carrion crows	59
Total			156

The collected samples were sent to Tottori University and, as the result of virus isolation tests, type A influenza virus was isolated from one mallard feces samples only (Kuritani Pond, Kami-Miyata, Tanba-cho). The isolated strain, however, was not found to be either subtype H5 or H7 and was not highly pathogenic avian influenza virus.

3.6. Isolation of virus from the wild (terrestrial) birds in the vicinity of the infected farm in Kyoto Prefecture and seroepidemiological surveys

We conducted a survey of wild (terrestrial) bird habitats within a radius of about 10km centered in the farm infected with highly pathogenic avian influenza and selected areas where samples collection would be possible. When making the selection, we were careful to select locations as close as possible to the site of the highly pathogenic avian influenza outbreak.

Based on the results of the survey, we captured birds with mist nets and collected feces, throat and leg swab and blood samples. The collection locations, captured bird species and the number of samples are given below.

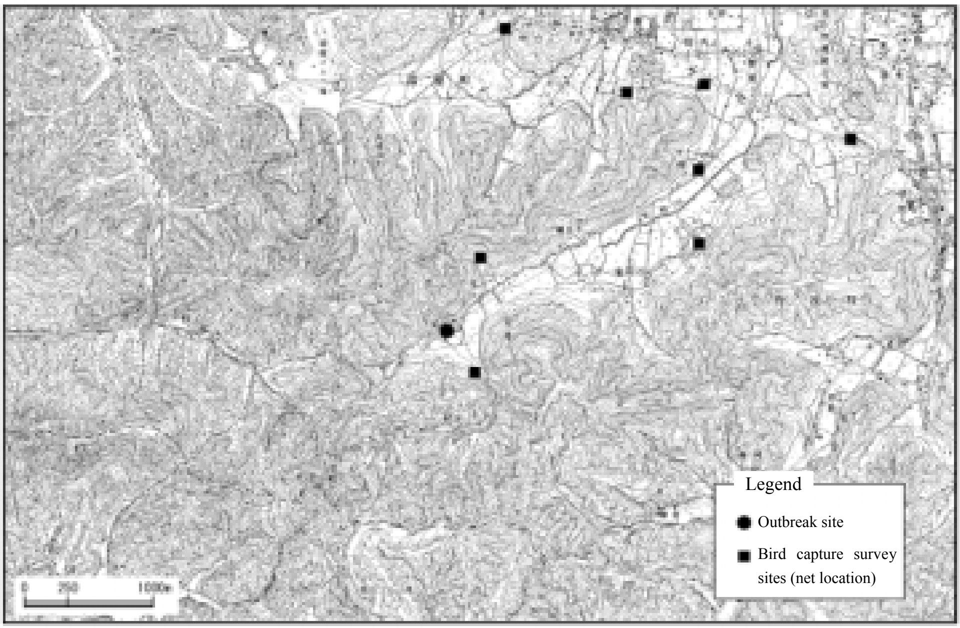


Fig. 3 Site of the outbreak of highly pathogenic avian influenza in Tanba-cho, Kyoto Prefecture, and bird capture survey location

Feces, respiratory and cloaca swab and blood samples were collected from these captured birds and were sent to Tottori University and, as the result of virus isolation tests, all of the samples tested negative. In addition, using the collected blood samples, HI tests were conducted and, as a result, all of the samples tested negative for H5 antigens.

Table 6 Terrestrial birds captured in the vicinity of the infected farm in Kyoto Prefecture

Order	Family	Species	No. of birds
Galliformes	Phasianidae	*Bambusicola thoracica*	1
Piciformes	Picidae	*Picoides major*	1
Passeriformes	Pycnonotidae	*Hypsipetes amaurotis*	15
	Laniidae	*Lanius bucephalus*	3
	Muscicapidae	*Erithacus cyanurus*	10
		Phoenicurus auroreus	3
		Zoothera dauma	1
		Turdus pallidus	7
		Turdus obscurus	1
		Turdus naumanni	18
		Cettia diphone	8
	Zosteropidae	*Zosterops japonicus*	1
	Emberizidae	*Emberiza cioides*	5
		Emberiza rustica	1
		Emberiza spodocephala	11
	Fringillidae	*Fringilla montifringilla*	4
		Carduelis cinica	1
		Uragus sibiricus	1
	Paridae	*Parus major*	5
	Aegithalidae	*Aegithalos caudatus*	2
	Corvidae	Crows	3
Total	11 families	21 species	102

3.7 Base sequences of subtype H5 low pathogenic virus from waterfowl flying into the Sanin area.

In the ongoing "Virus isolation survey of waterfowl flying into the Sanin area" being conducted at Tottori University, the base sequences were determined for the HA genes of 2 strains, namely, strain 150 strain isolated from teal feces collected at Nikko Pond in the suburbs of Ketaka-cho, Tottori Prefecture, in January 2002, and strain 580 isolated from whistling swan feces collected from a rice paddy field in the suburbs of Yasugi City, Shimane Prefecture, in December 2002, and their relationship to the present highly pathogenic avian influenza virus was examined using a phylogenetic tree (Fig. 4). As indicated in the diagram, both of these H5 low pathogenic virus strains originating in waterfowl belong to groups that are different from those of the present domestic virus strain A/chicken/Yamaguchi/7/04 (H5N1), the influenza virus strain HK/156/97 (H5N1) that spread to humans in Hong Kong in 1997 – 98 and the virus strain isolated from chickens in Hong Kong in 2001 (H5N1). Therefore, these subtype H5 virus strains originating in waterfowl are judged to have no relationship to the present three domestic outbreaks.

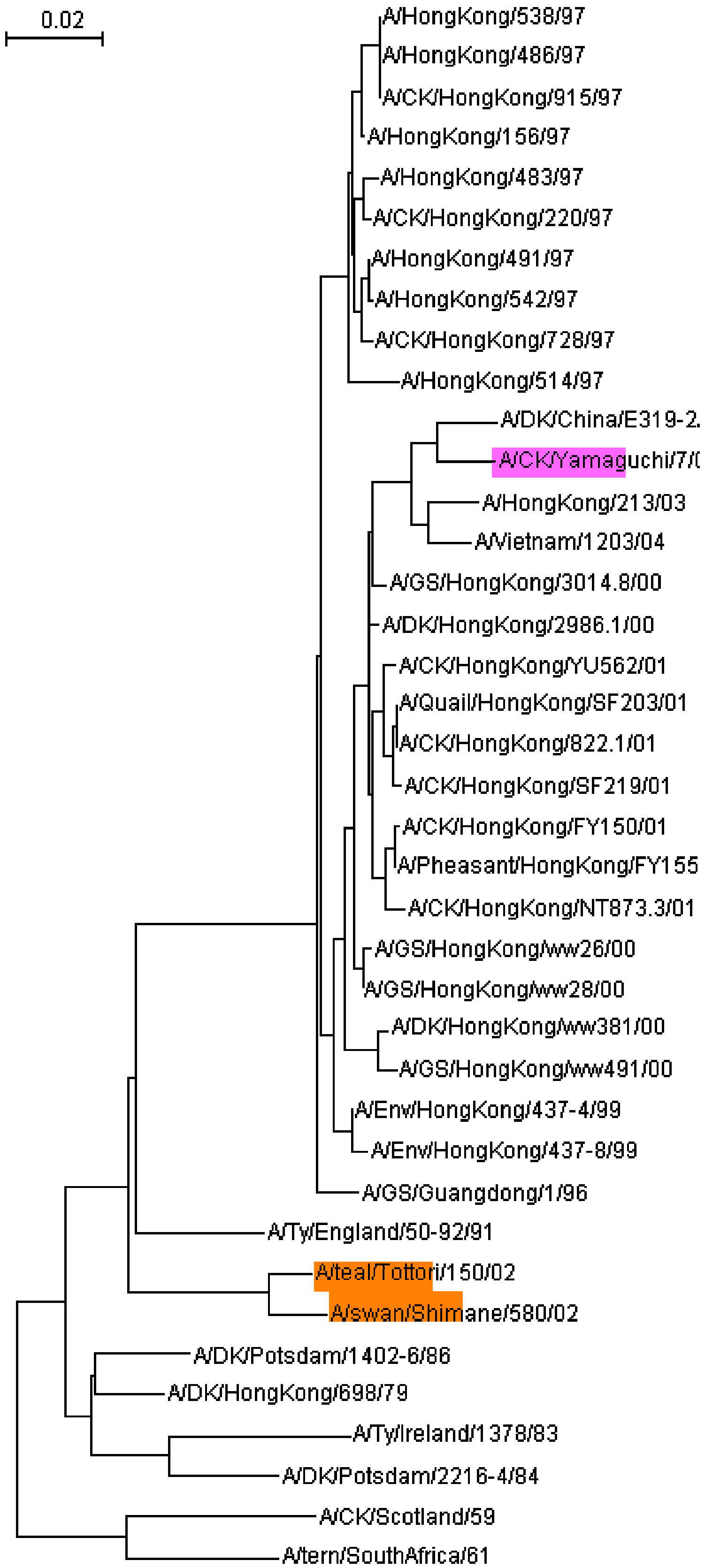


Fig. 4 Phylogenetic tree of HA genes of H5 avian influenza virus

4. Discussion

4.1. Possibility of wild birds as carriers

The fact that neither highly pathogenic virus nor serum antibodies were detected from the wild bird specimens that were collected in the vicinity of the outbreak sites (total 292 wild bird specimens, total waterfowl and crow feces samples: 577) in this survey does not necessarily negate the possibility that the viruses in the present domestic outbreaks were carried from the continent by wild birds. Nevertheless, it has been judged that there is little possibility that the virus became widely spread among wild birds at least in the vicinity of the outbreak sites. In other words, even if it is hypothesized that the virus was brought from the continent by wild birds, it did not become widely spread among wild birds in Japan prior to the infection of the chicken flocks and it is thought that a relatively limited number of bird species played the role of carrier.

Since it was not possible to examine the serum antibodies of waterfowl this time due to the limitations of this urgent survey, it is not possible to determine whether or not the waterfowl in the vicinity were infected with the virus. Consequently, even if there is little possibility that the wild waterfowl could directly enter the poultry houses, the possibility remains that, first of all, they brought the virus into Japan, which was then then brought into the poultry houses by some other route (humans, sparrows, mice, etc.).

4.2. Possibility of increased virulence in the low pathogenic virus carried in domestic wild waterfowl

As the result of genetic analysis of low pathogenic H5 virus isolated from wild waterfowl in the Sanin area the year before last, it was judged that there was no relationship to the present highly pathogenic virus. During the past few years, H5 virus was isolated only in 2002 and the same virus was likewise isolated this year in Hokkaido (Professor Hiroshi Yoshida, Hokkaido University, personal communication). Furthermore, H5 virus has not been isolated in virus isolation tests in the Sanin area and in Hokkaido last year or this year. Based on cases in Mexico, Italy and other countries, if low pathogenic H5 viruses carried by wild waterfowl are transmitted to chicken flocks, they always become more virulent within about 6 months to 1 year. Given these circumstances, it is thought that there is an extremely low possibility that low pathogenic H5 virus brought by wild waterfowl was transmitted to domestic chickens in recent years and thereby increased in virulence, culminating in the present outbreaks of highly pathogenic avian influenza.

Section 5 Wild bird surveys

(Yutaka Kanai, Kumiko Yoneda)

1. Wild bird ecology and avian influenza

(Yukata Kanai)

As bird species inhabiting Japan, the (2000) *Check-list of Japanese Birds: Sixth revised edition* published by the Ornithological Society of Japan lists birds in 18 orders, 74 families, 230 genera and 542 species plus 26 introduced species. Among them, there are many accidental visitors that are blown in by typhoons or otherwise come to Japan unintentionally but, other than these accidental visitors and sea birds, there are about 350 species that are constant inhabitants of the Japan mainland.

Since Japan is located in the mid-latitudes, there are many migratory bird inhabitants, which can be divided for the most part between summer visitors, passage birds and winter visitors. Summer visitors spend the winter in the area spanning from southern China to Southeast Asia and migrate to Japan to breed from April to May in the summer. Though these include terns, little ringed plovers, ruddy crakes and other wetland birds, the majority, some 80 species, consists of small birds that live in forests and grasslands. Passage birds are species that use Japan as a stopover when migrating in the spring from April to early May and in the autumn from August to October. Sandpipers and plovers are typical examples but forest dwelling species are also included among the total of about 40 species. Winter visitors breed in Siberia and the Arctic and migrate to Japan for wintering and there are about 100 species. Typical winter visitors are geese, ducks and swans but there are also many forest and grassland birds. Species that stay in Japan all year long are referred to as Residental species, of which there are about 130. Virtually all of the species that inhabit areas around human settlements are Residental species, though swallows, which build nests in homes, are summer visitors.

The habitats of these bird species are segregated due to differences in habitat location, foraging environment and food depending on the species.

The avian influenza virus primarily infects the digestive tract of geese, ducks, swans or sandpipers and plovers as the basic host and is transmitted by feces. Various subtypes of avian influenza virus have been isolated from 88 bird species of 12 orders, including geese, ducks, sandpipers and plovers as well as petrels, auklets, sea gulls and pheasants. The detection rate in bird species with the exception of freshwater ducks is usually not particularly high even in sandpiper and plover species. According to the *Field Manual of Wildlife Diseases: Birds* published by the USGS-National Wildlife Health Center, virus propagation is apparent in geese, ducks, sandpipers and plovers but seldom in other waterfowl and it is virtually never confirmed in the case of terrestrial birds. However, if artificially infected, many terrestrial birds do become infected and the reason why virus infection is seldom confirmed in terrestrial birds is thought to be because of little contact with infected waterfowl that would culminate in infection due to ecological and behavior differences.

Generally speaking wild birds seldom become ill even if infected and there has been only one case of large-scale deaths reported to date, which involved terns infected with H5N3 avian influenza virus in South Africa in 1961. In this case, avian influenza had spread through poultry farms in the vicinity two years prior to the large-scale death of terns.

Cited literature and references
Ornithological Society of Japan, Avian Influenza Problem Investigation Committee, 2004 Avian Influenza Problem Investigation Committee Report, *Current State of the Avian influenza Problem and Issues*, Ornithological Society of Japan
USGS-National Wildlife Health Center, *Field Manual of Wildlife Diseases: Birds*

2. Migratory bird migration routes and infection routes

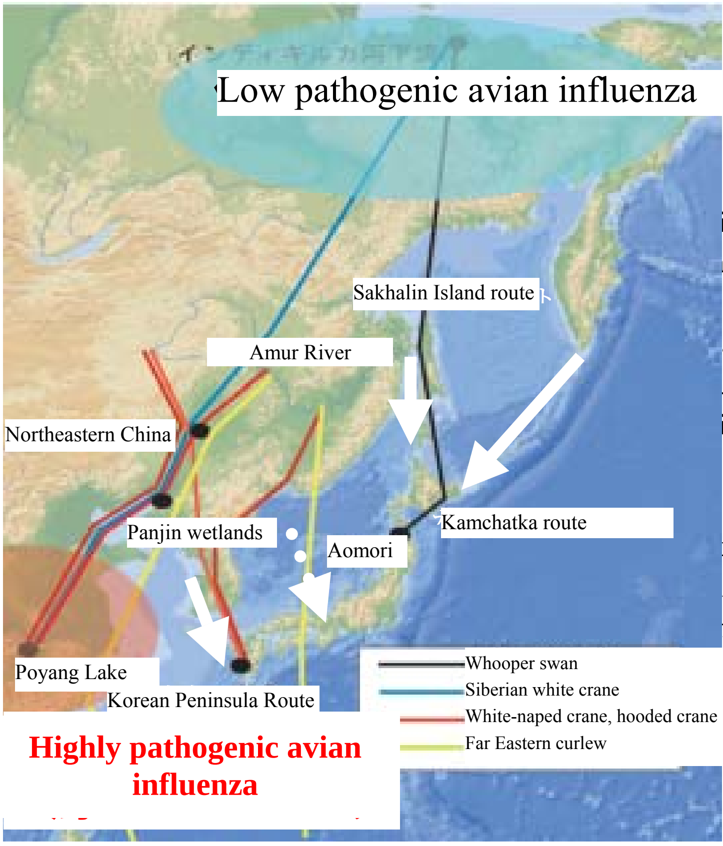
(Yutaka Kanai)

The migration routes of migratory birds are assumed to be the island-hopping route from Hokkaido to the Kamchatka Peninsula along the Kurile Islands, the route via Sakhalin Island, the route from the Korean Peninsula to Kyushu, the Nansei Island route and the Ogasawara Island route. However, extremely few attempts have been made to direct confirm migratory routes. The Sakhalin Island and Kamchatka routes have been confirmed using neck tags and satellite tracking in the case of geese and swans and satellite tracking in the case of eagles. In addition, the Korean Peninsula route has been indicated through the satellite tracking of cranes. Besides these routes, there are also suggestions of the existence of routes directly across the Japan Sea, which has been indicated through satellite tracking of the Far Eastern curlew.

Since the present cases of highly pathogenic avian influenza occurred during the winter, possibly be involved. Since the virus birds with possible involvement could ninsula or a route directly across the

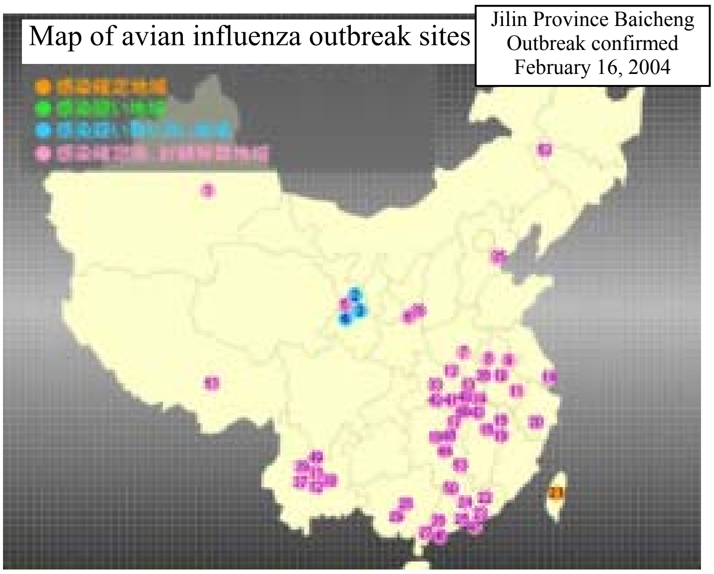
ember 12, 2003, the time of infection in Yamaguchi Prefecture is thought to pan after the outbreak in Korea, there

avian influenza, the Ministry of the urveys in Yamaguchi, Oita and Kyoto ion, virus confirmation tests were also brought to local government offices confirmed other than what would be d bird capture and feces tests were also n only one case, a magpie captured in ital birds with a limited activity range tbreak of avian influenza at a nearby



Migratory bird routes and avian influenza accumulation sites

Low pathogenic avian influenza is in the breeding grounds of geese, ducks, sandpipers and plovers and is brought to Japan by virus carriers. However, since highly pathogenic avian influenza occurs primarily south of the south central part of China, it deviates from the migratory route to Japan. The Korean Peninsula route passes over Korea (the white arrows indicate the conjectured routes).



Distribution of domestic avian influenza outbreaks in China

There was only one outbreak (no. 52), which occurred on February 16, 2004, in northeastern China, which is on the migratory route from the Korean Peninsula to Japan.

Cited literature and references

Higuchi, H. Ozaki, K., Fujita, G., Soma, M., Kanmuri, N., Ueta, M., 1992. Satellite-tracking of migration route of cranes from southern Japan. Strix11, 1 20.

Kanai,Y., Sato,F., Ueta,M. , Minton, J., Higuchi.H., Soma.M., Mita,N., h,,latsui,S., 1997.
The migration routes and important rest sites of Whooper swans satellite tracked from northern Japan. Strix

Driscoll, P.V., UETA. M., 2002. The migration route and behaviour of Eastern Curlews. Ibis 144:119-130

Ueta, M., Sato, F, Lobkov, E.G. & Mlita, N. 1998. Migration route of White-tailed Sea Eagles Haliaeetus albicillain northeastern Asia. Ibis 140: 684-696.

Ueta, M., Sato. F., Nakagawa. H. & Mita. N. 2000. Migration routes of Steller's Sea Eagles Haliaeetus pelagicusin northeastern Asia and differences of migration schedule between adult and young eagles. Ibis 142:35-39.

3. Wild bird habitats in the vicinity of the outbreak sites

(Kumiko Yoneda)

We prepared a list of bird habitats through a literature search and confirmed wild birds that reside in areas where outbreaks of highly pathogenic avian influenza have occurred through on-site surveys.

3.1. Method

3.1.1. Literature search

We prepared a list of bird species with habitats in Yamaguchi, Oita and Kyoto Prefectures where the outbreaks occurred based on available literature (Environment Agency, 1988a, Kyoto Prefecture, 1979; 1993; 2002, Oita Prefecture 1982; 2001; Yamaguchi Prefecture 1976; 2003; Yamashina Institute for Ornithology 1994; 1995; 1996; 1997; 1998). From among them, we extracted winter visitors and residental birds and furthermore excluded pelagic bird species that are known to seldom come to land.

3.1.2. On-site surveys

We conducted on-site surveys at about the same time as the capture tests for virus surveys during the following periods:

* Yamaguchi Prefecture: January 19 – February 27, 2004 (major survey period: about 1.5 months following the outbreak)
* Oita Prefecture: February 24 – March 9, 2004 (major survey period: about 20 days following the outbreak)
* Kyoto Prefecture: March 1 – 18, 2004 (major survey period: about 20 days following the outbreak)

Two types of surveys were conducted, namely, surveys to ascertain the avifauna in the area near the avian influenza outbreak and surveys to ascertain the conditions of wide-area duck habitats, which are considered to be possible virus carriers. Furthermore, on-site observations by the Ministry of the Environment and species that were observed at the time of preliminary inspections for the capture tests were also added to the results.

We conducted route censuses to ascertain the avifauna in the area near outbreak sites. We set 3 different census routes within 2-3km from avian influenza outbreak sites so that they would pass through differing environments such as outbreak site, rice paddy field and forestland, to assure that we were able to broadly ascertain the avifauna in the area of outbreak. In Kyoto, however, since epidemic prevention activities were implemented in the infected poultry houses and there were some areas where access was prohibited, it was not possible to set routes that passed through the outbreak site. The censuses were therefore conducted on four surveyable routes in the vicinity of outbreak site. The line length was set at 1km and the census width was 25m on each side (total 50m). While walking along the route at a speed of 1-2km/hr and using and 8-10 power binoculars, the species, count, confirmation time and other data were recorded for confirmed bird sightings. If it was not possible to identify the species, a notation was made such as “unknown species, genus Emberiza.” The route censuses were conducted in Yamaguchi Prefecture on February 24-25, 2004, in Oita Prefecture on March 8-9, 2004 and in Kyoto Prefecture on March 16-17, 2004, and the censuses were repeated four times on each route.

To ascertain duck species, an area with a radius of about 10km from the outbreak site was set as the survey area. We went around lakes, ponds, rivers and so forth within this range that were inhabited by ducks and other waterfowl and recorded the bird species and count using binoculars and telescopes. We conducted this survey in Yamaguchi Prefecture on February 16-25, 2004, in Oita Prefecture on March 7-9, 2004 and in Kyoto Prefecture on March 15-17, 2004.

3.2. Results and discussion

The species confirmed through the on-site surveys were recorded on the lists obtained through the literature searches (Tables 1-3). Species other than winter visitors and residents that were confirmed within the areas through the on-site surveys were also recorded. The conditions primarily of winter resident habitats were compiled for each area as indicated below. Since the surveys were conducted several weeks after the occurrence of the avian influenza outbreaks and the poultry houses had been disinfected with hydrated lime, etc., the time and environment differed from that of the outbreaks and there is a possibility that the results of the surveys differs from the avifauna at the time of the outbreaks.

3.2.1. Ato-cho, Yamaguchi Prefecture

The infected poultry house site was disinfected with hydrated lime and virtually no birds could be confirmed, probably indicating a considerable change in the avifauna. However, it was possible to confirm rustic buntings (*Emberiza rustica*), yellow-throated buntings *(Emberiza elegans*), long-tailed rosefinches *(Uragus sibiricus*) and other species on the west side separated somewhat from the poultry houses where idle rice paddy fields had become marshy grassland as well as dusky thrushes (*Turdus naumanni*) and pale thrushes (*Turdus pallidus*) on the forestland somewhat removed on the east side. A flock of about 30 Eurasian siskins (*Carduelis spinus*) was confirmed in the afforested area to the south and a flock of about 30 rustic buntings was confirmed in the paddy fields after reaping somewhat removed. There is a small pond near the poultry houses and ducks came there at night. Though they were probably mallards (*Anas platyrhynchos*), it was not possible to confirm since it was dark and they tended to fly away immediately.

Large flocks of several hundred mandarins ducks (*Aix galericulata*) were confirmed in dam lakes and other relatively expansive open bodies of water within a radius of about 10km from the outbreak site and many mallards and green-winged teals (*Anas crecca*) were also observed in rivers and the small ponds that are scattered here and there.

3.2.2. Kokonoe-machi, Oita Prefecture

Since the avian influenza outbreak site is in the middle of a human settlement with an unstaffed train station, many tree sparrows (*Passer montaus*) and brown-eared bulbuls (*Hypsipetes amaurotis*) are evident. There are homes near the outbreak site where chickens are being raised and we were able to confirm the entry of about 10 sparrows through gaps in the wire mesh of the poultry houses. Flocks with a few mandarin ducks, mallards and green-winged teals were seen in the river that flows on the north side of the site.

Many mallards, Eurasian wigeons (*Anas penelole*)and green-winged teals were seen in dam lakes and other relatively expansive open bodies of water within a radius of about 10km from the outbreak site. In addition, common pochards (*Aythya ferina*), falcated ducks (*Anas* *falcate*) and Northern shovelers (*Anas clypeata*) and other birds were also confirmed. A flock of about 200-300 rooks (*Corvus frugilegus*) was confirmed in paddy fields after reaping at a distance of about 5km to the northwest.

3.2.3. Tanba-cho, Kyoto Prefecture

Though it was not possible to enter the premises since the fowl were still being culled at the time when the on-site surveys were conducted, we were able to conduct a survey very close to the poultry houses. As in Ato-cho, Yamaguchi Prefecture, disinfection with hydrated lime had been carried out thoroughly and there was therefore the possibility that the avifauna was different than that at the time of the outbreak. Dusky thrushes were the dominant species in the paddy fields and other farm fields somewhat apart from the poultry houses while many Eurasian siskins, bramblings (*Fringilla montifringilla*) and other species were seen in the forestland.

There are many agricultural use ponds here and there that could be used by ducks for wintering within a radius of about 10km from the outbreak site. Ducks had already begun to fly north at the time that the survey was conducted and it was not possible to confirm any whatsoever but mandarin ducks, mallards and green-winged teals were confirmed in the survey conducted by the Ministry of the Environment after the outbreak.

References

Environment Agency (1988), Report of the 3rd National Survey on the Natural Environment, Animal Distribution Survey – Birds
Kyoto Prefecture (1979) Wild Birds of Kyoto
Kyoto Prefecture (1993) Wild Birds of Kyoto
Kyoto Prefecture (2002) Red Data Book of Kyoto Prefecture, 2002, Vol. 1, Wildlife (Plants and Animals)
Oita Prefecture (1982) Threatened Wildlife of Oita Prefecture
Oita Prefecture (2001) Red Data Book Oita, Wildlife in Danger of Extinction in Oita Prefecture
Yamaguchi Prefecture (1976) Wild Birds of Yamaguchi Prefecture
Yamaguchi Prefecture (2003) Red Data Book Yamaguchi
Yamashina Institute for Ornithology, Report on the Japanese Bird Banding Scheme for 1994
Yamashina Institute for Ornithology, Report on the Japanese Bird Banding Scheme for 1995
Yamashina Institute for Ornithology, Report on the Japanese Bird Banding Scheme for 1996
Yamashina Institute for Ornithology, Report on the Japanese Bird Banding Scheme for 1997
Yamashina Institute for Ornithology, Report on the Japanese Bird Banding Scheme for 1998

Table 3-1 State of Bird Populations in the Vicinity of Avian Influenza Outbreak Sites

Order	Family	Species	Ato-cho, Yamaguchi Prefecture Jan. 19 – Feb. 27			Kokonoe-machi, Oita Prefecture Feb. 24 – Mar. 9			Tanba-cho, Kyoto Prefecture Mar. 4 -18		
			Migratory bird category	Within a radius of 2km from the infected site	Within a radius of 10km	Migratory bird category	Within a radius of 2km from the infected site	Within a radius of 10km	Within a radius of 2km from the infected site	Within a radius of 10km	Migratory bird category
Podicipediformes	Podicipedidae	*Tachybaptus ruficollis*	Winter visitor Residental bird		•	Resident		•	Resident		•
		Podiceps nigricollis	Winter visitor			Winter visitor			Winter visitor		
		Podiceps auritus	?			Winter visitor			Winter visitor		
		Podiceps cristatus	Winter visitor			Winter visitor			Winter visitor		
Pelecaniformes	Phalacrocoracidae	*Phalacrocorax carbo*	?		•	Resident		•	?		•
Ciconiiformes	Ardeidae	*Botaurus stellaris*	?			Winter visitor			Passage visitor		
		Nycticorax nycticorax	Resident			Resident	•	•	Resident		
		Egretta alba	Summer visitor		•	Winter visitor, some Residental	•	•	Winter visitor (some Passage visitor)		•
		Egretta garzetta	Resident, Summer visitor			Resident	•	•	Resident		
		Egretta eulophotes	?			Winter visitor or Passage visitor			-		
		Egretta sacra	Resident			Resident			Resident		
		Ardea cinerea	Winter visitor		•	Winter visitor, some Residental	•	•	Resident	•	•
	Ciconiidae	*Ciconia boyciana*	Stray			-			Winter visitor		
	Threskiornithidae	*Platalea leucorodia*	Stray			Winter visitor			-		
		Platalea minor	Stray			Winter visitor			Stray		
Anseriformes	Anatidae	*Branta bernicla*	Winter visitor			Stray			Winter visitor		
		Anser albifrons	?			Winter visitor			Winter visitor		
		Anser fabalis	?			Winter visitor			?		
		Anser cygnoides	Winter visitor			-			Winter visitor		
		Cygnus cygnus	Winter visitor			Stray			Winter visitor		
		Cygnus columbianus	Winter visitor			Stray			Winter visitor		
		Tadorna ferruginea	-			Winter visitor			Winter visitor		
		Tadorna tadorna	Winter visitor			Winter visitor			Winter visitor		
		Aix galericulata	Winter visitor		•	Winter visitor	•	•	Passage visitor		•
		Anas platyrhynchos	Winter visitor		•	Winter visitor	•	•	Winter visitor	•	•
		Anas poecilorhyncha	Resident		•	Winter visitor, some Residental		•	Resident		
		Anas crecca	Winter visitor		•	Winter visitor	•	•	Winter visitor		•
		Anas formosa	Winter visitor		•	7Winter visitor			Passage visitor (some Winter visitor)		
		Anas falcata	Winter visitor			Winter visitor		•	Winter visitor		
		Anas strepera	Winter visitor			Winter visitor			Winter visitor		
		Anas penelope	Winter visitor		•	Winter visitor		•	Winter visitor		
		Anas americana	Winter visitor			Stray			Winter visitor		
		Anas acuta	Winter visitor		•	Winter visitor		•	Winter visitor		
		Anas querquedula	Winter visitor			Passage visitor			Passage visitor		
		Anas clypeata	Winter visitor			Winter visitor		•	Winter visitor		
		Aythya ferina	Winter visitor			Winter visitor		•	Winter visitor		
		Aythya baeri	-			Winter visitor			Stray		
		Aythya fuligula	Winter visitor		•	Winter visitor			Winter visitor		
		Aythya marila	Winter visitor			Winter visitor			Winter visitor		
		Bucephala clangula	Winter visitor			Winter visitor			Winter visitor		
		Mergus albellus	Winter visitor			Winter visitor			Winter visitor		
		Mergus squamatus	-			Winter visitor			-		
		Mergus merganser	?		•	Winter visitor		•	Winter visitor		

Order	Family	Species	Ato-cho, Yamaguchi Prefecture Survey Jan. 19 – Feb. 27			Kokonoe-machi, Oita Prefecture Survey Feb. 24 – Mar. 9			Tanba-cho, Kyoto Prefecture Survey Mar. 4 -18		
			Migratory bird category	Within a radius of 2km from the infected site	Within a radius of 10km	Migratory bird category	Within a radius of 2km from the infected site	Within a radius of 10km	Within a radius of 2km from the infected site	Within a radius of 10km	Migratory bird category
Falconiformes	Accipitridae	*Pandion haliaetus*	Resident		•	Resident	•	•	Resident		
		Pernis apivorus	Passage visitor			Summer visitor			Passage visitor		
		Milvus migrans	Resident	•	•	Resident	•	•	Resident	•	•
		Haliaeetus albicilla	-			-			Winter visitor		
		Haliaeetus pelagicus	?			-			Winter visitor		
		Accipiter gentilis	?			Passage visitor			Resident		
		Accipiter gularis	Passage visitor			Winter visitor			Resident		
		Accipiter nisus	?	•	•	Winter visitor	•	•	Resident		•
		Buteo lagopus	-			Stray			Winter visitor		
		Buteo buteo	Winter visitor, Resident	•	•	Resident	•	•	Winter visitor	•	•
		Spizaetus nipalensis	Winter visitor			Resident			Resident		
		Aquila chrysaetos	?			Resident			Passage visitor		
		Circus cyaneus	Winter visitor			Winter visitor			Winter visitor		
		Circus spilonotus	Winter visitor			Winter visitor			Winter visitor		
	Falconidae	*Falco peregrinus*	Winter visitor			Winter visitor			Winter visitor (some Resident)		
		Falco subbuteo	Winter visitor ?			Passage visitor			Passage visitor		
		Falco columbarius	Winter visitor			-			Winter visitor		
		Falco tinnunculus	Winter visitor			Winter visitor		•	Winter visitor		
Galliformes	Phasianidae	*Coturnix japonica*	Winter visitor			Winter visitor			Winter visitor		
		Syrmaticus soemmerringii	Resident			Resident			Resident	•	•
		Phasianus colchicus	Resident	•	•	Resident	•	•	Resident	•	•
Gruiformes	Gruidae	*Grus monacha*	Winter visitor			Stray			?		
		Grus vipio	Winter visitor			Winter visitor			?		
	Rallidae	*Rallus aquaticus*	Winter visitor			Winter visitor			Winter visitor		
		Gallinula chloropus	Summer visitor, Resident		•	Resident		•	Resident		
		Gallicrex cinerea	Winter visitor, Resident			Passage visitor			Passage visitor		
		Fulica atra	?			Winter visitor			Passage visitor		
Charadriiformes	Rostratulidae	*Rostratula benghalensis*	Summer visitor			Resident			Resident		
	Charadriidae	*Charadrius placidus*	Resident			Resident	•	•	Resident		•
		Charadrius alexandrinus	Resident			Resident			Resident		
		Vanellus cinereus	?			Winter visitor			Resident	•	•
		Vanellus vanellus	?			Winter visitor	•	•	Winter visitor		
	Scolopacidae	*Calidris alpina*	Passage visitor			Winter visitor			Passage visitor (some over-wintering)		
		Tringa ochropus	Passage visitor, Winter visitor			Winter visitor	•	•	Winter visitor		•
		Actitis hypoleucos	Passage visitor, Winter visitor			Winter visitor, some Residental		•	Resident		
		Scolopax rusticola	Winter visitor			Winter visitor			Winter visitor		
		Gallinago gallinago	Winter visitor	•	•	Winter visitor			Winter visitor (some Winter visitor)		
		Gallinago stenura	-			-			Winter visitor		
		Gallinago solitaria	?	*	•	-			Winter visitor		
	Laridae	*Larus ridibundus*	Winter visitor			Winter visitor			Winter visitor		
Columbiformes	Columbidae	*Columba janthina*	Resident			Resident			Resident		

Order	Family	Species	Ato-cho, Yamaguchi Prefecture Survey Jan. 19 – Feb. 27			Kokonoe-machi, Oita Prefecture Survey Feb. 24 – Mar. 9			Tanba-cho, Kyoto Prefecture Survey Mar. 4 -18		
			Migratory bird category	Within a radius of 2km from the infected site	Within a radius of 10km	Migratory bird category	Within a radius of 2km from the infected site	Within a radius of 10km	Within a radius of 2km from the infected site	Within a radius of 10km	Migratory bird category
		Streptopelia orientalis	Resident	•	•	Resident	•	•	Resident	•	•
		Sphenurus sieboldii	Winter visitor			Resident	•	•	Resident		
Strigiformes	Strigidae	*Asio otus*	Winter visitor			-			Winter visitor		
		Asio flammeus	?			Winter visitor			Winter visitor		
		Otus lempiji	Resident			Summer visitor			Resident		
		Strix uralensis	Resident			Resident	•	•	Resident		
Apodiformes	Apodidae	*Apus affinis*	-			Resident			Stray		
		Apus pacificus	Passage visitor			Summer visitor			Passage visitor		
Coraciiformes	Alcedinidae	*Ceryle lugubris*	Resident		•	Resident	•	•	Resident		
		Alcedo atthis	Resident		•	Resident			Resident		•
Piciformes	Picidae	*Jynx torquilla*	Winter visitor			Stray			Winter visitor		
		Picus awokera	Resident			Resident	•	•	Resident	•	•
		Dendrocopos major	Resident			Resident			Resident		
		Dendrocopos leucotos	Resident			Resident			Resident		
		Dendrocopos kizuki	Resident	•	•	Resident	•	•	Resident	•	•
Passeriformes	Alaudidae	*Alauda arvensis*	Resident			Resident	•	•	Resident	•	•
	Hirundinidae	*Hirundo rustica*	Summer visitor			Summer visitor	•	•	Summer visitor	•	•
		Delichon urbica	Passage visitor, Summer visitor			Summer visitor	•	•	Summer visitor		
	Motacillidae	*Motacilla flava*	Passage visitor			Winter visitor			Passage visitor		
		Motacilla cinerea	Resident			Resident	•	•	Resident	•	•
		Motacilla alba	Winter visitor			Winter visitor (some Resident)		•	Winter visitor		
		Motacilla grandis	Resident	•	•	Resident	•	•	Resident	•	•
		Anthus hodgsoni	Winter visitor			Winter visitor			Winter visitor		
		Anthus spinoletta	Winter visitor			Winter visitor	•	•	Winter visitor		
	Pycnonotidae	*Hypsipetes amaurotis*	Winter visitor, Resident	*	•	Resident	•	•	Resident	*	•
	Laniidae	*Lanius bucephalus*	Resident	*	•	Resident	*	•	Resident	*	•
		Lanius sphenocercus	-			-			Winter visitor		
	Bombycillidae	*Bombycilla garrulous*	Winter visitor			Winter visitor			Winter visitor		
		Bombycilla japonica	Winter visitor			Winter visitor			Winter visitor		
	Cinclidae	*Cinclus pallasii*	Resident			Resident	•	•	Resident		
	Troglodytidae	*Troglodytes troglodytes*	Winter visitor	•	•	Resident			Resident		•
	Prunellidae	*Prunella rubida*	Winter visitor			Winter visitor			Winter visitor		
	Turdidae	*Erithacus akahige*	Passage visitor			Summer visitor			Passage visitor		
		Luscinia cyane	Passage visitor			Summer visitor			Passage visitor		
		Tarsiger cyanurus	Winter visitor	*	•	Winter visitor	•	•	Winter visitor	*	
		Phoenicurus auroreus	Winter visitor	*	•	Winter visitor	*	•	Winter visitor	*	•
		Monticola solitarius	Resident			Resident			Resident		
		Zoothera dauma	Winter visitor, Resident			Resident			Resident	*	
		Turdus chrysolaus	Passage visitor, Winter visitor			Winter visitor			Passage visitor		
		Turdus pallidus	Winter visitor	*	•	Winter visitor	•	•	Winter visitor	*	•
		Turdus obscurus	Passage visitor			Passage visitor			Passage visitor	*	
		Turdus naumanni	Winter visitor	*	•	Winter visitor	*	•	Winter visitor	*	•

			Ato-cho, Yamaguchi Prefecture Survey Jan. 19 – Feb. 27			Kokonoe-machi, Oita Prefecture Survey Feb. 24 – Mar. 9			Tanba-cho, Kyoto Prefecture Survey Mar. 4 -18		
Order	Family	Species	Migratory bird category	Within a radius of 2km from the infected site	Within a radius of 10km	Migratory bird category	Within a radius of 2km from the infected site	Within a radius of 10km	Within a radius of 2km from the infected site	Within a radius of 10km	Migratory bird category
Passeriformes	Sylviidae	*Cettia diphone*	Resident	*	•	Resident	*	•	Resident	*	•
		Regulus regulus	Winter visitor	•	•	Winter visitor			Winter visitor	•	•
		Cisticola juncidis	Resident			Resident			Summer visitor (some Resident)		
	Aegithalidae	*Aegithalos caudatus*	Resident	*	•	Resident	*	•	Resident	*	•
	Remizidae	*Remiz pendulinus*	?			Winter visitor			Winter visitor		
	Paridae	*Parus montanus*	Winter visitor, Resident	•	•	Resident			Resident	•	•
		Parus ater	Winter visitor, Resident			Resident			Resident	•	•
		Parus varius	Resident	•	•	Resident	•	•	Resident	•	•
		Parus major	Resident	*	•	Resident	•	•	Resident	*	•
	Sittidae	*Sitta europaea*	Resident			Resident			Resident		
	Certhiidae	*Certhia familiaris*	Resident			Resident			Resident		
	Zosteropidae	*Zosterops japonicus*	Resident	•	•	Resident			Resident	*	•
	Emberizidae	*Emberiza cioides*	Resident	*	•	Resident	*	•	Resident	*	•
		Emberiza yessoensis	Winter visitor			Winter visitor			Winter visitor		
		Emberiza tristrami	Stray			-			Winter visitor		
		Emberiza fucata	Winter visitor, Resident			Resident			Winter visitor		
		Emberiza rustica	Winter visitor	•	•	Winter visitor	*	•	Winter visitor	*	•
		Emberiza elegans	Winter visitor	*	•	Winter visitor	*	•	Winter visitor		
		Emberiza sulphurata	Winter visitor			Winter visitor			Passage visitor		
		Emberiza spodocephala	Winter visitor	*	•	Winter visitor	*	•	Winter visitor	*	•
		Emberiza variabilis	Winter visitor	•	•	Winter visitor			Winter visitor		
		Emberiza pallasi	?			-			Winter visitor		
		Emberiza schoeniclus	Winter visitor			Winter visitor			Winter visitor		
		Plectrophenax nivalis	-			-			Winter visitor		
	Fringillidae	*Fringilla montifringilla*	Winter visitor			Winter visitor	•	•	Winter visitor	*	•
		Carduelis sinica	Resident	*	•	Resident	•	•	Resident	*	•
		Carduelis spinus	Winter visitor	*	•	Winter visitor	•	•	Winter visitor	•	•
		Carduelis flammea	-			-			Winter visitor		
		Leucosticte arctoa	Winter visitor			Winter visitor			Winter visitor		
		Carpodacus roseus	?			-			Winter visitor		
		Pinicola enucleator	-			-			Winter visitor		
		Loxia curvirostra	Winter visitor			-			Winter visitor		
		Loxia leucoptera	-			-			Winter visitor		
		Uragus sibiricus	Winter visitor	*	•	Winter visitor			Winter visitor	*	•
		Pyrrhula pyrrhula	Winter visitor	•	•	Winter visitor			Winter visitor	•	•
		Eophona migratoria	Winter visitor, passage bird			Passage bird			Winter visitor		
		Eophona personata	Winter visitor, Resident			Resident, some winter vistors			Resident		•
		Coccothraustes coccothraustes	Winter visitor			Winter visitor			Winter visitor		
	Ploceidae	*Passer rutilans*	Winter visitor			Winter visitor			Winter visitor		
		Passer montanus	Resident	•	•	Resident	•	•	Resident		•
	Sturnidae	*Sturnus cineraceus*	Winter visitor, Resident			Resident	•	•	Resident		•
	Corvidae	*Garrulus glandarius*	Resident, Winter visitor	•	•	Resident	•	•	Resident	*	•

Order	Family	Species	Ato-cho, Yamaguchi Prefecture Survey Jan. 19 – Feb. 27			Kokonoe-machi, Oita Prefecture Survey Feb. 24 – Mar. 9			Tanba-cho, Kyoto Prefecture Survey Mar. 4 -18		
			Migratory bird category	Within a radius of 2km from the infected site	Within a radius of 10km	Migratory bird category	Within a radius of 2km from the infected site	Within a radius of 10km	Within a radius of 2km from the infected site	Within a radius of 10km	Migratory bird category
		Nucifraga caryocatactes	Accidental visitor			Resident			Accident6al visitor		
		Corvus dauuricus	?			Winter visitor			Winter visitor	*	
		Corvus frugilegus	Winter visitor			Winter visitor		•	Accidental visitor		•
		Corvus corone	Resident	•	•	Resident	•	•	Resident	•	•
		Corvus macrorhynchos	Resident	•	•	Resident	•	•	Resident	•	•
Introduced species											
		Cygnus olor			•			•			
		Anser cygnoides domesticus						•			
		Anas platyrhynchos (Domestic)			•						
		Bambusicola thoracica		•	•		•	•		*	•
		Columba livia					•	•			•
		Garrulax canorus					•	•			
		Leiothrix lutea					•	•			
Total number of confirmed species			161	37	57	156	54	70	170	44	51

* Migratory bird categories in Yamaguchi Prefecture are based primarily on *Wild Birds of Yamaguchi Prefecture* (1976)

* Migratory bird categories in Oita Prefecture are based primarily on *Wild Birds of Oita Prefecture* (1982)

* Migratory bird categories in Kyoto Prefecture are based primarily on *Wild Birds of Kyoto Prefecture* (1979)
Migratory bird categories marked with “?” means that there are no notations regarding category in the literature.
Migratory bird categories marked with “-” means that there was no record of the species in the relevant prefecture.

• Confirmed species   * Captured species

4. Infection route from wild birds to domestic waterfowl

(Yutaka Kanai)

4.1. Possibility of infection by migratory birds

The primary habitat environment of those migratory bird species and habitation in the poultry farms were added to the list of species that could possibly have entered the outbreak locations via the Korean Peninsula, as indicated below. Among these migratory birds, there were no incursions into the poultry houses themselves by geese, ducks or other waterfowl. In addition, given the fact that bird species that inhabit forests or grassland are extremely wary and have different foraging environments, it is virtually inconceivable that they would enter the poultry houses.

Consequently, there is virtually no possibility of direct infection by migratory birds.

Relationship between Conjectured Migratory Bird Species and Poultry Houses
(Kubota et al.(2004), modified from urgent report)

Order	Family	Species	Habitat	Involvement in poultry houses	
				Incursions into poultry houses or	compost
Podicipediformes	Podicipedidae	*Podiceps nigricollis*	Broad water areas	×	×
		Podiceps auritus	Broad water areas	×	×
		Podiceps cristatus	Broad water areas	×	×
Ciconiiformes	Ardeidae	*Ardeola alba*	Shallow water areas	×	×
		Ardea cinerea	Shallow water areas	×	×
Anseriformes	Anatidae	*Aix galericulata*	Water areas	×	×
		Anas platyrhynchos	Water areas	×	×
		Anas poecilorhyncha	Water areas	×	×
		Anas crecca	Water areas	×	×
		Anas formosa	Water areas	×	×
		Anas falcata	Water areas	×	×
		Anas strepera	Water areas	×	×
		Anas penelope	Water areas	×	×
		Anas acuta	Water areas	×	×
		Anas querquedula	Water areas	×	×
		Anas clypeata	Water areas	×	×
		Aythya ferina	Broad water areas	×	×
		Aythya fuligula	Broad water areas	×	×
		Mergus albellus	Broad water areas	×	×
		Mergus merganser	Broad water areas	×	×
Falconiformes	Accipitridae	*Accipiter gentilis*	Forest & grassland	Δ	Δ
		Accipiter gularis	Forest & grassland	Δ	Δ
		Accipiter nisus	Forest & grassland	Δ	Δ
		Buteo buteo	Forest & grassland	×	×
		Circus spilonotus	Reed fields	×	×
	Falconidae	*Falco peregrinus*	Cliff areas	×	×
		Falco tinnunculus	Forest & grassland	×	×
Gruiformes	Rallidae	*Rallus aquaticus*	Wetlands	×	×
		Fulica atra	Water areas	×	×
Ciconiiformes	Charadriidae	*Vanellus cinereus*	Wetlands, paddy fields	×	×
		Vanellus vanellus	Wetlands, paddy fields	×	×
	Scolopacidae	*Tringa ochropus*	Wetlands, paddy fields	×	×

Order	Family	Species	Habitat	Involvement in poultry houses	
				Incursions into poultry houses or	compost
		Actitis hypoleucos	Wetlands, paddy fields	×	×
		Scolopax rusticola	Forest & grassland	×	×
Ciconiiformes	Scolopacidae	*Gallinago gallinago*	Wetlands, paddy fields		
Columbiformes	Columbidae	*Treron sieboldii*	Forest	×	×
Strigiformes	Strigidae	*Asio flammeus*	Wetlands, paddy fields	×	×
Passeriformes	Motacillidae	*Motacilla alba*	Grassland, upland fields	Δ	*
		Anthus hodgsoni	Forest & grassland	×	×
		Anthus spinoletta	Wetlands, paddy fields	×	×
	Bombycillidae	*Bombycilla garrulus*	Forest & grassland	×	×
		Bombycilla japonica	Forest & grassland	×	×
	Turdidae	*Erithacus cyanurus*	Forest & grassland	×	×
		Phoenicurus auroreus	Forest & grassland	×	*
		Zoothera dauma	Forest & grassland	×	*
		Turdus chrysolaus	Forest & grassland	×	*
		Turdus pallidus	Forest & grassland	×	*
		Turdus naumanni	Forest & grassland	×	*
	Sylviidae	*Regulus regulus*	Forest	×	×
	Remizidae	*Remiz pendulinus*	Cliff areas	×	×
	Emberizidae	*Emberiza yessoensis*	Cliff areas	×	×
		Emberiza fucata	Forest & grassland	×	×
		Emberiza rustica	Forest & grassland	×	×
		Emberiza elegans	Forest & grassland	×	×
		Emberiza sulphurata	Forest & grassland	×	×
		Emberiza spodocephala	Forest & grassland	×	×
		Emberiza variabilis	Forest & grassland	×	×
		Emberiza schoeniclus	Cliff areas	×	×
	Fringillidae	*Fringilla montifringilla*	Forest & grassland	×	×
		Carduelis spinus	Forest & grassland	×	×
		Uragus sibiricus	Forest & grassland	×	×
		Pyrrhula pyrrhula	Forest & grassland	×	×
		Eophona personatas	Forest & grassland	×	×
		Coccothraustes coccothraustes	Forest & grassland	×	×
	Passeridae	*Passer rutilans*	Forest & grassland	×	×
	Sturnidae	*Sturnus cineraceus*	Forest & grassland	Δ	*
	Corvidae	*Corvus frugilegus*	Wetlands, paddy fields	×	×

× : Virtually no incursions
Δ : incursion possible depending on the poultry house
* : frequent incursions

4.2. Infection via residental birds

Residental birds that could possibly enter the poultry houses are limited to a small number of terrestrial species including sparrows (*Passer montanus*), pigeons, rufous turtle doves (*Streptopelia orientalis*) and jungle crows (*Corvus macrorhynchos*). They would enter the houses for nesting or foraging but, since the outbreaks occurred during the winter, foraging would be the reason why they would enter the houses. For residental birds to be involved in the poultry outbreaks, it would be necessary for them to be infected by migratory birds or to come into contact with feces or other material contaminated by the virus.

Even if the species is different, water birds may use the same water areas as resting sites and there is thus a possibility of infection through the water. Terrestrial birds have different living areas depending on the species and there would therefore be few opportunities for close contact between different species. Residental birds with a strong possibility of entering poultry houses, in particular, which primarily areas near the human living environment, would have few chances to come into contact with migratory birds. Living spaces shared by both resident and migratory birds would be limited to water only and, since water in hillside areas in a natural state is mostly in the form of flowing spring water, it would be limited to places where they come to drink water.

Since jungle crows eat carrion, if an infected bird died, there would be the possibility of infection through the food chain. However, in the capture and feces tests targeting migratory and other birds at the outbreak sites, virus was not detected and there were no signs of wide-spread infection among sparrows, pigeons or other residental birds.

4.3. Outbreak conditions at the outbreak sites and wild birds

4.3.1. Yamaguchi Prefecture

Though wild bird habitats have been confirmed in the vicinity of Ato-cho, Yamaguchi Prefecture, the site of an outbreak, it is not an area with an especially high density of such habitats. In addition, since there are few residences and no feed was supplied at nearby ponds said to have been inhabited by ducks, there was probably little contact between sparrows and other resident birds that entered the poultry houses and ducks.

The infection began at the central passageway at the poultry house entrance, the place with the most active human and animal movement. Even sparrows would ordinarily avoid foraging in a place with much human traffic; however, they will forage if there is food and no human movement. There is evidence that the entrance doors were left open during the day after the conclusion of work and the possibility exists for incursion by sparrows, which are found in large numbers in the vicinity of the residences.

Japanese pied wagtails (*Motacilla grandis*) have been reported by farm employees. In addition, the existence of flies has also been reported in the poultry houses and in the manure and compost yards. Migratory thrushes and wagtails are thought to be among birds that are attracted to insects. It is entirely possible that insect-eating terrestrial birds other than the Japanese pied wagtail foraged for food in manure and compost yards and elsewhere on the farm premises.

4.3.2. Oita Prefecture

The site of the outbreak in Kokonoe-machi, Oita Prefecture, was a private residence and the houses where the bantams were raised were located in the residence garden, which is not an environment where migration-oriented birds would come to forage. There is the possibility, as with poultry farms, that sparrows, rufous turtle doves, pigeons, crows or other residental birds would come into the area where the houses are located; however, since the structure of the houses was thought to have prevented incursions, there was probably very little chance that wild birds could have carried the virus directly into the houses.

In mid-January 2004, the bantam raiser witnessed the deaths of about 30-40 birds that were not ordinarily seen. However, given the body color, time and region, they were not relevant birds and there is also the possibility of a lapse of memory or multiple species mixed together. It would be difficult to speculate upon a relationship between the witnessed evidence and the outbreak. It is regrettable that no confirmation was made at the time when the large-scale deaths occurred and, since there is also the possibility of illness and toxic contamination, it is necessary to establish a system for routine reporting and elucidation.

4.3.3. Kyoto Prefecture

Though there are many wild birds in the vicinity of the outbreak site in Tanba-cho, Kyoto Prefecture, it is ordinary forestland without an especially high density of wild bird habitats.

Since the place where the first infection occurred in poultry house no.8 is located in the center of the poultry farm, it is thought that the virus was brought in a carrier other than human. Since there were holes in the windows rear the outbreak location and incursion by sparrows was confirmed, involvement by sparrows is possible. However, that would necessitate infection by sparrows or contamination by the virus since sparrows are residential species that do not travel long distances.

Contacts between sparrows and other birds occur in ponds and also in chicken manure and compost yards. There are observation reports of crows coming to the manure and compost yards but there are no reports of other birds. However, birds came to the site of the 3rd outbreak, as in Yamaguchi Prefecture, with the intention of catching the insects that proliferate in the manure and compost yard seven in the winter. It would be possible for some migratory birds to be included among them.

In spite of the fact that the virus existed in the manure and compost sheds in large quantities, there were no reports of large-scale deaths of sparrows and there is no direct evidence that sparrows were virus carriers.

5. Infection of crows at the outbreak sites

(Yutaka Kanai)

A major characteristic of the outbreak in Tanba-cho in Kyoto Prefecture was the secondary infection of jungle crows. If secondary infection occurred at the infected farm, it is necessary to prevent secondary infection of wild birds since there is a strong possibility of spread of the virus into the surrounding area.

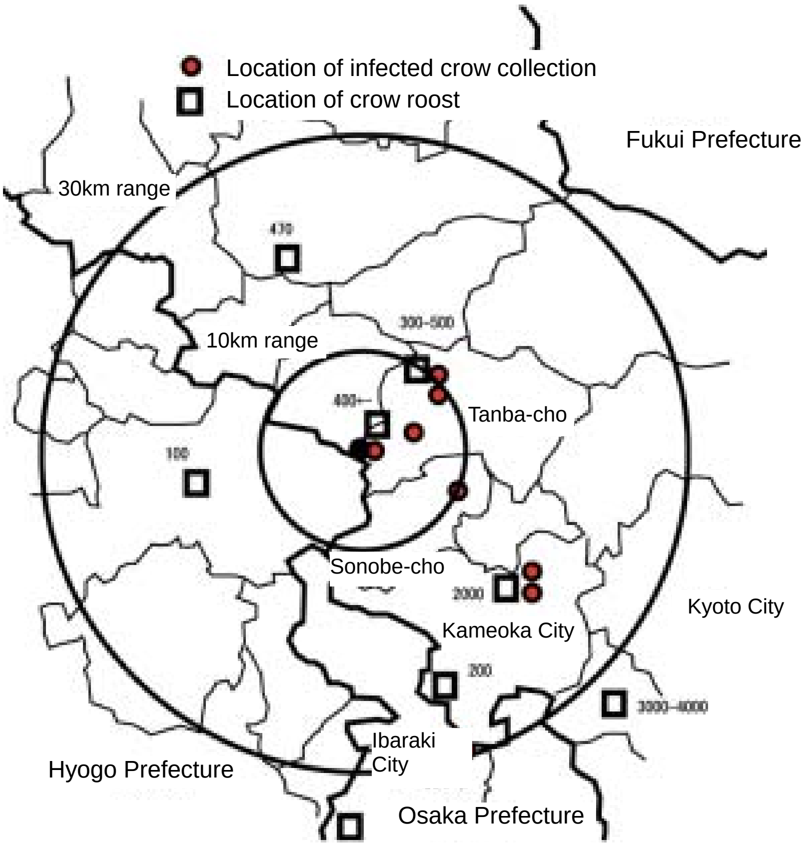
5.1. Cause of the infection

There are large manure and compost yards at the location of the 3rd outbreak and dead birds were discarded there. Jungle crows as well as other birds normally gathered there and there was the possibility of ingesting the virus and becoming infected when wild birds foraged there.

5.2. Dispersal of the crows through epidemic prevention measures

After the outbreak of avian influenza, about 1,000 crows had gathered at the site of the 3rd outbreak and they are thought to have dispersed into the surrounding area when the epidemic prevention measures were initiated. The home range of crows in the winter is often a distance of about 10km per day from their roost. However, since they also change their roost from one location to another, it is entirely possibility that they dispersed over a distance of several ten kilometers during a week or more. With the disturbance caused by media reporters and epidemic prevention measures and the loss of food, they may have dispersed farther than usual.

After the occurrence of the avian influenza outbreak, many crows were captured and many bodies were collected; however, there was a total of only 9 confirmed crow infections, all of which were within 30km of the 3rd outbreak site (Ministry of the Environment data). Large roosts with several dozen crows each are known to exist after the outbreak, 2 within a distance of10km from the outbreak site and 4 within 30km. After confirmation of the outbreak, almost all of the crows dispersed to those roosts and there were probably a few that dispersed even farther away.



Crow roosts and infected jungle crow collection sites

Large-billed crows with confirmed infection were collected within a range of 30km. The figures in the diagram indicate the number of crows in the roost.

Reference literature

Ornithological Society of Japan, Avian Influenza Problem Investigation Committee, 2004 Avian Influenza Problem Investigation Committee Report, *Current State of the Avian influenza Problem and Issues*, Ornithological Society of Japan

5.3. Infection conditions

The table below indicates the number of crows that were captured or collected each week. After March 9, when the infection of jungle crows became known, there has been a rapid increase in the number of crows captured or collected due to the increase in general concern and the total number of dead crows collected from March 1, when the outbreak was confirmed, through April 18 was 396 in Kyoto, Osaka and Hyogo Prefectures and 129 within an area of 30km from the site of the 3rd outbreak. In contrast, infection was confirmed in 7 of those captured and collected in Kyoto and Osaka on March 4-5.

The number of infected specimens that were collected was extremely small in light of the fact that about 1,000 crows were observed at the compost yard at the time of the outbreak,. Jungle crows frequently eat meal scraps and other food that originates in human activity. Since food in nature is especially scarce during the winter, they have a greater dependence on humans. Given this fact, though there are probably individuals that die in places that are unnoticed by humans, it is not conceivable that large-scale deaths occurred. It would be difficult for jungle crows to become infected through ordinary foraging activity and there is the possibility that, even if infected, only a few would actually become ill.

Transitions in the number of crows collected in the Tanba-cho area and the number of confirmed infections
(source: Ministry of the Environment data)

<Table 3> Transitions in virus test results in Kyoto Prefecture, etc.

Date of specimen discovery or capture	Overall area of Kyoto, Osaka & Hyogo Prefectures			Within a radius of 30km from the outbreak site		
	No. of virus tests	No. of tests with results known	No. testing positive	No. of virus tests	No. of tests with results known	No. testing positive
Mar. 1-7	8	8	5	7	7	5
Mar. 8-14	91	91	2	25	25	2
Mar. 15-21	105	105	1	36	36	1
Mar. 22-28	67	67	0	17	17	0
Mar. 29 - Apr. 4	74	74	1	26	26	1
Apr. 5-11	41	41	0	7	7	0
Apr. 12-18	10	7	0	10	7	0
Total	396	393	9	129	126	9

Note: This indicates the results of virus tests of wild crows (dead, feeble or captured specimens) conducted by the 3 prefectures, Tottori University and the Ministry of the Environment.

6. Other items

6.1. Imported wild birds

(Yutaka Kanai)

More than 100,000 birds are imported into Japan every year from around the world. According to Ministry of Finance trade statistics and other data, countries that supply most of the imports are Taiwan, Pakistan, Korea, Malaysia, Guinea, Indonesia and Myanmar. There are also imports from Vietnam and Singapore.

In the trade statistics, imported bird species are only categorized as raptors, parrots, pigeons or other birds and the individual species are unknown. However, 16,000 birds were imported from Korea in 2003. In a survey of birds sold at retail outlets from 2000 through 2003 conducted by the Wild Bird Society of Japan, 129 species of wild birds that inhabit Japan as well as about 270 species from other countries were recorded. In a survey of pet birds imported into the Japan, it was reported in 1997-98 that low pathogenic avian influenza (subtypes other than H5 and H7) was isolated from dead birds.

Though many birds among imported bird species that are also native to Japan were likely captured at wintering sites in the area spanning from China through Southeast Asia, in surveys to date, no relationship has been recognized between the infected farms and these imported birds.

References:

Wild Bird Society of Japan, 2004 Wild Bird Protection Collection, *Wild bird cultivation, sales and importation and related problem areas*
Ornithological Society of Japan, Avian Influenza Problem Investigation Committee, 2004 Avian

Influenza Problem Investigation Committee Report, *Current State of the Avian influenza Problem and Issues*, Ornithological Society of Japan

6.2. Surveys in Korea

(Kumiko Yoneda)

Since there were outbreaks of highly pathogenic avian influenza in Korea prior to the outbreaks in Japan, information was collected in Korea on March 11-17, 2004, in order to examine contact points.

6.2.1. Outbreak conditions in Korea

Interviews regarding the outbreak conditions affecting domestic fowl were conducted at the Avian Disease Division, National Veterinary Research & Quarantine Service of the Korean Ministry of Agriculture & Forestry. Information was also received at that time regarding the conditions of wild bird surveys, the content of which is recorded in 6.4.2.

The conditions of highly pathogenic avian influenza outbreaks among domestic fowl in Korea are indicated in Table 6-2-1, and the locational relationships between outbreak sites in Korea and in Japan are indicated in Fig. 6-2-1. Sites A-D in Korea in Fig. 6-2-1 correspond to the item “Region” in Table 6-2-1. Site A is the location of the initial outbreak and it is thought that the infection spread to sites B and C through the movement of domestic duck chicks. Site D is the location of the final outbreak.

A decline in the egg-laying rate of the domestic ducks was confirmed; besides that, however, no clinical symptoms were observed. Monitoring surveys of the conditions of duck infection are being continued.

6.2.2. Wild bird habitats in Korea

6.2.2.1. Topography of migratory routes to Japan and information regarding land use

Based on interviews with a number of bird experts, most migratory birds on the Korean Peninsula fly southward along the western coastline. Expansive tidal flats are found in Asanman, Chonsuman, Saemankeum and other locations and many migratory birds apparently use them as stopovers or for wintering. The Korean Ministry of the Environment conducts simultaneous fixed point surveys of migratory birds at 118 locations nationwide during the migration season.

Chungchong-bukto, where the first outbreak of highly pathogenic avian influenza occurred in Korea, is an area of concentrated poultry farms; however, since it lies inland and freezes in the winter, there are apparently few migratory birds.

Along with the drop in temperature, winter residing birds gradually move southward and there are even some that spend the winter in the southeastern part of the peninsula. Since that is the closest point between Korea and Japan, the question arises of whether or not there are any that cross over to Japan.

6.2.2.2. Conditions of wild bird habitats in the vicinity of highly pathogenic avian influenza outbreaks in Korea

The conditions of wild bird habitats in the vicinity of highly pathogenic avian influenza outbreak sites in Korea were observed on March 13-16, 2004. A total of 8 locations were observed, including 6 infected poultry houses (refer to Table 6-2-1) and an additional 2 adjacent houses where disposal and disinfection had been carried out.

The bird species confirmed at the site are listed in Table 6-2-2. Besides the record of observed poultry houses and the vicinity, a record of the estuary of the Nakdong River and a record of other birds confirmed while in transit are also included. The estuary of the Nakdong River is located in the suburbs of Pusan and is one of the largest migratory bird destinations; on the day of observation (March 16), however, Winter visitors had already flown north and it was still too early for the arrival of passage visitors and, therefore, both the number of species and bird count were not very large.

Table 6-2-1 Highly pathogenic avian influenza outbreaks in Korea

Farm no.	Report date	Location	Region	Infected bird	Observation site	Remarks
1	12/10	Eumsung County, Chungbuk Prov.	A	Chickens	(2)	
2	12/14	Eumsung County, Chungbuk Prov.	A	Ducks		
3	12/16	Eumsung County, Chungbuk Prov.	A	Chickens		
4	12/18	Eumsung County, Chungbuk Prov.	A	Ducks		
5	12/18	Eumsung County, Chungbuk Prov.	A	Ducks		
6	12/18	Cheonan City, Chungnam Prov.	A	Ducks		Detected through nationwide active surveillance
7	12/18	Cheonan City, Chungnam Prov.	A	Ducks		Detected through nationwide active surveillance
8	12/20	Naju County, Chonnam Prov.	B	Ducks		
9	12/20	Kyongju City, Kyongbuk Prov.	C	Chickens		
10	12/21	Kyongju City, Kyongbuk Prov.	C	Chickens	(5)	
11	12/21	Cheonan City, Chungnam Prov.	A	Chickens		
12	12/21	Jincheon County, Chungbuk Prov.	A	Ducks		Detected through nationwide active surveillance
13	12/23	Icheon City, Kyonggi Prov.	A	Chickens		
14	12/23	Ulju County, Ulsan Metropolitan City	C	Chickens & ducks	(6)	Detected through nationwide active surveillance
15	1/2	Cheonan City, Chungnam Prov.	A	Ducks		
16	1/11	Yangsan City, Kyongnam Prov.	C	Chickens	(8)	
17	1/25	Cheonan City, Chungnam Prov.	A	Chickens	(4)	
18	2/4	Asan City, Chungnam Prov.	A	Ducks	(3)	
19	3/20	Yangju County, Kyonggi Prov.	D	Chickens		

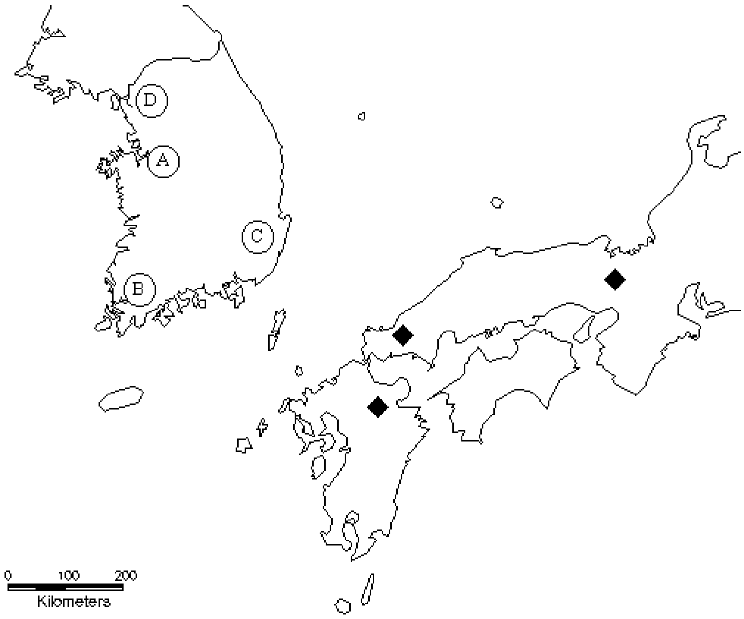


Fig. 6-2-1 Highly pathogenic avian influenza outbreak sites in Korea and Japan
(Source: Publicly released materials of the Avian Disease Division, National Veterinary Research & Quarantine Service, Korean Ministry of Agriculture & Forestry accessible at http://www.nvrqs.go.kr/extra/english/index.asp)

Table 6-2-2 Bird species observed in Korea

Species	Farm no.								Tidal flats[*1]	In transit[*2]
	1	2	3	4	5	6	7	8		
Tachybaptus ruficollis			●	○	○					
Podiceps cristatus					○					
Phalacrocorax carbo									⊙	
Egretta alba			○	●						⊙
Egretta garzetta	○	○								
Ardea cinerea			●	●					⊙	⊙
Anser albifrons					○					
Tadorna tadorna									⊙	
Aix galericulata	○	●	○	●			○	●		
Anas platyrhynchos	○	○	●	○	○		○	●	⊙	
Anas poecilorhyncha	○	○	●	●	○				⊙	
Anas crecca			●	●			○	●	⊙	⊙
Anas strepera			●	●						
Anas penelope									⊙	
Anas acuta			●	○						
Anas clypeata			○	○						
Aythya ferina					○					
Aythya fuligula			○	○						
Mergus merganser			○	○	○					
Pandion haliaetus									⊙	
Falco peregrinus									⊙	
Falco tinnunculus	○	○	●	○						
Charadrius dubius							○	●		⊙
Numenius madagascariensis									⊙	
Gallinago gallinago							○	●		
Larus argentatus			○	○					⊙	
Streptopelia orientalis	●	●	○	○	●	●	○	●		⊙
Upupa epops					●					
Motacilla alba	○	○			●					
Hypsipetes amaurotis	●	○			○					
Phoenicurus auroreus	○	●			●					
Turdus naumanni	○	●	●	○			○	●		⊙
Parus major	●	○	●	○						
Emberiza elegans										⊙
Carduelis cinica										⊙
Coccothraustes coccothraustes			●	○						
Passer montanus	●	●	●	○	●		○	●		⊙
Sturnus cineraceus			●	○			●	○		
Pica pica	●	●	●	○	●	●	●	●	⊙	⊙
Corvus dauuricus										⊙
Corvus frugilegus										⊙
Corvus macrorhynchos					○					
Phasianus chochicus	○	○								
Paradoxornis webbianus					○					

●: Confirmed within a 500m radius of the farm
○: Confirmed within a 10km radius of the farm
⊙: Confirmed
Note 1: Record of observations in the vicinity of the Nakdong River estuary in the outskirts of Pusan on March 16, 2004
Note 2: Record of observations during the trip other than farms and tidal flats

The poultry houses other than (no.6) were located on level land among rice paddy fields and were either floor feeding or cage type. There were also windowless houses (no.5).

House no.6 was located in area of mountainous terrain and almost no bird species were confirmed, though it was possible to confirm mallards (*Anas platyrhynchos*), oriental turtle doves (*Streptopelia orientalis*), tree sparrows (*Passer montanus*) and magpies (*Pica pica*).

The following characteristics are evident when the wild bird habitat conditions in the vicinity of outbreak sites in Korea are compiled.

* Bird species density is lower overall than in Japan.
* There are many magpies and frequently appear in the same manner as crows or tree sparrows in Japan.
* There were rivers close to all of the poultry houses. There were duck habitats nearby all except the one site in mountainous terrain.

6.2.2.3. Bird species that could possibility fly from Korea to winter in Yamaguchi Prefecture (literature search)

There is a registry of migratory birds in Korea and Japan in the Agreement between Japan and the Republic of Korea on Cooperation in the Field of the Environment, which includes 18 orders, 50 families and 278 species. This, in other words, is a list of species that could possibly travel between Korea and Japan.

Furthermore, in order examine the bird species that could possibly carry the virus from Korea to Japan, we extracted bird species that could fly to areas in the vicinity of the outbreak sites for the winter in the sequence given below.

We first of all prepared lists of birds in Yamaguchi, Oita and Kyoto Prefectures based on the existing literature (Environment Agency 1988a, Kyoto Prefecture, 1979; 1993; 2002, Oita Prefecture 1982; 2001; Yamaguchi Prefecture 1976; 2003; Yamashina Institute for Ornithology 1994; 1995; 1996; 1997; 1998). Based on that, we extracted species that were noted in the registry of migratory birds in Korea and Japan and that were described as Winter visitors in the literature. We then eliminated pelagic bird species and bird species that probably would only appear very rarely in areas in the vicinity of the avian influenza outbreak sites in Japan.

As a result, we identified 9 orders, 20 families and 67 species of birds (Table 6-2-3) that could probably fly from Korea to the vicinity of the outbreak sites in Japan as possible winter visitors.

There are 10 species in 18 records from past recovery records in banding surveys, a breakdown of which is given in Table 6-2-4. Of these, there are 7 species in 11 cases in which they were released or recovered in Japan in the winter (October through January).

Table 6-2-3 Possible winter visitors from Korea to the outbreak sites

Order	Family	Species
Podicipediformes	Podicipedidae	*Podiceps nigricollis*
		Podiceps auritus
		Podiceps cristatus
Ciconiiformes	Ardeidae	**Egretta alba*
		Ardea cinerea
Anseriformes	Anatidae	*Aix galericulata*
		Anas platyrhynchos
		Anas poecilorhyncha
		Anas crecca
		Anas formosa
		Anas falcata
		Anas strepera
		Anas penelope
		Anas acuta
		Anas querquedula
		Anas clypeata
		Aythya ferina
		Aythya fuligula
		Mergus albellus
		Mergus merganser
Falconiformes	Accipitridae	*Accipiter gentilis*
		Accipiter gularis
		Accipiter nisus
		Buteo buteo
		Circus aeruginosus
	Falconidae	*Falco peregrinus*
		Falco tinnunculus
Gruiformes	Gruidae	*Rallus aquaticus*
		Fulica atra
Charadriiformes	Rostratulidae	*Vanellus cinereus*
		Vanellus vanellus
	Scolopacidae	*Tringa ochropus*
		Actitis hypoleucos
		Scolopax rusticola
Charadriiformes	Scolopacidae	*Gallinago gallinago*
Columbiformes	Columbidae	*Sphenurus sieboldii*
Strigiformes	Strigidae	*Asio flammeus*
Passeriformes	Motacillidae	*Motacilla alba*
		Anthus hodgsoni
		Anthus spinoletta
	Bombycillidae	*Bombycilla garrulus*
		Bombycilla japonica
	Turdidae	*Tarsinger cyanurus*
		Phoenicurus auroreus
		Zoothera dauma
		Turdus chrysolaus
		Turdus pallidus
		* *Turdus naumanni*
	Sylviidae	*Regulus regulus*
	Remizidae	* *Remiz pendulinus*
	Emberizidae	*Emberiza yessoensis*
		Emberiza fucata
		Emberiza rustica
		* *Emberiza elegans*
		Emberiza sulphurata
		Emberiza spodocephala
		Emberiza variabilis
		* *Emberiza schoeniclus*
	Fringillidae	*Fringilla montifringilla*
		Carduelis spinus
		Uragus sibiricus
		Pyrrhula pyrrhula
		Eophona personatas
		Coccothraustes coccothraustes
	Ploceidae	*Passer rutilans*
	Sturnidae	*Sturnus cineraceus*
	Corvidae	*Corvus frugilegus*

*: Species with record of recovery

Table 6-2-4 Results of bird recovery in banding surveys

No.	Species	Mon/Yr Japan		Mon/Yr Korea
1	Streaked shearwater *(Calonectris leucomelas)*	10/79 Kyoto Pref.	→	6/83 Kangwon Prov.
2	Streaked shearwater *(Calonectris leucomelas)*	10/80 Kyoto Pref.	→	7/83 Cheju Prov.
3	Hooded crane *(Grus monacha)*	12/80 Kagoshima Pref.	→	3/85 Chungnam Prov.
4	White-naped crane *(Grus vipio)*	1/86 Kagoshima Pref.	→	2/89 Chungbuk Prov.
5	White-naped crane *(Grus vipio)*	10/84 Kagoshima Pref.	→	3/85 Kyonggi Prov.
6	White-naped crane *(Grus vipio)*	1/83 Kagoshima Pref.	→	12/83 Kyonggi Prov.
7	Dusky thrush *(Turdus naumanni)*	10/79 Fukui Pref.	→	2/81 Kyongnam Prov.
8	Penduline tit *(Remiz pendulinus)*	12/82 Fukuoka Pref.	→	2/84 Pusan Metropolitan City
9	Yellow-throated bunting *(Emberiza elegans)*	1/00 Nagasaki Pref.	←	5/98 Kangwon Prov.
10	Reed bunting *(Emberiza schoeniclus)*	11/87 Yamaguchi Pref.	→	3/89 Pusan Metropolitan City
11	Reed bunting *(Emberiza schoeniclus)*	10/88 Yamaguchi Pref.	→	3/89 Pusan Metropolitan City
12	Streaked shearwater *(Calonectris leucomelas)*	6/76 Iwate Pref.	→	6/79 Kyongnam Prov.
13	Great egret *(Egretta alba)*	6/80 Yamaguchi Pref.	→	6/81 Chonbuk Prov.
14	Little egret *(Egretta garzetta)*	6/90 Fukuoka Pref.	→	8/91 Kyongnam Prov.
15	Black-tailed gull *(Larus crassirostris)*	6/70 Aomori Pref.	→	12/70 Kyongbuk Prov.
16	Black-tailed gull *(Larus crassirostris)*	6/71 Aomori Pref.	→	12/71 Pusan Metropolitan City
17	Black-tailed gull *(Larus crassirostris)*	7/92 Hokkaido	→	3/93 Cheju Prov.
18	Black-tailed gull *(Larus crassirostris)*	7/85 Hokkaido	→	1/86 Cheju Prov.

References

Environment Agency (1988), Report of the 3rd National Survey of the Natural Environment: Animal Distribution Survey– Birds
Kyoto Prefecture (1979) Wild Birds of Kyoto
Kyoto Prefecture (1993) Wild Birds of Kyoto
Kyoto Prefecture (2002) Red Data Book of Kyoto Prefecture, 2002, Vol. 1, Wildlife (Plants and Animals)
Oita Prefecture (1982) Wild Birds of Oita Prefecture
Oita Prefecture (2001) Red Data Book Oita, Threatened Wildlife of Oita Prefecture
Yamaguchi Prefecture (1976) Wild Bird of Yamaguchi Prefecture
Yamaguchi Prefecture (2003) Red Data Book Yamaguchi
Yamashina Institute for Ornithology, Report on the Japanese Bird Banding Scheme for 1994
Yamashina Institute for Ornithology, Report on the Japanese Bird Banding Scheme for 1995
Yamashina Institute for Ornithology, Report on the Japanese Bird Banding Scheme for 1996
Yamashina Institute for Ornithology, Report on the Japanese Bird Banding Scheme for 1997
Yamashina Institute for Ornithology, Report on the Japanese Bird Banding Scheme for 1998

6.3. Migration speed of wild birds (literature search)

(Kumiko Yoneda)

Data is available regarding the flight speed of wild birds calculated based on actual measurements by following with vehicles or by radar. Generally speaking, larger birds travel faster than smaller birds and they are thought to fly about 10 hours a day while migrating. However, there are also breaks for foraging and resting and they are slower than the speed derived by multiplying flying time by transient speed measurements. In addition, it is also known that the speed when traveling to breeding grounds is faster than when traveling to wintering locations. There are examples of average migration speeds derived by dividing the distance from marking until recovery by the number of days, as indicated in Table 6-3, and the distance is thought to be in the range of about 60-200km per day. This is the average speed including rest and other breaks. It can be seen that the blackpoll warbler (*Dendroica striata*), which increases its speed during latter half of the migration by a factor six, has the strongest flying ability.

There is also data, given in Table 6-3, regarding the distance that can be flown nonstop. The straight line distance between Yamaguchi Prefecture and the highly pathogenic avian influenza outbreak sites in southeastern Korea is about 250km (refer to Fig. 6-2-1) and, as long weather and other conditions are not adverse, it is evident that this is a distance that can be covered within one day flying nonstop.

Table 6-3 Flight speed of wild birds

General flight speed	
Ducks and geese	64-80 km/h
Herons, hawks, horned larks, ravens, shrikes	35-45km/h
Flycatchers	16-27km/h

Flight speed during migration	
Lesser yellowleg (*Tringa flavipes*) (autumn)	507km/d, average of 6 days
Blue-winged teal (*Anas discors*) (spring)	123-160km/d; average of 30 days
American black duck (*Anas rubripes*) (spring)	130km/d; average of 12 days
Gray-cheeked thrush (*Catharus mimimus*) (spring)	208km/d, average of 30 days
Yellow warbler (*Dendroica petechia*) (spring)	178km/d, average of 15 days
American robin (*Turdus migratorius*) (spring)	62km/day, average of 78 days
Blackpoll warbler (*Dendroica striata*) (spring) (first half of migration)	48-56km/day, average of 30 days
Blackpoll warbler (*Dendroica striata*) (spring) (latter half of migration)	320km/d, average of 14 days

Nonstop flight distance	
Snow goose (*Anser caerulescens*) (spring)	2,500km
Barn swallow (*Hirundo rustica*)	560km
Shorebirds	Several hours at 72km/h

References

Lincoln FC, Peterson SR, Zimmerman JL. (1998) Migration of birds. U.S. Department of the Interior, U.S. Fish and Wildlife Service, Washington, D.C. Circular 16. Jamestown, ND: Northern Prairie Wildlife Research Center Home Page.
http://www.npwrc.usgs.gov/resource/othrdata/migratio./htm (Version 02APR2002).

6.4. Cases of avian influenza detection in wild birds (literature search)

(Kumiko Yoneda)

6.4.1. Examples of mass deaths of wild birds due to avian influenza

Reports of mass deaths of avian species other than chickens and turkeys due to avian influenza are given below.

(1) More than 1,300 deaths of common terns (*Sterna hirundo*) in South Africa in 1961 (detection of H5N3 highly pathogenic avian influenza virus) (Becker 1966)

(2) Mass deaths of young ostriches (*Struthio camelus*) being raised in South Africa in 1991 (detection of H7N1 virus; it has low pathogenicity to chickens and is not a highly pathogenic avian influenza virus). Other infestations leading to death caused by several different types of low pathogenic avian influenza viruses involving young ostriches being raised in South Africa and Zimbabwe have been confirmed (Verwoerd 2000).

(3) Deaths of about 150 waterfowl in Hong Kong in November-December 2002 (detection of H5N1 avian influenza virus) (OIE 2003). There had been an outbreak involving chickens 9 months prior to that but the cause was a virus that had mutated further and readily propagated in duck brains (Sturm-Ramirez et al. 2004).

(4) Deaths of about 700 open-billed storks (*Anastomus oscitans*) in Thailand in February 2004 (detection of H5N1 avian influenza virus) (newspaper reports: Asahi Shimbun,

February 18, 2004, etc.). There were also media reports that the number of deaths was much smaller. There are reports of avian influenza outbreaks involving chickens in Thailand in January 2004.

6.4.2. Wild bird virus tests following avian influenza infection in waterfowl

There are a number of examples of wild birds being subjected to virus isolation and antibody testing at the sites of highly pathogenic avian influenza outbreaks that affect domestic fowl due to concerns that the virus could have been carried and spread by wild birds. As a result, the only examples of virus detection appear to be those involving secondary infection from the domestic fowl. Comparing the four cases with the detailed reports in the U.S., Italy and the present cases in Korea and Japan (Table 6-4-1), it can be seen that the 9 jungle crows that tested positive represent the second highest detection rate after Italy.

(1) Occurrence of H5N2 highly pathogenic avian influenza in the U.S. in 1983-84 (Nettles et al. 1985)

The survey subjects in the case in the U.S. were wild and domestic geese and ducks, birds that had contact with the infected farm, chicken feces or dead birds, mice in the vicinity of the infected farm and wild birds in the quarantine zone that appeared feeble or were discovered dead as well as road-kill ring-necked pheasants and hunted wild turkeys.

While testing more than 1,000 specimens, H5N2 highly pathogenic avian influenza virus was isolated from only a single specimen, a chukar partridge (*Alectoris chukar*, a member of the pheasant family somewhat larger than a quail) being raised at the infected farm. It had escaped when the culling of the chickens began and was later found dead at the farm. Highly pathogenic avian influenza virus was not detected in any other specimens also being raised there. In addition, low pathogenic avian influenza virus was isolated from 4 waterfowl. Though not included in the report, H5N2 low pathogenic avian influenza virus was also isolated from a ring-necked pheasant that had been hunted during the outbreak.

(2) Outbreak of H7N1 highly pathogenic avian influenza in Italy in 1999-2000

H7N1 highly pathogenic avian influenza virus was isolated from two of 103 specimens in the testing of dead and captured birds discovered in the vicinity of the infected farm. In one of the cases, the virus was detected in the combined organs of two dead house sparrows (*Passer domesticus*) discovered at the farm and the other was a dead collared dove (*Streptopelia decaocto*) found within 1km from the outbreak site (Capua et al., 2000).

H7N1 highly pathogenic avian influenza virus was also isolated from a saker falcon (*Falco cherrug*) being raised in captivity that had died during the outbreak in the area of the infestation. Three other falcons raised together with the other all tested negative and virus was not isolated from cloaca swabs or feces (Magnino et al., 2000).

(3) Outbreak of H5N1 highly pathogenic avian influenza in Korea in 2003-04 (Japan Wildlife Research Center, 2004)

More than 5,000 waterfowl specimens were tested in Korea. Almost all of the specimens were feces collected on migration routes separated from the outbreak sites and, though low pathogenic avian influenza was detected, no highly pathogenic avian influenza virus was isolated.

The one specimen from which H5N1 highly pathogenic avian influenza virus was isolated was a magpie (*Pica pica*) that had been captured for testing about two months after the outbreak.

Table 6-4-1 Results of wild bird studies following outbreaks of highly pathogenic avian influenza among domestic fowl

Country	Virus Type	Test	Area	Waterfowl[1]	Pheasants	Crows	Other birds	Rodents	Total	Collection time
U.S.A. 1983-84	H5N2 Chickens, turkeys	Isolation	PA	0/1,498	1/429	0/201	0/1,735	0/269	0/4,132	During outbreak – 6 months later
			VA	0/4	0/84		0/223		0/313	
			MD	0/1,511						
			Total	0/3,013	1/513	0/201	0/1,958	0/269	1/4,445	
		Antibody	PA	92/953 (318/953)[2]	0/396	0/174	0/1,452	0/125	92/2,147 (318/2,417)[2]	During outbreak – 6 months later
Italy 2000	H7N1 Chickens, etc.	Isolation	Northern Italy	0/28			2/75		2/103	During outbreak
Korea 2003-04	H5N1 Chickens, ducks	Isolation	Migration routes	0/5,460					0/5,460	During outbreak
			Cheonan, Jincheon, Eumsung	0/29			0/11		0/40	Abt. 1 month after outbreak
			Yangsan City[3]			0/1	1/99		1/100	Abt. 2 months after outbreak
			Total	0/5,489		0/1	1/110		1/5,600	
		Antibody	Chonan, Jincheon	0/29			0/11		0/40	Abt. 1 month after outbreak
			Total	0/29			0/11		1/40	
Japan 2004	H5N1 Chickens	Isolation	Yamaguchi	0/140		0/50	0/109		0/299	Abt. 2 months after outbreak
			Oita	0/123		0/108	0/99		0/330	Abt. 1 month after outbreak
			Kyoto	0/50	0/1	0/107	0/101		0/259	Abt. 2 weeks after outbreak
			Kyoto-Osaka			9/396[4]			9/396[4]	Abt. 10 days – 2 months after outbreak
			Nationwide			0/386	0/274[5]		0/660	Abt. 1 – 3 months after outbreak
			Nationwide (dead birds)						0/4,866[6]	Abt. 1 – 3 months after outbreak
			Total	0/313	0/1	9/1,047	0/583		9/7,962	
		Antibody	Yamaguchi	0/1			0/84		0/85	Abt. 2 months after outbreak
			Oita				0/81		0/81	Abt. 1 month after outbreak
			Kyoto		0/1	0/1	0/83		0/85	Abt. 2 weeks after outbreak
			Total	0/1	0/1	0/1	0/248		0/251	

1) Waterfowl include geese and ducks, sandpipers and plovers and seagulls.
2) Numbers include those having either H5 or N2 antibodies.
3) Samples in Yangsan city were collected by shooting and not tested for antibody.
4) Total of dead, feeble and captured specimens. The positive specimens were dead jungle crows
5) Subjects are feral pigeons
6) Excluding 9 crows of Kyoto and Osaka

(4) Japan (Ministry of the Environment, 2004)

Isolation of virus was attempted using a total of 888 specimens obtained through captures and feces collection in the three areas where highly pathogenic avian influenza outbreaks occurred. All tested negative. However, H5N1 highly pathogenic avian influenza was isolated from 9 dead jungle crows (*Corvus macrorhynchus*) in the area surrounding Kyoto where a large outbreak involving chickens had occurred. More than 4,000 dead wild bird specimens that had been collected with the cooperation of local government agencies nationwide were furthermore subjected to virus isolation testing, though all tested negative.

(5) Other cases

H7N7 highly pathogenic avian influenza virus was isolated from cloaca swabs of one common starling (*Sturnus vulgaris*) captured in a poultry house during an outbreak of H7N7 highly pathogenic avian influenza in Australia in 1985 (Cross 1987).

In addition, wild waterfowl were tested 6 weeks after the first outbreak in the outbreak of H7N4 highly pathogenic avian influenza in 1997 and type A influenza virus antibodies were detected. However, type H7 antibodies were not detected nor was the virus isolated (details including sample number are not shown) (Selleck et al. 2003).

Type H5N1 highly pathogenic avian influenza virus was isolated from a dead peregrine falcon in Hong Kong in January 2004. In a wild bird virus carrier monitoring survey in 2003, more than 6,000 swab samples were subjected to virus isolation testing but H5N1 virus was not isolated (OIE 2004). H5N1 highly pathogenic avian influenza in Hong Kong occurred in 1997 and 2002 involving chickens and at the end of 2002 involving wild waterfowl.

In Thailand, H5N1 highly pathogenic avian influenza was detected in two dead crows at the Bangkok Zoo in January 2004 and capture testing of crows and pigeons in the city was conducted later. Twenty-three crows and 55 pigeons were tested but no virus was detected (newspaper reports: Bangkok Post, February 18, 2004).

(6) Cases in which the same type of virus was observed in wild birds prior to outbreaks of avian influenza involving domestic fowl

Type H7N2 virus was isolated from 15 dead wild mallards in Israel in 1979 and, at the same time, outbreaks of the same type involving turkeys were observed in an adjacent area (Lipkind et al., 1979).

In tests conducted in the U.S. in 1980-81, when mallards that had tested negative were put together with turkeys, virus (H4N8, H4N2, H5N2) was isolated from the mallards before or at the same time that avian influenza of the same type viruses was detected in the turkeys. However, there were also cases in which, though detected in the turkeys, the virus was not detected in the mallards (Halvorson et al., 1983).

In Cambodia, H5N1 virus was first detected at the Wildlife Rescue Center (combined with the zoo) in the suburbs of Phnom Penh in December 2003. Nine days later, an outbreak occurred in Phnom Penh affecting chickens (FAO 2004). The first species affected was reported to be a grey heron (*Ardea cinerea*). Later, however, the infection spread to raptors, egrets, parrots and wild crows at the zoo and deaths were also observed. Infection by chicken meat given as food is suspected and there is also the possibility of infection from chickens in the first case of infection.

6.4.3. Detection of avian influenza virus in wild birds

Surveys of the conditions of wild birds as avian influenza virus carriers consist primarily of those targeting waterfowl and those conducted as a part of the import quarantine procedures for small pet birds. Bird species in which avian influenza virus has been defected based on a number of reference works reviewing the results of such surveys are indicated in Table 6-4-2. Pathogenicity has not been confirmed for most of the viruses that have been detected or they have low pathogenicity for chickens.

Avian influenza virus isolated in the natural state has been isolated mostly from ducks (Order Anseriformes), followed by seagulls (Order Charadriiformes). Avian influenza virus is also often isolated from small birds of the Orders Passeriformes and Psittaciformes in captivity, though retention of type H5 or H7 virus, which potentially can cause highly pathogenic avian influenza, is almost never seen. Since small pet birds are kept in dense conditions when raised and shipped, infection probably spreads readily and there are also likely to be many cases of detection.

Table 6-4-2 Wild birds species in which avian influenza virus has been detected

Order	In the wild	In captivity	Source
Struthioniformes	-	Ostrich, emu	3, 25, 29
Timamiformes	-		
Sphenisciformes	Adelie penguin (antibodies only)		6
Gaviiformes	Red-throated loon (*Gavia stellata*), Arctic loon (*Gavia arctica*)		27
Podicipediformes	Pied-billed grebe (*Podilymbus podiceps*)		27
Procellariiformes	Wedge-tailed shearwater (*Puffinus pacificus*)		27
Pelecaniformes	Great cormorant (*Phalacrocorax carbo*), American plain pelican (*Pelecanus erythrorhynchos*)		25, 27
Ciconiiformes	Grey heron (*Ardea cinerea*), little egret (*Egretta garzetta*), glossy ibis (*Plegadis falcinellus*), hadeda ibis (*Bostrychia hagedash*), squacco heron (*Ardeola ralloides*), open-billed stork (*Anastomus oscitans*)		22, 27, 28
Phoenicopteriformes	-	Greater flamingo (*Phoenicopterus ruber*), Chilean flamingo (*Phoenicopterus chilensis*) (antibodies only)	2, 28
Anseriformes	Whistling swan (*Cygnus bewickii*), Mallard (*Anas platyrhynchos*), Spot-billed duck (*Anas poecilorhyncha*), Green-winged teal (*Anas crecca*) and many others		3, 9, 14, 22, 25, 27
Falconiformes	Peregrine falcon (*Falco peregrinus*), Buzzard (*Buteo buteo*) (antibodies only)	Saker falcon (*Falco cherrug*)	3, 9, 21
Galliformes	Rock partridge (*Alectoris graeca*), Ring-necked pheasant (*Phasianus colchicus*)	Chachalaca, chukar	2, 18, 25, 27
Opisthocomiformes	-		
Gruiformes	Common moorhen (*Gallinula chloropus*), Eurasian coot (*Fulica atra*), American coot (*Fulica Americana*)		9, 27

Charadriiformes	Eastern dunlin (*Calidris alpine*), Eurasian woodcock (*Scolopax rusticola*), Herring gull (*Larus argentatus*), Common tern (*Sterna hirundo*), Common murre (*Uria aalge*) and many others		3, 25, 27
Pterocliformes	-		
Columbiformes	Collared turtle dove (*Streptopelia decaocto*)		7, 27
Psittaciformes	(Macaw, *Charmosyna josefinae*)	Budgerigar (Melopsittacus undulates), Great alexandrine (*Psittacula eupatria*), Banded parakeet (*Psittacula alexandri*), Sulphur-crested cockatoo (*Cacatua galerita*) and many others	1, 2, 3, 14, 17, 19, 24
Cuculiformes	-	(Touraco, Tauraco corythaix)	24
Strigiformes	(owl species in Italy)		9
Caprimulgiformes	-		
Apodiformes	-		
Coliiformes	-		
Trogoniformes	-		
Coraciiformes	-		
Galbuliformes	-		
Piciformes	Great-spotted woodpecker (*Picoides major*)		27
Passeriformes	House sparrow (*Passer domesticus*), Carrion crow (*Corvus corone*), Common crow (*Corvus brachyrhynchus*), European starling (*Sturnus vulgaris*), etc.	Hill-mynah (*Gracula religiosa*), Siberian meadow bunting (*Emberiza cioides*), Blue-and-white flycatcher (*Cyanoptila cyanomelana*), Zebra finch (*Poephila guttata*), etc.	3, 7, 8, 12, 14, 19, 24, 27

- : No test reports found
For the species in parentheses, only reports with negative test results are found. The list is not exhaustive.
The source indicates the reference number.

References

1. Alexander DJ, Allan WH. Harkness JW. (1974) Isolation of influenza virus from Psittacines. Research in Veterinary Science 17: l25-127.
2. Alexander DJ. (1982) Isolation of influenza A viruses from birds in Great Britain during 1980 and 1981. The Veterinary Record 111: 319-321.
3. Alexander DJ. (2003) Report on avian influenza in the Eastern hemisphere during 1997-2002. Avian Dis. 47(3 Suppl.) : 792~797.
4. Allwright DM. Burger WP, Geyer A, Terblanche AW. (1993) Isolation of an influenza A virus from ostriches (*Struthio camelus*). Avian Pathology 22: 59-65.
5. Becker WB. (1966) The isolation and classification of Tern virus: Influenza Virus A/Tern/South Africa/196l. The Journal of hygiene 64: 309-320.
6. Briggs LC, Ashton RM, Metcalf P. (2003) Development of a highly sensitive screen for influenza A in guano and its application in the search for ancient RNA preserved under antarctic adelie penguin colonies. Avian Dis. 47(3 Suppl.) : 1200-1202.
7. Capua I, Crossele B, Bertoli E, Cordioli P. (2000) Monitoring for highly pathogenic avian influenza in wild birds in Italy. The Veterinary Record 147: 640.

8. Cross GM. (1987) The status of avian influenza in poultry in Australia. Proceedings of the Second International Symposium on Avian Influenza, Sept. 1986 Atlanta, Georgia. pp. 96-103.
9. De Marco MA, Foni E, Campitelli L, Raffini E, Delogu M, Donatelli I. (2003) Long-term monitoring for avian influenza viruses in wild bird species in Italy. Veterinary Research Communications Sup. 1: 107-114.
10. FAO (2004) Avian influenza control in Cambodia. FAOAIDEnews Issue no. 16.
11. Halvorson D, Karunakaran D, Senne D, Kelleher C, Bailey C, Abraham A, Hinshaw V, Newman J. (1983) Epizootiology of avian influenza - simultaneous monitoring of sentinel ducks and turkeys in Minnesota. Avian Dis. 27 (1): 77-85.
12. Johnson DC, Maxfield BG, Moulthrop JI. (1977) Epidemiologic studies of the 1975 avian influenza outbreak in chickens in Alabama. Avian Dis. 21(2): 167-177.
13. Ministry of the Environment (2004), press releases (March 30, 2004, April 20, 2004)
14. Japanese Society on Poultry Diseases (2002), *Transmission of poultry diseases by wild birds and its prevention*, Journal of Japanese Society on Poultry Diseases Report 38 (1): 10-19
15. Lipkind MA, Weisman Y, Shihmanter E, Shoham D. (1979) Identification of avian influenza viruses isolated from wild mallard ducks in Israel. The Veterinary Record 105: 558.
16. Magnino S, Fabbi M, Moreno A, Sala G, Lavazza A, Ghelfi E, Gandolfi L, Pirovano G, Gasperi E. (2000) Avian influenza virus (H7 serotype) in a saker falcon in Italy. The Veterinary Record 146: 740.
17. Nerome K, Nakayama M, Ishida M, Fukumi H, Butterfield WK, Webster RG, Campbell CH. (1978) Isolation and serological characterization of influenza A viruses from birds that were dead on arrival at Tokyo airport. Archives of Virology 57: 261-270.
18. Nettles VF, Wood JM, Webster RG. (1985) Wildlife surveillance associated with an outbreak of lethal H5N2 avian influenza in domestic. poultry. Avian Dis. 29(3) : 733-741.
19. Nishikawa F, Sugiyama T, Yamamoto K, Yonemura H. (1977) Isolation of influenza type A viruses from imported pet birds. Jpn. J. Med. Sci. Biol. 30: 31-36.
20. OIE (2003) Highly pathogenic influenza in Hong Kong, Special Administrative Region of the People' s Republic of China: in waterfowl. Disease Information (OIE) 16(4): 21-22.
21. OIE (2004) Avian influenza in Hong Kong, Special Administrative Region of the People's Republic of China in a wild bird. Disease Information (OIE) 17(5): 18-19.
22. Pfitzer S, Verwoerd DJ, Gerdes GH, Labuschagne AE, Erasmus A, Manvell RJ, Grund Ch. (2000) Newcastle disease and avian influenza A virus in wild waterfowl in South Africa. Avian Dis. 44(3): 655-660.
23. Selleck PW, Arzey G, Kirkland PD, Reece RL, Gould AR, Daniels PW, Westbury HA. (2003) An outbreak of highly pathogenic avian influenza in Australia in 1997 caused by an H7N4 virus. Avian Dis. 47(3 Suppl.) : 806-811.
24. Senne DA, Pearson JE, Miler LD, Gustafson GA. (1983) Virus isolations from pet birds submitted for importation into the United States. Avian Dis. 27: 731-744.
25. Senne DA. (2003) Avian influenza in the Western hemisphere including the Pacific islands and Australia. Avian Dis. 47(3 Suppl.): 798-805.
26. Japan Wildlife Research Center (2004), Report of Urgent Study on Korean Migratory Birds in 2003
27. Stallknecht DE, Shane SM. (1988) Host range of avian influenza virus in free-living birds.

Veterinary Research Communications 12: 125-141.
28. Sturm-Ramirez KM, Ellis T, Bousfield B, Bissett L, Dyrting K, Rehg JE, Poon L, Guan Y, Peiris M, Webster RG. (2004) Reemerging H5N1 influenza viruses in Hong Kong in 2002 are highly pathogenic to ducks. Journal of Virology 78(9): 4892-4901.
29. Verwoerd DJ. (2000) Ostrich diseases. Revue scientifique et technique (International Office of Epizootics) 19(2): 638-61.

6.5. Pathogenicity of avian influenza virus to wild birds (literature search)

(Kumiko Yoneda)

There are not many reports that examine the pathogenicity of highly pathogenic avian influenza virus by infecting wild birds with it and the susceptibility of wild birds is not well understood. Tests involving respiratory inoculation of the H5N1 highly pathogenic avian influenza virus of chicken origin that caused infestation in Hong Kong in 1997 (Perkins and Swayne 2003) are shown in Table 6-5.

The "Virus Propagation" item in the table indicates where the virus primarily propagates. Group 1 had a high death rate as a result of propagation throughout the body and Group 2 demonstrated neurological symptoms and movement disorders as the result of propagation in the brain. Meanwhile, it would be difficult to say that infection itself occurred in the members of Group 4. Consequently, there is probably little possibility that the species in these three groups could be long-distance carriers of highly pathogenic avian influenza virus. In contrast to these, the species in Group 3 did not demonstrate symptoms for a long time though they were infected and the virus propagated internally. They are therefore probably capable of being long-distance carriers of the virus.

Table 6-5 Differences in the susceptibility of bird species by experimental infection of H5N1 highly pathogenic avian influenza virus

Classification	Virus propagation	Species	Order
Group 1	Full body propagation	Chickens Turkeys Japanese quails (*Coturnix japonica*), bobwhite quails Pearl guineafowl (*Numida meleagris*) Ringneck pheasants (*Phasianus colchicus*) Chukar partridges (*Alectoris chukar*) Zebra finches (*Poephila guttata*)	Galliformes Galliformes Galliformes Galliformes Galliformes Galliformes Passeriformes
Group 2	Propagation in brain	Emus Domestic geese Budgerigars (*Melopsittacus undulates*) House finches (*Carpodacus mexicanus*)	Struthioniformes Anseriformes Psittaciformes Passeriformes
Group 3	Low propagation rate	Domestic ducks Laughing gulls (*Larus atricilla*) House sparrows (*Passer domesticus*)	Anseriformes Charadriiformes Passeriformes
Group 4	No propagation	Pigeons European starlings (*Sturnus vulgaris*)	Columbiformes Passeriformes

However, there are also reports that, in the experimental infections using H7N7 highly pathogenic avian influenza virus, a reaction was observed in the European starling (*Sturnus vulgaris*) similar to the reactions of the members of Group 1 in Table 6-5 (Nestorowicz et al., 1987) and, in the experimental infections of the laughing gull (*Larus atricilla*) using H5N3 virus from the infestation of common terns (*Sterna hirundo*) in 1961, a greater pathologic change was observed than with chicken-origin H5N1 virus (Perkins and Swayne 2002). It is thought that the Order Galliformes generally is highly susceptible while it is thought that ducks do not become ill when infected. However, there were many deaths among the ducks and egrets that were infected by H5N1 highly pathogenic avian influenza virus in Hong Kong at the end of 2002 and it has been confirmed in infection tests with mallards that the virus propagates throughout the body and especially in the brain (Sturm-Ramirez et al., 2004). There are thus thought to be differences in susceptibility even within the same species due to differences in the virus type or lineage (if within the same type). In addition, there are many species in the Order Passeriformes and there appears to be an abundance of differences in susceptibility and there is therefore probably a need for more meticulous research in order to be able to conjecture the susceptibility of specific species.

References

Nestorowicz A, Kawaoka Y, Bean WJ, Webster RG. (1987) Molecular analysis of the hemagglutinin genes of Australian H7N7 influenza viruses: role of Passerine birds in maintenance or transmission? Virology 160(2): 411-418.

Perkins LE, Swayne DE. (2002) Susceptibility of laughing gulls (*Larus atricilla*) to H5N1 and H5N3 highly pathogenic avian influenza viruses. Avian Dis. 46(4): 877-885.

Perkins LE, Swayne DE. (2003) Comparative susceptibility of selected avian and mammalian species to a Hong Kong-origin H5N1 high-pathogenicity avian influenza virus. Avian Dis. 47(3 Suppl.): 956-967.

Sturm-Ramirez KM, Ellis T, Bousfield B, Bissett L, Dyrting K, Rehg JE, Poon L, Guan Y, Peiris M, Webster RG. (2004) Reemerging H5N1 influenza viruses in Hong Kong in 2002 are highly pathogenic to ducks. Journal of Virology 78(9): 4892-4901.

Section 6 Comprehensive discussion

1. Route of infections

(Nobuyuki Terakado, Toshiyuki Tsutsui)

1.1. Characteristics of outbreaks

1.1.1. Raising configuration at the infected farms

There were significant differences between the raising configurations at the infected farms in the four locations in three prefectures. In Yamaguchi Prefecture, it was an egg farm with 35,000 fowl, in Oita Prefecture, it was the home of a bird fancier (13 long-tailed bantams and 1 duck) and, in Kyoto Prefecture, it was a large-scale egg farm with 225,000 fowl and broiler farm with 15,000 fowl. There were great differences in scale and poultry raising methods and mutual relationships are not evident.

1.1.2. Characteristics of the outbreak locations

The location in Yamaguchi Prefecture where an outbreak was first confirmed is separated from the outbreak locations in Oita and Kyoto Prefectures by straight-line distances of 140km and 360km, respectively. The location of a confirmed outbreak at the southern tip of the Korean Peninsula (Kyongsang-namdo Province) is separated from Oita and Yamaguchi Prefectures by more than 350km and from Kyoto Prefecture by more than 700km. The outbreak locations are in mountainous areas and the infected farm in Yamaguchi Prefecture and the location of the first outbreak in Kyoto Prefecture, in particular, were the most secluded separated from human settlements and there were ponds and bodies of water nearby that served as waterfowl habitats. Meanwhile, the home where the outbreak occurred in Oita Prefecture was located in a community in mountainous terrain and, therefore, a location surrounded by mountains is a characteristic that it shares with the other outbreak locations, though the surrounding environment is different.

1.1.3. Characteristics of the time of outbreak occurrence

There were reports of outbreaks of avian influenza in Korea, Hong Kong, China, Vietnam, Thailand, Indonesia, Cambodia and Laos beginning in December 2003 and into 2004 by the same serum subtype H5N1 virus that was isolated in Japan.

In Korea, the nearest country with infestations and with confirmed affinity to the isolated virus, there were reports of 14 outbreaks from December 10 to December 23, 2003, while 5 additional outbreaks were reported to have occurred sporadically until March 20 of the next year.

It was on December 23, 2003, that the first clinical symptoms among chickens was confirmed in Yamaguchi Prefecture and, subsequently, the first fowl deaths were confirmed in Oita Prefecture on February 4 and in Kyoto Prefecture on February 17. There was a time lag of about one month and a half between them.

The incubation period with avian influenza is thought to differ depending on the virus strain, the amount of virus exposure and route of exposure but it is generally considered to be in the range of several hours to 7 days (USDA). In nasal inoculation tests of chickens using the Yamaguchi isolated strain, it took an average of 48 hours until death. Even if the amount of infection virus were small and the incubation period were long in the first case to appear, it is thought that the incursion of virus to the chicken flocks occurred in late December in Yamaguchi Prefecture and in mid-February in Oita and Kyoto Prefectures.

1.2. Characteristics of the isolated avian influenza virus

1.2.1. Homology of isolated virus strains

As a result of the genetic analysis of isolated virus strains from the outbreak locations in Yamaguchi, Oita and Kyoto (first outbreak) Prefectures, it was revealed that the mutual homology of the strains is extremely high and that there are slight differences in the base sequences. This indicates that the strains are from a single source but that they are different virus strains that mutated relatively early. Therefore, the outbreaks in Yamaguchi, Oita and Kyoto Prefectures were not mutual direct infections or outbreaks from the same infection source but rather there is the possibility that the infections originated from different virus sources. In addition, the virus strain isolated form the second infected farm in Kyoto Prefecture was the same as in the first outbreak, while a portion of the virus strains isolated from the dead jungle crows that were found in the Kyoto area was slightly different from the strains that had already been isolated, including those from Yamaguchi and Oita Prefectures. Incidentally, there was a high homology between all of these strains and those isolated in Korea.

1.2.2. Pathogenicity of the isolated virus strains and the susceptibility of the host

1.2.2.1. Chickens

The virus strains isolated from fowl at the time of the present avian influenza outbreaks (originating in Yamaguchi, Oita and Kyoto) indicated strong pathogenicity to chickens and indicated propagation throughout the body with nasal inoculation with the minimum infection dose of about 300 virus particles. There were even chickens that died suddenly without exhibiting any characteristic clinical symptoms.

1.2.2.2. Other birds

Infection was confirmed in 9 dead jungle crows found within a radius of about 30km from the vicinity of the outbreak in Kyoto. Meanwhile, in infection tests of jungle crows by oral inoculation, no deaths were observed during the observation period and no health impairment or other symptoms were recognized in three of the four specimens. Nevertheless, antibodies were detected in these crows and it is thought that infection was established. There were reports that more than 1,000 crows gathered at the compost yard where dead chickens had been discarded at the first outbreak site in Kyoto and, based on these reports, there were fewer crows with confirmed virus infection than estimated. Taking the results of infection tests also into account, there is the possibility that crows are not highly susceptible to the virus and that there are many that are able to endure it even if infected. There is a need to examine the role of crows in the spread of avian influenza to determine whether or not the feces of infected crows becomes a new source of infection or if infection becomes established among crows taking further research results into consideration.

The susceptibility of ducks to isolated virus strains has been confirmed through infection tests. Virus was recovered from all organs in the body of mallards after nasal inoculation, though they endure the infection without symptoms or death, suggesting the possibility that they become carriers of the virus and sources of infection. In addition, infection also becomes established in budgerigars and gray starlings. In the case of budgerigars, in particular, all of the specimens in the infection test died and lethality is considered to be high. In infection tests with sparrows, high concentrations were recovered from the brain and respiratory organs and a high death rate was confirmed.

1.2.2.3. Mammals

In infection tests with mice, 14 of 18 specimens died and virus was isolated at a high rate from the brain and lungs, though not detected in the feces. It is consequently thought that, if rodents other than mice were to indicate similar clinical conditions, the virus would not readily spread via the feces. In virus inoculation tests with miniature hogs, virus infection did not become established, which suggests that the hogs have resistance to the virus strain.

1.2.3. Virus transmittability

At the first outbreak site in Kyoto, the infection was revealed after many live chickens and processing residue had been shipped out and the virus was detected at related broiler processing plants and rendering facilities, though no spread of the virus was evident at farms along the transport routes. In addition, there is also the possibility that the environment surrounding the farm was infected with high concentrations of the virus not only due to the many dead chickens that were discarded in the outdoor compost yard by the time the infection was reported but also by virus excreted by the many infected chickens. In spite of the fact that the farm was accessible to crows and other wildlife, humans and vehicles, the infection spread to only one other nearby farm. In cohabitation infection tests, there were cases in which the virus did not spread to other chickens in the same cage or adjacent cages if there were few infected chickens.

There is no data for comparing transmittability of other virus strains and nothing definite can be said; however, the characteristic of the present virus infestation is that, rather than spreading rapidly between farms through the movement of humans or objects, the spread of the virus to new farms probably does not readily occur unless the chickens are exposed to a given volume of virus. This is also surmised in the spread of the virus between farms in Korea, which is thought to have been caused primarily by the introduction of ducks.

Meanwhile, the modality of infestation in the proliferation of the virus between chickens in the same poultry house is thought to differ depending on chicken density, poultry house structure and other factors in the poultry raising environment.

1.3. Possibility of mutation from low to highly pathogenic virus strains in Japan

It is argued that low pathogenic avian influenza spread among chicken flocks in outbreaks in Italy, U.S. and elsewhere and, while with infection repeatedly spreading from one to the other (for more than 6 months), the virus acquired high pathogenicity. In the present outbreaks in Japan, (1) no avian influenza virus or anti-avian virus antibodies were detected in surveys of nearby farms, (2) low pathogenic virus (not H5 or H7) was isolated from one of 762 wild birds in the surrounding environment; however, it is not conceivable that this strain mutated and became highly pathogenic; in addition, no anti-H5 antibodies were detected in 295 terrestrial birds, (3) subtype H5N3 low pathogenic virus strains isolated in Tottori and Shimane Prefectures in 2002 are genetically different from the present isolated virus strains and (4) there is homology between the virus strains isolated from the three outbreak sites and it is unlikely that mutation from low to high pathogenicity happened to occur at any of these locations. These facts do not support the conjecture that low pathogenic avian influenza virus proliferated prior to the outbreaks of highly pathogenic avian influenza virus.

Given the above, it would be difficult to consider that low pathogenic avian influenza virus mutated to highly pathogenic virus in Japan.

1.4. Possibility of incursion from overseas and the route of incursion

In light of the inconceivability of a mutation of already existing low pathogenic avian influenza virus to highly pathogenic virus as well as the fact that outbreaks were confirmed in Korea in mid-December 2003 prior to the outbreak in Yamaguchi Prefecture and that there is genetic affinity between the strains isolated in Japan and those isolated in Korea, it is probably reasonable to consider that the virus was carried from the Korean Peninsula to Japan by one means or another.

In epidemic prevention related surveys of the infected farms, there was no confirmation of movement by directly related persons or objects to Korea or other countries where avian influenza had occurred. In addition, it would likewise be difficult to think that imported birds or livestock products that could serve as the source of infection were involved in the outbreaks at the three farms, which had greatly differing poultry raising configurations. In order for infection to become established by direct contact of humans or objects coming from overseas, it is probably necessary for the virus to be brought by humans contaminated by the virus in a given density or by imported birds or animals that are carrying the virus; however, such movement of humans or animals has not been confirmed to date. It would therefore be unlikely that virus brought to the farms from overseas through the medium of humans or objects led directly to the outbreaks at the three sites.

The involvement of wild birds, especially waterfowl, is generally suspected as the route of incursion to the site of the initial outbreak of avian influenza. Waterfowl and other migratory birds are known to fly from the Korean Peninsula and other locations where outbreaks have occurred to Japan. Since wild ducks have especially strong resistance to avian influenza virus, they are known to be carriers that excrete the virus in their feces. They fly to Japan with the peak in late October to early November and, depending on the species and weather conditions (cold waves on the Korean Peninsula, etc.), they may arrive later than that. It is furthermore said that the virus generally survives in feces for a period of about 35 days at 4°C and, taking into account the suitability of the low temperature environment of winter for virus survival and the possibility that the virus is also carried by waterfowl, it cannot be denied that the virus may possibly have been brought to Japan somewhat earlier than the outbreaks.

Meanwhile, thrushes, wagtails and other terrestrial birds came directly to the farm compost yards and there are some among them that have migratory behavior. Since gray starlings, sparrows and other terrestrial birds are infected by the virus strains based on outbreaks that have occurred to date and infection tests, the possibility cannot be denied that terrestrial birds with migratory behavior were infected in Korea and brought the virus to Japan.

In surveys of migratory birds in the vicinity of the outbreak sites, highly pathogenic avian influenza virus has not been isolated but, given the total number of birds that fly to Japan, it cannot be automatically concluded based on these results that they had no involvement in carrying the virus to Japan. It has been confirmed that there are rivers, ponds and other bodies of water in the vicinity of the outbreak sites that are actually frequented by waterfowl.

In light of the above, we conclude that there is a possibility that the virus was brought to Japan from the Korean Peninsula or other location by migratory birds. Taking into account differences in the base sequences of the isolated virus strains in this case, there is probably a strong possibility that the virus was brought from the Korea Peninsula or other location to each of the three infected farms separately.

1.5. Route of incursion to the farms and poultry houses

Even if we were to conjecture that the virus was brought to Japan by migratory birds, the infection would not have occurred unless it reached the chickens. It is generally difficult to imagine that ducks or other waterfowl would enter directly into the poultry houses and these species were not confirmed in farm interview surveys. Nevertheless, since it is known that the virus propagates in the intestinal tract of ducks and that the feces contain large quantities of the virus, there is the possibility that the feces of ducks and other migratory birds are the source of infection and that the virus is brought into the poultry houses by resident birds, mice or other animals that inhabit areas near human settlements or by humans or other means.

1.5.1. Resident birds inhabiting areas near human settlements

Though there are thought to be few opportunities for resident birds to come into contact with migratory birds, if resident birds are infected or contaminated through the medium of water that is contaminated by feces or virus in feces, they could enter the poultry house and cause the infection of chickens.

Crows, pigeons, sparrows and wagtails were confirmed in the poultry houses in Yamaguchi Prefecture, sparrows and other birds were also confirmed in the poultry houses in Kyoto Prefecture and sparrows and other birds were confirmed in the vicinity of the outbreak site in Oita Prefecture.

1.5.2. Vehicles, humans and objects

Though there is a possibility that humans or vehicles that had had contact with migratory birds or their feces were the route of infection into the poultry houses, there has been no confirmation to date of persons who were engaged in bird watching or otherwise had contact with wild birds. In addition, there has also been no confirmation of apparatus, equipment or other materials that were brought purposefully only into the poultry house where the infection occurred first at the farm in Yamaguchi Prefecture. Vehicles used for feed shipments and egg deliveries at the farm in Yamaguchi Prefecture were disinfected while, at the farm in Kyoto Prefecture, a system had been set up that enabled the direct delivery of feed to feed tanks lined up along the town road outside of the farm. Though it cannot be completely denied that these delivery trucks or the vehicles of employees may have become contaminated while in operation and then brought the virus into the farm, if there were the possibility of a source of infection that could cause such strong contamination, many other farms in the area would also likely have been infected. Consequently, there is a probably little possibility that they carried the virus from wild bird habitats into the farm area.

On the other hand, there is the possibility of involvement by humans in bringing the virus into the poultry houses if we assume that the area surrounding the poultry houses was first contaminated by migratory or other birds. The poultry house first infected in Yamaguchi Prefecture was the one that was entered first in the sequence of egg collection and cleaning tasks and the area near the entrance, where work tasks began, was the location where the infection occurred first inside the poultry house. Not all of the infected farms were equipped with boot disinfection tanks and exhaustive epidemic prevention measures had not been adopted and workers may therefore have carried the virus into the poultry houses attached to their boots or clothing.

1.5.3. Drinking water and feed

Water from a pond where the presence of ducks has been confirmed was used only in poultry houses nos. 8 and 9 of the farm infected first in Kyoto Prefecture. Even so, the first outbreak was confirmed in a group of chickens gathered in the center of house no.8 and infection along the water supply line has not been confirmed. Moreover, since the outbreak in house no.9, which used the same water source, occurred 5-6 days later, it would be difficult to say that the pond water was the direct source of infection. It is a fact that, in Oita Prefecture, avian influenza occurred only in the poultry house that had been supplied with pond water the previous evening; however, water flows in and out of the pond constantly and it would be difficult to imagine that the water flow had been contaminated by virus in sufficient quantities to cause the death of the bantams in less than 20 hours after drinking the water. Still, given the fact that none of the other bantams being raised in other houses were infected, the possibility that the water supplied to the one house was contaminated by virus cannot be ignored.

If the feed itself were contaminated, infection would likely occur simultaneously throughout the poultry houses and, if feed in a specific automatic feed tank were contaminated, the infection should occur along the feed supply line. However, infection modalities of that sort were not evident ether in Yamaguchi Prefecture or in the first outbreak in Kyoto Prefecture. However, the possibility still exists that outbreaks could occur due to partial contamination of the feed in the feed tanks by infected wild bird feces or infected wild animals. In Oita Prefecture, the possibility of infection through the feed is slight since the same feed was given to the bantams in all three houses and the feed was stored in a tin container isolated from wild birds or mice.

1.5.4. Rodents and insects

The infected farms in Yamaguchi Prefecture and at the first outbreak site in Kyoto Prefecture had a structure that readily allowed incursion by mice into the poultry houses and evidence of rodent activity has been confirmed at both farms. Meanwhile, the infectivity of the isolated virus strains in mice was confirmed through infection tests, though the virus has not been isolated from the intestinal tract and it is thought that the virus is not excreted in feces. Though differences in susceptibility of test mice and mice in the wild is unknown, if the virus is indeed not excreted in the feces, their role in virus infestation would be limited, though there is the possibility that they could have brought the virus from the surrounding area into the poultry houses with the virus attached to their bodies through contacts with infected wild birds both living and dead in the area surrounding the farm. It would also not be possible to completely deny that flies or other insects could have brought the virus from the surrounding area into the poultry houses attached to their bodies.

1.6. Virus spread between farms in Kyoto Prefecture

In Kyoto Prefecture, an outbreak following the first was confirmed at a broiler farm at a distance of about 4km from the first site. Since the gene base sequences of the isolated viruses from the two farms coincided, it is thought to have spread from the first outbreak site in Kyoto

Prefecture to the second. Epidemic prevention measures, including prevention of wild bird incursion into the poultry houses, were strengthened at this farm at the time when the outbreak was confirmed in Yamaguchi Prefecture in January and the outbreak occurred here just as they were taking steps to limit access to the farm prompted by the outbreak at the farm nearby.

As the route of proliferation from the first outbreak site in Kyoto Prefecture, the movement of humans or objects between the two farms has not been confirmed but considerable contamination of the surrounding environment may have occurred due to virus excreted by large numbers of infected chickens. Given the fact that humans and vehicles used the nearby public road even though no measures had been devised to prevent the spread of the virus outside of the farm during a period of about ten days between the time when deaths began to increase and the authorities were notified and that wild birds and other wildlife had free access to the compost yard where dead chickens had been discarded for a period of about ten days after the outbreak, it is thought that this caused the spread of the virus into the surroundings. The farm is actually in a location that can be overlooked from the road linking it to the first outbreak site and the first house to be infected is in a location facing a wooded area adjacent to that road. In addition, since dead chickens at the first infected farm had been discarded in a location allowing contact by wild animals, crows and other wild birds from the surrounding environment, there is the possibility that these wild animals carried the virus into the vicinity of this farm over a distance of about 4km. Moreover, since the first outbreak site was visited by media reporters before movement restrictions were instituted and it has also been confirmed that they stopped by other poultry farms in the area, it cannot be denied that they or their vehicles brought the virus into the vicinity of the farm. Though it is difficult to identify the direct route of incursion into the poultry houses, there is possible involvement by sparrows, mice or other wild animals as well as insects.

2. Recommendations for infestation and proliferation preventive measures

(Nobuyuki Terakado)

Above, we have pointed out the problem areas that have been experienced, remedies and so forth through the process of elucidation of the route of infection of highly pathogenic avian influenza, which should contribute to the prevention of outbreaks and proliferation in the future.

2.1. Epidemiological surveys

When an infectious disease such as highly pathogenic avian influenza occurs, measures to prevent proliferation are imposed pursuant to the Domestic Animal Infectious Diseases Control Law and a series of epidemic prevention measures is implemented primarily by the local Livestock Hygiene Service Center. The basic policy at that time is to exterminate the pathogen as quickly as possible and thereby prevent the proliferation of the infestation. Specifically, it is necessary to rapidly dispose of diseased animals carrying the pathogen as well as other animals, feed, feces and so forth and implement disinfection. To achieve that, once an outbreak has been confirmed, the situation unfolds at the site just as if it were a battlefield through the implementation of epidemic prevention measures. That tendency becomes stronger the larger the facility is in scale and, actually, the disposal of more than 200,000 egg hens was required at the site of the third outbreak in Kyoto Prefecture, even eventually requiring the mobilization of the Self-Defense Forces.

Meanwhile, detailed epidemiological surveys based on scientific data are absolutely essential in the elucidation of the route of infection. To liken an infectious disease outbreak to a fire disaster, a survey team is set up after firefighting activities are completed at the site and interviews of related persons and on-site inspections are initiated. At this point, however, the physical evidence itself, which would be useful in the elucidation, is frequently destroyed as a result of the epidemic prevention measures.

In the case of the second outbreak in Oita Prefecture, the domestic duck that was raised in the same garden was culled along with the infected bantams. Domestic ducks are generally thought to have strong resistance to this disease and detailed data regarding the duck was essential in examining the possibility of virus transmission from wild birds to the duck and from the duck to the bantams. Likewise, in regard to data concerning rodents and other wild animals that were thought to be permanent residents of the poultry houses, capture itself would be difficult after thoroughgoing firefighting activities and direct data regarding the existence of virus was not obtained.

Furthermore, in interviews with employees or others at the site, surveys themselves become confused perhaps due to human memories that fade with the passage of time.

The following are possible remedies to resolve these problem areas.

(1) Epidemiological surveys should be initiated by launching epidemiological survey teams at the time of an outbreak, collecting data as promptly as possible, gathering materials for the preparation of required scientific data, etc., with the assumption that an outbreak of infectious disease could occur at any time.

(2) The epidemiological survey teams should include not only veterinary science specialists but also experts in wild birds, wild animals, harmful pests and other fields.

(3) It is necessary for the epidemiological survey teams to go to the site and give instructions relating for the epidemiological surveys in cooperation with the local Livestock Hygiene

Service Center and also play a role as advisers to prevent proliferation later.

There are few researchers who specialize in the area of livestock hygiene but it is necessary to further reinforce the training of epidemiology experts to promote the enhancement of epidemiological surveys in the field of livestock hygiene.

2.2. International collaboration and cooperation

International collaboration and cooperation centered in the WHO functioned effectively at the time of the outbreak of human SARS infection that occurred primarily in Southeast Asia last year and developed into a major problem of global scale. In other words, the most recent information regarding the state of disease infestation, virus properties and so forth was shared by all countries and effective epidemic prevention measures were devised. As a result, it did not develop into a global infestation, enabling a reaffirmation of the need for international collaboration and cooperation in the prevention of infectious diseases, which are not stopped by national borders. The increase in the volume of traffic and exchanges between countries also at the same time promotes the proliferation and transmission of infectious diseases and the sharing of data and other forms of collaboration and cooperation between countries in measures to prevent infectious diseases have now become essential.

Highly pathogenic avian influenza, which occurred in Japan for the first time in 79 years, is one of those borderless infectious diseases. Since there were multiple outbreaks that occurred simultaneously both in Japan and in neighboring countries, much interest was shown in the molecular epidemiology of the disease viruses isolated in each of the countries. In Japan, the genes of the viruses isolated in Japan were analyzed primarily by the National Institute of Animal Health and notification of the outcome was sent to the WHO and relevant institutions in Korea and China.

The isolated virus strains from the outbreaks in Korea, which occurred earlier than those in Japan, were obtained rather quickly by researchers at a certain domestic research institution but, based on a promise between the researchers involved, they were not permitted to share the sample with any other institutions including the National Institute of Animal Health or release genetic properties and any other information. The National Institute of Animal Health therefore obtained samples of isolated strains from Korea by a different route and, as the result of a comparison of virus properties, a strong affinity was revealed between the genotypes of the Japan strains and Korea strains.

Meanwhile, as the result of a comparative examination of genetic data using Internet information regarding the strains isolated from infected humans in Vietnam, where deaths occurred, and samples obtained by the National Institute of Animal Health, originating in domestic fowl in Thailand, it was found that the strains originating in Southeast Asia and strains originating in Japan are genetically different.

Since there were more than 20 deaths due to H5N1 highly pathogenic avian influenza at the time in Vietnam and Thailand, the news media and others showed strong interest in the differences between the strains isolated in Southeast Asia and those from Japan and Korea. As a result, it could probably be said that the early release of information made it possible to avoid unnecessary apprehension and confusion.

Considering the future development of vaccines and so forth for new diseases and pathogenic agents, it becomes a matter of the priorities of the researchers who first discover them as well as intellectual property rights. However, in order to effectively carry out epidemic prevention measures for this disease, at least the international sharing of information is extremely important. It will thus probably be necessary to examine how best to coordinate the balance between international collaboration and cooperation and the intellectual property rights and so forth of individual researchers.

Meanwhile, in Japan, urgent research relating to highly pathogenic avian influenza is being conducted with the participation of the research organs of the Ministry of Education, Culture, Sports, Science and Technology, the Ministry of Agriculture, Forestry and Fisheries, the Ministry of Health, Labor and Welfare and the Ministry of the Environment. The results as well as the results of infection tests of wild birds using the isolated virus strains conducted at Hokkaido University have become valuable basic data in providing scientific support for the wild bird theory in the elucidation of the route of infection. This is a major accomplishment resulting from collaboration and cooperation relating to this disease.

Since the disease is a borderless infectious disease, it is a problem that would be difficult for Japan working alone to resolve. Exchanges of information, technical cooperation and so forth with the other countries of Asia and throughout the world will ultimately lead to the prevention of outbreaks in Japan. Collaboration and cooperation in epidemic prevention measures in Japan and between countries as well are essential.

2.3. Promotion of research

Of the three types of viruses, A, B and C, that exist in the natural world, type A influenza is the only type that infects birds. Wild waterfowl (primarily ducks) are considered to be the natural host of the virus. The virus carried by ducks propagates in the intestinal tract and is harmless to the host ducks.

However, when the virus enters the world of chickens and other domestic fowl and, while repeating the process of infection and propagation there, mutated strains (highly pathogenic virus) emerge that indicate high pathogenicity to chickens. In light of this, highly pathogenic virus likely originates in low pathogenic virus that ducks and other birds naturally carry.

Even so, many aspects of highly pathogenic avian influenza virus still remain unknown. Various questions arise, for example, of whether or not only low pathogenic strains actually exist in ducks in the wild, whether or not mutations from low pathogenicity to high pathogenicity occur internally in ducks, why the virus indicates strong pathogenicity to chickens and other domestic fowl and why highly pathogenic virus appears only in subtypes H7 and H7. While the virus strains that spread in Southeast Asia and Japan are the same subtype H5N1, why are there differences in the incidence of infection in humans? Where is it that the virus detected in Japan originally acquired high pathogenicity?

There are especially strong demands for the development and use of effective vaccines from persons involved in raising chickens. Killed vaccines existing in the world today prevent a decline in the pathological conditions of infected chickens and cause a reduction in the volume of virus excreted from the intestinal tract but they are not able to prevent open-air infection itself. Therefore, the epidemic prevention policies of the government are based on the principle of detection and culling; vaccines, however, are held in reserve by the government for use on an emergency basis only if the infection spreads and prevention is not possible through ordinary epidemic prevention measures. Joint initiatives have recently been launched through government, industry and academia collaboration with the aim of developing new vaccines and we look forward with anticipation to the results.

There is a need for the further promotion of research in highly pathogenic avian influenza and the avian influenza virus strains used in the research must be handled with exhaustive safety management. The virus demonstrates high pathogenicity not only to chickens but also it is also thought to incur damage in humans in some cases.

2.4. Preventive measures for the future

The basic principle of infectious disease prevention is to block one or more of the three factors involved in outbreaks, namely, (1) pathogenic agent, (2) susceptible animals and (3) route of infection. Preventive measures for the future centered in blocking the route of infection are noted below.

(1) Since the disease is an infectious disease with incursion across national boundaries, it is necessary to pursue close exchanges of information with other countries and contribute to the prevention of outbreaks of the disease in all countries through joint research, technological cooperation and other activities with China, Korea, Vietnam, Thailand and other countries of Southeast Asia.
(2) It is necessary to strengthen wild bird measures at poultry farms. It is especially necessary to give the utmost attention to range-fed poultry, which have many opportunities for contact with wild birds. In addition, in the case of open-type poultry houses, bird nets are to be installed over the windows to prevent entry into the house by sparrows, crows and other wild birds, while making every effort to assure full control of feed in the houses and reinforcing measures to prevent the incursion to chicken feces, compost yards, etc.
(3) The importation of birds, including pet birds, should be prohibited from countries where outbreaks of the disease have occurred.
(4) Attention should be given to wild animal countermeasures. Measures should be reinforced especially for rodents, weasels and other animals as well as flies, cockroaches and other harmful pests on the farm premises.
(5) Only water that is suitable as drinking water or that has been disinfected should be provided for drinking purposes. At least, unboiled water that may possibly have come into contact with wild birds or animals should not be given directly to poultry.
(6) Boot disinfection tanks should be permanently installed at entrances to poultry houses and at internal entrances inside poultry houses. Every effort should be made to disinfect vehicles, equipment, the employees themselves, etc., and access to the farm by outsiders should be strictly limited.
(7) Employees who work at poultry farms should be fully trained in hygiene management, the workflow in poultry farms should be improved and work records aggressively promoted. The most recent information should constantly be provided relating to the conditions of disease outbreaks and caution aroused.
(8) In the case of the first and third outbreaks, the farms themselves were totally disserted during the night and poultry houses were even left unlocked. Even if located in secluded areas, it is necessary to be fully attentive to crime prevention measures.
(9) Early detection is extremely important in effectively preventing the spread of the disease. To that end, it is necessary to conduct appropriate inspections (monitoring) in Japan throughout the year and also to constantly observe health conditions at the farms and ascertain abnormalities in the poultry as early as possible.

As indicated above, diseases with a clearly known pathogenic agent are basically prevented by eliminating opportunities for contact between the pathogenic agent and susceptible animals.

The farm where the first outbreak occurred did not install boot disinfection tanks at the entrances to the poultry houses and sparrows and other wild birds were seen to come and go freely with the doors of the houses left wide open throughout the day. Furthermore, there were holes that would probably allow entrance by mice and there were also marks of chewing by mice on the ceiling inside the poultry houses. Therefore, these wild animals and birds could possibly have transmitted the virus to the poultry if they had carried the virus or had become infected.

Meanwhile, in the poultry house where the infection was observed first at the farm in Kyoto Prefecture at the time of the third outbreak, water from the pond outside was used as drinking water without disinfection. Though it was a small pond of spring water located next to the house, wild ducks came there regularly to rest. Dead chickens were casually discarded in the unattended compost yard located near the poultry houses and wild crows, sparrows and other birds flocked there with the aim of foraging. Though natural, it was an environment in which mice as well as weasels, raccoon dogs and other wildlife came and went without restriction.

Shortcomings were thus evident in day-to-day hygiene management at the farms where the disease occurred and it cannot be denied that that could be what triggered the outbreaks. Though we humans and other animals certainly do not live in an aseptic environment, as long as hygiene management is carried out thoroughly, it should be possible to prevent even this disease, an infectious disease that knows no boundaries.

Conclusion

Highly pathogenic avian influenza, which occurred in Japan for the first time in 79 years, ultimately concluded with four outbreaks and the culling of about 275,000 chickens. Compared to the circumstances in Korea, China, Thailand, Vietnam and other countries, which also experienced outbreaks of the disease due to infection by the same subtype H5N1 virus, the epidemic prevention measures that were implemented in Japan after the outbreaks occurred could probably be described as extremely effective. Nevertheless, we must remain steadfastly alert to incursions of the disease from other Asian countries surrounding Japan. It is necessary to pay special attention to countermeasures for wild animals and birds in addition to early detection of the disease though appropriate monitoring activities.

To reiterate, the basic principle of infectious disease prevention is to block any one of the three factors involved in outbreaks, namely, (1) pathogenic agent, (2) susceptible animals and (3) route of infection. Though this may seem to be a matter of "easier said than done," we must not forget that diseases with clearly known pathogenic agents are basically prevented by eliminating or reducing opportunities for contact between the disease and susceptible animals and that that is possible only through the practical application of basic livestock hygiene management. I earnestly hope that this report will contribute to the formulation of measures to prevent future incursions of highly pathogenic avian influenza.

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