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An outbreak of H7N6 low pathogenic avian influenza in quails in Japan

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Summary

In February and March 2009, a total of seven quail farms in the Aichi Prefecture in Japan were found to be infected with an avian influenza (AI) virus. Low pathogenic AI viruses, subtype H7N6, were isolated from three of these farms. The infection was eliminated through the destruction of susceptible birds on the infected premises, movement controls of quail and other poultry in areas around infected premises, accompanied by intensive clinical, serological and virological surveillance. Sentinel quails were used to verify that the infected farms were free from AI virus before they were restocked. An epidemiological study revealed that the virus was likely to have been introduced into the infected area some time ago. Economic losses amounted to 874 million yen (US$9.75 million), mainly accounting for costs incurred by control and eradication measures and financial support for the infected farms and farms in the movement control areas.

Keywords

Avian, Bird flu, Eradication, Flu, Influenza, Japan, Low pathogenic avian influenza, Quail, Surveillance.

Focolaio di influenza aviaria H7N6 a bassa patogenicità in quaglie in Giappone

Riassunto

Nei mesi di febbraio e marzo 2009, nella Prefettura di Aichi in Giappone, 7 allevamenti di quaglie sono risultati infetti dal virus dell'influenza aviaria (IA). In 3 di queste aziende è stato isolato il virus dell'influenza aviaria H7N6 a bassa patogenicità. L'infezione è stata eradicata con la distruzione degli uccelli suscettibili presenti nelle aziende infette, il controllo della movimentazione delle quaglie e del pollame nelle aree degli allevamenti infetti e un'intensa sorveglianza clinica, sierologia e virologica. Prima di procedere al ripopolamento, sono state utilizzate quaglie sentinella per verificare che le aziende fossero indenni dal virus IA. Lo studio epidemiologico ha permesso di evidenziare la probabilità che il virus sia stato introdotto nell'area infetta da molto tempo. La perdita economica è stata di 874 milioni di yen (pari a 9,75 milioni di dollari), principalmente dovuta al costo delle misure di controllo ed eradicazione e al supporto finanziario per le aziende infette e per quelle delle aree soggette a movimentazione.

Parole chiave

Eradicazione, Giappone, Influenza, Influenza aviaria, Influenza aviaria a bassa patogenicità, Pollame, Quaglia, Sorveglianza.

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Introduction

The Japanese quail (*Coturnix japonica*) (Fig. 1) is a species of quail found in East Asia. It is a migratory species, but is domesticated in Japan and is used mainly for table egg production. Quail is an important poultry species, second to chickens in terms of the number of birds kept in Japan. There were 112 commercial quail farms that raised 5.9 million quails in Japan in February 2008. The Aichi Prefecture is the leading quail-producing prefecture with 35 quail farms raising a total of 3.9 million quails (2).

Figure 1
Japanese quail (*Coturnix japonica*)
Adults are approximately 20 cm in length

Eradication measures against exotic animal diseases are taken in accordance with the Domestic Animal Infectious Diseases Control Law (Law No. 166, 1951). The law defines notifiable avian influenza (NAI) as a disease of chickens, ducks, quail, turkey, pheasant (*Phasianus versicolor*), ostrich (*Struthio camelus*) and helmeted guinea-fowl (*Numida meleagris*), caused by an infection of an avian influenza virus type A which is identified as highly pathogenic in accordance with the *Manual of standards for diagnostic tests and vaccine*s of the World Organisation for Animal Health (*Office International des Épizooties*: OIE) (9) and other avian influenza virus subtypes H5 and H7. In accordance with the law, NAI Guidelines, which describe the specific eradication measures to be taken, were established (3). In accordance with both the law and the guidelines, the eradication measures that should be taken in the event of an NAI outbreak are based on the following principles:

- immediate destruction of susceptible birds on infected farms
- movement controls of poultry within a radius of 5 km to 30 km around infected farms
- intensive surveillance of farms in the movement control areas and on other farms that are epidemiologically related to the infected farms.

Most of these measures are implemented by prefecture veterinary inspectors (veterinarians), under guidance and instructions from the Ministry of Agriculture, Forestry and Fisheries (MAFF). There are 172 prefecture Livestock Hygiene Service Centres (LHSCs) located across Japan, which are responsible for taking samples or making a preliminary diagnosis of an exotic disease. Confirmatory diagnosis of exotic diseases is made by the National Institute of Animal Health (NIAH).

Recently, Japan has had four outbreaks of avian influenza, as follows:

- an outbreak of highly pathogenic notifiable AI (HPNAI) was recorded in early 2004, the first for 79 years, with four farms infected with HPNAI virus subtype H5N1 (6, 7)
- in 2005, 41 farms were found to be infected with low pathogenic NAI (LPNAI) virus subtype H5N2 (7)
- in early 2007, four farms were infected with HPNAI virus subtype H5N1 (7)
- in early 2009, seven quail farms were infected with LPNAI subtype H7N6.

This paper describes the details of the outbreak of LPNAI recorded in quail farms in Japan in 2009.

Details of the outbreak

Following the outbreak of LPNAI in 2005, all layer chicken farms with over 1 000 birds were subjected to monitoring by a LHSC every year. After the outbreak of AI in the Republic of Korea in 2007 and isolation of HPNAI viruses from whooper swans (*Cygnus cygnus*) in early 2008 in the northern part of Japan, the number of birds was lowered to 100 and target species were expanded to include broiler chickens,

ducks, quails and turkey in September 2008. The target species were further expanded to include pheasant, ostrich and helmeted guinea-fowl in December 2008. Monitoring activities included clinical examination of the flocks and antibody testing of serum samples from 10 randomly selected birds per farm. As a result of this surveillance, one quail farm in the Aichi Prefecture was found to be infected with LPNAI virus subtype H7N6 in February 2009. Further surveillance conducted after the detection of this infected farm identified six additional as being farms infected with the AI virus in March 2009.

The seven infected farms were named A, B, C, D, E, F and G in chronological order of detection. Information concerning the number of susceptible birds kept and the number of birds destroyed on the farms, the date of diagnosis and the duration of movement controls are presented in Table I. The map in Figure 2 shows the location of these infected farms.

Farm A

Samples were taken on 18 February 2009 from two quail farms in Toyohashi City and from one quail farm in Tokoname City in the Aichi Prefecture under the regular surveillance programme for AI conducted by the Prefectural government. On 25 February, an antibody against an AI virus type A was detected using an agar-gel immunodiffusion (AGID) test conducted by a prefecture LHSC on serum samples taken from one of these farms (Farm A). On the same day, tracheal swab samples from Farm A were subjected to a reverse transcription-polymerase chain reaction (RT-PCR) test which gave a positive result for influenza virus type A. Farm A was placed under voluntary quarantine. On 26 February, a virus with haemagglutination activity was isolated and sent to the NIAH for subtype identification. On 27 February, the isolated virus was confirmed to be subtype H7 in a haemagglutination inhibition (HI) test. On 1 March, the virus was identified to be N6

Table I
Number of quails and the results of laboratory investigations of low pathogenic avian influenza infected quail farms in Toyohashi City, Aichi Prefecture, in 2009

Infected farms	Number of susceptible birds	Number of birds affected	Date of diagnosis	Stamping-out completed	Number of birds destroyed	Movement control area applied	Clinical signs	Laboratory verification
A	257 437 layer quails	10	27 Feb 2009	5 March	257 437	27 Feb-27 Mar	No clinical signs	Subtype H7N6 (virus isolation)
B	204 358 layer quails	20	4 Mar	13 March	204 358	4 Mar-4 Apr	No clinical signs	Subtype H7N6 (virus isolation)
C	109 018 layer quails	10	10 Mar	29 March	109 018	10 Mar-20 Apr	No clinical signs	Subtype H7N6 (virus isolation)
D	669 207 layer quails	0	18 Mar	19 Apr	669 207	18 Mar-11 May	No clinical signs	Subtype H7 (antibody detection)
E	134 956 layer quails	0	29 Mar	18 Apr	134 956	29 Mar-11 May	No clinical signs	Subtype H7 (antibody detection)
F	129 006 layer quails	0	29 Mar	14 Apr	129 006	29 Mar-11 May	No clinical signs	Subtype H7 (antibody detection)
G	91 595 layer quails	0	29 Mar	19 Apr	91 595	29 Mar-11 May	No clinical signs	Subtype H7 (antibody detection)

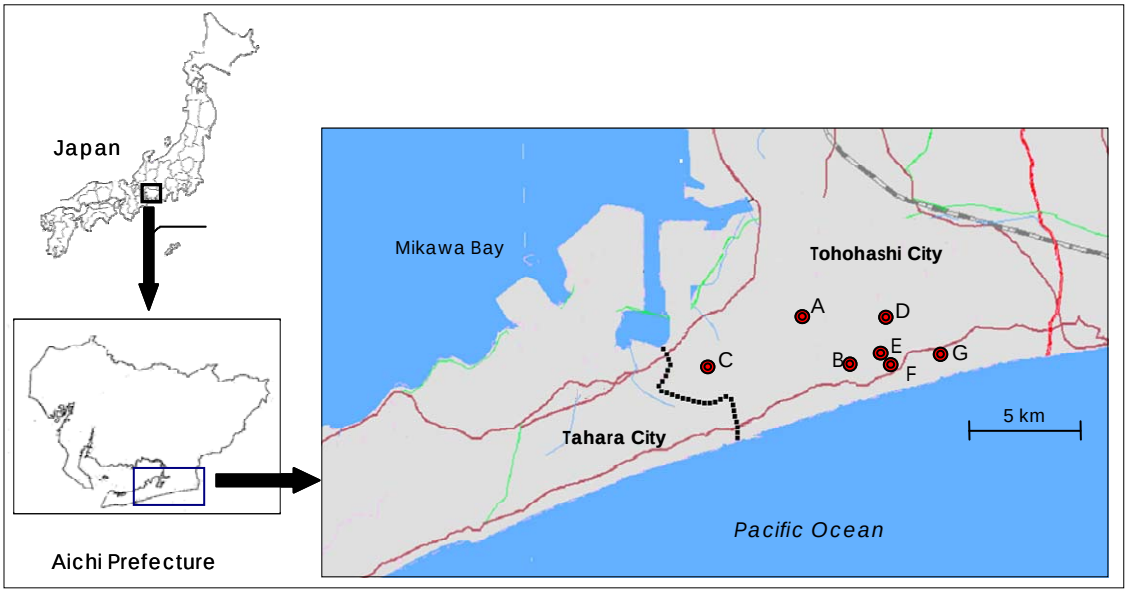

Figure 2
Location of the avian influenza-infected quail farms in 2009
The red lines indicate national highways
The striped line is a Shinkansen railroad

using the conventional neuraminidase inhibition (NI) assay. The isolated virus was identified to be of low pathogenicity based on the hemagglutinin 1 (HA1) and HA2 cleavage site amino acid sequence on 27 February. The infective allantoic fluid was inoculated to eight 7-week old chicks, in accordance with OIE standard procedures (9); none of these chicks died 10 days after inoculation. The putative HA cleavage site amino acid sequence of the isolated virus suggested that the virus had mutated from the low pathogenic H7 strains isolated from wild birds.

Farm B

On 1 March 2009, a RT-PCR test reacted positive on tracheal swab samples taken from another quail farm (Farm B) during an emergency surveillance of all poultry farms located within a 5-km radius around Farm A. On 3 March, a virus with haemagglutination activity was isolated from a tracheal swab sample and sent to the NIAH for subtype identification. On 4 March, the isolated virus was identified to be subtype H7. On 6 March, the virus was confirmed as N6.

Farm C

On 9 March, a virus with haemagglutination activity was isolated from a tracheal swab sample taken on 7 March from Farm C located within a radius of 5 km around Farm A. The isolated virus was sent to the NIAH for subtype identification. On 10 March, the virus was identified as H7. On 11 March the virus was confirmed as N6.

Farm D

On 17 March, an antibody against an AI virus type A was detected as a result of an AGID test conducted by a prefecture LHSC using serum samples taken from another quail farm (Farm D) located within a 5-km radius around Farm A. The antibody was identified to be subtype H7 on 18 March using an HI test conducted by the NIAH. Virus isolation from tracheal and cloacal swab samples taken from Farm D was attempted but no AI virus was isolated.

Farms E, F and G

On 28 March, an antibody against an AI virus type A was detected after an AGID test was conducted by a prefecture LHSC on three other quail farms (Farms E, F and G). Farms E and F were located within a 5-km radius around Farm A. Farm G was located within a 5-km radius around Farm B. The antibody was identified to be subtype H7 on 29 March, following an HI test conducted by the NIAH.

Virus isolation from samples taken from these three farms was attempted but failed.

Measures taken

Depopulation of infected farms

After diagnosis of AI, all quails kept on the seven infected farms were destroyed. Depopulation was followed by cleaning and disinfection of the quail houses. Depopulation and disinfection of Farms A, B and C were completed on 5 March, 13 March and 29 March, respectively. Depopulation and disinfection of Farms D, E, F and G were completed on 19 April, 18 April 14 April and 19 April, respectively. All quails on Farms A and B were humanely euthanised and buried on site. All quails on Farms C to G were incinerated. A total of 1 595 577 quails were destroyed.

Movement controls

Farm A was voluntarily quarantined on 25 February 2009 when the presence of AI virus was suspected. On 27 March, a movement control area, with a radius of 10 km, was established around Farm A. The radius was immediately reduced to 5 km as the virus involved was found to be of low pathogenicity based on the HA1 and HA2 cleavage site amino acid sequence. In the movement control area, the movement of poultry and all goods that had the potential to spread the disease was prohibited. Movement controls were lifted on 27 March, after 21 days had elapsed after the completion of cleaning and disinfection.

Likewise, a movement control area with a 5-km radius was established around Farms B to G from the day of diagnosis until 21 days had elapsed after the completion of cleaning and disinfection.

In the movement control areas around Farms D, E, F and G, which were only serologically positive, only the movement of live and dead quails and other quail-related goods that had the potential to spread the disease was prohibited.

There were some exceptions to the movement controls, as follows:

- shipment of poultry eggs to grading and packaging plants (GP plants) was allowed on condition that the farm of origin was free from AI virus based on clinical inspection, PCR test and virus isolation, and that the eggs were transported without going near poultry farms
- shipment of manure to compost plants was allowed on condition that the farm of origin was free from AI virus based on clinical inspection, PCR test and virus isolation, and that the manure was transported without going near poultry farms
- shipment of poultry other than quails to processing plants outside the movement control area was allowed on condition that the farm of origin was free from AI virus based on clinical inspection, AGID test, PCR test and virus isolation, and that the poultry were transported without going near poultry farms
- movement of chicks, other than quail chicks, from a farm outside the movement control area to a chicken farm within the movement control area was allowed on condition that the farm of origin and the receiving farm were both free from AI virus based on clinical inspection, AGID test, PCR test and virus isolation.

Surveillance in movement control areas

All poultry farms in movement control areas were visited by prefecture veterinary inspectors for clinical surveillance. All 24 poultry farms (8 quail farms, 10 layer chicken farms, 4 broiler chicken farms and 2 Aigamo duck (mallard and domestic duck cross) farms in the movement control area around Farm A were subjected to sampling for serological examination and virus isolation; 5 quail farms (Farms B to F) were serologically positive. All 9 poultry farms (1 quail farm, 4 layer chicken farms, 2 broiler chicken farms and 2 Aigamo duck farms) in the movement control area around Farm B were also subjected to sampling for serological examination and virus isolation; 1 quail farm (Farm G) was serologically positive. There

were 2 farms (layer chicken farms) and 5 farms (2 quail farms and 3 layer chicken farms) in the movement control areas around Farms C and D, respectively. No infected farms were detected in the movement control areas around Farms C to G.

The poultry farms in the movement control areas were subjected to sampling for serological examination and virus isolation for a second time, after the completion of cleaning and disinfection of the infected farms and after an interval of 10 days from the first sampling; no farm was serologically or virologically positive.

After the last movement control was removed on 11 May, all poultry farms in the movement control areas were placed under surveillance for three months, as follows: 34 farms located in the movement control areas and 9 epidemiologically related farms were subjected to clinical inspection, serological examination and virus isolation; all farms were free from the presence of clinical signs, antibodies or AI virus.

Nationwide surveillance

Considering the fact that the virus involved was of low pathogenicity, causing only subclinical infection and that the quail subpopulation was highly compartmentalised, all quail farms in Japan (a total of 173 quail farms in 38 prefectures, including those that were keeping quails as companion or laboratory animals) were visited by veterinary inspectors in their respective prefectures and subjected to clinical and serological examination and virus isolation by 28 March 2009; no farm was found to be infected with AI virus subtype H7 (5). The result of this nationwide surveillance indicated that the infection was confined to the quail farms in Toyohashi City in Aichi Prefecture.

Verification of virus freedom using sentinel quails

After the initial completion of cleaning and disinfection that followed the destruction of susceptible birds, the infected farms were disinfected on two additional occasions. Between 27 April and 14 May 2009, swab samples were taken from the floors, walls and ceilings of the quail houses of the infected farms and subjected to virus isolation; no virus was isolated. Between 12 May and 19 May, sentinel quails were placed on the infected farms before restocking; the sentinel quails were subjected to clinical, serological and viral examinations between 26 May and 23 June; all sentinel quails were free of the presence of clinical signs, antibodies against AI or AI virus (Fig. 3).

Source of infection

An epidemiological study team formed by the MAFF visited the infected premises four times between 7 March and 22 April 2009. The NIAH conducted a phylogenetic analysis of the virus strains isolated from the infected farms. The NIAH, Hokkaido University and Tottori University examined the susceptibility of chickens, mouse and pigs to the isolated virus. To date, it has been found that:

- no virus has been isolated recently in the rest of the world that is genetically similar to any of the virus strains isolated from the infected quail farms (Fig. 4)
- the similarity of the HA gene between the three strains isolated from the infected quail farms was approximately 96%
- the infected farms were epidemiologically linked in terms of the movement of people and farm supplies.

Based on these facts, the preliminary conclusions of the the epidemiological study team was that the virus was most likely to have been introduced into the infected area in the Aichi Prefecture several years ago through people, farm supplies or wild animals, spread among farms by the same route and it continued to exist on these farms (4).

Economic losses and consequences

Due to the nature of the disease (no clinical signs, no mortality), there were no direct economic losses arising from the outbreak but there were indirect consequences arising from control and eradication measures taken during

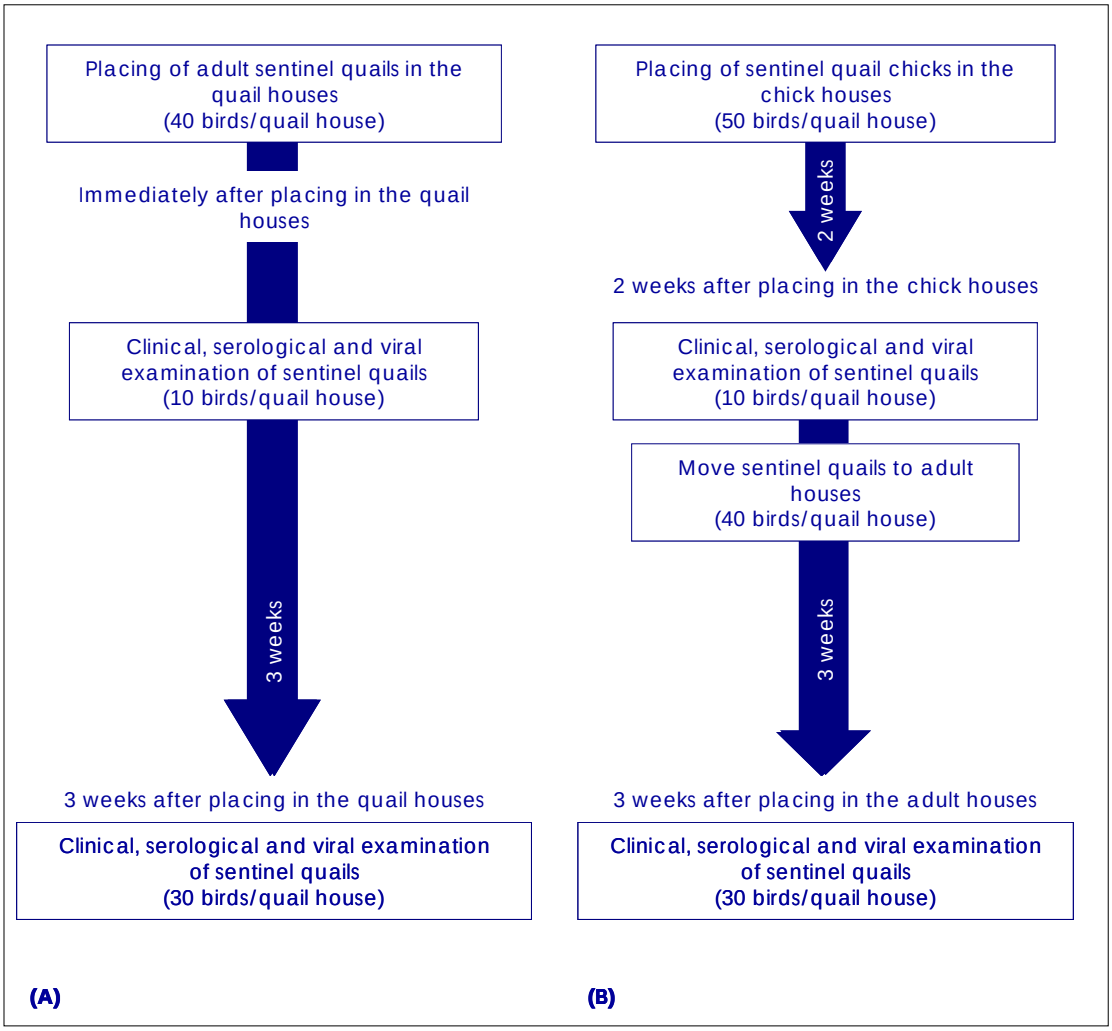

Figure 3
Virus freedom verification programmes using sentinel quails
The infected farms were restocked after the absence of AI virus was verified using these programmes
Program A applied to Farm A, where only adult quails were raised
Programme B applied to Farms B to G, where both chick and adult quails were raised
These programmes were applied to the infected farms after the completion of depopulation, cleaning and disinfection, and viral examination of the environment (floors, walls and ceilings of quail houses)

the outbreak and their effect on supply and demand for quail products.

Compensation to farmers for the destruction of quails on the seven infected farms totalled 397 million yen (US$4.43 million). Cleaning and disinfection of infected premises cost 129 million yen (US$1.44 million). In addition, 113 million yen (US$1.26 million) was paid to farmers located in the movement control areas to compensate them for their losses.

As a result of the eradication campaign, 27% of the quails in Japan were destroyed. A total of 235 million yen (US$2.72 million) of financial support was provided to the owners of the infected farms to help them swiftly restock their quails and resume their acitivity. To minimise the adverse effect on the consumption of quail products, efforts were made by the Food Safety Commission and other agencies to provide information to consumers stating that AI was not a foodborne disease (1). During the course of the outbreak, to keep the public informed of the disease situation, the Aichi Prefectural government issued a total of 103 press releases, updating the situation on the spread of the disease, the results of surveillance and the number of birds depopulated. In addition, local MAFF officials visited a total of 52 823 supermarkets and other retailers to check if there was improper

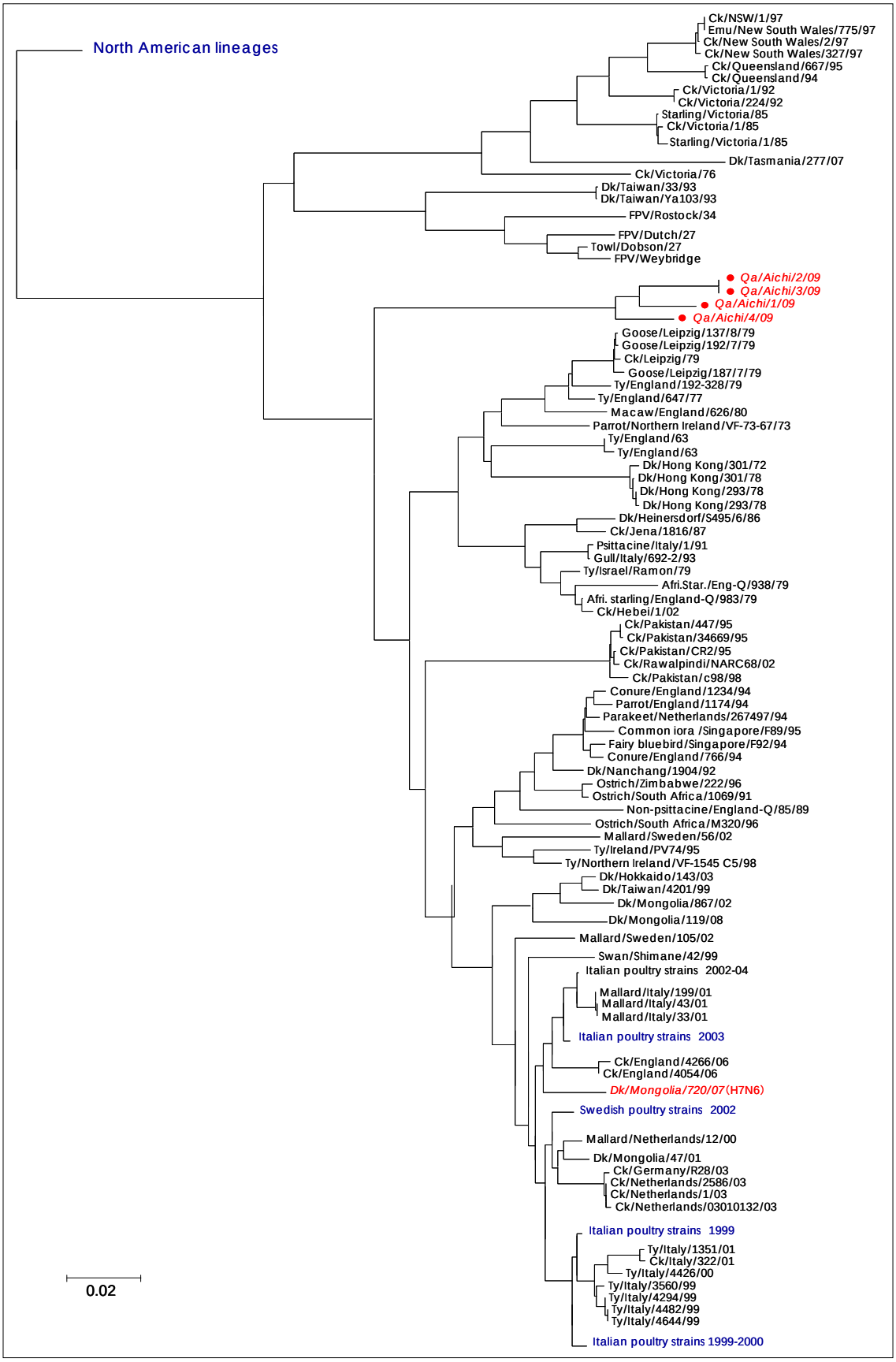

Figure 4
A phylogenetic tree created using the neighbour joining method based on hemagglutinin gene base sequence

labelling of quail products; 309 retailers (0.6%) had quail products improperly labelled, but this was corrected immediately. As a result of these efforts, there was not much decline in demand for quail products but there was a shortage of supply, leading to some hikes in the prices of quail products for several months.

Discussion and conclusion

The outbreak of AI in quails in early 2009 was successfully eradicated in a relatively short period of time. Japan declared its freedom from notifiable AI on 19 July 2009, according to Article 10.4.3 of the OIE *Terrestrial Animal Health Code* (10).

After the outbreak of LPNAI in 2005 and following the outbreak of AI in Korea in 2007 and the isolation of AI virus from whooper swans in northern Japan in early 2008 (8), AI surveillance had been enhanced to include small-scale farms and duck, quail, turkey, pheasant, ostrich and helmeted guinea-fowl farms. The detection of an AI-infected quail farm in February 2009 indicates that this enhanced surveillance was effective in detecting even a small number of infected farms.

The NAI eradication guidelines had been amended based on the experience of the LPNAI outbreak in 2005 (3). However, while implementing the measures following the detection of AI on a quail farm, it turned out that the virus involved was of low pathogenicity and the virus was only circulating on quail farms. Not only the minimum radius of 5 km was applied in establishing movement control areas, but also other deviations from the guidelines were made, including exemptions from movement controls. These flexible decisions enabled the eradication measures to be taken with little disruption to economic and social activities.

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