For discussion
on 10 March 2009

LegCo Panel on Food Safety and Environmental Hygiene

Epidemiology Report of the Highly Pathogenic Avian Influenza H5N1 Outbreak in December 2008 in a Chicken Farm in Ha Tsuen, New Territories

Purpose

This paper briefs Members on the "Epidemiology Report of the Highly Pathogenic Avian Influenza H5N1 Outbreak in December 2008 in a Chicken Farm in Ha Tsuen, New Territories (the Report)" released on 5 March 2009.

Detection of avian influenza virus in a local farm in December 2008

2. On 9 December 2008, a local chicken farm located at Ha Tsuen, Yuen Long (the index farm) was declared as an infected place after dead chickens found in the farm were tested positive of avian influenza. A series of response actions were taken immediately to prevent the spread of virus including the culling operations in the index farm, another chicken farm located within three-kilometre radius of the index farm and Cheung Sha Wan Temporary Poultry Wholesale Market, suspension of local and imported live poultry including day-old chicks and pet birds supply for 21 days, thorough cleansing and disinfection in retail markets, etc. To understand the cause of outbreak and better prepare our farms against the risks of any possible avian influenza outbreak in future, the Administration set up two investigation groups responsible for conducting an epidemiological study of the avian flu outbreak on the index farm and a vaccine study respectively on 12 December 2008. The vaccine study is still underway and is expected to be completed by end 2009.

The Investigation Group on Epidemiological Study

3. The convenor of the Investigation Group on Epidemiological

Study was the Assistant Director of Agriculture, Fisheries and Conservation (Inspection and Quarantine) and members included experts from the University of Hong Kong and representatives of the Agriculture, Fisheries and Conservation Department (AFCD), Department of Health and Food and Environmental Hygiene Department.

4. The Investigation Group has conducted an in-depth examination of the biosecurity measures of the index farm and attempted to identify the source of virus as well as the channel that the virus could have entered into the farm. The Investigation Group has also reviewed the biosecurity measures of the index farm and other local farms and recommended remedial and improvement measures so as to assist the farmers in enhancing their vigilance against avian influenza outbreak. The Investigation Group submitted the Report to the Secretary for Food and Health on 5 March 2009, which has also been uploaded onto the website of AFCD for public viewing on the same day. The full report, including an executive summary is at Annex A.

Way forward

5. AFCD has taken on board the recommendations of the Report and will facilitate the implementation of the enhanced biosecurity measures in the index and other local farms.

Food and Health Bureau
March 2009

Annex A

Epidemiology Report of the Highly Pathogenic Avian Influenza H5N1 Outbreak in December 2008 in a Chicken Farm in Ha Tsuen, New Territories

Investigation Group on Epidemiological Study

(March 2009)

Epidemiology Report of the Highly Pathogenic Avian Influenza H5N1 Outbreak in December 2008 in a Chicken Farm in Ha Tsuen, New Territories

EXECUTIVE SUMMARY

Following a report of a suspected notifiable disease in chickens on 8 December 2008, highly pathogenic avian influenza (HPAI) caused by a virus of the H5N1 subtype was confirmed on 11 December 2008 in a chicken farm in Ha Tsuen, New Territories (the index farm). The Government immediately raised the alert level for avian influenza (AI) to Serious Response Level and took a series of actions in a bid to prevent the spread of the virus in Hong Kong, including the culling operations and temporary suspension of imported and local poultry supply. In addition, the Food and Health Bureau announced on 12 December 2008 the setting up of two investigation groups (one on epidemiological study and the other on vaccine study) related to this outbreak. This is the report of the Investigation Group on Epidemiological Study (the Investigation Group).

2. The investigation was conducted against the background that there had been no HPAI outbreak on farm since 2003 until this one, whilst Hong Kong is located in a region where HPAI is endemic.

3. The Investigation Group noted that many measures to prevent infection in commercial poultry have been introduced since the last virus detection in poultry retail markets in June 2008, including the reduction in the number of farms, poultry wholesalers and retail outlets, and no overnight keeping of live poultry in remaining retail outlets would have substantially reduced the risk of persistent infection in the markets and therefore the potential for subsequent spread of infection back to farms through contaminated material or personnel.

4. The Investigation Group also noted that HPAI viruses of the H5N1 subtype have been isolated every year in Hong Kong since 1997 (apart from 1998) either from commercial poultry and poultry retail markets (1997, 1999-2003, June 2008) or from dead wild birds (since 2002). The most recently detected case of a dead wild bird found with an Asian-lineage H5N1 HPAI virus prior to the December 2008 outbreak was on 16 October 2008 but this virus belonged to a clade (2.3.2) which was different to the one found on the index farm.

5. The outbreak was confined to this single farm. The viruses were only found in samples from chickens from two of the nine sheds (i.e. shed nos. 9 and 17) which housed chickens in the farm at the time of the outbreak, and there was no widespread exposure of all the poultry on site. Investigations also found no evidence of infection on any other farms, indicating no spread of infection to any other premises.

6. Genetic analyses of the virus isolates from the chickens show that they belong to Clade 2.3.4, a clade that is currently circulating among poultry in southern China and northern Vietnam and detected in Hong Kong previously. The virus was most closely related to and shared a common ancestry with isolates detected in markets in Hong Kong in June 2008 and from a peregrine falcon in March 2008. All of these share the same constellation of genes (i.e. they belong to the same genotype). The hemagglutinin (HA) gene of the farm isolates shared approximately 97 percent similarity with these virus strains.

7. As with all other Asian-lineage H5N1 viruses detected since 1997 the current virus was considered to be a highly pathogenic (HP) strain when introduced to the farm (i.e. the disease was not the result of introduction of a low pathogenicity strain and subsequent conversion to a HP strain on the farm). The genes of the virus are all of avian origin. The majority of dead and infected poultry on 8 and 9 December 2008 were unvaccinated sentinels and a significant number of poultry in the ageing breeder flock in shed 17 had low H5 antibody titers.

8. For HPAI to occur on a farm, the virus must have entered the farm through some means and subsequently infected the poultry there. The Investigation Group therefore first studied the possible sources of the virus and then went on to consider how the virus, once introduced onto the farm, could have entered the poultry sheds and spread among the poultry. As with many epidemiological studies of this nature, it is extremely difficult to pinpoint the exact cause of the outbreak and the precise course of events. While no biosecurity measures can be so strong as to be completely foolproof against the introduction of the virus whether in Hong Kong or elsewhere, the adoption of reasonably practicable biosecurity measures and vigilance should go a long way towards containing the risks of the introduction or spread of the virus. The Investigation Group therefore also looked carefully into the biosecurity situation of the index farm. The investigation revealed some biosecurity vulnerabilities and breaches on the farm, including issues with

wild bird protection nets, presence of sites that are potentially attractive to wild birds and non-compliance by staff with biosecurity measures (including hand/glove hygiene) relating to entry into the chicken sheds.

9. Some fifteen events were recorded between 29 November and 6 December 2008 involving the introduction of people or other items from outside the farm onto the farm premises. All represent low level risks and would require the person or item introduced to be contaminated with the virus before entry and would only result in the introduction of the virus if the biosecurity measures at the farm gate were not implemented. Without independent observation of procedures implemented at the time, it is not possible to prove or disprove whether biosecurity breaches occurred during these introductions. In addition, wild birds were detected on the farm premises and within the poultry sheds, despite the use of bird protection nets. This points to the need to further improve protection against wild birds.

10. Samples were collected for virus detection/isolation on the farm from dogs and cats on the premises, from the farm environment, from wild bird droppings and from feed used on the farm, all with negative results. However, due to the limits associated with such testing, negative results do not rule out the presence of infection at the time of the outbreak or it may have been present at a level below the limits of detection when the samples were collected.

11. A few of the possible pathways of virus introduction onto the farm could be largely excluded as a result of the investigation, including introduction by contaminated fertile eggs (based on the evidence that suggested breeders on the farm were producing more than enough eggs to meet demand) and introduction by drinking water (as the water used for this purpose is reticulated town water).

12. The Investigation Group's conclusions are as follows:

(1) Introduction of virus into farm

(i) The virus could have been introduced to the farm by droppings of infected wild birds given the presence of aquatic birds and other birds on and around the farm and the presence of trees on the farm and uncovered soak away pits that are attractive to birds, including large aquatic species (herons and egrets). Part of the

aisle of the infected shed no. 17, although covered by bird protection nets, was under some branches of the trees near its entrance. This design of the shed could have allowed the droppings of wild birds to contaminate the exposed area inside the shed.

(ii) The strong winds and gust from the north and north-east from 4 to 6 December 2008 could have deposited potentially contaminated dust and leaves from the trees into the nearby shed no. 17 via its north opening. These contaminated materials could then have gathered at the corner of the shed where the initial high mortality in poultry occurred.

(iii) Introduction of the virus from outside the farm through contaminated fomites or people is considered less likely.

(iv) Introduction of the virus from drinking water can be ruled out as all chickens are supplied with reticulated water.

(2) Spread of virus on the farm

(i) Once the virus gained entry to the farm from outside, a number of possible routes of introduction of virus to the infected sheds could not be ruled out.

(ii) The farm owners and the six farm workers are likely to have contributed to the spread of virus on the farm via contaminated hands/gloves and/or clothing. The Investigation Group considers that inadequate glove/hand hygiene could have been an important means of spread of infection on the farm.

(iii) Small wild birds (e.g. sparrows) and rodents were both found in poultry sheds and could have acted as short term carriers of the virus and/or mechanical transfer agents of contaminated material into and between sheds.

(iv) While the introduction of the virus to poultry sheds via contaminated feed or wandering cats could not be ruled out entirely, it was an unlikely means of spread on the farm,

(3) Time of introduction

The virus was probably introduced to the farm sometime between 29 November and 6 December 2008.

(4) Illegal activities

There is no evidence that the infection was introduced via illegally imported poultry or poultry products or any activities associated with such imports.

(5) Sentinels

The purpose of having unvaccinated sentinel chickens in a flock is to allow early detection of disease. However, the concentration of susceptible unvaccinated sentinel chickens near the entrances to the infected sheds in this farm would have resulted in significant amplification of the virus once it was introduced and infection occurred in these birds.

13. Taking into account the findings of the investigation, as well as the location and configuration of structures on the index farm, the Investigation Group recommends the following measures for improving the biosecurity on that farm:

(1) Modify the structure of shed no. 17 so that the shed area is fully covered by its roof and not directly exposed to the tree and add a solid partition on the side facing north to protect against wind gusts.

(2) Cover all uncovered soak away pits and wells to avoid gathering of aquatic birds on farm.

(3) Ensure that the bird protection facilities on farm are sufficient to prevent small birds from entering the farm sheds, e.g., instead of woven meshes which may easily degrade, metal meshes may be used.

(4) Implement the other enhancement measures recommended for all chicken farms in paragraph 14 below.

14. The Investigation Group also recommends the following improvement measures in respect of all chicken farms to strengthen the prevention of avian influenza infection -

(1) Further tighten up biosecurity

(i) Each farm to develop a tailor-made biosecurity plan (covering such issues as bird protection, rodent control and farm management practices) in consultation with AFCD, taking into account the circumstances of the farm;

(ii) Each farm to keep records related to chicken movements, vaccinations, medications and mortalities according to the format specified by AFCD.

(iii) Each farm to provide hand washing / cleaning facilities at designated locations inside the production area for use by anyone prior to entering the poultry sheds;

(iv) AFCD to facilitate compliance by conducting refresher courses periodically for farmers, farm workers and other allied workers on the concepts and practices of biosecurity, for example, the proper use of gloves, and devising templates on record keeping and vaccination;

(2) Facilitate early detection

(i) AFCD to increase the inspection frequency of poultry farms from once every week to once every 5 days with stepped up veterinarian audit;

(ii) AFCD to increase the sampling size of the blood test for vaccinated chickens for enhancing the precision of flock immunity estimates;

(3) Prevent virus spread on introduction

(i) AFCD to require the further scattering of sentinel chickens among the vaccinated flock.

(ii) AFCD to consider, in consultation with the farmers, how best to segregate the operations relating to the rearing of breeder flocks and broiler flocks to minimize the risk of spread of infection.

1. Introduction

1.1 An outbreak of a disease in domestic fowl on a farm in Ha Tsuen was reported to the Agriculture, Fisheries and Conservation Department (AFCD) on 8 December 2008.

1.2 This outbreak was caused by a highly pathogenic avian influenza (HPAI) virus of the H5N1 subtype. It was the first such outbreak reported on a local farm since 2003 despite the many enhancements to the control and prevention of AI in Hong Kong in the past few years, including farm biosecurity measures, reduction in the number of poultry and poultry farms in Hong Kong, reduction in the number of market stalls and traders, and the implementation of "no overnight stocking of poultry" in retail markets.

1.3 To assist in preventing further outbreaks in the future, the Food and Health Bureau set up two Investigation Groups to undertake a detailed investigation to ascertain the most plausible routes for virus introduction in this case and to review the efficacy of the current vaccine respectively. The terms of reference of this Investigation Group on Epidemiological Study are at Annex 1. The Investigation Group comprises experts from the University of Hong Kong, representatives of AFCD, Department of Health, Department of Food and Environmental Hygiene, and the Food and Health Bureau. The membership list is at Annex 2. The Investigation Group on Vaccine Study will separately report on the suitability of the current vaccine in due course. Thus, aspects relating to vaccination and immunity relevant to the index farm are examined and discussed only briefly in this report.

Annex 1

Annex 2

1.4 The index farm and the affected sheds were inspected by members of the Investigation Group. In addition, other veterinary professionals and field officers and an in-house ornithologist of AFCD inspected the farm on different occasions and collected information to facilitate the Investigation Group's consideration.

1.5 The investigations comprised

a. Current regulatory regime on poultry farms

b. Details of the outbreak

c. Detailed examination of the index farm and affected sheds

d. Detailed review of the index farm's inspection records

e. Interviews with the workers on the index farm

f. Ornithological analysis of wild bird activity on and around the index farm

g. Analysis of the HPAI virus recovered from the index farm and comparison with viruses of the same subtype identified from previous domestic and wild bird AI cases.

h. Inspection of and tests on samples taken from all other local poultry farms

i. Review of recent surveillance data

1.6 Each of these aspects is described in detail below along with the significance of the findings followed by a discussion on the findings and conclusions that can be drawn.

2. Current regulatory regime on poultry farms

2.1 The Investigation Group has reviewed the current regulatory regime for monitoring farm hygiene and preventing disease outbreaks in local poultry farms.

2.2 In Hong Kong, only licensed farms may raise live poultry. An integral part of the licence conditions is the need for appropriate biosecurity measures to reduce the risk of AI infection. Examples of the specified biosecurity measures include the installation of bird protection nets for chicken sheds and disinfection facilities for incoming personnel and vehicles. It is also a requirement that the chickens be tested for AI H5 antibody before being sold. The conditions also specify the trigger points for reporting abnormal mortalities to AFCD. AFCD runs a computer system to record the number and movement of the chickens on each poultry farm. The introduction of day-old chickens and sale of marketable poultry are updated on a daily basis under the current system. Among other things, this reduces the incentives for smuggling chickens into the farms. The system also contains records of sample collection and the blood test results of each batch of chickens to ensure that overall they have a satisfactory H5 titer level before dispatch to the market.

2.3 All poultry farms are inspected at least once every 7 days. During these inspections, the farm's biosecurity measures, including bird protection, disinfectant pools and footbaths, as well as the health of the chickens are checked. Any dead chickens found during the visit would be sent to the AFCD veterinary laboratory for examination and testing. These complement the requirements for reporting abnormal mortalities to AFCD (see paragraph 2.2 above).

2.4 All farmers are requested to vaccinate their chickens with a vaccine containing an oil emulsion adjuvant and a killed H5N2 antigen (Intervet ®) at 9 to 11 days of age and with a booster four weeks later. From time to time, AFCD staff visit the farms to collect blood samples of vaccinated chickens for testing the H5 immune response; conduct pre-sale tests if there are chickens for sale; and collect routine environmental swabs for disease monitoring. If things are in order, blood test certificates and poultry transportation authorizations would be issued to the farmer for sale of the chickens during their visits.

2.5 Random blood sample collection is conducted monthly for testing H5 antibody titer for chicken flocks over 120 days of age and another injection would be required if these chickens are found with insufficient H5 antibody titer. AFCD staff monitor the procedure of egg disinfection and also issue movement permits for farms to send their eggs to off-farm hatcheries.

2.6 The on-farm hatchery records are reviewed, egg count conducted regularly and the number of chicken counted monthly. AFCD staff would look for any unusual signs in the cages and count sentinels during the chicken count. They also conduct inspections for potential mosquito breeding sites every month. During the inspections, AFCD staff would take the opportunity to enquire about the general running of the farm, see if there is anything untoward and answer any questions that the farmer and / or workers may have.

2.7 In short, HPAI disease prevention and detection in local poultry farms is achieved through requiring the use of H5 vaccine and monitoring the H5 immune response of vaccinated chickens by conducting routine tests on blood samples, faecal swabs and environmental samples as well as inspecting the bird protection facilities, farm's general condition and the health status of chickens on farm. The requirements introduced over the

years have resulted in improvements in farm practices and biosecurity, and seek to strike a balance between prevention and practicability / enforceability.

3. Outcome of Investigations

3.1 Details of the outbreak

Detection and Confirmation of H5N1

3.1.1 A local chicken farmer on a farm located in Ha Tsuen reported unusual mortality in a breeder shed (shed no. 17 – see Figure 1 and details of the farm described in section 3.2 below) to the AFCD in the morning of 8 December 2008. Some 60 breeders were found dead by the farmer that morning. In response to the report, a team of AFCD staff immediately conducted an inspection at the farm and found other sentinel chickens from two batches in a nearby chicken shed (shed no. 9) were also dead or dying along with smaller numbers of vaccinated poultry (see distribution of dead chickens in Figure 1). The main signs seen were depression, dyspnoea, cyanosis and oedema of the comb and wattle with yellow creamy nasal discharge.

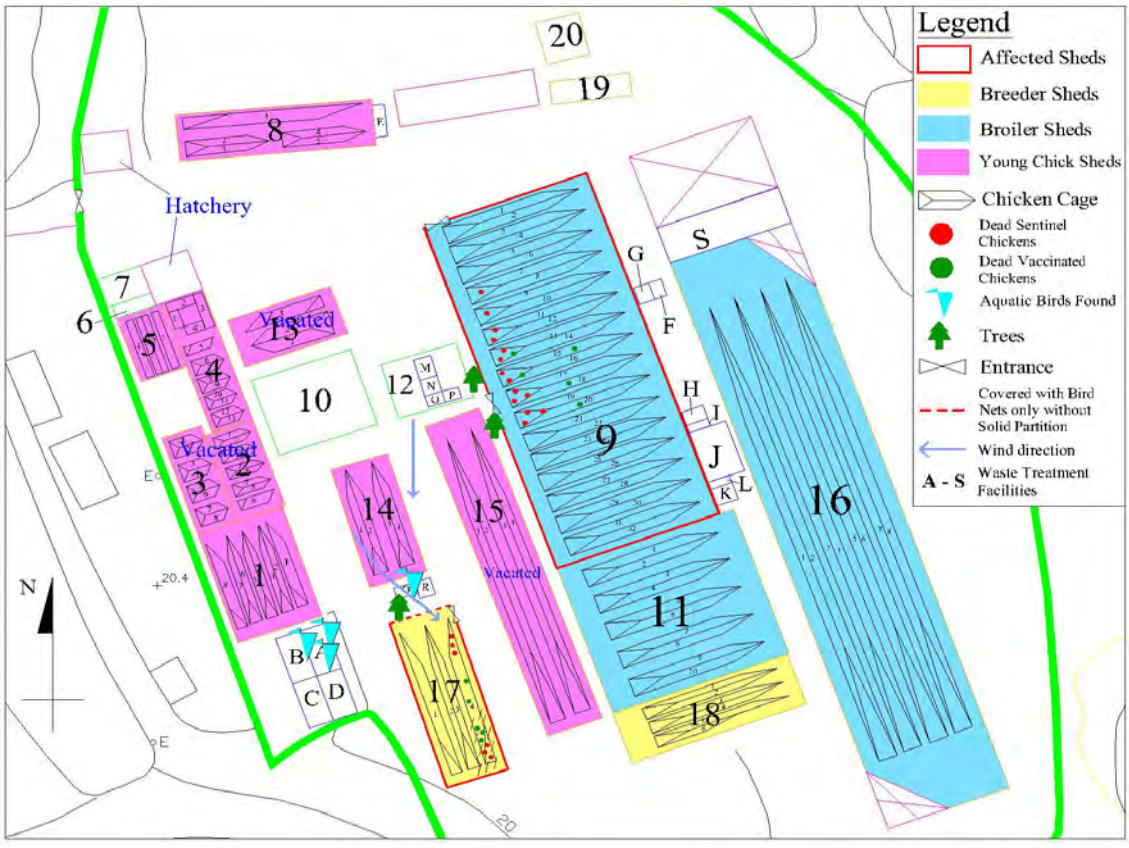

Figure 1 Farm layout and distribution of dead chickens

3.1.2 Three chicken carcasses (1 vaccinated chicken and 2 sentinel chickens) and 120 sentinel chicken faecal samples were collected from the affected sheds of the farm for testing for the avian influenza virus. On 9 December 2008 all pools of samples tested positive for the H5 virus by RRT-PCR test and tissues from the dead birds were positive using immunohistochemistry.

3.1.3 The outbreak was confined to this farm. It appears that the case was identified and reported sufficiently early to prevent spread of infection to other farms.

Culling Operations

3.1.4 A culling operation was performed on the farm in the same afternoon and completed in the late evening of 10 December 2008. A total of 67 683 chickens were culled and 25 856 fertilized eggs were destroyed.

3.1.5 All local farms were ordered to stop dispatching live poultry to the wholesale poultry market starting from 9 December 2008. Importation of live poultry and birds from the Mainland was also suspended from noon, 9 December 2008.

3.1.6 There was one farm present within a 3 km zone around the infected farm and a total of 17 960 chickens on that farm were also culled on 11 December 2008 as a precautionary measure (see Figure 2).

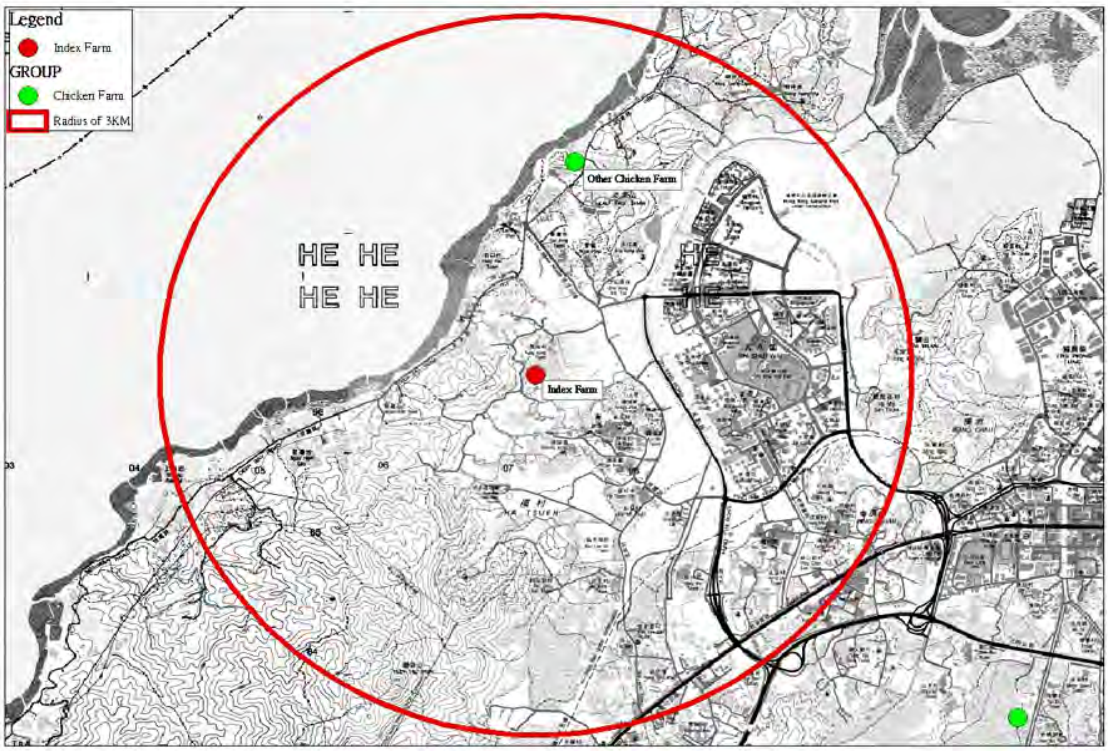

Figure 2 Location of the infected farm and the farm within a 3 km radius

3.1.7 Although no live poultry were sent from the infected farm to the Cheung Sha Wan Temporary Wholesale Poultry Market on 8 and 9 December 2008, some chickens from the other farm within a 3 km radius of the infected farm were found in the wholesale market on 9 December 2008. As a precautionary measure, all live poultry including 10 704 chickens and 7 790 minor poultry in the wholesale market were culled.

Mortality Records

3.1.8 The farm manager reported that no marked increase in mortality was found in either shed 9 or 17 until 6 December 2008 when 5 vaccinated breeder birds were found dead (see Table 1). Although the farm did not have formal written records of mortality (as required in the biosecurity requirement under the licence conditions), the reported mortality was found to be in line with the result of AFCD's chicken count conducted on 9 December 2008 juxtaposed against the results of previous counts. The cumulative mortality rate (number dead of a particular type/total number of birds of that type) for sentinel and vaccinated breeder chickens in shed no. 17 as determined in the afternoon of 9 December 2008 was 83.3% (50/60) and 7.2% (40/548) respectively. The cumulative mortality rate for sentinel and vaccinated broilers in shed 9 was 43% (78/180) and 0.2% (27/10,480) respectively.

Date	2/12	3/12	4/12	5/12	6/12	7/12	8 & 9/12
Shed no. 17	0	0	0	0	5 v	2v	50 s + 40 v
Shed no. 9	0-2v	0-2v	0-2v	0-2v	0-2v	0-2v	78 s + 27 v

Table 1 Daily mortality in affected sheds (v- vaccinated chicken; s – sentinel chicken)

Pre-culling Tests on the Index Farm

3.1.9 To investigate the spread of the virus on the farm and the immune response of the vaccinated chickens, AFCD staff conducted pre-culling sample collection from the chickens on the farm. Altogether 14 blood samples from each batch of chickens aged over 50 days (see Table 2), and 60 oro-pharyngeal and cloacal swabs from each chicken shed were collected on 9 December 2008. Of the total of 360 swab samples

collected from chickens in different sheds other than shed no. 9 and shed no. 17, all tested negative for the H5 virus (see Table 3). The serological findings revealed that the breeder chickens in the affected row of cages in shed no. 17 did not have satisfactory H5 antibody titers. The virological findings suggested that the disease was confined to shed nos. 17 and 9 as of 9 December 2008, before the culling operation commenced.

Case No.	Age (days)	Shed no.	No. of Samples	Geometric mean	No. of samples with HI > 16	% of samples with HI > 16
S-08-6988	214	18	14	74.25	13	93%
S-08-6989	256	18	14	70.66	13	93%
S-08-6987	64	9	14	35.33	10	71%
S-08-6990	57	9,16	14	35.33	10	71%
S-08-6993	78	9	14	35.33	11	79%
S-08-6995	89	9,11	14	35.33	11	79%
S-08-6991	70	9	14	27.58	10	71%
S-08-6994	632	17	14	17.67	8	57%

Table 2 Serological results of vaccinated chickens aged over 50 days (samples taken on 9 December 2008)

Case No.	Location	Vaccinated chicken sample details	H5 PCR	H5 virus isolation
SV-08-3162	Shed no.14	60 cloacal + oro-pharyngeal swabs/12 vials	Neg	Neg
SV-08-3163	Shed no. 11, 18	60 cloacal + oro-pharyngeal swabs/12 vials	Neg	Neg
SV-08-3164	Shed no. 5	60 cloacal + oro-pharyngeal swabs/12 vials	Neg	Neg
SV-08-3165	Shed no. 8	60 cloacal + oro-pharyngeal swabs/12 vials	Neg	Neg
SV-08-3166	Shed no. 1	60 cloacal + oro-pharyngeal swabs/12 vials	Neg	Neg
SV-08-3167	Shed no. 16	60 cloacal + oro-pharyngeal swabs/12 vials	Neg	Neg

Table 3 Samples collected from other batches of poultry on 9 December 2008

3.2 The infected farm and its mode of operation and management

Figure 3 Farm location

Location and rearing capacity of the index farm

3.2.1 The infected farm is located at San Wai, Ha Tsuen which is approximately 1 km from the coastline of Deep Bay and is within 3 km of wetland areas where a wide range of aquatic birds including resident and migratory species congregate (see Figure 3.) Coordinates of the location under WGS84 system are Latitude N 22 27 19.5 and Longitude E 113 59 06.7. The farm is set on agricultural land rented by the farmer since 1994. The whole site occupies an area of approximately 12 000 square meters and comprises 20 licensed structures. The farm has a licensed rearing capacity of 102 000 chickens and was holding 67 683 chickens before the culling operation.

Mode of operation

3.2.2 There were 14 batches of chickens including 11 batches of broilers and 3 batches of breeders on the farm on 8 December 2008 (see Table 4). The farm is managed using a batch-in batch-out system, with chickens sent to the market at 10 to 12 weeks of age.

Origin	Date Introduced	Age (Days)	Number*	Shed No.	(Shed) and Cage No. as in Figure 1
Guangzhou	18-Mar-07	632	610	17	1-4
Zhongshan	28-Mar-08	256	1300	18	1-6
Guangzhou	9-May-08	214	490	18	1-6
Local	11-Sep-08	89	600	11	10
Local	22-Sep-08	78	1400	9	19,20
Local	30-Sep-08	70	5850	11	1-7
Local	6-Oct-08	64	3140	9	16-18
Local	13-Oct-08	57	6020	9, 16	(9)14,15; (16)8
Local	20-Oct-08	50	4780	16	7,8
Local	29-Oct-08	41	7800	9	7-13
Local	10-Nov-08	29	9700	1,9	(1)1-8; (9)1
Local	17-Nov-08	22	7700	8, 14	(8)1,2,3; (14)1-4
Guangzhou	23-Nov-08	16	5000	8	1,4,5
Local	1-Dec-08	8	5100	5	1-4
Local	9-Dec-08	0	8200	Hatchery	

Table 4 Location of the chickens on the index farm as at 9 Dec 2008. (Rounded figures)*

3.2.3 The farm usually has a mix of meat chickens (broilers) and breeders and has a small hatchery attached. Most day old chicks on the farm are hatched locally and chicks hatched at the on-farm hatchery are not sold to other farms. As with many Hong Kong farms, the poultry sheds are located close together and hold poultry of different ages. In the main broiler shed (shed no. 9), multiple batches of poultry are kept. Once the poultry reach market weight they are sent to the market over several days rather than in a single batch (due in part to limits of market demand).

Biosecurity situation of index farm

3.2.4 The farm is fully fenced preventing free access from outside by visitors. The farm has a disinfectant wheel bath for vehicles that enter the farm. The arrangement of the farm is such that the main control points for preventive measures beyond the farm gate are at the entrances to individual sheds where disinfectant is available for boots. Hand washing facilities are available inside the production area, but not at the entrance to individual sheds and workers do not change clothing or gloves when they move from one shed to another, after they enter the production area.

3.2.5 All poultry are reared in cages, including breeders (which are inseminated using artificial insemination). This housing system minimizes the likelihood of virus introduction via footwear coming into contact with poultry.

3.2.6 The feed store is not fully enclosed and farm staff operating in different sections of the farm all used the same feed sheds for collecting feed for the poultry under their charge.

3.2.7 Metal or woven nets to keep out aquatic birds are installed in all chicken sheds; however, sparrows were found in some of the sheds on the day when the Investigation Group visited the farm. The presence of wild birds on the farm, despite the use of bird protection nets, points to the need to further improve protection against wild birds.

3.2.8 The farm owner failed to observe the biosecurity requirement on preparation of disinfectant. Instead of following the manufacturer's instruction to apply disinfectant, he mixed bleach with detergent for filling the disinfectant pool and all foot baths. Quick lime powder was used for ground disinfection from time to time and after vehicles entered the farm.

3.2.9 Buckets of the self-formulated disinfectant mixture for shoe dipping were found placed outside the entrance of each chicken shed. Upon questioning, the farm manager admitted that it was possible that the farm workers did not always dip their boots before entering the chicken sheds.

Vaccination of chickens

3.2.10 The farm did not have full vaccination records. According to the farmer, all chickens are vaccinated with a vaccine containing an oil emulsion adjuvant and a killed H5N2 antigen (Intervet ®) at 9 to 11 days of age and with a booster four weeks later. If broilers are selected as future breeders, the farmer will give them another booster at 150 days of age.

3.2.11 The farmer placed unvaccinated sentinel poultry (60 per flock) in groups at the ends of rows of cages to reduce the danger of inadvertent vaccination (which farmers have complained occurs easily if the sentinels are scattered throughout the shed).

The Affected Sheds

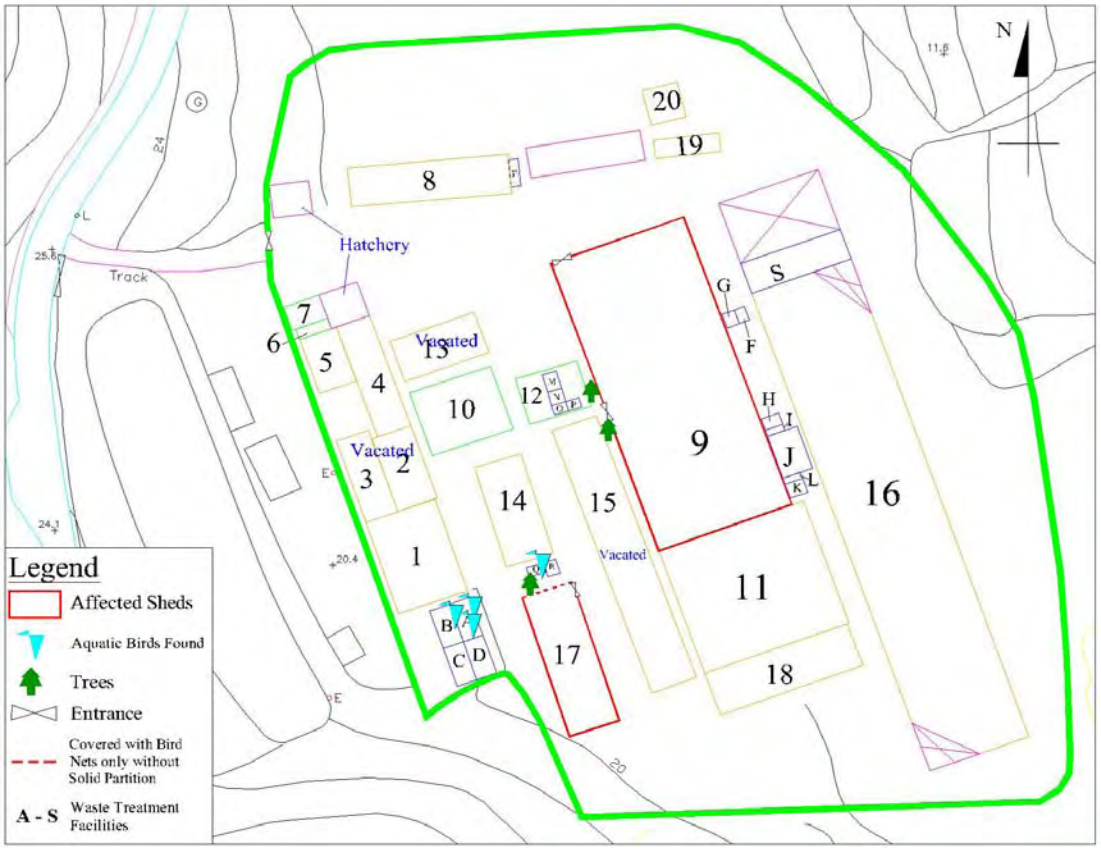

Figure 4 Farm layout and affected chicken sheds, shed nos. 6,7and 10 are feed stores; shed no. 12 is agricultural store.

3.2.12 There are 20 licensed chicken sheds on the farm with 16 chicken sheds, 3 feed stores and 1 agricultural store. Shed nos. 9 and 17 are the infected sheds where mortalities were found. (See figure 4).

3.2.13 Shed nos. 9, 11 and 18 are under the same roof and share the same side entrance on the western side. The structure is close to shed no. 17 (the breeder shed where the unusual mortality was first detected by the owner). There is another shed (shed no. 15) located in between shed no. 9 and shed no. 17; however, shed no. 15 held no chicken on 8 December 2008.

3.2.14 Shed no. 9 is of metal sheet construction outside and is covered with either metal mesh or woven nets as bird protection. The roof of the shed contains thermal insulation material and ventilation is provided by fans. Although the bird protection net was found to be intact, some sparrows were found flying inside the shed during the investigation visits. As reported by the farmer, some small size birds could squeeze through the 2.5 cm X 2.5 cm mesh.

3.2.15 Shed no. 17 was constructed in 2004 and designed for keeping breeders. The shed has a metal roof with three sides covered with a thick plastic sheet leaving a north-facing opening. There are woven nets covering the shed with the opening side hung obliquely from the top of the roof towards the branches of the trees, leaving an “exposed” concreted aisle under the open area. This potentially allows the droppings of wild birds to contaminate the covered pathway inside the shed (e.g. via workers’ shoes). An uncovered soak-away pit which is attractive to wild birds is located next to the trees (see Figure 5).

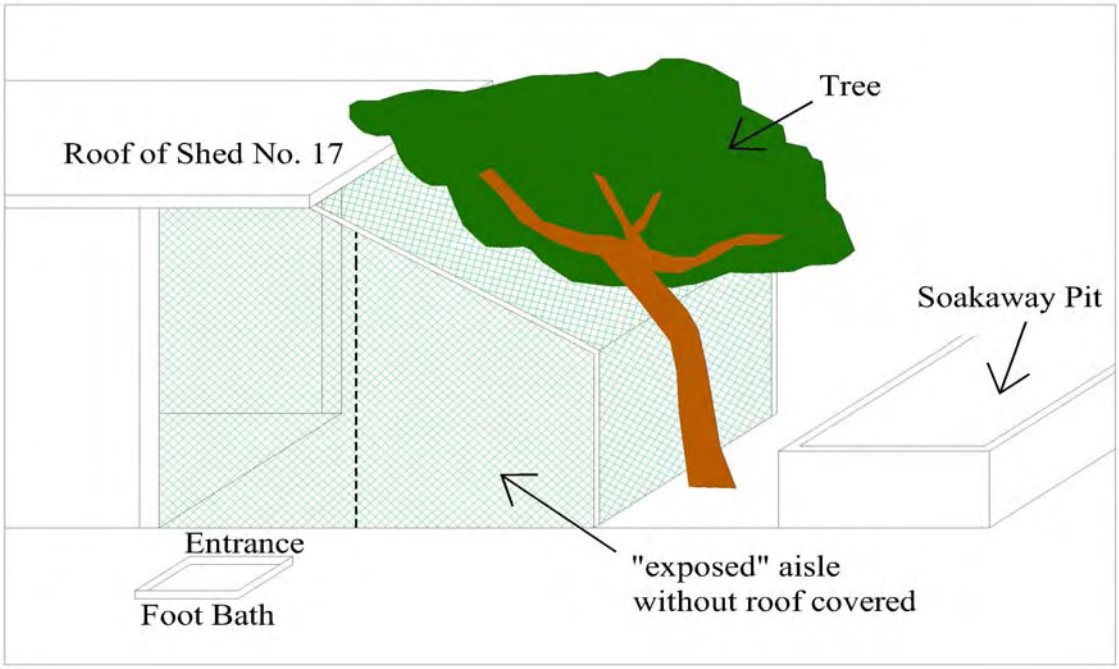

Figure 5 Entrance of shed no. 17

3.3 Farm inspection records

Testing results in the past

3.3.1 The farm was inspected at least once a week. Farm inspection records in the past year revealed some vaccinated broiler chicken flocks with unsatisfactory blood test results which needed to be revaccinated. Overall, the farm did not perform poorly. However, the test results varied from time to time, and were not always consistently satisfactory.

3.3.2 Breeders were bled every month to test for H5 antibody titer and they were last bled on 28 November 2008, before the outbreak. The results of the last test showed that the breeders at 203 days of age and 245 days of age in shed no. 18 as at 28 November 2008 did not have satisfactory H5 titers and required a booster vaccination. The 621-day old breeders in shed no. 17 at that time appeared to have satisfactory results contrasting with the poor results on 9 December 2008 (only 11 days later). (See the blood test results of the infected breeder flock at Annex 3.) The reason for the different results in late November and early December 2008 is not known. One possibility is the sampling variation. Not all the same chickens were tested again on 9 December 2008.

Annex 3

Production of eggs and day old chicks

3.3.3 The farm had 2 498 breeders for fertile egg production as of 8 December 2008. Staff of AFCD have recorded the quantity of egg production periodically (see Table 5). The rate of egg production by the breeder flock, calculated from Table 5 and Table 6 below (taking into account the number of eggs in the incubator and egg storage room plus chicks hatched, and the composition of the breeder flock), ranges from approximately 40% to 50%. This rate of production could be readily achieved by the breeders on the farm. Therefore we found no evidence to support the suggestion that unauthorized eggs from outside the farm were introduced during the two months before the outbreak.

LK	Date of egg count	No. of eggs in hatchery	No. of eggs in egg room	Breeder age <160 days	Breeder aged >160 days*
1114	3-10-2008	25000	5000	500	2240
1114	14-10-2008	22000	8000	500	2240
1114	29-10-2008	22000	4000	0	2740
1114	7-11-2008	22000	10000	0	2500
1114	13-11-2008	19000	4000	0	2500
1114	24-11-2008	21000	5000	0	2500
1114	5-12-2008	21000	5500	0	2500

*Table 5 Egg production record in the 2 months prior to the outbreak. (*rounded figures)*

3.3.4 The majority of day old chickens were produced on farm with only one batch of day old chickens introduced from Guangzhou on 23 November 2008 (See Table 6). This batch of chicks was located at shed no. 8 and appeared clinically healthy prior to the culling operation. Sixty oro-pharyngeal and cloacal swabs were collected from this batch on 9 December 2008 and tested negative for the H5 virus.

Day of introduction	Batch origin	Number of day-old-chicks
6-10-2008	Local	3200
13-10-2008	Local	6200
20-10-2008	Local	4800
29-10-2008	Local	7860
10-11-2008	Local	9700
17-11-2008	Local	7700
23-11-2008	Guangzhou	5000
1-12-2008	Local	5100
9-12-2008	Local	8200

Table 6 On farm day old chicks production in the 2 months prior to the outbreak

3.4 Interviews with the farm owner, workers and bird catcher

3.4.1 All personnel working in the farm were interviewed regarding their daily activities to investigate any potential sources of infection.

According to the interviews, each worker is assigned to work on specific work procedure in specified sheds (see distribution of duties in Figure 6) and follows biosecurity measures that the farmer and farm manager have taught him / her. However, if there are manpower constraints on the farm, workers may need to enter other sheds to support other colleagues with vaccination.

3.4.2 The workers usually stayed on farm even on holidays. During the past few months before the outbreak, they observed no outsiders entering any chicken sheds except the bird catcher on the nights when chickens were sold and AFCD staff conducting farm inspections. They stated that no eggs were introduced to the farm during the month before the outbreak and only one batch of day old chicks from the Mainland was received on 23 November 2008. No unusual mortality had occurred on the farm during the 6 months before the outbreak of HPAI in December 2008. The workers did not know if the farmer had any other kind of business other then raising chickens. One worker had just arrived in Hong Kong from Hunan on 29 November 2008 and another worker advised that she had gone to Shenzhen for shopping on 28 November 2008. Both of them claimed that they did not have any contact with any chicken before they came to or returned to the index farm.

3.4.3 The bird catcher had not visited any poultry farms other than the index farm during the period from 4 to 7 December 2008. She did not notice any sick chicken during her catching of chickens from the farm.

3.5 Ornithological findings

3.5.1 Staff of AFCD conducted a wild bird survey of the farm site. The subject site is typical of farms in a village-type environment (i.e. buildings, trees and shrubs are mixed together) and is surrounded by abandoned agricultural land. Since it is close to the coastline of Deep Bay and within 3 km of the Wetland Park, where migratory birds gather, aquatic bird activities around the index farm are not unexpected.

3.5.2 In fact, aquatic birds were found on the farm during the investigation visits (at a time when there were no poultry on the farm). Two little egrets *(Egretta garzetta)* were found roosting in a tree on top of the entrance of shed no. 17 on 16 December 2008 and five or six Chinese pond herons *(Ardeola bacchus)* were seen seeking food at the uncovered soak-away pits (A, B, C, D as marked in figure 1) on 18 December 2008.

3.5.3 An on site survey of wild birds was conducted by an in-house ornithologist of AFCD on 17 and 18 December 2008. All the individual birds either seen or heard (calling) at the subject site and adjacent area (within about 100m) were recorded. Results of the survey are listed at Table 7.

Common Name	Relative abundance	Hong Kong status	Latest H5N1 records	Water bird
White Wagtail	2	Common winter visitor		
Spotted Dove	12	Common Resident		
Great Tit	7	Common Resident		
Yellow-bellied Prinia	3	Common Resident		
Tree Sparrow	10+	Common Resident		
Chinese Pond Heron*	6	Common Resident	Jan 2005	Yes
Red-whiskered Bulbul	4	Common Resident		
Crested Myna	4	Common Resident	Jan 2006	
Black Kite	1	Common Resident/winter visitor		
Oriental Magpie Robin	2	Common Resident	Mar 2008	
Olived-backed Pipit	1	Winter visitor/passage migrant		
House Swift	3	Resident/spring migrant		
Common Koel	1	Common Resident		

*Table 7 Bird survey results at the index farm and adjacent area on 18 December 2008. *The six Chinese pond herons were found next to the infected shed no. 17*

3.5.4 The survey and one of our investigation visits showed that four species of birds that had been found infected with H5N1 viruses in the past were detected in or around the infected farm. A total of 17 environmental swabs and wild bird fecal samples were collected from the index farm on 17 and 18 December 2008. All samples tested negative for the H5 virus (see Table 8).

Case No.	No. of environmental swab	Source of sample	H5 PCR	H5 virus isolation
D-08-9079	1	Incubator 1 - wall & trays	neg	neg
D-08-9079	1	Hatchery	neg	neg
D-08-9079	1	Egg storage room - tray & floor	neg	neg
D-08-9079	1	Incubator 2 - wall & trays	neg	neg
D-08-9079	1	Shed no. 17 - bird protection net	neg	neg
D-08-9079	1	Shed no. 16 - area with chickens	neg	neg
D-08-9079	1	Shed no. 16 - area without chickens	neg	neg
D-08-9079	1	Bird droppings near water well	neg	neg
D-08-9079	1	Worker resting area	neg	neg
D-08-9079	1	Shed no. 5	neg	neg
SV-08-3254	1	Soak away pit near shed no. 17	neg	neg
D-08-9147	6	Wild bird droppings inside the farm	neg	neg

Table 8 Virological results on environmental swabs collected in the index farm

3.5.5 The presence of wild birds on the farm, despite the use of bird protection nets, points to the need to further improve protection against wild birds.

3.6 Molecular analysis

3.6.1 Genetic studies, involving the sequencing of all eight genes of a cultured virus, were conducted at the University of Hong Kong and by the Department of Health. The results of the genetic sequencing suggest the isolates are most closely related but not identical to the wild bird isolate from a peregrine falcon found dead in March 2008 and viruses isolated from a few poultry retail markets in June 2008. The phylogeny suggests that the recent viruses are not direct descendents of the viruses from poultry retail markets in June 2008 but they do have a common ancestor. These recent viruses (including the current strain) belong to Clade 2.3.4, a Clade that is circulating among poultry in southern China and northern Vietnam. The majority of the passerine isolates from Hong Kong fall within this Clade (2.3.4) although the majority of viruses from dead wild birds in 2008 were Clade 2.3.2 viruses.

3.6.2 Preliminary laboratory testing, based on comparative haemaggutination inhibition (HI) tests using the standard antigen and the new virus as antigen, suggests that the virus differs antigenically from other H5N1 viruses isolated prior to 2008. Similar results were obtained with H5N1 viruses isolated in retail markets in June 2008. The Investigation Group on Vaccine Study will separately look into the related issues in greater detail.

3.7 Testing conducted in other farms in Hong Kong

AFCD called all poultry farmers by phone to inform them of the outbreak immediately after the laboratory results from the index farm samples were available on 9 December 2008. A special AFCD team was deployed to inspect these poultry farms (29 farms in total) and conduct swab sample collection from sentinels of these farms on 9 and 10 December 2008. Chickens on the 29 farms were confirmed clinically healthy. A total of 2 538 swab samples (from 1 530 sentinels and 1 008 vaccinated chickens) were collected from these farms after the outbreak and all tested negative for the H5 virus.

3.8 General surveillance results in the six months prior to the outbreak

Chicken farms

3.8.1 All chicken farms are inspected at least once a week for chicken health inspection, sample collection and checking of bird protection facilities. A total of 8 080 chicken swab samples were collected under routine chicken farm surveillance system since the H5 positive samples were found in retail outlets in June 2008. All tests were negative for the H5 virus before 8 December 2008. Over 19 000 blood samples were also collected from local chickens during the same period to ensure the H5 titer of vaccinated chickens were up to a satisfactory level.

Retail and wholesale markets

3.8.2 Market surveillance at the wholesale and retail markets has not revealed any H5 viruses since viruses were detected in retail stalls in June 2008.

4. Assessment of potential pathways for introduction of infection

4.1 Water

Chickens at the farm are provided with municipal reticulated water for drinking. Well water is used for the cleaning of cages and washing the floor only. Although the well water source was not properly covered during the investigation visit, the cages were not rinsed with well water after being cleaned and disinfected. No H5 virus was found in the three water samples collected from the drinking system, the well and a koi pond at the entrance to the farm.

4.2 Feed

The farmer imported cornmeal from the Mainland and mixed it with formulated feed concentrate (from a local feed company) on farm. All feed is delivered in sealed bags. There was feed delivery to the farm on 1 and 2 December 2008. Trucks for feed delivery pass through the disinfectant pool at the entrance before they can off load their feed to the feed store near the entrance. The feed stores are not fully enclosed. There could therefore be a risk of contamination of feed by wild bird droppings or rodents during the mixing of feed ingredients. Eleven feed

samples were collected from feed troughs in affected sheds and the feed store and no H5 virus was found in the samples.

4.3 Dead chickens

Some mortality is normal in the chicken rearing process. For the index farm, the arrangement is that dead chickens are sealed in plastic bags with disinfectant powder and sent on a daily basis to a carcass collection point using a specific cart in the evening after work. The worker after sending any carcasses to the collection point does not enter any chicken shed until the following day.

4.4 Personnel on the farm

Duties of farm workers

4.4.1 There are eight personnel (farm owner, his wife and six imported workers) working on the farm who are responsible for the day-to-day management of the hatching facilities and the chickens. The workers have been working on the farm for varying periods, with the shortest being just 10 days (arriving on the farm on 29 November 2008) and the longest being five years at the time of the outbreak. Each worker is designated to perform specific work procedures or look after chickens of specific age(s) (See Figure 6). Before the outbreak, Worker A was deployed to take care of the young chickens. Worker B was deployed to take care of the breeder chickens in shed nos. 17 and 18. He usually worked on shed no.18 first before entering shed no. 17. The duties of staff members C and D included taking care of the medium size to marketable size broilers. Staff members E and F were deployed to work on vaccination and to take care of the hatchery and day old chickens. These workers vaccinated the breeders in shed nos. 17 and 18 in the morning of 2 December 2008 before their routine work on hatchery and day old chicks. The farmer's wife acts as the manager of farm and supervises all work performed by the workers.

Workers' observance of biosecurity measures

4.4.2 All workers live on the farm with meals and daily living necessities provided by the farmer. They seldom leave the farm and none of them had any known contact with other chickens outside the farm in the month before the outbreak.

4.4.3 The farm workers are trained in farm biosecurity by the farm manager. They are required to put on a face mask, working clothes, gum boots, cotton gloves then outer rubber gloves before they enter the chicken sheds. AFCD staff have observed that the workers generally did so on their inspection visits before the outbreak. If the workers have left the farm, they would shower and change their clothes before entering any chicken shed. The farm manager claims that workers are paired up to work on poultry waste collection in the chicken shed, with one collecting the waste and the other transporting it to the waste collection point. However, one of the workers has revealed that he handled the work by himself including collecting poultry waste into waste bins and transporting these waste bins to the waste collection point. It is possible that the disease may have spread from one shed to the other by contaminated gloves or clothing if this worker failed to properly clean and disinfect his outer gloves or change his clothing before entering other sheds.

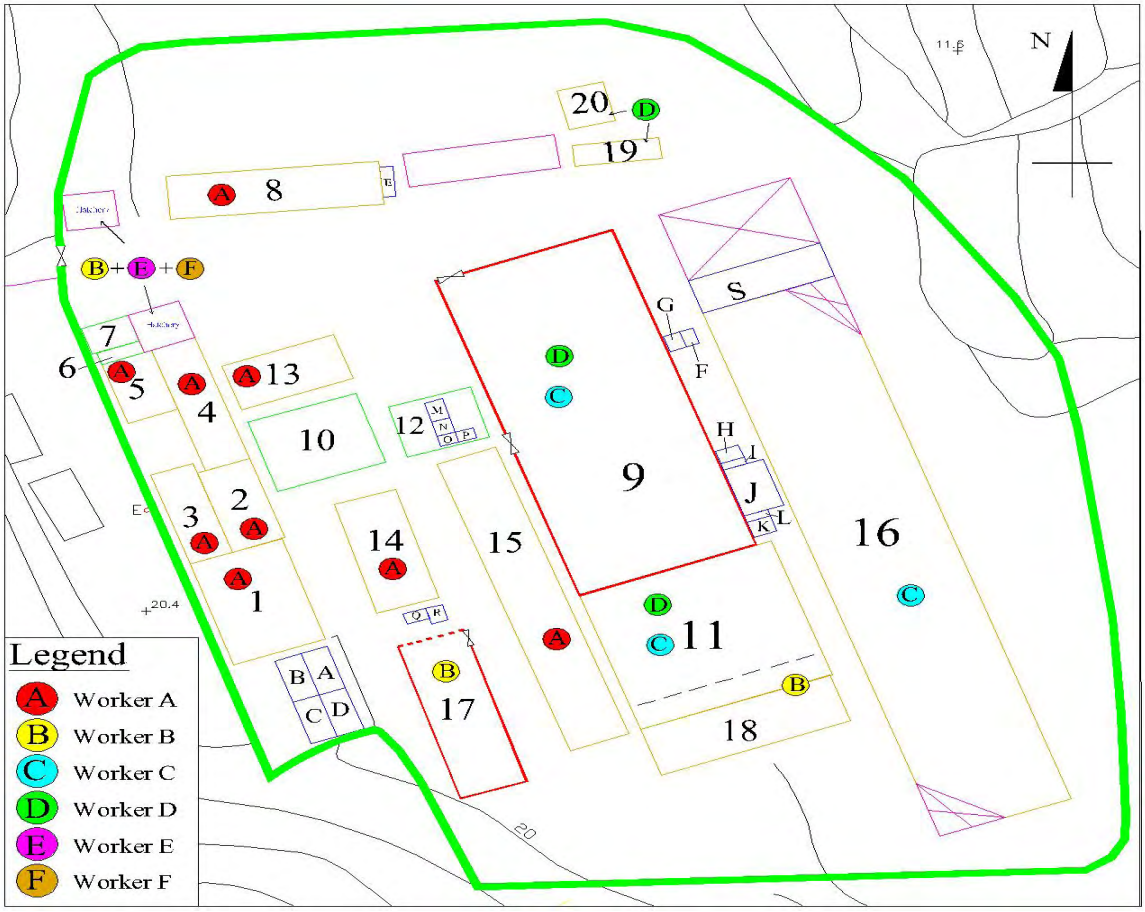

Figure 6 Areas looked after by individual workers

4.5 Introduction of fertile eggs

Routine inspection visits found that the number of fertile eggs produced and the number of breeders on the farm were in balance, thus the likelihood that smuggled eggs had been introduced to the farm is considered very low.

4.6 Introduction of day old chicks

Only one batch of day old chicks was introduced from Guangzhou on 23 November 2008. This batch of chicks appeared clinically healthy prior to the culling operation and tested negative for the H5 virus.

4.7 Movement of people and other items into the farm

Farm workers

4.7.1 Worker F went on a shopping trip to Shenzhen on 28 November 2008 and returned on the same day. He claimed to have had no contact with live chicken before he went back to the farm. Worker A commenced working on the farm on 29 November 2008; he stated that he had not worked on chicken farms before he came to Hong Kong. After they come back from outside, all workers need to take a shower and change clothes before they enter any chicken shed.

The bird catcher

4.7.2 One bird catcher entered the farm to collect chickens for sale to the market on 4 to 7 December 2008. She entered shed no. 9 from the entrance facing north, for bird catching. She had no contact with the breeders in shed no. 17. The bird catcher can only visit one farm per night and she claimed to have put on her own working clothes, face mask, gum boots and gloves before entering the farm.

The staff of AFCD

4.7.3 Staff of AFCD conducted two farm inspections (on 3 and 5 December 2008) in the week before the outbreak. A full set of personal protective clothing and equipment was worn by all staff. The chance of the virus being introduced to the farm via AFCD staff is considered to be very low.

Livestock waste collection truck

4.7.4 A livestock waste collection truck entered the farm on 3 and 5 December 2008 to collect poultry waste. The truck had driven through a disinfectant pool containing disinfectant at the entrance to the farm and the ground where the truck had driven over was disinfected with hydrated lime powder immediately after the waste collection.

Major events involving entry of material or people from outside farm

4.7.5 The following table in reverse chronological order provides details on the major events involving the entry of material or people from outside the farm from 28 November to 8 December 2008.

Date	Feed delivery	Worker	Bird catcher	AFCD staff	Faeces collection
8/12	Nil	Nil	Nil	Disease investigation	Nil
7/12	Nil	Nil	Catch birds at shed no. 9	Nil	Nil
6/12	Nil	Nil	Catch birds at shed no. 9	Nil	Nil
5/12	Nil	Nil	Catch birds at shed no. 9	Farm inspection	Faeces collection
4/12	Nil	Nil	Catch birds at shed no. 9	Nil	Nil
3/12	Nil	Nil	Nil	Farm inspection	Faeces collection
2/12	Feed delivery	Nil	Catch birds at shed no. 9	Nil	Nil
1/12	Feed delivery	Nil	Catch birds at shed no. 9	Nil	Nil
30/11	Nil	Nil	Catch birds at shed no. 9	Nil	Faeces collection
29/11	Nil	One new worker from Hunan	Nil	Nil	Nil
28/11	Nil	One worker went to Shenzhen	Nil	Nil	Nil

Table 8 Personnel and Material Movement Record

4.8 Animals kept on the farm

Under the current licence conditions, no dogs and cats are allowed to be brought into the chicken sheds. Two dogs were kept on the farm but they were confined all the time, and had no access to the chicken sheds. Two kittens were found wandering around the worker's dormitory. The dogs and kittens appeared clinically healthy and are considered to present a low risk for spreading the disease to the chickens. No other pets were kept on the premises. Nasal swabs samples were collected from these animals and no H5 virus was isolated.

4.9 Wild birds

Wild birds were seen on the farm and if infected could potentially introduce the virus onto the farm. Please see section 3.5 for details.

4.10 Airborne particles

AI virus is not normally spread over long distances by airborne particles, but there is a very low possibility that recently contaminated material on the farm could be blown into the affected shed and this could potentially infect susceptible poultry. Any dust and dirt on the trees at the entrance of the affected sheds, if containing droppings contaminated with the virus, could reach the chickens inside the shed on windy days and accumulate in the corner of the shed.

4.11 Illegally kept poultry outside the infected farm

Patrols to detect and deter the illegal keeping of poultry have been in place since 2006. The location of the index farm and nearby region had been patrolled from 14 to 27 November 2008, with no unusual findings. AFCD re-deployed staff to conduct additional patrols around the Ha Tsuen region after the culling operation on 10 December 2008. No backyard poultry farming was detected around the infected farm during the patrols.

5. Discussion

5.1 The Investigation Group considered three main questions –

(i) when the virus was introduced into the farm (Section5.3);

(ii) the source(s) of the virus (Section 5.4); and

(iii) once the virus entered the farm, how it then infected the poultry therein (Section 5.5) and spread within the farm (Section 5.6)

5.2 For HPAI to occur on the index farm, the virus must have entered the farm through some means and subsequently infected the poultry and spread through the sheds involved. Like many epidemiological studies of this nature, it is not possible to identify the exact cause of the outbreak, the precise course of events, the exact date of introduction, or the precise source of virus. Nonetheless, the investigation has narrowed the likely time of introduction to the period from 29 November to 6 December 2008. It has also identified a number of potential routes of introduction that could not be ruled out, with some more unlikely than others.

5.3 Date of introduction

Based on the mortality records of the farm, the Investigation Group focused on events between 29 November and 6 December 2008, and believed that this was the most likely period of the introduction of the virus.

5.4 Source of the virus

5.4.1 The Investigation Group recognized that, like many other places, there is a constant threat of H5N1 virus incursion in Hong Kong. HPAI viruses of the H5N1 subtype have been isolated every year in Hong Kong since 1997 (apart from 1998) either from commercial poultry and poultry retail markets (1997, 1999-2003, June 2008) or from dead wild birds[1]. The most recently detected case of a dead wild bird infected with an Asian-lineage H5N1 HPAI virus prior to the December 2008 outbreak was

[1] This includes isolates from a little egret in Penfold Park in 2002, black-headed gull in Chek Lap Kok in 2003, grey heron in Lok Ma Chau in 2004, Chinese pond heron in Lok Ma Chau in 2005 and others isolated through dead bird surveillance since 2006. For details of virus positive dead wild birds identified through wild bird surveillance since 2006 see http://www.afcd.gov.hk/english/quarantine/qua_vetlab/qua_vetlab_ndr/qua_vetlab_ndr.html

on 16 October 2008 but this virus was found to belong to a different clade (2.3.2) to the one found on this farm. While compared to many other places the incidence rate may not be particularly high[2], it does indicate that the risk is not negligible. And given the very serious consequences should the virus affect humans, continued vigilance is of paramount importance.

5.4.2 The investigation of the source of the virus in this outbreak was not helped by the absence of reports of an identical virus elsewhere in Hong Kong or in the wider region. The closest known relatives to this particular strain of virus are the isolates from a peregrine falcon in March 2008 and in live poultry markets in June 2008. Although these viruses are similar they are not identical and it appears from genetic studies that they share a common ancestor.

5.4.3 Potential sources of the virus (i.e. pathways of introduction) in this case include the following:

(A) Wild birds

(i) Wild birds could be a potential source of infection given that large aquatic birds and other birds were found on and around the farm and were attracted to uncovered soak away pits and trees at the entrance to the two infected sheds.

(ii) It is now generally accepted that wild birds have played a role in the spread of Asian-lineage H5N1 viruses. In Hong Kong, H5N1 viruses, predominantly Clade 2.3.4 and Clade 2.3.2, with viruses from the former clade, have been found mainly in small passerines and scavenger or predator birds. Those in the latter clade have been found in a range of species, including herons and egrets. Against this, however, the extremely rare reports of detection of these viruses in healthy wild birds, despite the intensive surveillance conducted globally, demonstrate that the prevalence of infection in healthy wild birds is extremely low.

[2] Annual report of the EU wild bird surveillance 2006 and preliminary results of the first quarter 2007 available at http://ec.europa.eu/food/committees/regulatory/scfcah/animal_health/ai_premres_en.pdf

(iii) The current virus falls within Clade 2.3.4 but apart from the isolate from a peregrine falcon found in March 2008, no other Clade 2.3.4 viruses were detected in dead wild birds in 2008. The current isolate is some distance away genetically from earlier wild bird isolates.

(iv) The genetic evidence suggests that this particular strain is not derived from viruses detected in the live poultry markets in June 2008 but that these viruses share a common ancestry. The findings from intensive surveillance in Hong Kong suggest that this strain and others are likely to be circulating in the wider region.

(B) Other activities that could have resulted in introduction of the virus

Some 15 events were recorded during the period from 29 November and 6 December 2008 (the period when the virus was considered most likely to have been introduced) involving the introduction of people or items onto the farm from outside. These included a person catching poultry for transport to the market, vehicles delivering feed, vehicles collecting faecal material and staff from AFCD conducting farm inspections. Appropriate biosecurity measures such as changes of clothing or cleaning and disinfection are required to ensure that even if the virus is present on the footwear, clothing or vehicles, it no longer represents a threat when personnel or vehicles enter the farm / chicken sheds. Although measures are in place on the farm to prevent virus from being carried into the farm, some of the inconsistent statements made by the farm owner, manager and workers tended to cast doubt on whether all the required measures were applied appropriately at all times.

(C) Illegal keeping and illegal imports

(i) No evidence of illegal keeping of chickens was found around the infected farm, suggesting that illegal keeping activities were an unlikely source of infection.

(ii) The Investigation Group found no evidence to support the suggestion that infection was introduced through the import of contaminated fertile eggs smuggled onto the farm. The expected

breeder production and the number of chicks hatched during the 2 months prior to the outbreak tally with each other. The egg production record for the 2 months indicated that the farm had more than enough eggs for day old chicks production thus the farmer had no incentive to introduce eggs from outside.

(D) Water and Feed

(i) Since all chickens are supplied with reticulated water as drinking water and only two sheds experienced mortality on 8 December 2008, the possibility that the infection was related to drinking water appears to be extremely low.

(ii) Although the feed store is "open" in design, the distribution of mortality in only two sheds does not support feed contamination as the source of infection. However, the feed store represents a point that workers from different sheds visit and potentially cross contamination of workers could occur at this point.

5.5 Infection of Poultry on the Farm

5.5.1 Once the virus gains entry to a farm it also has to infect the poultry before it can cause disease. Infection of the poultry is made more difficult on farms in Hong Kong because virtually all the poultry are reared in cages and therefore the chickens do not have much direct contact with farm workers (in contrast to poultry reared on the floor). The exception is the breeder flock which is handled by the farm workers regularly for artificial insemination.

5.5.2 The arrangement of having the sentinels placed at the ends of rows of cages means that if one sentinel is infected, the probability of transmission to other sentinels is much higher (and helps to explain why so many sentinels were infected in this case), than if the sentinels are scattered through the flock. Such distribution of sentinels has the effect of amplifying the virus and therefore generating a large dose of virus for the surrounding vaccinated poultry. This arrangement provides a strong signal of a viral incursion, which is likely to trigger a report from the farm owner, as occurred in this case.

5.5.3 There are a number of potential pathways for the introduction of the virus to the first shed and spread of the virus to the other shed. These include the following:

(i) Windborne dust particles

The Investigation Group considered the possibility of wind borne contaminated dust particles as a source of infection. Spread of H5N1 HPAI by contaminated dust or infected feathers has not been proven to occur over extended distances but there have been some suggestions from an outbreak in Canada of HPAI that contaminated dust particles might have played a role in the spread of infection.

According to the meteorological records provided by the Hong Kong Observatory, there were strong winds and gust from the north and north-east from 4 to 6 December 2008. The north wind could have carried potentially contaminated dust particles from outside the shed and blown them into the infected shed no. 17. The slope beside shed no. 17 would likely alter the course of the north-east wind allowing it to then change direction to north-west. The new course of the wind might have also blown potentially contaminated dust and leaves into the shed where they would accumulate in the part of the shed which experienced the highest mortality. (See Figure 1)

(ii) Rodents

Rats and mice can be infected with H5N1 viruses experimentally but not known to be infected naturally. Nonetheless, they cannot be ruled out as potential short term carriers of the virus (or mechanical transfer agents of contaminated material).

(iii) Wild birds

Sparrows were seen within some of the poultry sheds on the infected farm during the investigation visits and farm workers in their interviews suggested that this also occurred in the past.

The role of sparrows in the carriage and transmission of H5N1 HPAI viruses has been investigated in Hong Kong and elsewhere. One H5N1 virus positive dead sparrow was detected in Kowloon Park in early 2003

at a time when infection was present in the zoological collection in that park but no positive dead sparrows have been found in Hong Kong subsequently despite intensive surveillance since October 2005. In total over 3 000 dead sparrows have been tested from October 2005 to December 2008 with none testing positive for the H5N1 avian influenza virus.

Experimental studies with the original H5N1 isolates from Hong Kong in 1997 demonstrated that sparrows were relatively resistant to infection. However a recent experimental study[3] demonstrated that tree sparrows can be infected with and excrete (for a short period of time) a range of recent H5N1 HPAI viruses after inoculation with a large dose of the virus. This study included two isolates from dead birds from Hong Kong (both Clade 2.3.4 viruses isolated from dead wild birds in 2006). Five of the six sparrows infected with these Hong Kong viruses died within 6 days of being infected. However, under the conditions of the experiment, transmission from infected to in-contact sparrows did not occur.

H5N1 HPAI viruses were isolated from four out of 38 captured tree sparrows in Henan Province in 2004[4]. The viruses isolated from these sparrows belonged to Clades (Clades 6 and 7)[5] different to those found so far in Hong Kong.

Based on the above it is apparent that sparrows have the potential to excrete H5N1 HPAI viruses at least for a few days if infected but the absence of positive results from recent dead bird surveillance suggests that infection of these birds occurs infrequently in Hong Kong.

It is also theoretically possible for wild birds to act as mechanical carriers of contaminated material into poultry sheds but this has never been proved as a source of infection in any other outbreaks.

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[3] Boon et al (2007) Emerg Infect Dis13(11):1720-24 available at http://www.cdc.gov/eid/content/13/11/1720.htm

[4] Kou et al 2005 j. Virol 79(24) 15460-15466 available at http://jvi.asm.org/cgi/content/full/79/24/15460?view=long&pmid=16306617

[5] WHO (2007) http://www.who.int/csr/disease/influenza/tree_large.pd

(iv) Bird catcher (pre-sale)

Chicken collection, for sale of chickens to the market, occurred in shed no. 9 from 4 December to 7 December 2008. The same bird catcher worked in shed no. 9 for the four nights and dead chickens were detected the day after (8 December). The catching of birds from cages generates significant quantities of dust and feathers (which if derived from infected chickens can be contaminated with H5N1 virus). The Investigation Group could not rule out the possibility that she might have contributed to the spread of the virus in shed no. 9.

(v) Staff movements

Staff movements could have been responsible for the introduction and spread of the virus to the infected sheds. In addition, it was evident from the investigation visits and interviews that not all the biosecurity and hygiene requirements requested by the farm manager were implemented or observed fully at all times, and that there were some deficiencies in these measures especially those relating to hand/glove hygiene and disinfection.

5.6 Possible spread of infection in the farm after infection had occurred

5.6.1 The mode of management and concern of disease control for breeder chicken and broiler chicken are different. In the case of this farm, the level of immunity in the batch of old breeders on the farm was falling, as is often the case with older poultry and therefore there was a higher number of susceptible chickens in this flock. Once the flock was infected, the potential for spread to other poultry flocks on the farm increased.

5.6.2 The mortality of chickens was first found in the breeder shed no.17 then later on the same day in the broiler shed no. 9. Worker B is designated to work on all breeder sheds including shed no. 17 and shed no. 18. Shed no. 9 and shed no. 18 share the same entrance. Though this worker does not need to work on the broilers in shed no. 9, we could not rule out the possibility that contaminated particles from shed no. 17 could have been carried to shed 9 via means such as workers' movement or windborne dust particles and then infected the sentinel chickens located near the side entrance. As the sentinels were all placed at the ends of each row of cages and the ends of these cages all face the side entrance of shed no. 9, once a sentinel was infected, the virus load could have quickly amplified and spread to other sentinels.

5.6.3 The mortality was concentrated at the side entrance of shed no. 9 with scattered mortality inside the shed. There are a number of possible explanations for this, including contact with workers if their gloves were contaminated with the H5 virus and possibly introduction by air movement, with sentinels serving as the amplifier.

5.6.4 Our on-farm investigation pointed to considerable room for improvement in biosecurity. The workers mix bleach with detergent for filling the disinfectant pool and all foot baths, which is inappropriate as it may reduce the effectiveness of the disinfectant. The farm manager admitted that there was a possibility that the farm workers might not always dip their boots in the foot bath before entering the chicken sheds (which, without cleaning of their boots, is not an adequate biosecurity measure) and the farm workers might have left the entrance to the sheds open to facilitate their routine operation. This could create "biosecurity breaks" and allow potential vectors like rodents and wild birds to enter the chicken sheds. The lack of formal written visitor records, proper vaccination records and chicken mortality records are also areas that require improvement.

5.6.5 The discrepancy between the manager's account and that of the workers regarding the way waste material was handled, discovered during the investigation, showed that there is room for improvement on the concept and understanding of good farm practice and biosecurity vigilance of individual workers.

6. Conclusion

The main conclusions of the Investigation Group are:

6.1 There is a constant threat of infection by the H5 virus for poultry farms in Hong Kong, especially in winter.

6.2 The virus on the farm belongs to Clade 2.3.4 but no identical virus has been detected elsewhere. It shares a common ancestry with viruses detected elsewhere in Hong Kong in 2008.

6.3 The outbreak was caused by a combination of factors – presence of the virus in the vicinity of the index farm, its introduction into the farm and its spread among the poultry of the two sheds involved.

6.4 The exact source of infection or route of introduction to the farm and infected sheds could not be identified. There are a number of possible routes of introduction of infection, including wild birds and the entry of items or people from outside the farm, coupled with or facilitated by biosecurity lapses. Given that an identical virus was not detected outside the farm, it is not possible to pinpoint one single pathway as the most likely one, but some possibilities such as contaminated drinking water have a lower probability and can largely be ruled out.

6.5 The strong winds that prevailed a few days before the outbreak might have contributed to the introduction of the virus to the poultry in the infected sheds although other routes could not be totally eliminated including rodents, bird catchers, contaminated hands/gloves or clothing of farm staff and small wild birds.

6.6 The keeping of breeders and broilers on the same premises requires careful management. In this case, the infected breeders were almost 2 years old and the percentage of immune poultry in this flock was lower than in other flocks on the farm. It was not unexpected that once this flock was infected some vaccinated poultry would succumb to infection (i.e. flock immunity was insufficient to prevent the spread of infection and many of the chickens had insufficient titers to protect them from the disease once infected).

6.7 Unvaccinated sentinel chickens played an important role in early detection in this case. However, since the sentinel chickens were not evenly distributed throughout the flock, they could have acted as an amplifier in this outbreak which led to a heavy challenge of vaccinated chickens. To strike a balance between early detection while reducing the amplifying effect to an appropriate extent, further scattering of sentinels among vaccinated chickens should be carefully considered.

6.8 The structure of the infected shed no. 17 facilitated the introduction and accumulation of potentially contaminated material in the shed.

6.9 Inconsistent observance of biosecurity by individual workers may have created “biosecurity breaks” and possibly helped spread the disease from one shed to another.

6.10 The Investigation Group noted that many improvements in the biosecurity of the entire production and marketing chain have been implemented in Hong Kong and recognized that it is not possible to aim for zero risk either in Hong Kong or elsewhere. Nonetheless, this case points to room for improvement. The improvement measures recommended below are aimed at further reducing the risk of infection. The Investigation Group recognizes the importance of ensuring the practicability / enforceability of the improvement measures - no measure would be effective if it solely relied on the regulator or the regulated. On the part of the regulator, enforcement should be accompanied by compliance facilitation and on the part of the industry, cooperation and the right mindset would be critical. Effective prevention of AI requires continued vigilance and a combination of measures by all the parties concerned.

7. Recommendations

7.1 Recommendations for improvement of biosecurity measures for the index farm

The Investigation Group identified a number of “biosecurity breaks” that might have made the outbreak in the index farm possible. Taking into account the findings of the investigation, as well as the location and configuration of structures on the index farm, the Investigation Group recommends the following measures to improve the biosecurity on that farm:

(i) Modify the structure of shed no. 17 so that the shed area is fully covered by its roof and not directly exposed to the tree and add a solid partition on the side facing north to protect against wind gusts.

(ii) Cover all uncovered soak away pits and wells to avoid gathering of aquatic birds on farm.

(iii) Ensure that the bird protection facilities on farm are sufficient for preventing small birds from entering the farm sheds, e.g., instead of woven meshes which may easily degrade, metal meshes may be used.

(iv) Implement the other enhancement measures recommended for all chicken farms in paragraph 7.2 below.

7.2 Recommendations to strengthen the prevention of avian influenza infection in chickens in local poultry farms

Though many of the improvements in paragraph 7.1 are specific to the index farm, the Investigation Group believed there were lessons that could be drawn for other poultry farms. The Investigation Group suggested the Administration to consider further strengthening the AI prevention programme on local poultry farms through implementation of the following recommendations -

(i) Further tighten up biosecurity

- Each farm to develop a tailor-made farm biosecurity plan (covering such issues as bird protection, rodent control and farm management practices) in consultation with AFCD, taking into account the circumstances of individual farms;

- Each farm to keep records related to chicken movements, vaccinations, medications and mortalities according to the format specified by AFCD;

- Each farm to provide hand washing / cleaning facilities at designated locations inside the production area for use by anyone prior to entering the poultry sheds;

- AFCD to facilitate compliance by conducting refresher courses periodically for farmers, farm workers and other allied workers on the concepts and practices of biosecurity, for example, the proper use of gloves, and devising templates on record keeping and vaccination[6];

(ii) Facilitate early detection

- AFCD to increase the inspection frequency of poultry farms from once every week to once every 5 days with stepped up veterinarian audit;

- AFCD to increase the sampling size of the blood test for vaccinated chickens for enhancing the precision of flock immunity estimates;

[6] The Investigation Group noted that AFCD has already introduced new templates or updated previous ones since the December 2008 outbreak. An example is the template on vaccination / medication/ movement / mortality / sale numbers (at Annex 4).

(iii) Prevent virus spread on introduction

- AFCD to require the further scattering of sentinel chickens among the vaccinated flock; and

- AFCD to consider, in consultation with the farmers, how best to segregate the operations relating to the rearing of breeder flocks and broiler flocks to minimize the risk of spread of infection.

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Annex 1

Investigation Group on Epidemiological Study

Terms of Reference

(1) To do an in-depth examination of the biosecurity measures of the index farm in Yuen Long;

(2) To take a thorough look at the preventive measures taken by the farm, as well as the management and operation of the farm to see if there are any shortcomings;

(3) To identify the source of infection and the channel that the virus could have got into the farm; and

(4) To submit a preliminary report to the Food and Health Bureau in two to three weeks, followed by a full report in two to three months with recommendation on remedial measures.

Annex 2

Investigation Group on Epidemiological Study
Membership List

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Secretary:

Mr Peter HO
Deputy Administrative Manager (Special Duties)
AFCD

Annex 3

Blood test results of the infected breeder flock in shed no. 17

Date of Submission : **9-12-2008**
Case Number : S-08-06994
Test Type : Haemagglutination Inhibition for Vaccination Programme
Flock information : Age 632 days
Introduction Date : 18-03-2007

Specimen Number	Result (HI titer)	Specimen Number	Result (HI titer)
1	16	8	32
2	<16	9	16
3	<16	10	64
4	<16	11	128
5	32	12	<16
6	256	13	<16
7	64	14	<16

Date of Submission : **28-11-2008**
Case Number : S-08-06849-F-V1
Test Type : Haemagglutination Inhibition for Vaccination Programme
Flock information : Age 621 days
Introduction Date : 18-03-2007

Specimen Number	Result (HI titer)	Specimen Number	Result (HI titer)
1	128	8	32
2	64	9	256
3	256	10	256
4	16	11	128
5	128	12	256
6	64	13	256
7	16	14	256

Annex 4

Example of Template for Farmers [Vaccination / Medication / Movement/ Mortality / Sale Numbers]

給農友記錄每批雞隻疫苗、用藥、換籠、死亡及出售的紀錄範本

雞苗引入日期:__________________________

日期	日齡	用藥/疫苗詳情	死亡數量	出售數量	雞舍編號
x/x	0				
	1				
	2				
	3				
	4				
	5				
	6				
	7				
	8				
	9				
	10				
	119				
	120				