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Vaccination of chickens against H5N1 avian influenza in the face of an outbreak interrupts virus transmission

Trevor M. Ellis[1,*], Connie Y. H. C. Leung[2], Mary K. W. Chow[3], Lucy A. Bissett[1], William Wong[1], Yi Guan[2] and J. S. Malik Peiris[2]

[1]Tai Lung Veterinary Laboratory, Agriculture Fisheries and Conservation Department, Lin Tong Mei, Sheung Shui, Hong Kong SAR, China, [2]Department of Microbiology, The University of Hong Kong, SAR, China, and [3]Livestock Farm Division, Agriculture Fisheries and Conservation Department, Lin Tong Mei, Sheung Shui, Hong Kong SAR, China

Vaccination of chickens with a commercially available killed H5N2 vaccine was being evaluated as an additional tool to enhanced biosecurity measures and intensive surveillance for control of highly pathogenic avian influenza subtype H5N1 disease in Hong Kong in 2002. In December 2002 to January 2003, there were outbreaks of H5N1 disease in waterfowl in two recreational parks, wild water birds, several poultry markets and five chicken farms. In addition to quarantine, depopulation of the affected sheds and increased biosecurity, vaccination of the unaffected sheds and surrounding unvaccinated farms was undertaken on three farms. In at least two farms, infection spread to the recently vaccinated sheds with low rates of H5N1 mortality in sheds when the chickens were between 9 and 18 days post-vaccination. However, after 18 days post-vaccination no more deaths from H5N1 avian influenza occurred and intensive monitoring by virus culture on these farms showed no evidence of asymptomatic shedding of the virus. This provides evidence that H5 vaccine can interrupt virus transmission in a field setting.

Introduction

Outbreaks of H5N1 highly pathogenic avian influenza (HPAI) have occurred in Hong Kong in chickens and other gallinaceous poultry in 1997, 2001, 2002 and 2003 (Sims *et al*., 2003; Ellis *et al*., 2004a). High mortality rates were seen in gallinaceous birds on farms (1997, 2002 and 2003) and/or in poultry markets (1997, 2001, 2002, 2003) in all outbreaks and in wild or captive waterfowl (geese, ducks and swans) in outbreaks in two bird parks during December 2002 to January 2003. Deaths also occurred in other wild or captive water birds (Little Egrets, *Egretta garzetta*; Greater Flamingo, *Phoenicopterus ruber*; Grey Heron, *Ardea cinerea*; Black-headed Gull, *Larus ridibundus*) during these outbreaks (Ellis *et al*., 2004a). Outbreaks of H5N1 HPAI were also detected in five chicken farms in Hong Kong in late December 2002 and January 2003. These were detected after the outbreaks were detected in water birds in the two bird parks and the detection of H5N1 HPAI in the two wild Grey Herons.

The 1997, 2001 and early 2002 H5N1 outbreaks had substantial economic impacts in Hong Kong due to costs of partial or total depopulation of poultry, closure of live poultry markets, reductions in tourism and the costs of a comprehensive H5N1 testing and surveillance system for local and imported poultry. After the outbreak in 2001, the poultry farm and market biosecurity measures and monitoring systems in place since 1998 were enhanced. Following a detailed epidemiological study of the February to April 2002 H5N1 HPAI

*To whom correspondence should be addressed. Tel: +852 2455 2156. Fax: +852 2461 6412. E-mail: ellis_trevor@afcd.gov.hk
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outbreak, further measures were introduced to improve farm and market biosecurity. However, due to the large daily movement of poultry into retail live poultry markets of Hong Kong from farms in Hong Kong and elsewhere in southern China, together with the possibility of H5N1 virus infections occurring in the wider region, the Hong Kong SAR Government investigated the use of H5 avian influenza vaccination as an additional control measure for H5N1 avian influenza.

A field evaluation trial using Nobilis® Influenza H5, an inactivated avian influenza Type A H5N2 virus (A/Chicken/Mexico/232-CPA/94) water-in-oil emulsion vaccine (Intervet International, Boxmeer, The Netherlands), on chicken farms was commenced in April 2002 in the district where the last four farm outbreaks of H5N1 had occurred, and evaluation continued until March 2003. The evaluation trial showed that acceptable flock H5 antibody responses were generated by the vaccine and vaccinated chickens were protected from high-dose laboratory challenge with H5N1 HPAI viruses from the February to April 2002 outbreaks (Ellis *et al*., 2004b). In December 2002, the H5N1 outbreaks in waterfowl and wild birds and the detection of viruses in several retail markets, together with the encouraging vaccine trial results, led to the decision to broaden the chicken farm vaccination area to cover those areas most exposed to wild bird movements. H5N1 outbreaks occurred in five unvaccinated chicken farms in late December 2002 and January 2003. Strict quarantine and movement controls were initiated immediately, followed on two farms by total depopulation with ring vaccination of surrounding farms. In three other affected farms only sheds showing high or rising mortality rates were depopulated together with vaccination of unaffected sheds and surrounding farms. The effect of killed H5N2 vaccination in the face of these outbreaks was monitored in terms of protection from disease and ability to interrupt transmission of H5N1 virus.

Materials and Methods

Outbreak history. The three chicken farms referred to in this report rear chickens in semi-enclosed sheds in A-frame cage banks, as do most of the 154 chicken farms in Hong Kong. Farm sizes in Hong Kong vary between approximately 20 000 and 100 000 chickens and they rear slow-growing yellow–brown broiler chickens, which are imported as one-day-old chickens from Mainland China and are usually marketed at between 80 and 100 days of age.

Vaccinations on the affected farms were given using a single standard 0.5 ml dose of the Nobilis® Influenza H5 vaccine using the subcutaneous route at the base of the neck. Separate farm staff members were used to vaccinate the different sheds and all staff wore their standard working uniform. Disinfectant footbaths containing Virkon™ were located at the entrance of each shed and hand washing facilities were available and were used by staff. Experienced teams of Agriculture, Fisheries and Conservation Department (AFCD) staff were involved in depopulation of chicken sheds and they wore impervious protective overalls with built-in hoods, boots, gloves and masks and were not permitted into other sheds on the farms.

Farm 1. A moderate sized farm (51 000 chickens) in Tai Kong Po (see farm plan in Figure 1) reported increased mortalities (about 35 chickens) on 6 January 2003, and follow-up inspection by AFCD staff detected further deaths in three sheds (shed 7 had 107 dead/6000 in 98-day-old birds, shed 8 had 44 dead/1500 in 65-day-old birds and shed 11 had eight dead/2600 in 77-day-old birds) by that afternoon. Rapid testing (real-time reverse transcriptase polymerase chain reaction [RRT-PCR] for H5) detected HPAI H5 virus that evening. Movement control and strict attention to biosecurity was initiated immediately and the farm was officially quarantined on 7 January 2003. However, unresolved issues with compensation resulted in culling of chickens in the affected sheds being delayed. Vaccination with the killed H5N2 vaccine was started in the six unaffected sheds on the farm on 7 January 2003. Mortalities commenced in sheds 3, 6 and 10, which were adjacent to the three initially affected sheds, on 8 January 2003. Subsequently mortalities started to rise rapidly in sheds 7, 8 and 11, then in sheds 3, 6 and 10 and by 15 January 2003 in sheds 4 and 5 (Table 1). The 8000 remaining chickens in sheds 7, 8 and 11 were killed on 10 January 2003 and the 12 000 remaining chickens in sheds 3, 4, 5, 6 and 10 were killed on 15 January 2003.

On 16 January 2003 (day 9 post-vaccination) deaths, confirmed as H5N1 HPAI after postmortem examination, H5 RRT-PCR and virus culture in chicken embryos, were detected in the remaining large shed on the farm (shed 2 containing 19 000 chickens). This shed was physically well isolated from the other sheds on the farm and staffed by a separate group of workers. Mortalities confirmed as H5-positive continued at a low level until 25 January (day 18 post-vaccination) (Table 1). Sequential measurements of haemagglutination inhibition (HI) test antibody levels to H5 avian influenza were conducted for chickens from this shed (days 15, 22, 28, 34, 38 and 42 post-vaccination). Cloacal swabs were collected from 60 randomly selected chickens in this shed for virus culture on days 15, 22 and 28 post-vaccination by AFCD. A larger cross-sectional sampling of chickens in the shed for virus culture (300 throat and 300 cloacal swabs each time) was conducted by the Department of Microbiology, University of Hong Kong on days 28, 33 and 38 post-vaccination.

Farm 2. A second farm (35 000 chickens) in Tai Kong Po reported increased mortalities (about 150 chickens in two batches of chickens) in two adjacent sheds (shed 1 with 46-day-old chickens and shed 3 with 39-day-old chickens) on 20 January 2003. Rapid testing (postmortem examination and H5 RRT-PCR) diagnosed H5 HPAI. Movement controls and strict adherence to biosecurity procedures were instigated immediately. The farm was placed in quarantine and the 5300 chickens in those sheds were killed on 21 January 2003. This farm was near the first outbreak farm at Tai Kong Po and had been included in the ring vaccination programme around that farm. Shed 3 was vaccinated on 8 January 2003 and Shed 1 on the 14 January 2003. Other sheds were vaccinated on 9 to 15 January 2003.

The remaining chickens on the farm were checked daily for mortalities that were investigated to determine the cause of death. On 22 to 23 January 2003 AFCD staff collected 180 chicken cloacal swabs and 120 fresh faecal droppings the trays under the cages from sheds 2, 4 and 5 for virus culture. On 11 February 2003 60 cloacal swabs were randomly collected from the market-aged chickens (about 100 days old) and on 20 February 2003 100 swabs of fresh faecal droppings from the cage trays were also collected and tested by virus culture from the market aged birds. Subsequently, the seven batches of market-aged chickens between February and April 2003 all had 60 cloacal swabs randomly collected and tested by H5 RRT-PCR and virus culture.

Serum antibody levels were measured by H5 HI tests on 14 birds from shed 4 at 10 days post-vaccination and 14 birds from shed 2 at 13 days post-vaccination on 22 January 2003, then 30 birds each from shed 2 and 4 (30 to 33 days post-vaccination) on 11 February 2003.

Farm 3. A third farm (20 700 chickens) in Shek Kong Tsuen reported increased mortalities (about 100 chickens of 38 days old) in a single shed on 20 January 2003. Rapid testing (post-mortem examination and H5 RRT-PCR) detected HPAI H5 virus that day. This farm was in a separate valley 1.5 km away from the Tai Kong Po farms. The farm was immediately placed in quarantine and the 5600 chickens in the affected shed were killed on 21 January 2003. All remaining chickens on the farm between 8 and 55 days old were vaccinated and ring vaccination

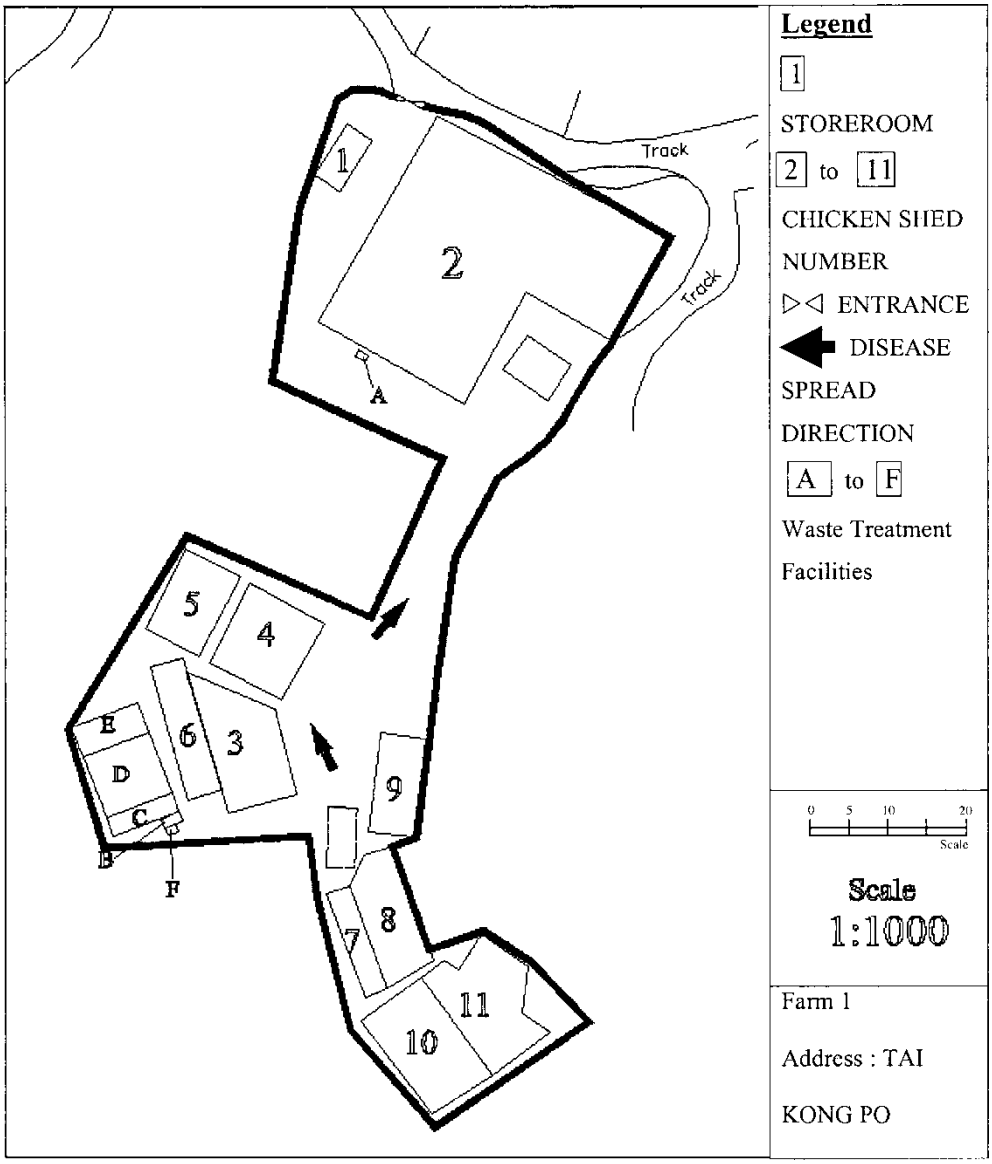

Figure 1. *Location plan of chicken sheds on Farm 1.*

was conducted on the nine farms nearby (involving 212 000 chickens) on 23 January 2003.

The remaining chickens on the farm were checked daily for mortalities, which were investigated immediately to determine the cause of death. Blood samples were collected from 14 chickens in all batches of market age chickens to measure H5 antibody responses. From each batch of market-aged chickens between February and April 2003, 60 cloacal swabs were randomly collected for H5 RRT-PCR testing and virus culture.

Laboratory test procedures. Dead birds were subjected to postmortem examination as described for previous H5N1 outbreak investigations (Ellis *et al*., 2004a). Pooled cloacal and tracheal swabs from each dead bird were suspended in antibiotic containing viral transport media and subsequently inoculated into the allantoic cavity of 9-day-old to 11-day-old specific pathogen free chicken embryos following standard procedures (Alexander, 2000). Cloacal swabs, throat swabs and swabs of faecal droppings from clinically healthy chickens that were collected for avian influenza virus surveillance in the chicken sheds were similarly tested. Allantoic fluid from eggs with dead embryos and all eggs at 4 days post-inoculation were tested for presence of haemagglutinins (HA) of chicken red blood cells, and HA-positive allantoic fluid was routinely subjected to HI tests using reference antisera (Veterinary Laboratory Agency, Weybridge, UK and USDA, Ames, IA, USA) to avian influenza subtypes H5 and H9 and Newcastle disease virus by standard procedures (Alexander, 2000).

Cloacal and faecal dropping swabs for surveillance testing of chicken sheds were also tested for presence of the H5 HA gene by the RRT-PCR test described by Spackman *et al*. (2002). HA-positive allantoic fluids from the virus cultures were also tested for the H5 gene by the RRT-PCR. Any isolated H5 virus was further characterized by sequencing of virus gene segments at the Department of Microbiology, University of Hong Kong using procedures described previously (Guan *et al*., 2002).

Antibody levels to H5 avian influenza virus in chicken sera were tested by the standard HI test procedures described by Alexander (2000) using avian influenza A/chicken/Hong Kong/97 (H5N1) virus antigen.

Results

Affected dead chickens from all three farms had gross and microscopic pathology changes consistent with previous H5N1 HPAI cases in chickens in Hong Kong. The chickens shown as H5N1 positive in Tables 1 and 2 gave positive results by H5 RRT-PCR tests on cloacal swabs and H5N1 virus was isolated by chick embryo allantoic cavity inoculation. Partial gene sequencing of eight gene

Table 1. *Record of the number of dead chickens in respective sheds on Farm 1 (Tai Kong Po)*

Date	Sheds 7, 8, 11	Shed 10	Sheds 3, 6	Sheds 4, 5	Shed 2
Number of chickens (age)	10 300 (65 to 98 days old)	8000 (26 days old)	3000 (62 days old)	10 300 (28 days old)	19 000 (62 to 74 days old)
3/1/03	7 to 8				
4/1/03	5 to 6				
5/1/03	11 to 12				
6/1/03	194				
7/1/03	403	Vaccinated	Vaccinated	Vaccinated	Vaccinated
8/1/03	318	36	7	0	0
9/1/03	493	0	4	0	0
10/1/03[a]	819	700	15	0	0
11/1/03	Depopulated	210	0	0	7 (negative)[b]
12/1/03		220	12	0	0
13/1/03		250	0	0	4 (negative)
14/1/03		600	18	0	0
15/1/03[c]		2500	26	6000	2 (negative)
16/1/03		Depopulated	Depopulated	Depopulated	10 (H5-positive)[d]
17/1/03					4 (H5-positive)
18/1/03					4 (H5-positive)
19/1/03					3 (H5-positive)
20/1/03					6 (negative)
21/1/03					8 (H5-positive)
22/1/03					10 (H5-positive)
23/1/03					11 (H5-positive)
241/03					12 (H5-positive)
25/1/03					6 (H5-positive)
26/1/03					6 (negative)
27/1/03					4 (negative)
28/1/03					5 (negative)
29/1/03					5 (negative)
30/1/03					4 (negative)
31/1/03					6 (negative)
1/2/03 to 7/2/03					All 0

[a]A total of 8000 chickens were culled from sheds 7, 8 and 11 on 10 January 2003.
[b](negative), negative for H5 virus isolation.
[c]A total of 12 000 chickens were culled from sheds 3, 4, 5, 6 and 10 on 15 January 2003.
[d](H5-positive), H5N1 avian influenza virus was isolated from dead chickens.

segments from representative viruses from these farms showed that all viruses were similar and they were also similar to H5N1 viruses that had been detected from the outbreak in waterfowl at one water bird park and in wild Grey Herons in December 2002.

Farm 1 investigation. H5N1 infection was detected in shed 2 on 16 January, 9 days post-vaccination, and dead chicken continued to be detected until 25 January (day 18 post-vaccination). However, subsequently no sick or dead birds were diagnosed as H5 avian influenza in shed 2 (Table 1). No subclinical infection with H5N1 virus was detected by virus culture of random samples of cloacal and throat swabs from 60 clinically normal chickens from this shed by AFCD on days 15, 22 and 28 post-vaccination or from cloacal and throat swabs collected from 300 clinically normal chickens on days 28, 33 and 37 post-vaccination in the cross-sectional sampling in this shed by University of Hong Kong.

The sequential antibody responses to H5 avian influenza in vaccinated chickens in shed 2 are indicated in Table 3. By day 22 post-vaccination, 81.7% chickens had H5 antibody titre ≥ 16 and the overall GMT for these birds was 33.9. By this time there had been no mortalities due to H5 avian influenza or H5 virus isolations for 4 days.

Farm 2 investigation. The dead bird monitoring in sheds 2, 4 and 5 showed that a small number of chickens in each of these sheds died of H5N1 HPAI (diagnosed by postmortem examination, RRT-PCR and virus culture) on 25 to 26 January 2003, indicating that these sheds had also been exposed to H5N1 virus. Two other more isolated sheds of younger chickens on the farm showed no H5N1 HPAI (Table 2).

By the time the affected chickens were detected in sheds 2, 4 and 5, these batches were already 13 to 17 days post-vaccination and there was only limited disease in these sheds over a 2-day period.

Prior to marketing of the first batch of chickens from this farm, virus culture testing of cloacal swabs collected from 60 randomly sampled chickens and swabs of 100 randomly collected fresh faecal droppings from the cage trays were shown to be negative for H5N1 viruses. Subsequently, no H5N1 virus was detected by H5 RRT-PCR or by virus culture from 60 randomly sampled cloacal

Table 2. *Record of the number of dead chickens in respective sheds on Farm 2 (Tai Kong Po)*

Date	Shed 1	Shed 3	Shed 2	Shed 4	Shed 5	Sheds A, B
Number of chickens (age)	2800 (46 days old)	2 500 (39 days old)	5000 (95 to 102 days old)	5800 (52 to 88 days old)	10 200 (59 to 81 days old)	8700 (16 to 32 days old)
Date vaccinated	14/1/03	8/1/03	9/1/03	12-13/1/03	10-11/1/03	15/1/03
20/1/03	150 (14 H5-positive pools)[a]			0		0
21/1/03	Depopulated			0		0
22/1/03				0		0
23/1/03				0		0
24/1/03				0		0
25/1/03				2 (1 H5 +ve pool)		0
26/1/03				8 (2 H5 +ve pools)		2 (−ve)[b]
27/1/03				0		5 (−ve) (1 NDV +ve)[c]
28/1/03 −1/2/03				0		0
2/2/03				0		20 (−ve) (1 H9 +ve)[d]
3/2/03				0		3 (−ve)
4/2/03				0		3 (−ve)
5/2/03 to 6/2/03				0		0
7/2/03				0		2 (−ve)

[a](H5-positive pool), H5N1 avian influenza virus was isolated from pooled cloacal and throat swabs from the dead chickens.
[b](negative), negative for H5 virus isolation.
[c]NDV, Newcastle disease virus.
[d]H9, avian influenza H9N2 virus.

swabs from the next seven batches of market-aged chickens from this farm.

H5 HI antibody titre ≥ 16 was detected in six of 14 (42.9%) of chickens in shed 4 at 10 days post-vaccination, four of 14 (28.6%) of chickens in shed 2 at 13 days post-vaccination and in all chickens from both shed 2 and 4 at 30 to 33 days post-vaccination (57 of 60 had H5 HI titre ≥ 32 and three chickens had titre $= 16$).

Farm 3 investigation. No H5N1 HPAI occurred in other sheds on the farm or in the nearby nine farms subsequent to this outbreak. H5N1 virus was not detected by H5 RRT-PCR or by virus culture from 60 randomly sampled cloacal swabs from all batches of market-aged chickens from this farm between February and April 2003.

The first batch of vaccinated chickens was marketed on day 37 post-vaccination, and blood tests on these chickens showed that all (14/14) had H5 HI antibody titres ≥ 32.

Discussion

One of the concerns in the use of vaccine to control HPAI in poultry farms is the possibility that while vaccine may protect from disease, asymptomatic virus circulation may continue, resulting in spread of infection to other farms. The monitoring and surveillance conducted on these three chicken farms showed that use of this killed H5N2 vaccine in the face of HPAI H5N1 virus challenge was able to protect chickens from disease and interrupt virus transmission. The protective effect of vaccine became apparent after day 18 post-vaccination. On farms 1 and 2, clear evidence of H5N1 infection was demonstrated in sheds of vaccinated chickens, and subsequently extensive surveillance by clinical inspection and virus detection tests, both H5 RRT-PCR and virus culture, showed that the virus transmission had been interrupted. For farm 3, the rapid depopulation of the affected shed and strict biosecurity measures applied combined to minimize the level of challenge to other sheds. No

Table 3. *Antibody responses to H5 virus[a] in vaccinated chickens on Farm 1*

Number of days post-vaccination (date)	Number of chickens	Titre 4	Titre 8	Titre 16	Titre 32	Titre 64	Titre 128	Titre 256	% birds positive	Geometric mean titre
15 (22/1/03)	60	28		11	15	4	2		53.3	11.7
22 (29/1/03)	60	11		6	16	15	7	5	81.7	33.9
28 (4/2/03)	60		6	5	49[b]				90	n/a
34 (10/2/03)	60		4		56 [b]				93.3	n/a
38 (14/2/03)	14				14 [b]				100	n/a
42 (18/2/03)	14				14 [b]				100	n/a

[a]Antibody responses were detected by HI tests using A/chicken/Hong Kong/97 (H5N1) antigen.
[b]Tested in a three-dilution test (8, 16, 32) only.
n/a indicates geometric mean titre was not calculated because end-point titres were not available.

evidence of clinical disease or H5N1 infection was demonstrated in the sheds of vaccinated chickens so it is possible that the other sheds on this farm may not have received significant exposure to the H5N1 virus from the initial infected shed.

Vaccines have been used in other countries to assist in the control of avian influenza. Countries that have used vaccines for avian influenza control include Italy (Capua *et al.*, 2002), the US (Halvorson, 2002), Mexico (Villarreal & Flores, 1998) and Pakistan (Naeem, 1998). Mostly vaccination has been directed against low pathogenic strains of avian influenza virus but Mexico and Pakistan have successfully used vaccine against highly pathogenic H5 or H7 avian influenza viruses. Experimental studies have shown that commercially available H5 avian influenza vaccines could protect poultry from 1997 Hong Kong strains of H5N1 HPAI virus (Swayne *et al*., 2001).

On Farm 2, avian influenza H9N2 virus was detected in the sheds containing 16-day-old to 32-day-old chickens. Recent experimental studies have suggested that infection with H9N2 virus may stimulate cell-mediated immune responses that could cross-protect chickens from intranasal H5N1 virus challenge that was lethal in uninoculated controls (Seo & Webster, 2001). This cross-protectivity was effective at 15 days after intranasal inoculation with H9N2 virus given in a low challenge dose (10 50% lethal chicken doses), but its effectiveness was diminished by 30 days post-inoculation. Infection of chickens with H9N2 avian influenza viruses is quite common in chickens in Hong Kong based on monthly serological surveillance conducted by our laboratories on local and imported chickens between 1999 and 2001. The H9N2 viruses isolated from chickens in Hong Kong belong to a lineage of viruses related to A/Duck/Hong Kong/Y280/97 (H9N2) (Guan *et al*., 2000), which generally causes mild or inapparent infections of the upper respiratory tract in chickens. On local farms where H9N2 infection has been monitored, it generally occurs in chickens under 30 days that are reared on litter. By the time they are moved to the A-frame cages infection is less common and chickens of multiple ages on affected farms are H9N2 antibody positive. On farm 2 with H9N2 infection circulating in the 16-day-old to 32-day-old birds it would be highly probable that the older birds (39 to 46 days old) in sheds 1 and 3 would have been exposed to this virus, but this did not prevent the H5N1 outbreak in these sheds. During the 2002 H5N1 outbreak on chicken farms in Hong Kong there appeared to be no correlation between exposure to H9N2 virus, measured by serology, and the severity of the outbreak. The H9N2 AI virus exposure and resulting immunity had no protective effect against the field challenge by H5N1 AI virus possibly because of either short-lived cross-protective cellular immunity or a high environmental challenge dose of H5N1 AI virus.

Avian influenza vaccination has generally been used in uninfected flocks in control areas around but not including infected flocks. From this investigation we are definitely not suggesting that the use of vaccination to assist in the control of an avian influenza outbreak could be delayed in the control area until evidence of spread from infected farm(s) occurs. Nor do we recommend the use of partial depopulation plus vaccination on an infected farm as a normal practice. In the first Tai Kong Po farm, five sheds with 22 000 chickens had to be killed before vaccination had a chance to work in the final shed, and in the meantime outbreaks occurred on two nearby farms that were ring vaccinated at the same time as the initial farm. Generally, when ring vaccination is used for avian influenza control, the infected farm and high-risk contact farms within an epidemiologically sustainable perimeter (usually several kilometres) are quarantined, monitored and possibly depopulated. Ring vaccination is used outside this zone where there is a good chance for immunity to develop to the virus before exposure occurs. The close proximity of farms and limited land availability makes this approach difficult in Hong Kong. For the three farms involved in this investigation the individual circumstances at the time, together with expanding use of preventative vaccination throughout Hong Kong, led to an unusual control strategy involving quarantine, partial depopulation and vaccination of unaffected sheds and surrounding farms. As part of this strategy very strict attention had to be paid to movement control of birds, people and materials onto and from the farm and strict biosecurity practices had to be maintained. This was combined with an intensive monitoring programme on the vaccinated sheds and the surrounding farms to rapidly detect any spread of the infection. This strategy was very resource intensive and would have been very difficult to sustain in a more widespread outbreak.

Another factor that should be considered with vaccinating in the face of an outbreak is the possibility of selection of variant viruses when the virus is replicating rapidly in the presence of partial or incomplete flock immunity. The chance of this occurring will clearly be lower if virus is introduced to a fully vaccinated flock that has had time to develop its immunity. However, concerns expressed about the risk of enhanced H5N1 virus evolution in the presence of a vaccinated antibody-positive chicken population needs to be kept in perspective. If you do not vaccinate, all exposed chickens have the potential to become infected with H5N1 viruses that will replicate to high titres and shed large quantities of virus in faeces and respiratory secretions that will infect further chickens. Each replication cycle increases the number of mutations and

the potential for antigenic variation. There are also many examples of emergence of HPAI avian influenza viruses from low or medium pathogenic avian influenza viruses without any influence from vaccination (Alexander *et al*., 2000). Inactivated oil emulsion avian influenza vaccines have given good protection despite variation of up to 10.9% in haemagglutinin-deduced amino acid sequence (Swayne *et al*., 1999, 2000). Avian influenza vaccination has been most widely practiced in Mexico, beginning in January 1995, and it continues to be used. Over 1.4 billion doses of inactivated vaccine and 500 million doses of fowlpox-AI-H5 recombinant vaccine have been used and the vaccines are still considered protective (Villarreal-Chavez & Rivera-Cruz, 2003).

The ultimate goal of any control programme for avian influenza should be to eradicate HPAI. This was also the goal in Hong Kong during this outbreak, and this goal was achieved. With the presence of these viruses in wild water birds in the region and the large daily cross-border movement of poultry the risk of H5N1 virus incursions infection in Hong Kong is very high. A comprehensive package of measures including enhanced biosecurity programmes for farms, wholesale and retail poultry markets, the use of rest days in markets to break cycles of infection and a comprehensive monitoring and surveillance programme for early detection of any H5 avian influenza virus incursions have been in place since 2001 and were enhanced after the February to April 2002 outbreak. As stressed by international animal health authorities (Alexander *et al*., 2000), avian influenza vaccination in Hong Kong is used to complement the strict biosecurity measures and a comprehensive monitoring and surveillance programme already in place. Comprehensive vaccination of all chicken farms supplying the local retail markets was introduced as an additional layer of protection after a one year long vaccination evaluation trial (Ellis *et al*., 2004b). This investigation showed that the use of killed H5N2 vaccine on three farms undergoing H5N1 HPAI outbreaks was able to protect chickens against disease and also to interrupt asymptomatic virus shedding. This is particularly relevant when dealing with viruses such as H5N1 where the virus also poses a significant risk to human health.

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