9

Evaluation of vaccination to support control of H5N1 avian influenza in Hong Kong

T.M. Ellis[#], L.D. Sims, H.K.H. Wong, L.A. Bissett, K.C. Dyrting, K.W. Chow, C.W. Wong

Abstract

In 1997, 2002 and 2003 highly pathogenic avian influenza (HPAI) was diagnosed on chicken farms in Hong Kong. Following the February-April 2002 outbreak, vaccination using a killed oil-adjuvanted H5N2 avian influenza vaccine was evaluated as an additional control measure on 22 farms within a 2-km radius of the four farms that were depopulated following infection with HPAI H5N1 virus. Vaccination produced satisfactory flock antibody responses. The serological response was improved following a second dose of vaccine and the response to vaccination was poorer when delivered to older birds compared to birds first vaccinated at 8 days of age. Infection with field virus was not detected in any of these vaccinated flocks so the protective effect of the vaccine was tested under secure laboratory conditions on vaccinated and unvaccinated chickens challenged with HPAI H5N1 virus. Vaccinated birds were protected from disease, virus excretion was not detected in eight of ten vaccinated birds and the two birds that did excrete virus excreted much less virus than unvaccinated controls (> 1000 fold reduction). In December 2002 HPAI H5N1 outbreaks in 2 waterfowl parks and deaths in wild water birds in Hong Kong were followed by outbreaks on five previously unvaccinated chicken farms. Vaccination used in the face of outbreaks on three of these farms, coupled with selective culling, resulted in elimination of H5N1 virus infection from these farms. These investigations showed that the killed H5N2 vaccine, used in conjunction with enhanced biosecurity measures on chicken farms and in poultry markets, reduced the risk of H5N1 avian-influenza outbreaks in Hong Kong and consequently the risk of spread to humans.
Keywords: avian influenza H5N1; killed H5N2 vaccine; chickens; Hong Kong; evaluation

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. High mortality rates were seen in gallinaceous birds on farms (1997, 2002, 2003) and/or in poultry markets (1997, 2001, 2002, 2003) in all outbreaks, but not in wild or captive water birds until late 2002. Outbreaks of H5N1 HPAI occurred in waterfowl (geese, ducks and swans) and other wild water birds (Little Egrets *Egretta garzetta*, and in captive Greater Flamingo *Phoenicopterus ruber*) at two waterfowl parks in

[#] Corresponding author: Dr T.M. Ellis, Agriculture, Fisheries and Conservation Department, 303 Cheung Sha Wan Road, Kowloon, Hong Kong SAR. E-mail: ellis_trevor@afcd.gov.hk

Hong Kong in December 2002. HPAI H5N1 virus was also isolated from two dead wild Grey Heron (*Ardea cinerea*) and a Black–headed Gull (*Larus ridibundus*).

In the 1997 avian-influenza outbreak a strain of H5N1 HPAI virus spread directly to humans, causing 18 influenza cases with death in six people (Shortridge et al. 2000). This had a dramatic effect on the perception of avian influenza worldwide and a substantial economic impact in Hong Kong. The commercial chicken population of Hong Kong, (1.3 million birds) was killed in December 1997 (Shortridge 1999), live poultry markets remained closed for several months, there was a significant drop in tourism to Hong Kong and a comprehensive H5N1 testing and surveillance system had to be introduced for local and imported poultry. The 2001 retail poultry market H5N1 HPAI outbreak resulted in culling of 440,000 birds in poultry markets, closure of the markets and culling of 800,000 unaffected older market-age chickens on farms. The H5N1 HPAI outbreaks on farms in early 2002 resulted in the culling of 900,000 chickens. Although no human cases of disease occurred, disruption to the poultry trade and effects on tourism to Hong Kong (due to perceived risks by tourists) caused significant economic and social costs.

After the 2001 outbreak the poultry farm and market biosecurity measures and monitoring systems in place since 1998 were further enhanced. Detailed epidemiological study of the February-April 2002 H5N1 HPAI outbreak by an Investigation Team recommended further measures to improve farm and market biosecurity. All these measures have now been included in individual farm biosecurity plans for all local poultry farms and these form part of the poultry farms' licence conditions. Due, in part, to the large daily movement of poultry into Hong Kong from Southern China and the possibility of H5N1 virus infections occurring in the region the H5 avian-influenza vaccine was introduced as an additional control measure.

Vaccines have been used in other countries to assist in the control of avian influenza. Countries using vaccines against influenza viruses include Italy (Capua et al. 2003a), USA (Halvorson 2002), Mexico (Villarreal and Flores 1997) and Pakistan (Naeem 1997). 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).

Field evaluation of a commercial killed H5N2 vaccine on chicken farms in Hong Kong in terms of adequate H5 antibody response and protection from challenge with current Hong Kong H5N1 HPAI viruses is reported. The effect of vaccination in the face of an outbreak was also examined in three chicken farms which were not included in the original vaccination evaluation trials.

Materials and methods

Evaluation procedures for an H5N2 avian-influenza vaccine in Hong Kong

The vaccine used in these evaluations was Nobilis® Influenza H5, an inactivated avian-influenza Type A H5N2 virus (A/Chicken/Mexico/232/94/CPA) water-in-oil emulsion vaccine (Intervet International, Boxmeer, The Netherlands). The dose, 0.5 ml, was administered subcutaneously into the neck in young chickens and intramuscularly into the breast muscle of older chickens by farm workers after instruction from Agriculture, Fisheries and Conservation Department (AFCD) staff.

Initially (Phase-1 vaccination programme), an evaluation trial was conducted on chicken farms in the district involved in the last four cases of H5N1 HPAI in the

February-April 2002 outbreak. After H5N1 HPAI outbreaks in waterfowl parks and wild birds in December 2002 the vaccination programme was extended to farms considered at higher risk from wild-bird transmission (Phase 2). Subsequently, with H5N1 HPAI outbreaks on five unvaccinated chicken farms, vaccination was used during the outbreak on three farms and its effect was evaluated (Phase 3).

Chicken farms in Hong Kong are broiler farms rearing yellow meat chickens favoured by consumers in Hong Kong. They receive day-old chickens from breeder farms in Mainland China. Chickens are reared in cages and are marketed as a batch at around 90-100 days of age. There were about 150 active chicken farms in Hong Kong at the time, producing 8 million chickens per year (approximately 20% of consumption) for sale in Hong Kong. The remaining birds are derived from the Mainland.

Phase-1 vaccination programme

Phase-1 vaccine evaluation was conducted on 22 chicken farms in the Pak Sha district of the New Territories in Hong Kong and located within 2 km of the last four chicken farms infected with H5N1 virus in the February-April 2002 outbreak. Vaccinations commenced in April 2002 and the programme continued on these farms until March 2003. In the first round all chickens between 8 and 55 days of age were vaccinated and revaccinated 4 weeks later. Subsequently, all new batches of chickens were vaccinated at 8-10 days of age and again four weeks later. Each batch had a group of 30 individually identified chickens left unvaccinated (sentinels).

Blood was collected from the 30 unvaccinated sentinel chickens and 30 individually identified vaccinated chickens 4 weeks after the first and second dose of vaccine and again within 5 days of sale. Serum was tested by standard haemagglutination-inhibition test (Alexander 2000) for antibody to H5 avian influenza using avian-influenza A/chicken/Hong Kong/97 (H5N1) virus antigen. Sentinel or vaccinated chickens that died were subjected to necropsy examination and tested for the presence of avian influenza virus by standard procedures (Alexander 2000). Prior to sale a sample of 60 chickens per batch had cloacal swabs collected and tested for presence of H5 virus by NASBA (Collins et al. 2002) or real-time RT-PCR (Spackman et al. 2002).

Statistical analysis was conducted on post-vaccination antibody responses on the vaccinated flocks from these farms using analysis of covariance with repeated measures (Wong 2003).

The criteria empirically set for successful use of the vaccine in this farmer-administered vaccination programme were that at least 90% of batches developed a measurable antibody response (HI titre $\geq$ 16) after one vaccination, that $\geq$ 70% of chickens per batch had a HI titre $\geq$ 16 after two doses of vaccine and that the geometric mean titre (GMT) by HI test of the batch after two doses was $\geq$ 20.

Experimental challenge procedure

An experimental challenge with a current H5N1 virus was conducted in a biosafety level 3 laboratory using 71-day-old vaccinated and sentinel chickens from a single batch of chickens from one vaccinated farm. The virus used was a H5N1 ("Z" genotype) virus isolated from a dead chicken from a retail poultry market in April 2002. The virus was inoculated via the eye, nose and beak into 10 vaccinated chickens and 10 sentinel chickens such that each chicken was challenged with approximately 15,000 ($10^{4.2}$) egg infectious doses (EID) of virus per chicken.

After challenge all chickens were examined daily for signs of disease and swabs were collected daily from cloaca and throat of all surviving chickens for the duration of the trial (10 days). Surviving chickens were tested for antibody at 10 days post-challenge.

Phase-2 vaccination programme

In late December 2002 a decision was taken to extend the H5N2 vaccination programme to areas around the initial 22 farms and to farms deemed to have a high potential risk of exposure to wild birds (Phase-2 vaccination programme). This was done in response to waterfowl wild-bird and retail poultry-market outbreaks.

Vaccination commenced on 53 farms from 23 December 2002. As with the Phase-1 (Pak Sha) vaccination trial, initially all chickens up to 55 days of age were vaccinated twice at a monthly interval and then subsequent batches of chickens were vaccinated at 8 and 36 days of age. Antibody response was measured a month after the second vaccination to determine the proportion of batches with ≥ 70% of chickens with HI antibody titre ≥ 16 and the overall percentage of chickens with HI antibody titre ≥ 16.

Phase 3 – vaccination of chicken flocks during an outbreak

In late December 2002 to end of January 2003 outbreaks of HPAI caused by H5N1 virus occurred on five previously unvaccinated chicken farms. Immediate quarantine and movement control was initiated and two farms were completely depopulated. On three farms affected sheds were depopulated and strict biosecurity procedures, vaccination and close daily monitoring of other sheds and surrounding farms started. In two farms (TKP1 and TKP2) infection spread to adjacent sheds before vaccination had time to induce an immune response. This enabled monitoring of the effect of the vaccine in the face of field challenge with virulent H5N1 virus.

On farm TKP1, HPAI was detected in eight sheds (30,000 chickens) in relatively close proximity between 7 and 16 January 2003. These were depopulated in two stages, three on 11 January and five on 16 January. A single large, more isolated shed (20,000 birds) that had been vaccinated 9 days previously was closely monitored.

Farm TKP2 was adjacent to TKP1 and various sheds had been vaccinated between 8 and 14 January 2003 as part of the control programme around TKP1. Low-level mortality was detected in two adjacent sheds on TKP2 on 20 January 2003 and these sheds (5,300 birds) were depopulated immediately with close monitoring of other sheds.

Sequential measurements of serum H5 antibody levels and cross-sectional virus culture of cloacal and throat swabs from chickens in affected sheds were conducted on TKP1 until 14 February 2003 (38 days p/v) and on TKP2 until 20 February 2003 (42 days p/v) using procedures referred to above. Subsequently all ongoing batches from these farms were checked for absence of H5 antibody in unvaccinated sentinel chickens and virus-testing using RRT-PCR for H5 virus was conducted using procedures referred to above on 60 chickens per batch until May 2003.

A third farm (SKT) situated over 1 Km away had one affected shed (5,600 chickens) on 20 January 2003 that was depopulated next day. The other sheds on the farm and nine nearby farms were vaccinated on 23 January and all were subjected to daily monitoring for H5N1 HPAI-affected birds. Unvaccinated sentinel chickens were tested for H5 antibody and 60 random cloacal swabs were tested for virus from each batch of market-aged chickens.

Results

Antibody responses and virus monitoring on Phase-1 farms

In total 248 batches of chickens involving 1.35 million birds were vaccinated and fully tested, by 31 March 2003. No clinical outbreaks of disease associated with H5N1 virus were detected on any of these vaccinated farms. Nor was any H5N1 virus detected in tests conducted on the chickens from these farms prior to sale or on dead sentinel chickens. Ninety-eight percent of batches had detectable antibody after the first dose of vaccination and 80% of the 248 batches of chickens developed satisfactory antibody levels after two doses of vaccination. The monthly breakdown of results for field vaccinations is shown in Table 1.

Table 1. Results of Phase-1 vaccination in Pak Sha based on the month when chickens received the first dose of vaccine

Month given first dose	Number of successful batches	Total batches vaccinated	Proportion successful batches (%)	Mean no. antibody-positive *	Mean antibody titre **
April 2002	21	42	50%	70%	31.0
May 2002	14	21	67%	76%	37.7
June 2002	17	27	65.4%	70%	39.3
July 2002	17#	23	73.9%	82%	49.3
Aug. 2002	16	17	94.2%	86%	50.6
Sept. 2002	23	25	92%	87.7%	75.2
Oct. 2002	25	28	89.3%	84.8%	69.6
Nov. 2002	25	29	86.2%	84.4%	66.4
Dec. 2002	16	20	80.0%	77.5%	43.4
Jan. 2003	13	16	81.3%	85.2%	53.8
Total	188	248	75.8%	80.5% $^{+}$	50.9 $^{+}$

* % of birds with HI titre ≥ 16 at one month after second vaccination
** Mean geometric titre of batches at one month after second vaccination
# Five of these batches were very marginally below the success target
$^{+}$ Weighted mean allowing for numbers of batches per month

Of the 60 batches that did not meet the success targets, 16 batches (first vaccinated in April 2002) were vaccinated as older chickens (>21 days), 15 batches were marginally outside targets, 12 batches occurred on three farms on which batches of birds vaccinated in the early part of the trial responded poorly but the response in later batches improved subsequently, and 17 were individual batches within farms that otherwise had a good response to vaccination in other batches of chickens.

Statistical analysis of the antibody-response data confirmed that the GMT and the proportion of seropositive chickens after two vaccinations were significantly higher than after one vaccination ($P < 0.001$ for both). Older birds had a significantly decreased GMT ($P = 0.007$) and a significantly reduced percentage of seropositive birds ($P < 0.001$) after second vaccination. Vaccination of birds early in the programme was also associated with significantly lower GMT after second vaccination than vaccination later in the programme ($P < 0.001$). The latter is most probably related to proportions of flocks vaccinated at an older age in the first one to two months of the programme. By the third month all birds were being vaccinated at 8-10 days and 36-38 days of age. There was also a significant association ($P = 0.001$) between farm of origin and GMT after second vaccination (Wong 2003).

In total 202 dead sentinel or vaccinated chickens from 67 batches of birds from the vaccine trial farms were necropsied and cultured for H5N1 virus. Most deaths involved individual sentinel or vaccinated chickens in the 20-50-day-old range. Several farms had more than four sentinel deaths in a batch on more than one occasion caused by virulent Newcastle disease virus (ND). Known immunosuppressive diseases diagnosed in necropsied chickens included infectious bursal disease (IBD), ND, Marek's disease, infectious laryngo-tracheitis and chronic respiratory disease (CRD) complex (infectious bronchitis virus +/or ND virus + *Mycoplasma* spp.). Other parasitic and bacterial diseases encountered included coccidiosis, airsacculitis, peritonitis, coryza and colibacillosis.

Experimental challenge

The H5 HI titres in the vaccinated chickens at challenge ranged from 32 to 256. No sentinel chickens had antibody to H5 influenza virus. After challenge, none of the vaccinated chickens became ill whereas all the unvaccinated sentinel chickens died within 3 days. This indicated a highly significant level of protection (Chi-square = 20, p = 0.00000). Vaccinated chickens excreted markedly reduced titres of virus via cloaca or throat. Throat swabs from unvaccinated chickens contained an average of 18,000 EID of virus. No vaccinated chickens had detectable virus in throat or cloaca on day 2 or any other day except for day 4 post-infection when two vaccinated chickens had low levels of virus in the throat ($10^{1.0}$ EID) (see Table 2).

Table 2. Summary of results of experimental H5N1 challenge

Type of chicken	Proportion infected (mean days to 1st signs)	Proportion dead (mean death time)	HI antibody-positive 10 days post-challenge	Cloacal virus (titre on day 2)*	Throat virus (titre on day 2)*
Not vaccinated	10/10 (2)	10/10 (2.3)	None survived	7/10 ($10^{2.03}$)	10/10 ($10^{4.25}$)
Vaccinated (GMT = 119)	0/10	0/10	10/10 (GMT= 239)	0/10	0/10**

* Virus titre = Log_{10} embryo infectious doses (EID) per swab via chicken embryo allantoic route.
** virus was detected in two vaccinated birds at low level ($10^{1.0}$ EID) on day 4 only.

Phase-2 vaccination programme

A total of 60 batches of chickens received two doses of vaccine. Thirty-two batches (53.3%) had ≥ 70% of chickens with HI antibody titre ≥ 16 and overall 69.2% of the chickens had HI antibody titre ≥ 16.

No clinical outbreaks of disease associated with H5N1 virus infection were detected on any of the Phase-2 vaccinated farms, no H5N1 virus was detected in tests conducted on any dead or sick chickens submitted from these farms as part of the sentinel and surveillance programme and none of the sentinel chickens gave positive H5 antibody results. Some 1.55 million vaccine doses were given to chickens on these farms with no adverse effects apparent from use of this killed vaccine.

The vaccination response in Phase 2 was not significantly different from that for the first round of Phase 1, which also involved vaccination of birds ranging from 8 to 55 days of age. In Phase 2 nearly half (28/60) the batches received their first dose of vaccine when older than 21 days of age and only 19 batches received their first vaccination at 8-10 days of age. Problems of vaccination technique with older chickens and immunosuppressive diseases are likely to have had a similar effect on the first round of vaccination on these farms as they did with the Phase 1 farms.

Phase 3 – vaccination of chicken flocks during an outbreak

Use of the killed H5N2 vaccine in the face of a H5N1 outbreak on Farms TKP1 and TKP2 showed that it was able to provide significant protection from disease and shut down virus excretion by 13-18 days post-vaccination. On the third farm (SKT) where vaccination was used and in the nine vaccinated surrounding farms no H5N1 HPAI or H5 virus infection was detected by serological monitoring of sentinels or virus culture of 60 random cloacal swabs per batch. In the latter farms it is possible that by rapid depopulation of the affected shed and by strict attention to biosecurity other sheds on the farm or surrounding farms may not have been exposed to H5N1 virus.

Discussion and conclusions

The vaccine evaluation studies showed that the killed H5N2 vaccine produced a satisfactory flock antibody response against H5 haemagglutinin antigen and could protect vaccinated chickens against highly pathogenic avian influenza that is caused by current Hong Kong strains of H5N1 virus. Moreover, vaccination produced a substantial reduction (>1000-fold) in excretion of infectious H5N1 virus in vaccinated compared with unvaccinated chickens and was able to protect chickens and shut down the virus excretion on infected farms by 13-18 days post-vaccination.

Post-immunization antibody measurement in humans vaccinated with A/New Caledonia/20/99 (H1N1) vaccines showed 78% of adult and 66% of elderly vaccinees responded, and for recent human isolates, including H1N2 viruses, the responses were similar with 70% of adults and 55% of elderly vaccinees positive. Vaccines containing influenza A/Panama/2007/99 (H3N2) and recent similar H3N2 viruses gave antibody responses in 66-71% of adult and 72-78% of elderly vaccines (Studies with inactivated influenza virus vaccines 2003). These population antibody responses are in line with the empirical success criteria used in these trials (≥ 70% of the flock antibody-positive with GMT of ≥ 20). The overall result of 80.5% antibody-positive birds with GMT of 50.9 supports our conclusion that the vaccine produced a satisfactory flock antibody response. While no studies have been conducted to determine the level of herd immunity that is protective for poultry against HPAI, the results of the phase-3 trial and the absence of HPAI in any of the fully vaccinated farms in 2002-03 in the face of circulating HPAI virus suggests that the levels achieved provide adequate flock immunity.

Commencing vaccination at an older age and farm of origin was significantly associated with poor antibody response. At the commencement of the programme batches of chickens up to 55 days of age were vaccinated. Experience in Hong Kong generally and in the current vaccination evaluation study was that birds in the 20 to 50-day age range are exposed to and are often susceptible to immunosuppressive effects from diseases such as IBD and ND. Initial vaccination at that age in immunosuppressed birds is likely to give inadequate immune-system priming with consequent poor antibody response from secondary vaccination. The farm-of-origin effect may also relate to the fact that multiple batches of chickens on individual farms, including older birds, were vaccinated at one time. Farmers in Hong Kong have limited experience of vaccinating older birds (there are no broiler breeders in Hong Kong).

Independent evaluation of the ability of the killed H5N2 vaccine to protect against experimental challenge with current Hong Kong H5N1 virus was conducted by

Professor Robert Webster's group at the WHO Reference Laboratory for Avian Influenza in Memphis, Tennessee, USA. They showed that chickens vaccinated at the same ages as for the field vaccination programme and challenged 3 weeks later with another highly pathogenic Hong Kong strain of H5N1 virus ('Z' genotype from the index farm in the February 2002 Hong Kong outbreak) were significantly protected from disease and showed reduced levels of virus excretion compared with unvaccinated controls (R.G. Webster, personal communication).

Although vaccination in the face of an outbreak was tried and on 2 farms was able to protect birds and shut down virus replication, this is not a suitable option in Hong Kong where all farms are in relatively close proximity due to limited land availability. It is very difficult to define and control an epidemiologically sustainable perimeter for infected and dangerous contact areas around which ring vaccination could be used. This would also require vaccination of older birds which may give sub-optimal antibody responses, and there is also potentially a greater chance of selection of variant viruses if virus is replicating rapidly in the presence of partial or incomplete flock immunity than there would be if virus is introduced to a fully vaccinated flock that has had time to develop its immunity.

While the ultimate goal should be to eradicate highly pathogenic avian influenza viruses when they occur, the presence of these viruses in wild birds in the Southern-China region means that this is not possible at present and the risk of infection in Hong Kong is very high. Enhanced biosecurity can reduce this risk but vaccination provides an additional layer of protection and its use is fully justified under these special circumstances.

Concerns have been raised that the use of influenza vaccines in poultry may accelerate antigenic drift of the virus necessitating frequent changes of vaccine composition. In addition, it has been suggested that there may be prolonged but undetected virus shedding in vaccinated chickens, that there will be delays in detecting emerging strains and that vaccination may undermine the push for enhanced biosecurity.

Vaccination elsewhere does not appear to have increased the risk of selection of new strains of virus. In sequential characterization of H5N2 viruses from Mexico, where AI vaccination has been practiced most widely, the Southeast Poultry Research Laboratory, USDA (SEPRL) has not demonstrated any acceleration of 'drift' and the vaccines are still protective (D.E. Swayne, personal communication). In experimental studies at SEPRL, H5 avian-influenza vaccines could protect against H5 viruses isolated from four continents over a 38-year period, despite variation of up to 10.9% in deduced amino-acid sequence of the haemagglutinins (Swayne et al. 1999; 2000).

In fact, evolution and selection of highly pathogenic avian influenza viruses occurs in the absence of vaccination. Low or mildly pathogenic avian influenza viruses in USA (1983-84), Mexico (1994-95) and Italy (1999-2000) have mutated to highly pathogenic influenza viruses without the influence of vaccination (D.E. Swayne, personal communication).

Field experience in a H7 outbreak in turkeys in Utah in 1995 (Halvorson et al. 1997) and on two farms in the 2003 H5N1 outbreak in Hong Kong indicated that new cases of disease stopped in vaccinated flocks and the virus was eliminated. There is no evidence to suggest that vaccination led to prolonged undetected shedding of virus. As vaccinated birds exposed to H5N1 viruses shed far less virus than their non-vaccinated counterparts it is likely that infection in properly vaccinated flocks will be self-limiting.

The monitoring programme instigated on vaccinated chicken farms in Hong Kong involves 60 individually identified unvaccinated sentinel chickens within each batch of chickens. The sentinels are monitored serologically, clinically and if required virologically over the 90 to 100-day production cycle for evidence of H5 avian influenza virus infection. This is relatively easy to achieve with the cage-rearing system used in Hong Kong. 'DIVA' (differentiating infected from vaccinated animals) serological testing has been implemented elsewhere as an aid to detecting avian-influenza infection in vaccinated flocks (Capua et al. 2003b). However, DIVA testing is likely to be less sensitive than sentinel-bird monitoring when dealing with a highly pathogenic influenza virus that kills virtually all infected non-immune birds.

The results from this study demonstrate that killed H5N2 influenza-virus vaccine is suitable for inclusion into control programmes for H5N1 avian influenza in Hong Kong. However, vaccination will only be used as part of a 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 rapid detection of any H5 avian influenza virus incursions. The latter includes dead-bird testing from farms, wholesale and retail markets and a regular programme of viral culturing of cage swabs from farms and retail markets. The farm monitoring also includes antibody testing to ensure vaccinated flocks maintain adequate H5 antibody levels.

Acknowledgments

We thank the staff of the Avian Influenza Serology, Avian Virology, Molecular Biology, Histology and Bacteriology laboratories at Tai Lung Veterinary Laboratory for their excellent technical support and field staff of Livestock Farm Division for the supervision of the on-farm vaccination, bird identification, clinical inspection of vaccinated and sentinel birds, blood and virology sample collection, which were all conducted in a highly competent manner. The assistance provided in the data analysis of the Phase-1 vaccination trial by Professor T.B. Farver, Department of Population Health and Reproduction, University of California, Davis is gratefully acknowledged.

References

Alexander, D.J., 2000. Highly pathogenic avian influenza. *In: OIE manual of standards for diagnostic tests and vaccines*. 4th edn. World Organisation for Animal Health OIE, Paris, 212-220.

Capua, I., Marangon, S., Dalla Pozza, M., et al., 2003a. Avian influenza in Italy 1997-2001. *Avian Diseases,* 47 (Suppl. S), 839-843.

Capua, I., Terregino, C., Cattoli, G., et al., 2003b. Development of a DIVA (Differentiating Infected from Vaccinated Animals) strategy using a vaccine containing a heterologous neuraminidase for the control of avian influenza. *Avian Pathology,* 32 (1), 47-55.

Collins, R.A., Ko, L.S., So, K.L., et al., 2002. Detection of highly pathogenic and low pathogenic avian influenza subtype H5 (Eurasian lineage) using NASBA. *Journal of Virological Methods,* 103 (2), 213-225.

Halvorson, D.A., 2002. The control of H5 or H7 mildly pathogenic avian influenza: a role for inactivated vaccine. *Avian Pathology,* 31 (1), 5-12.

Halvorson, D.A., Frame, D.D., Friendshuh, A.J., et al., 1997. Outbreaks of low pathogenicity avian influenza in USA. *In:* Slemons, R.D. ed. *Proceedings of the 4th international symposium on avian influenza, held May 29-31, 1997*. US Animal Health Association, Georgia Center for Continuing Education, The University of Georgia, Athens, 36-46.

Naeem, K., 1997. The avian influenza H7N3 outbreak in South Central Asia. *In:* Slemons, R.D. ed. *Proceedings of the 4th international symposium on avian influenza, held May 29-31, 1997*. US Animal Health Association, Georgia Center for Continuing Education, The University of Georgia, Athens, 31-35.

Shortridge, K.F., 1999. Poultry and the influenza H5N1 outbreak in Hong Kong, 1997: abridged chronology and virus isolation. *Vaccine,* 17 (Suppl. 1), S26-S29.

Shortridge, K.F., Gao, P., Guan, Y., et al., 2000. Interspecies transmission of influenza viruses: H5N1 virus and a Hong Kong SAR perspective. *Veterinary Microbiology,* 74 (1/2), 141-147.

Spackman, E., Senne, D.A., Myers, T.J., et al., 2002. Development of a real-time reverse transcriptase PCR assay for type A influenza virus and the avian H5 and H7 hemagglutinin subtypes. *Journal of Clinical Microbiology,* 40 (9), 3256-3260.

Studies with inactivated influenza virus vaccines, 2003. *Weekly Epidemiological Record,* 78 (9), 60. [http://www.who.int/entity/wer/2003/en/wer7809.pdf]

Swayne, D.E., Beck, J.R., Garcia, M., et al., 1999. Influence of virus strain and antigen mass on efficacy of H5 avian influenza inactivated vaccines. *Avian Pathology,* 28 (3), 245-255.

Swayne, D.E., Beck, J.R., Perdue, M.L., et al., 2001. Efficacy of vaccines in chickens against highly pathogenic Hong Kong H5N1 avian influenza. *Avian Diseases,* 45 (2), 355-365.

Swayne, D.E., Garcia, M., Beck, J.R., et al., 2000. Protection against diverse highly pathogenic H5 avian influenza viruses in chickens immunized with a recombinant fowlpox vaccine containing an H5 avian influenza hemagglutinin gene insert. *Vaccine,* 18 (11/12), 1088-1095.

Villarreal, C.L. and Flores, A.O., 1997. The Mexican avian influenza H5N2 outbreak. *In:* Slemons, R.D. ed. *Proceedings of the 4th international symposium on avian influenza, held May 29-31, 1997*. US Animal Health Association, Georgia Center for Continuing Education, The University of Georgia, Athens, 18-22.

Wong, H., 2003. *Evaluation of a chicken vaccination programme with an inactivated H5N2 avian influenza vaccine to prevent outbreaks of highly pathogenic H5N1 avian influenza in Hong Kong*. Thesis University of California, Davis.