Use of a tetanus toxoid marker to allow differentiation of infected from vaccinated poultry without affecting the efficacy of a H5N1 avian influenza virus vaccine

C. M. James-Berry, D. Middleton, J. P. Mansfield, S. G. Fenwick, T. M. Ellis

Tetanus toxoid (TT) was assessed as a positive marker for avian influenza (AI) virus vaccination in chickens, in a vaccination and challenge study. Chickens were vaccinated twice with inactivated AI H5N2 virus vaccine, and then challenged three weeks later with highly pathogenic AI H5N1 virus. Vaccinated chickens were compared with other groups that were either sham-vaccinated or vaccinated with virus with the TT marker. All sham-vaccinated chickens died by 36 hours postinfection, whereas all vaccinated chickens, with or without the TT marker, were protected from morbidity and mortality following exposure to the challenge virus. Serological testing for H5-specific antibodies identified anamnestic responses to H5 in some of the vaccinated birds, indicating active virus infection.

RECENT epizootics of highly pathogenic avian influenza (HPAI) H5N1 virus in many countries in Asia, Europe, the Middle East and Africa have led to HPAI becoming endemic in some poultry populations. Subclinically infected wild birds shed virus into watery environments and contaminate other terrestrial birds and domestic flocks, leading to potential transmission to human beings (Sturm-Ramirez and others 2004). Effective control of AI is hampered by many social and economic issues at local, regional and national levels (Macan-Marker 2007). Most Asian countries with poultry industries consisting of household, small farm and village flocks cannot control AI using stamping-out as the principal means. The closure of five animal markets reduces income and animal protein supplies for families in rural communities. Inadequate or no compensation to farmers with infected flocks results in unwillingness to report outbreaks to government authorities. Furthermore, trade sanctions related to countries with AI virus (AIV) infection place increased pressure on some of the vaccinated birds, indicating active virus infection.

Vaccination, in combination with vigilant monitoring and biosecurity measures, has been recommended for effective HPAI control programmes (Swayne 2003, Lee and Suarez 2005). However, AI disease outbreaks still occur, probably due in part to incomplete vaccination of entire flocks and poor monitoring for AIV infection. Vaccination complicates serosurveillance for AIV based on haemagglutinin (H)-specific antibody testing, as both vaccinated and naturally infected birds produce similar antibodies. It would be valuable to be able to distinguish vaccinated birds that are protected due to the presence of antibodies resulting from previous H5 vaccination from vaccinated birds that are silently infected by wild H5 AIV (Halvorsen 2002). Furthermore, monitoring flocks would also check that vaccination has been carried out with an approved/effective vaccine and delivered appropriately to the birds. Various strategies have been developed but have limitations for differentiating infected from vaccinated animals (DIVA), including testing for antibodies to heterologous neuraminidase (N), and internal viral antigens as negative endogenous markers. These strategies have been conceived to enhance virus surveillance, prevent unnecessary culling and regulate poultry vaccination, movement and trade (Grogan and others 2007). At present, the heterologous N DIVA approach has been validated for use only with low pathogenicity AI (LPAI) virus surveillance in chickens and turkeys (Suarez 2005, Capua and Alexander 2006), and this strategy is affected by concurrent circulation of AIVs with the same N subtype in the poultry population. Many recent HPAI disease outbreaks have occurred in China, Vietnam and Indonesia, which have concurrent circulation of different AIV strains, making heterologous N DIVA approaches impractical. Also, the varying extent to which poultry vaccination is practised in these regions, coupled with poor biosecurity, currently sustains susceptible populations as reservoirs for the virus.

This paper describes a practical approach to virus surveillance based on the observation that avian species are naturally resistant to tetanus and are not seropositive for antibodies to tetanus toxoid (TT) (Hagan and Bruner 1961). A positive exogenous marker for vaccination of birds, which allows testing by a cost-effective ELISA independent of circulating AIV strains, was developed by the authors. Recently, the authors reported 100 per cent seronegativity for naturally acquired antibodies to TT in over 2250 chickens and ducks, but upon TT vaccination both species produced strong antibody responses to TT antigen (James and others 2007, 2008). In ducks, the authors have recently found that the efficacy of vac...
Vaccine efficacy

All chickens received a priming vaccination at 19 days of age and a booster four weeks later, at 47 days of age. The AIV vaccine was a commercial inactivated AIV A/chicken/Mexico/232-CPA/94 H5N2 (A/ch/Mex/94) water-in-emulsion preparation (Nobilis Influenza H5, Intervet). The TT vaccine was a water-in-oil emulsion consisting of a commercial prevacuum formulation of TT (estimated total protein 30 mg/dose; Pfizer) mixed 1:1 (v/v) with Montanide ISA 70 VG (Seppic) as previously described by James and others (2007). One group of 10 chickens was given inactivated AIV H5N2 vaccine (H5N2) alone and another group of 10 birds was covaccinated with TT and inactivated AIV H5N2 vaccine (TT/H5N2), given as two separate injections delivered at the same site. A control group of 10 chickens was sham-vaccinated with PBS at 19 and 47 days of age. The vaccines and PBS were administered as water-in-oil emulsions given subcutaneously in the dorsal midline at the lower end of the nape of the neck near the anterior dorsal thorax, in a volume of 0.5 ml for the priming dose and 1 ml for the booster dose. Three weeks after the second vaccination or sham vaccination, the chickens in all groups were challenged via the intranasal, intraocular (eye drop) and intraoral routes with 0.5 ml of an AIV suspension containing 10^{5.2} EID_{50} of HP AI H5N1 A/chicken/Vietnam/02/2004 H5N1 (A/ch/VN/04). A 1 ml syringe was used to administer the virus into the nares, the mouth and on to the conjunctiva. The challenge isolate was prepared from infected allantoic fluid (passed three times in SPF chicken eggs). Back-titration of the inoculum confirmed that the dose administered to each chicken was 10^{5.2} EID_{50} H5N1 virus. Fourteen days after the challenge, all the surviving chickens were euthanased.

Morbidity and mortality

After challenge, the chickens were observed twice daily throughout the study for clinical signs of HPAI.

Antibody responses

Blood samples were obtained from each chicken before the first and second vaccinations, immediately before the challenge, and from each chicken that was still alive 14 days after the challenge. Blood samples were collected by venepuncture from the wing veins. Oropharyngeal and cloacal swabs from each challenged bird were taken on days 2, 3, 4 and 7 after challenge. The swabs were placed in isotonic PBS (pH 7.0 to 7.4) containing the antibiotics penicillin and streptomycin. The samples were frozen at −20°C before processing for AIV isolation.

H5-specific antibody titres were determined by haemagglutination inhibition (HI) assay using procedures previously described (WHO 2002) and homologous AI challenge virus. The HI titre was determined as the highest dilution of serum giving complete inhibition of haemagglutination, and data were expressed as the reciprocal end-point dilution of sera from individual chickens and mean reciprocal titre log_{10} (+ standard error of the mean) (se) for each group.

Serum antibody levels to TT were determined by ELISA (James and others 2007). Briefly, 0.012 µg purified TT antigen (List Biological Laboratories) was used as the coating antigen. Primary antibody was assayed in duplicate (diluted 1:200) from individual chickens at each time point, and rabbit anti-chicken F(ab')_{2}, antibody conjugated to horseradish peroxidase (diluted 1:40,000, Chemicon International) was used to label the primary antibody. The optical density (OD) of the plates was read at 450 nm with 630 nm reference wavelengths using a spectrophotometer (Bio-Rad Model 680). Controls included in each plate were six replicates of the negative control (pooled pre-bleeds [control sera taken before vaccination] from six naive Hy-Line Brown chickens, diluted 1:200) and six replicates of the positive control (pooled sera, diluted 1:200, collected at week 6 from the same six chickens, which had been vaccinated twice with 1 ml of the commercial tetanus vaccine for horses, Equivac T (CSL) at weeks 0 and 4. The level of TT antibody present in each serum sample was calculated using the formula:

\[
\%\text{TT antibody positive} = \left( \frac{\text{Mean OD test} - \text{Mean OD negative control}}{\text{Mean OD positive control} - \text{Mean OD negative control}} \right) \times 100
\]

Materials and methods

Animals

Thirty 10-day-old SPF chickens (both males and females) were obtained from SPAS Australia and housed in the Biosafety Level 3 containment animal facility of the Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australian Animal Health Laboratories (AAHL), Geelong, Australia. The chickens were supplied with commercial compound feed and tap water ad libitum. TT-unvaccinated female Hy-Line Brown layer pullets (six to seven weeks old) were supplied by Altona Hatchery to provide sera used in the ELISA. Animal experimentation was given prior approval by the CSIRO/AAHL and Murdoch University Animal Experimentation Ethics Committees.

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\[
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\]
of three embryonated chicken eggs at nine to 11 days’ incubation. The eggs were incubated at 35 to 37°C for four to seven days. Allantoic fluids from the eggs were tested for haemagglutinating activity. Samples positive by this test were further titrated in embryonated chicken eggs by serial 10-fold dilution of residual supernatant. Endpoint titres expressed as EID₅₀/0.1 ml represented the last reciprocal dilution of allantoic fluid causing infection of 50 per cent of the eggs, demonstrated by dying or dead embryos.

Haemagglutination titres from allantoic fluids were determined as described previously (OIE 2009). Briefly, chicken red blood cells were mixed with serial dilutions of allantoic fluid and incubated at 4°C for 60 minutes. The highest dilution giving complete haemagglutination was calculated as the endpoint titre.

Statistical analysis
The two-tailed unpaired Student’s t test assuming unequal variance between means was used to determine statistical significance (set at P<0.05).

Results
Efficacy of TT and inactivated AIV (H5N2) covaccination in chickens challenged with HPAI virus (H5N1)
Following challenge with a 10⁻⁵ EID₅₀ dose of HPAI virus isolate A/ch/VA/04 (H5N1), several chickens in the control group showed slight depression 24 hours after exposure to infectious virus. By 36 hours after challenge, all the controls had died, and their deaths were attributed to paroxysmic infection with AIV. In contrast, all the vaccinated chickens survived and remained clinically healthy following the viral challenge. Specifically, depression, respiratory signs, head oedema, cyanosis, subcutaneous haemorrhages, inappetence and diarrhoea were not observed during the two-week observation period following

Table 1: Haemagglutination inhibition (HI) antibody titres to H5 in groups of 10 chickens vaccinated twice at weeks 0 and 4 with inactivated H5N2 avian influenza virus (AIV) or tetanus toxoid (TT)/H5N2 marker vaccine, or left unvaccinated, and challenged with live HPAI H5N1 virus at week 7

<table>
<thead>
<tr>
<th>Week</th>
<th>Control</th>
<th>H5 HI antibody titre*</th>
<th>TT/H5N2 vaccinated</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&lt;4, &lt;4, &lt;4, &lt;4, &lt;4, &lt;4, &lt;4, &lt;4, &lt;4, &lt;4</td>
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</tr>
<tr>
<td>4</td>
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<td>512, 1024, 512, 256, 128, 128, 64, 128, 128, 512</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>&lt;4, &lt;4, &lt;4, &lt;4, &lt;4, &lt;4, &lt;4, &lt;4, &lt;4, &lt;4</td>
<td>512, 1024, 512, 256, 128, 128, 64, 128, 128, 512</td>
<td></td>
</tr>
<tr>
<td>9 NA</td>
<td>128, 64, 256, 32, 128, 256, 64, 128, 128, 256</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Not available for testing as all chickens had died

Table 2: Virus isolation from oropharyngeal swabs from groups of 10 chickens vaccinated twice with inactivated H5N2 avian influenza virus (AIV) or tetanus toxoid (TT)/H5N2 marker vaccine after challenge with live HPAI H5N1 virus

<table>
<thead>
<tr>
<th>Day</th>
<th>Virus isolation and titre (EID₅₀/0.1 ml)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>5/10</td>
</tr>
<tr>
<td>3</td>
<td>2/10</td>
</tr>
<tr>
<td>4</td>
<td>3/10</td>
</tr>
<tr>
<td>7</td>
<td>0/10</td>
</tr>
</tbody>
</table>

* Virus isolation was performed using embryonated chicken eggs and expressed as positive (+) or negative (−) for individual chickens 1 to 10 (left to right in rows), corresponding to Table 1. The EID₅₀ titres for positive virus isolations were 10¹ EID₅₀/0.1 ml except for the positive sample shown in parentheses for day 2 after challenge in the TT/H5N2 group.

Virus shedding in chickens covaccinated with TT and inactivated AIV H5N2 and challenged with HPAI virus (H5N1)
Virus was not reisolated from the cloacal swabs from any vaccinated chicken on days 2, 3, 4 or 7 after challenge (data not shown). In the group vaccinated with inactivated AIV (H5N2), HPAI virus was reisolated from five of 10 oropharyngeal swabs on day 2 postchallenge (Table 2). Interestingly, these positive samples were from four of the five chickens in this group that displayed an anamnestic response to H5. Further virus isolations were obtained from three of these chickens on either day 3 or day 7 after challenge.

In the TT/H5N2-vaccinated group, HPAI virus was reisolated from eight of 10 oropharyngeal swabs on day 2 after challenge (Table 2). The two birds that showed an anamnestic response to H5 were among the birds that were positive for virus isolation. Further virus isolations were made from three of these chickens on either day 3 or day 7 postchallenge.

Challenge virus was detected intermittently in 10 of 40 oropharyngeal swabs from the inactivated AIV H5N2-vaccinated group and in 11 of 40 oropharyngeal swabs from the TT/H5N2-vaccinated group throughout the seven-day sampling period after challenge. In both groups of vaccinated chickens, the titre of virus isolated from the oropharyngeal swabs from virus-positive chickens after challenge was very low, 10⁶ EID₅₀/0.1 ml for 12 chickens and 10⁶ EID₅₀/0.1 ml for one TT/H5N2-vaccinated bird (Table 2). There was no significant difference in the geometric mean titres of virus shed intermittently in the oropharynx between the inactivated AIV H5N2-vaccinated and the H5N2/TT-vaccinated groups after challenge.

Discussion
This study demonstrated that vaccination of chickens with inactivated AIV H5N2 or covaccination with TT/H5N2 resulted in all vaccinated chickens being protected from morbidity and mortality following challenge with a HPAI H5N1 virus that was rapidly and uniformly lethal in unvaccinated chickens. Although all the vaccinated chickens survived challenge with the HPAI H5N1 virus isolate, several chickens vaccinated with either the inactivated AIV H5N2
vaccine or the TT/HSN2 vaccine did become infected after the challenge. This was evident as a very low level of intermittent viral shedding from the oropharynx (10^3.2 EID50/0.1 ml) and boosting of H5 antibody titres, suggesting an anamnestic response in some chickens after challenge with heterologous AIV H5N1. However, there were no significant differences in the frequency of shedding or the titres of virus shed intermittently from the oropharynx between the groups of vaccinated birds with the AIV H5N2 and TT/HSN2 vaccines. Virus was not reisolated from the cloacal swabs from any vaccinated chicken, suggesting that faecal shedding was negligible and that environmental contamination from infected faeces would have been substantially reduced. Although the level of virus shedding in unvaccinated chickens two days after challenge could not be determined because all of these chickens had died by 36 hours after the challenge, the level of virus shedding from the oropharynx of vaccinated chickens was approximately 100-fold lower than the infectious dose required to infect chickens (10^5 EID50/0.1 ml) under standard laboratory conditions with this virus (D. Middleton, personal communication). In other studies of inactivated AIV H5N2 vaccines in chickens that were challenged with the same lineage of H5N1 AIV, the level of virus shedding detected by cloacal swabs (mean titre of 10^5.8 EID50/ml) and oropharyngeal swabs (mean titre of 10^6.6 EID50/ml) in unvaccinated control chickens two days after challenge was substantially higher (10^6 to 10^6-fold) than the virus shedding found in the present study. Furthermore, a similar proportion of vaccinated chickens shed virus from the oropharynx on day 2 after challenge with titres of 10^3.5-7.0 EID50/ml in the oropharynx and 10^3.0-7.0 EID50/ml in the cloaca (Swayne and others 2006).

Al can be a serious threat to animal and public health, as shown by the current epizootic of HPAI H5N1 viruses (Capua and Alexander 2007). It is of particular concern that all 16 H and nine N AIV subtypes continue to circulate in wild Anseriformes and Charadriiformes. To date, H5 and H7 subtypes have spread to domestic poultry, and with increased virulence and transmission some have developed into a problem. Before vaccination, all 30 chickens in the present study were negative for antibodies to TT. This finding supports the authors’ earlier observations of 100 per cent seronegativity for antibodies to TT in chickens two days after challenge with various strains and at various geographical locations (James and others 2007, 2008, Chua and others 2010). This brings the total number of poultry screened for antibodies to TT to 2294, with all being seronegative. These results provide a significant basis for the use of TT as a marker of vaccination in poultry.

A marker vaccine containing TT for inactivated AIV vaccines in poultry has many advantages over current DIVA approaches and has attributes that would enhance AIV surveillance and control in small-scale and village poultry systems. Although the inclusion of TT in vaccines adds incrementally to the cost, which is very low (approximately AU$0.20 per bird), the benefits are in the considerable cost savings for the serological tests, as the ELISA for antibodies to TT is substantially cheaper than the neuraminidase inhibition test. Thus, the use of the TT marker undermines a more economical serological DIVA approach than current alternatives used in the field. Addition of the TT marker to approved H5 AIV vaccines for poultry would allow serological identification of vaccinated flocks in farming systems where record keeping is poor or non-existent. Moreover, the increased use of unapproved vaccines and uncontrolled vaccination with improper distribution and administration of vaccines poses a significant future threat for AIV outbreaks in poultry (Peyre and others 2009). This situation for vaccination programmes can be regulated with the TT marker approach, limiting the potential of the virus to mutate and become a pandemic pathogen. Concurrent H5 antibody testing (by HI) could establish that vaccinated flocks were achieving H5 antibody titres that could protect them from challenge and significantly reduce virus excretion. The TT marker strategy could be used as a tool to monitor compliance with H5 AIV vaccination programmes and to check on the vaccine quality and coverage for poultry entering live bird markets. Serological evidence of compliance with the use of approved/effective vaccines could be regulated, as birds would need to show positive antibody responses to TT with concurrent H5 antibody levels that could be expected to protect them from AIV challenge and could significantly reduce virus excretion before they could be sold at market.

Investigation of deaths in AIV H5-vaccinated flocks could incorporate testing for antibodies to the TT marker. If dead birds in vaccinated flocks were confirmed to be infected with HPAI H5N1 virus, testing for TT antibody could establish whether they had been vaccinated in the past. Testing the dead birds’ cohort for TT and H5-specific antibody responses could determine if the level of vaccination coverage in the flock was poor, and, depending on the H5-specific antibody titres present, whether vaccination was suboptimal or the vaccine did not elicit protection against the circulating field H5N1 AIV. The authors have observed that antibodies to TT can be detected in heart blood of dead vaccinated chickens kept at 4°C for 80 hours after death and that dead chickens kept at ambient temperature (24°C to 30°C) for at least 30 hours (data not shown).

Furthermore, field use of the TT marker with inactivated AIV H5 vaccines could establish normal response curves for TT- and H5-specific antibodies in vaccinated uninfected flocks. The authors have previously reported that the TT antibody response in chickens vaccinated twice with a combined inactivated LPAI virus H6N2 and TT vaccine is sustained to 53 weeks after vaccination (the duration of the study), and that the titre at week 53 was not significantly different from the titre obtained at week 6, but that the H6 HI antibody response peaked at week 6 (9 log2) and then declined (week 53, 5 log2) (James and others 2007). These antibody responses could establish a standard profile of the ratio of TT/H5 HI antibody titre at any given time after vaccination. If altered ratios of antibodies to TT/H5, with higher than expected H5 antibody titres, were found, then this could indicate an anamnestic response to recent AIV H5N1 infection, requiring further virological investigation of the flock.

The authors conclude that chickens vaccinated twice with either inactivated AIV H5N2 vaccine or H5N2/TT vaccine are fully protected from morbidity and mortality associated with challenge by a lethal dose of a heterologous HPAI H5N1 virus. Moreover, co-vaccination with TT and H5N2 AIV antigens did not interfere with the antibody responses to each of the antigens individually. Although low levels of intermittent viral shedding occurred after the viral challenge, this was similar in terms of both the proportion of chickens shedding virus and the virus titres shed to the results of other studies with such
vaccines (Swayne, and others 2006), and the titres of virus shed were not significantly different between the H5N2-vaccinated and H5N2/TT-vaccinated groups. The benefits of the TT marker approach for AIV vaccination in poultry include specific antibody response data allowing evaluation of both vaccine coverage and effectiveness at flock or village level after virus exposure. Furthermore, concurrent HI testing and TT serology will probably provide information on altered ratios of these antibodies and assist in identifying exposure to field strains of AIV in vaccinated poultry flocks. Thus, the TT marker may be used as a tool for the identification of AIV vaccination of poultry and assist veterinary authorities in serosurveillance of HPAI H5N1 virus in countries using vaccination for the control of recurrent H5N1 AIV outbreaks. An integrated effort from the veterinary and medical communities in the control of zoonotic HPAI viruses is urgently required for improved global health (Capua and Cattoli 2010).

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References


