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Maternal immunity against avian influenza H5N1 in chickens: limited protection and interference with vaccine efficacy

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Running title: Maternal immunity against avian influenza H5N1

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Abstract

After avian influenza (AI) vaccination hens will produce progeny chickens with maternally derived AI specific antibodies. In this study we examined the effect of maternal immunity in young chickens on the protection against highly pathogenic AI H5N1 virus infection and on the effectiveness of AI vaccination. The mean haemagglutination inhibition (HI) antibody titre in sera of 14-day-old progeny chickens was approximately 8-fold lower than the mean titre in sera of vaccinated hens. After H5N1 infection at the age of 14 days, chickens with maternal antibody titres lived a few days longer than control chickens. However, only a low proportion of chickens with maternal immunity survived challenge with H5N1. In most progeny chickens with maternal immunity, high virus titres (>$10^4\text{ EID}_{50}$) were present in the trachea during the first 4 days after H5N1 infection. In the cloaca only low virus titres were present in most chickens. In 14-day-old progeny chickens with maternal immunity the induction of antibody titres by vaccination was severely inhibited, with only a few chickens showing responses similar to the control chickens. It is concluded that high maternal antibody titres are required for clinical protection and reduction of virus titres after infection of chickens, whereas low antibody titres already interfere with vaccine efficacy.
Introduction

Highly pathogenic avian influenza (HPAI) viruses can spread rapidly within and between poultry flocks and can cause morbidity and mortality in a high percentage of infected chickens. Based on the presence of antigenic differences between the viral haemagglutinin (H) and neuraminidase (N) surface proteins, 16 H and 9 N subtypes of AI have been identified. AI viruses of H5 and H7 subtypes can become highly virulent in poultry and can cause epizootics that have a large economic impact. Since 2003 there have been continuing outbreaks of HPAI H5N1 in Asia. This H5N1 AI virus was characterized by its high pathogenicity in chickens and the ability to cause infection not only in a number of avian species (Desvaux et al., 2009) but also in mammalian species including humans (Kuiken et al., 2004; Maas et al., 2007; Lipatov et al., 2009). To date, more than 250 people have died due to infection with H5N1 (WHO, 2010). The ongoing outbreaks of HPAI H5N1 in Asia and the subsequent spread of this virus to Africa and Europe has led to the start of vaccination campaigns in different countries in an attempt to prevent more H5N1 outbreaks.

Most commercially available AI vaccines are oil emulsions containing inactivated whole influenza virus. The efficacy of these inactivated vaccines depends on the antigen content, the antigenic similarity between vaccine virus and field viruses and, to a lesser extent, the composition of the oil emulsion (Swayne et al., 1999; 2000). It has been convincingly shown that vaccination can be effective in the prevention of disease, reduction of virus shedding after HPAI infection, and reduction of virus transmission (Webster et al., 2006; Van der Goot et al., 2008). Vaccinated chickens will develop antibody titres that mediate protection against infection with HPAI H5 or H7 viruses. As a consequence, progeny chickens from vaccinated hens will have maternally derived antibodies in the first weeks after hatch.
Maternally derived antibodies can protect young chickens against viral diseases (Otaki et al., 1992; Mondal & Naqi, 2001; Nemeth & Bowen, 2007). However, maternal antibodies can also interfere with vaccination by increased clearance of vaccine antigens and thereby preventing optimal exposure to the immune system (Naqi et al., 1983; Van Eck et al., 1991). For the primary vaccination of young chickens against viral poultry diseases such as Newcastle disease and Gumboro disease, live vaccines are mostly used. Depending on the antibody titres and the virulence of vaccine viruses, it can be predicted at what age young chickens can be vaccinated efficiently (Solano et al., 1986). The results of studies demonstrating reduced effectiveness of live vaccines in chickens with maternal immunity, can not be extrapolated to AI vaccination, since AI vaccines contain inactivated virus. Inactivated poultry vaccines are normally used as booster before the laying period to induce high and uniform antibody titres. It is therefore important to compare the benefits of maternal immunity, i.e. protection against H5N1, with the negative impact maternal immunity may have on vaccine efficacy. With this knowledge it can be determined whether there is a time period in which young chickens are susceptible for HPAI H5N1 infection and can not yet be vaccinated. If there is such a period, alternative vaccination strategies must be considered, or other measures must be taken, in addition to vaccination, in order to prevent circulation of H5N1 in young chickens.
Materials and Methods

Virus  Avian influenza H5N1 virus A/turkey/Turkey/1/05 was used as challenge virus. This virus was obtained from the Veterinary Laboratories Agency (UK).

Chickens  SPF-WLA chickens were obtained from Charles River Laboratories, SPAFAS (Sulzfeld, Germany).

Preparation of vaccines  Experimental oil emulsion vaccines were made containing either the A/turkey/Wisconsin/68 H5N9 strain or an H5N7 strain that was constructed using reverse genetics and which contained all genes from influenza A/chicken/Netherlands/621557/03 H7N7 except for the HA gene, which was derived from influenza A/turkey/Turkey/1/05 H5N1 (B. Peeters, Central Veterinary Institute of Wageningen UR; unpublished data). Avian influenza viruses were cultured in the allantoic cavity of embryonated eggs from SPF hens (Charles River Laboratories, SPAFAS, Sulzfeld, Germany) (WHO manual on animal influenza diagnosis and surveillance). Virus was inactivated by treatment with 0.1% formalin at 4°C for 72 h. Oil emulsion vaccines were prepared by thorough mixing of inactivated virus with Stimune (Prionics, Lelystad, The Netherlands) in a ratio of 4:5 (v/v). Vaccine haemagglutinin (HA) antigen was quantified by denaturing gel electrophoreses followed by quantification of the HA-band, as has been described by Van der Goot et al. (2005). Haemagglutinating units (HAU) were measured in a standard agglutination test using 25 µl virus and 25 µl 1% chicken red blood cells. Both vaccines contained 64, 16 or 4 HAU per 25 µl virus corresponding to 2.4, 0.6 or 0.15 µg HA protein.
**Generation of chickens with maternal immunity**  Sixty chickens were allotted into 6 groups, groups 1, 2, and 3 were inoculated intramuscularly with 0.5 ml of the H5N7 vaccine in doses of 64 HAU, 16 HAU and 4 HAU respectively. Groups 4, 5, and 6 were inoculated with 0.5 ml of the H5N9 vaccine in doses of 64 HAU, 16 HAU, and 4 HAU respectively. An uninoculated group of 10 chickens was kept as unvaccinated negative control. Chickens in groups 1 – 6 were vaccinated twice, initially at 4 weeks of age and for the second time at 7 weeks old. Antibody titres were monitored for a period of one year after vaccination. Fertilised eggs were collected 5 months after the last vaccination in order to obtain maternal antibody titres of intermediate values that represented field titres. After hatching, progeny chickens from five groups of hens that had been vaccinated with H5N7 (64 HAU, 16 HAU or 4 HAU) or H5N9 (64 HAU or 16 HAU), were housed separately. Hens vaccinated with 4 HAU H5N9 were not included because of a lack of antibody titres. Blood samples were taken from the hens in the same week that the fertilised eggs were collected.

**Challenge experiment**  Two-week-old chickens were challenged with $10^6 \text{EID}_{50}$ HPAI H5N1 virus (A/turkey/Turkey/1/05) in 0.2 ml. Thirty progeny chickens from hens that were vaccinated with different doses of H5N7 and twenty progeny chickens from hens that were vaccinated with different doses of H5N9 vaccines were challenged. Ten progeny chickens from unvaccinated hens were used as controls. Half of the challenge dose was administered intranasally and half intratracheally. The presence of H5N1 virus was monitored by taking tracheal and cloacal swabs on days 1, 2, 3, 4, 7 and 10 after challenge. In addition, blood samples were taken on days 7 and 14 after challenge.

**Vaccination experiment**  Two-week-old chickens were vaccinated intramuscularly with inactivated H5N7 or H5N9 vaccines (0.5 ml). Thirty progeny chickens from hens that were
vaccinated with different doses of H5N7 were vaccinated with H5N7 and twenty progeny chickens from hens that were vaccinated with different doses of H5N9 vaccines were vaccinated with H5N9. Twenty progeny chickens from unvaccinated hens were used as controls: 10 for the H5N7 vaccination and 10 for the H5N9 vaccination. Blood samples were taken on the day of vaccination and day 28 after vaccination.

ELISA  A double antibody sandwich blocking ELISA, using anti-nucleoprotein (NP) monoclonal antibody HB65 (ATCC, Manassas, VA, US) was used to measure antibodies against avian influenza (De Boer *et al*., 1990). The NP-protein that is used in this ELISA was prepared from influenza strain A/chicken/Italy/ 1067/V99 (H7N1).

Haemagglutination Inhibition assay  In the haemagglutination inhibition (HI) test 25 µl PBS containing 8 HAU H5N1, H5N7 or H5N9 virus, was incubated in V-bottom 96-well plates with 25 µl of diluted sera at room temperature for 60 min. Thereafter, 25 µl of 1% chicken red blood cells were added, the resulting mix was incubated at 4°C for 60 min, and haemagglutination was measured. HI antibody titres were expressed as the reciprocal of the highest serum dilution giving complete inhibition of haemagglutination. Sera had been heat inactivated at 56°C for 30 minutes prior to testing. HAU were determined before each assay using twofold dilutions of 5 different pre-dilutions (1/2, 1/3, 1/5, 1/7 and 1/9).

Micro neutralisation assay  All micro neutralisation assays were performed with Madin Darby Canine Kidney (MDCK) cells (ATCC, Manassas, VA, US). Sera had been heat inactivated at 56°C for 30 minutes prior to testing. Twofold serial dilutions of the chicken sera were made in GMEM/EMEM medium containing 1% w/v bovine serum albumin and antibiotics in 96-well plates. The diluted sera (50 µl/well) were mixed with 100 TCID<sub>50</sub> HPAI
H5N1 (range 50 – 200 TCID\textsubscript{50}) virus (50 µl) and incubated at 37°C and 5% CO\textsubscript{2} for 1 h. Thereafter, 150 µl of 2x10\textsuperscript{5} MDCK cells/ml were added to each well. The plates were incubated at 37°C and 5% CO\textsubscript{2} for 48 h. The monolayers were washed with PBS, frozen at -20°C and fixed with 4% cold (4°C) paraformaldehyde for 10 min. After washing, cells containing viral NP-protein were stained using HRPO-conjugated monoclonal antibody HB65 and 3-amino-9-ethyl-carbozole (AEC; Sigma-Aldrich, The Netherlands) as a substrate for HRPO. A lack of staining was scored as complete neutralisation. VN-antibody titres were expressed as the reciprocal of the highest serum dilution giving complete neutralisation.

**Quantitative Real-time RT-PCR** Real-time RT-PCR and subsequent data analysis were performed using the MX4000 Quantitative PCR System (Stratagene) equipped with Version 4.20 software. The reactions were carried out in a 96-well plate. Each 25 µl PCR reaction contained 5 µl of Qiagen 1-step RT PCR buffer, 1 µl Qiagen dNTP mix and 1 µl of Qiagen 1-step RT PCR enzyme mix (OneStep RT-PCR kit, Qiagen), 0.38 µl ROX Reference dye (1:500 dilution) (Stratagene), 0.4 µM Forward primer AI-M-F45 (5’-CTTCTAACCGAGGTCGAAACGTA-3’), 0.4 µM Reverse primer AI-M-R251 (5’-CACTGGGCACCGTGAGC-3’), 0.3 µM Taqmanprobe AI-M-Tqmn1 (5’-6FAM-CTCAAGGCGAGATCGCGCAGA-XT-PH) (TIBMolBiol), 1.25 mM MgCl\textsubscript{2}, 0.1 µl RNAsin (40U/µl) (Promega) and 5 µl of sample cDNA. After incubation for 30 min at 50 °C and 15 min denaturation at 95 °C, 45 cycles of amplification (5 s at 95 °C, 15 s at 58 °C, 20 s at 72 °C) were performed. A calibration curve was generated using serial dilutions of a standard batch of H5N1 virus (A/ Turkey/Turkey/1/05) with a known EID\textsubscript{50} titre, in the RNA isolation procedure as described above. Quantification of H5N1 virus present in each sample was based on the calibration curve generated by plotting the cycle threshold ($C_T$) value against
the known H5N1 virus titres. Titres of experimental samples are expressed as EID$_{50}$ equivalents.
Results

HI-antibody titres against H5N1 were detected in hens that were vaccinated twice with H5N9 or H5N7 vaccine, and in their progeny chickens. The antibody titres in the sera of 14-day-old progeny chickens varied from 0 to $2^6$. Mean HI-titres in these progeny chickens were approximately $2^3$ lower than in the hens, if the mean HI titres in the hens was sufficiently high (Table 1).

Only 3 of 32 chickens with maternally derived antibody titres survived the challenge with $10^6$ EID$_{50}$ HPAI A/turkey/Turkey/AV1194/05 (H5N1) virus: 1 of 13 chickens with an HI titre of $2^3$, 1 of 7 chickens with an HI titre of $2^4$ and the chicken with an HI titre of $2^6$ (Figure 1). A limited effect of maternal immunity on the survival time was observed. Chickens with antibody titres survived a few days longer than control chickens and the survival time was dependent on the HI antibody titre (Figure 1). However, also most progeny chickens from vaccinated hens with undetectable antibody titres survived one or two days longer than control chickens. The sera of these chickens did not have detectable levels of antibodies in the HI- and VN-assay or in the NP-ELISA.

At different time points after challenge of control chickens and chickens with maternal immunity, virus titres were estimated in tracheal and cloacal swabs using quantitative RT-PCR and expressed as EID$_{50}$ equivalents. In the tracheas of chickens with HI-antibody titres of $2^0$ to $2^3$, mean virus titres higher than $10^4$ EID$_{50}$ were measured in the first 4 days after challenge, with maximum virus titres between $10^6$ and $10^7$ EID$_{50}$ (Table 2). Chickens with HI-antibody titres of $2^4$ had mean virus titres around $10^3$ EID$_{50}$ on the first days after challenge, whereas in the trachea of the chicken with an HI titre of $2^6$ hardly any virus was found. No virus was found in surviving chickens after day 7 post challenge. In the cloaca, mean virus titres were much lower than in the trachea (Table 3). However, although mean virus titres
were below $10^2$ EID$_{50}$ in the cloaca of chickens with an antibody titre of $2^3$ or higher, virus titres of $10^4$ – $10^{4.5}$ EID$_{50}$ were still detected in some individual chickens with an HI-titre of $2^3$ on days 3 and 4 after challenge. In chickens with HI-titres of $2^4$ or higher, only low virus titres (<$10^2$ EID$_{50}$) were detected in the cloaca.

Fourteen-day-old chickens with different levels of maternal immunity were vaccinated with the same inactivated H5N7 and H5N9 oil emulsion vaccines that were used to vaccinate their hens. In control chickens without maternal immunity, vaccination generated mean HI-antibody titres of $2^{9.1}$ and $2^{7.6}$ against H5N7 (Figure 2) and H5N9 (Figure 3) vaccine respectively. However, in the chickens with maternal immunity the efficacy of the vaccines was strongly diminished. Of 21 chickens with maternally derived antibody titres against H5N7 of $2^2$ or more, only 3 chickens had antibody titres at day 28 after H5N7 vaccination that were in the same range as those in the controls ($2^7$-$2^{10}$). Eight of 21 chickens did not have a measurable HI-titre at all (Figure 2). The efficacy of the H5N9 vaccine was also strongly reduced in chickens with maternal immunity (Figure 3), with only a few chickens with a measurable HI-titre at day 28 after vaccination. In this case, even in maternally immune chickens without measurable HI titres, vaccine efficacy was strongly diminished. Measurement of virus neutralizing antibody titres gave essentially the same results (data not shown).
Discussion

Maternally derived antibodies can protect young chickens against various viral diseases (Otaki et al., 1992; Mondal & Naqi, 2001; Nemeth & Bowen, 2007). Maternal antibody titres in chickens are the highest directly after hatching and decrease to zero in three or four weeks. We studied protection against H5N1 HPAIV by maternal immunity in 14-day-old chickens. In order to be protected against clinical disease and to prevent high virus excretion upon infection, these chickens should have HI-antibody titres higher than $2^4$. This means that vaccinated hens must have HI antibody titres higher than $2^7$, since we demonstrated that antibody titres in 14-day-old chicks are approximately $2^3$ lower than the titres in their hens. These hens were vaccinated with either an inactivated H5 vaccine with an haemagglutinin protein identical to the H5 protein of the challenge virus or with a haemagglutinin protein that only had 90% amino acid homology with the the challenge virus. Both vaccines contained neuraminidase proteins with a different subtype to the challenge virus. This means that the vaccines used are representative of vaccines that are used in the field. Considering the variation in antibody titres after vaccination and the kinetics of the antibody response, it will be very difficult to maintain such high titres in the majority of the animals for a long period of time. Trani et al. showed that a mean HI-antibody titre of $2^8$ is only present for a few weeks after one vaccination and for 8-18 weeks after two vaccinations, depending on the vaccine antigen content (Di Trani et al., 2003). Tian et al. (2005) showed that a mean serum antibody titre of $2^8$ is present between week 3 and week 13 after a single vaccination. However, in a population of vaccinated chickens with a mean antibody titre of $2^8$, a considerable number of hens will have antibody titres below the mean. This means that progeny chickens from these hens might be protected in the first week after hatching but not at the age of 14 days.

Recently, the limited value of maternal immunity for protection against HPAI H5N1 was also
reported for broiler chickens (De Vriese et al., 2010). Challenge with H5N1 at 11 days of age resulted in 83% mortality of broiler chickens hatched from vaccinated breeders.

In studies with unvaccinated maternally immune chickens, the protective capacity of antibodies against HPAI can be studied in the absence of a primed immune system. It is our experience that vaccinated chickens with measurable HI-antibody titres are usually protected against clinical disease after infection with HPAIV, as has been demonstrated for vaccination against H7N7 (Maas et al., 2009). However, morbidity and mortality can be seen in some chickens with HI antibody titres of up to $2^3$ after challenge with a high dose of HPAIV H5N1. Since an antibody titre of at least $2^5$ is required to obtain significant clinical protection in chickens with maternal immunity, this suggests that in addition to the presence of serum antibodies, other immune mechanisms contribute to protection against avian influenza virus infection in immunized chickens. This conclusion is supported by a study of van der Goot et al., (2005) who reported good protection against virus transmission between chickens after challenge with a high dose of HPAI H7N7 at one week after vaccination, when antibodies could not yet be detected. Furthermore, the potential role of the stimulated innate immune system in the defence against H5N1 infection is also illustrated by the protective effect of the non-specific immune stimulant polyinosinic:polycytidylic acid (poly I:C) (Wong et al., 2009).

Breeder chickens are routinely vaccinated against viral diseases using live vaccines at a young age, followed by a second vaccination with inactivated vaccines just before onset of production. In particular this is practised to prevent diseases such as Newcastle disease and Gumboro disease (infectious bursal disease). In the case of avian influenza, however, only the use of inactivated vaccines is allowed. Inactivated vaccines mainly stimulate a systemic antibody response, whereas live vaccines stimulate a more broader immune response by infection of permissive cells and stimulation of the immune system in a way that is similar to a natural infection. It is well known that the efficacy of live vaccines can be negatively
affected by the presence of maternal immunity (Naqi et al., 1983; Solano et al., 1986; Van Eck et al., 1991). In the case of vaccination against Gumboro disease, the optimal time point of vaccination can be determined based on the maternally derived antibody titre in the chickens (Solano et al., 1986). Only below a specific antibody titre, is vaccination effective. Little is known about the efficacy of inactivated vaccines in chickens with maternal immunity. We therefore studied the efficacy of inactivated AI vaccines in the offspring of AI vaccinated hens. In our study the induction of antibodies after AI vaccination of maternally immune chickens was markedly inhibited even by low maternal antibody titres. In some of these chickens the induction of antibodies by vaccination was impaired even in the absence of measurable antibody titres. De Vriese et al. (2010) also reported poor induction of antibody titres after vaccination of broiler chickens with maternally derived antibodies. In this study, chickens with maternal immunity that were vaccinated at 10 days of age and challenged at day 34 were clinically protected against H5N1 virus (De Vriese et al. 2010). Furthermore, a recent study demonstrated that passive transfer of H5N1 antibodies to chicks suppresses the efficiency of subsequent active vaccination (Kim et al., 2010). It was hypothesized that the poor results of the vaccination campaign in Egypt could be partially explained by the presence of maternal immunity.

We conclude that there is a poor antibody induction after vaccination with inactivated influenza virus in young chickens with maternally derived antibodies. During the period in which the young chickens have low antibody titres (below $2^5$-$2^6$), strict biosecurity measures must be implemented to prevent introduction of avian influenza virus. Alternatively, the use of live vector vaccines may be considered in these chickens, since it has been demonstrated that live fowlpox vectored H5 vaccine efficacy was not inhibited by maternal antibodies (Bublot et al., 2006).
Acknowledgements

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References


Table Legends

**Table 1.** HI antibody titres against H5N1 in vaccinated hens and their progeny chickens.

Sera were collected from hens 5 months after two vaccinations with inactivated H5N9 or H5N7 vaccines. Sera of progeny chickens were collected at the age of 14 days. All groups contained 10 chickens.

**Table 2.** Serum antibody titres and H5N1 virus titres in the trachea of chickens with maternal immunity and of control chickens.

Antibodies against H5N1 were measured using the haemagglutination inhibition test in 14-day-old chickens at the day of challenge. Virus titres (PCR equivalents) were measured at different days after challenge. In groups of chickens with the same HI-titres, the mean virus titres are presented together with the highest and lowest virus titres per group (in parentheses).

**Table 3.** Serum antibody titres and H5N1 virus titres in the cloaca of chickens with maternal immunity and of control chickens.

Antibodies against H5N1 were measured using the haemagglutination inhibition test in 14-day-old chickens at the day of challenge. Virus titres (PCR equivalents) were measured at different days after challenge. In groups of chickens with the same HI-titres, the mean virus titres are presented together with the highest and lowest virus titres per group (in parentheses).

**Figure Legends**
**Figure 1.** Antibodies against H5N1 were measured using the haemagglutination inhibition test in 14-day-old chickens on the day of challenge. The survival of groups of chickens with the same HI-antibody titres is shown.

**Figure 2.** Fourteen-day-old control chickens and chickens with maternal immunity after vaccination of the parent hens with H5N7 vaccine, were vaccinated with H5N7 vaccine. Antibody titres against H5N7 were measured using the haemagglutination inhibition test on the day of vaccination and 4 weeks later (day 28). All sera were tested twice and the means are presented.

**Figure 3.** Fourteen-day-old control chickens and chickens with maternal immunity after vaccination of the parent hens with H5N9 vaccine, were vaccinated with H5N9 vaccine. Antibody titres against H5N9 were measured using the haemagglutination inhibition test on the day of vaccination and 4 weeks later (day 28). All sera were tested twice and the means are presented.
Figure 1.

Survival of chickens with maternal immunity after H5N1 challenge

![Graph showing survival of chickens over time with different HI titers]
Figure 2.

**Antibody titers after Al-vaccination (H5N7) of chickens with maternal immunity**

- **Day of vaccination**
- **Day 28 after vaccination**

The graph shows the HI-titer (2log) for individual chickens over the days of vaccination. Control chickens and chickens with maternal immunity are distinguished.
Table 1. *HI antibody titers in vaccinated hens and their progeny chickens.*

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<th>Vaccine</th>
<th>Mean HI antibody titer (log2) ± SD</th>
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<th>Chickens</th>
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<td>0.7 ± 1.1</td>
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<td>1.0 ± 1.1</td>
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<td>H5N9, 16 HAU/0.6 µg HA</td>
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<td>0.6 ± 1.0</td>
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<tr>
<td>Control</td>
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Table 2. Serum antibody titers and H5N1 virus titers in the trachea of chickens with maternal immunity and control chickens.

<table>
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<tr>
<th>Antibody titer</th>
<th>Number of chickens</th>
<th>Mean and range of H5N1 titers in trachea (10log EID50)</th>
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<td></td>
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<tr>
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<td>18</td>
<td>4.8 (3.5 - 6.0)</td>
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<td>5.3 (4.3 - 5.9)</td>
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<tr>
<td>HI-titer 3</td>
<td>13</td>
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<tr>
<td>HI-titer 4</td>
<td>7</td>
<td>3.7 (1.1 - 4.5)</td>
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<tr>
<td>HI-titer 6</td>
<td>1</td>
<td>0.9</td>
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Table 3. Serum antibody titers and H5N1 virus titers in the cloaca of chickens with maternal immunity and control chickens.

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<thead>
<tr>
<th>Antibody titer</th>
<th>Number of chickens</th>
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<tr>
<td>Control</td>
<td>10</td>
<td>1.0 (0.0 - 4.5)</td>
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<td>HI-titer 0</td>
<td>18</td>
<td>0.5 (0.0 - 2.6) 2.8 (1.0 - 5.3) 3.3 (1.7 - 5.5)</td>
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<td>HI-titer 3</td>
<td>13</td>
<td>0.0 0.7 (0.0 - 2.2) 1.5 (0.0 - 4.0) 1.0 (0.0 - 4.5) 1.6 (0.8 - 2.5)</td>
</tr>
<tr>
<td>HI-titer 4</td>
<td>7</td>
<td>0.0 0.1 (0.0 - 0.6) 0.3 (0.0 - 1.9) 0.1 (0.0 - 0.7) 0.2 (0.0 - 0.6)</td>
</tr>
<tr>
<td>HI-titer 6</td>
<td>1</td>
<td>0.0 0.0 0.0 0.0 0.0</td>
</tr>
</tbody>
</table>