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RESEARCH

Interfering of maternal derived antibodies with the protection of local inactivated reassortant H5N1 Avian influenza vaccines with antigenic content of 300 HA units in commercial broiler chickens

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ABSTRACT

Background*:* Influenza virus is an Orthomyxovirus that known as one of the causes of significant numbers of natural infections and disease, mainly infecting the upper respiratory tract, in humans, domestic pigs, horses, and numerous bird species. Maternal derived antibodies could interfere with the efficacy of vaccination against avian influenza in early age in chicks.

Methods: Eight groups of one-day old commercial broiler chicks were kept in isolators along the whole period of the study. Groups 1, 2 and 3 were vaccinated with the prepared vaccine; group 4, 5 and 6 were vaccinated with one of the imported reassortant H5N1 containing 300 HAU at 1, 5 and 10 days of age; respectively. Groups 7 and 8 were positive and negative groups for the challenge trial. Blood samples were collected weekly for 4 weeks of age and tested by HI test. Post challenge, tracheal and cloacal swabs were collected at 3, 5, 7, and 10 days and tested by both real time RT-PCR (rRT-PCR) and virus titration in SPF eggs.

Results: HI test revealed no significant difference between groups in the first 3 weeks post vaccination and group 2 showed lower significant statistical difference. Results of the challenge trial revealed 0, 14, 80 % and 0, 14 and 86 % of protection in groups 1,2,3 and groups 4, 5, and 6; respectively. RT-PCR and virus isolation revealed that all chicken groups vaccinated at 1 and 5 days of age demonstrated 100% shedding at 3, 5, 7 and 10 days post challenge. However, groups 3 and 6 which were vaccinated at 10 days of age revealed difference in shedding pattern where group 3 (vaccinated with local prepared vaccine) showed 100 shedding by rRT-PCR and 100%, 60% and 60 % of the chickens in tracheal swabs and 100%, 80% and 60%in cloacal swabs when tested by virus isolation in eggs at 3, 5 and 7 days post challenge; respectively. Swabs of 10 days post challenge of group 3 were positive by rRT-PCR and negative by virus isolation. On the other hand, group 6 (vaccinated with imported vaccine) demonstrated shedding % at 3 and 5 days post challenge by both rt-RT-PCR and virus isolation were positive in 60% and 20% for tracheal and 80% and 20% for cloacal swabs ; respectively. At 7 and 10 days shedding of all chickens in group 6 were negative by both rRT-PCR and virus isolation.

Conclusion*:* Indeed, there is evidence of interfering of maternal antibodies to vaccination at 1, and 5 days. Also, the 300 HAU of antigen in the prepared avian influenza H5N1 vaccine are not enough in reducing of virus shedding post challenge.

Keywords:highly pathogenic avian influenza; immunity; antigen content; vaccination; challenge.

BACKGROUND

Highly pathogenic avian influenza (HPAI) viruses can spread rapidly among poultry flocks with morbidity and mortality in a high percentage of infected chickens (Ducatez *et al.,* 2008). To date, based on the antigenic differences between the viral hemagglutinin (H) and neuraminidase (N) surface proteins, there are 18 H and 11 N subtypes of avian influenza (AI). The viruses of H5 subtype with highly pathogenicity can cause large economic losses in poultry industry (Tong *et al.,* 2013). Since 2003 there have been continuous outbreaks of HPAI H5N1 in Asia. This H5N1 AI virus is highly pathogenic in chickens and cause infection in several avian species (Desvaux *et al,* 2009) and in mammalian species including humans (Lipatov *et al.,*2009).

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The HPAI H5N1 in Asia, Africa and Europe has led to the start of vaccination programs in some countries in a trial to control H5N1 outbreaks. The efficacy of these inactivated vaccines depends on the antigen content, the antigenic similarity between vaccine virus and field viruses and the type of the oil emulsion to lesser extent (Swayne *et al.,* 2000). It has been shown that vaccination can be effective in the prevention of disease, reduction of virus shedding post HPAI infection, and reduction of virus transmission (Van der Goot *et al.,* 2008). Maternally derived antibodies can protect young chickens against viral diseases (Nemeth and Bowen, 2007). It can be predicted at what age young chickens can be vaccinated efficiently depending on the antibody titers and the virulence of vaccine seed viruses (Solano *et al.,* 1986). However, maternal antibodies can interfere with the vaccine immune response with negative impact in case of high titers at one day old which can affect the vaccine efficacy if applied in the presence of such titers (Maas *et al.,* 2011). The time of vaccination affects greatly the success of vaccination strategies or other measures taken in order to prevent circulation of H5N1 in young chickens (Maas *et al.,* 2011). Previous studies have shown that chickens with maternal immunity which were vaccinated at 10 days of age and challenged at day 34 were clinically protected against H5N1 virus (De Vriese *et al.* 2010). On the other hand, passive transfer of H5N1 antibodies to chicks suppresses the efficiency of subsequent active vaccination (Kim *et al.,* 2010). During the period in which the young chickens have low antibody titers below 25, strict biosecurity measures must be taken in consideration to control the introduction of avian influenza virus (Bublot *et al.,* 2006). The use of live vector vaccines may be considered in these chickens, since it has been demonstrated that live fowl pox-vectored H5 vaccine efficacy was not inhibited by maternal antibodies (Bublot *et al.,* 2006).

The aim of the present work is to study effect of maternal derived antibodies on the protection of local inactivated reassortant H5N1 Avian influenza vaccines with antigenic content of 300 HA units against the challenge HPAI H5N1 official strain in commercial broiler chickens.

MATERIALS AND METHODS

Viruses and Vaccine preparation:

Reassortant Avian Influenza virus A/Chicken/Egypt/Q1995D/2010(H5N1) and A/Duck/Egypt/M2583D/2010 (H5N1) which were developed by national research center and used in preparation of local influenza vaccines in Veterinary Serum and Vaccine Research Institute, Newcastle disease unit, Abbasia, Cairo-Egypt. The viruses were propagated in 9-11 days old specific pathogen free-embryonated chicken eggs (SPF – ECE) (Beard *et al,* 1989) via their inoculation of 300 EID50 into allantoic cavity and incubation at 36°C for 36-38 hours. The virus harvests were inactivated with formalin solution 0.1%. To confirm complete inactivation, samples from the inactivated virus before addition of adjuvant were tested by at least two passages in 9-11-day old SPF embryonated eggs (0.1 ml /egg) via the allantoic cavity. All embryos that died or remained alive after 24 hours and up to 6 days were examined for the presence of virus by the rapid HA on the allantoic fluid. The vaccine was prepared by mixing oil adjuvant Montanide ISA-70 (Seppic, France) at ratio (70/30) yielding stable white emulsion. In addition, the vaccine was supplemented with gentamicin (200 mg/ml) and thimerosal (0.102 mg/ml). The entire process of preparing laboratory specimens of the inactivated vaccine with use of the production strain was carried out under conditions of the Biological Safety Laboratory 3. Virus stock was amplified in SPF ECE and virus titer was determined by 50% Egg Infectious Dose (EID50). The titer used in the prepared vaccine was 10^{11} and 10^{12} for chicken and duck seed viruses in a percentage of 50/50; respectively.

Challenge trial:

Two hundred one – day-old broiler chickens (Cobb breed) were obtained from Commercial Hatcheries-Egypt, and housed in separate isolators all over the challenge trial. The chicks were reared under proper Hygienic conditions ventilated under positive pressure with HEPA- filtered air and maintained under continuous lightening, feed and water supplied ad libitum. Birds were randomly divided into groups (n = 25/group). Birds in Groups 1–6 were vaccinated subcutaneously (SQ) with 0.5 mL of the H5N1 vaccines. Groups 1, 2 and 3 were vaccinated with the prepared vaccine; groups 4, 5 and 6 were vaccinated with one of the imported reassortant H5N1 containing 300 HAU at 1, 5 and 10 days of age; respectively. Groups 7 and 8 were positive and negative groups for the challenge trial. Four weeks post vaccination, each bird was challenged intra-nasally (I.N.) with 10^6 EID50 of HPAIV/bird (A/Duck/Egypt/CLEVB-24-N00238/2015); Accession no: EPI579780 on GISAID obtained from viral strain bank of CLEVB which used for challenge tests). All chickens were daily observed and monitored for 10 days post challenge (DPC) in order to report the clinical sings as well as record mortalities and detection of virus shedding for each group. Swabs (oropharyngeal and cloacal) were taken from live birds at day 3,5,7 and 10 post challenge in all groups for quantification of virus shedding using real-time RT-PCR (OIE, 2015) and for virus re-isolation in ECE. RNA was extracted from the oropharyngeal and cloacal swabs using QIAmp Viral RNA Mini Kit that supplied from (QI Amp Viral RNA Mini Kit (QIAGEN) catalogue No. 52904. Virus isolation and detection in embryonated chicken egg were carried out as previously described (OIE, 2015). Both (oropharyngeal and cloacal swabs) should be placed in isotonic phosphate-buffered saline (PBS), pH 7.0–7.4 with antibiotics. Penicillin (2000 units/ml), streptomycin (2 mg/ml), gentamycin (50 µg/ml) and mycostatin (1000 units/ml) for oropharyngeal swabs, but at five-fold higher concentrations for cloacal swabs (OIE, 2015). For virus inoculation in ECE, these suspensions filtered through 0.22µm filter. Five 9-day-old SPF EGE were inoculated and candled daily for embryo viability for 7 days (Beard *et al.,* 1989). Eggs died within 24 h were discarded. Allantoic fluid from embryos dead 24 h p.i. was collected aseptically and tested for the presence of AI H5 virus by rapid slide Hemagglutination test (Anon *et al.,* 1971).

Serological monitoring of antibodies:

Blood samples were collected from jugular vein and kept in a slope position at 37 ^{o}C for one hour then at 4 ^{o}C overnight. Sera were then separated by centrifugation at 3000 rpm for 10 minutes and stored at -20 ^{o}C. Sera were inactivated at 56 ^{o}C for 30 minutes before testing. Ten serum samples were collected from each group (1-8) at 7th, 14th, 21th and 28th days PV for post vaccination monitoring in the first experiment, while it was collected at 1st, 5th, 7th, 14th, 21st and 28th day old and from non-vaccinated non challenged group (gp7) to follow up maternally derived antibodies. Serum samples were subjected for hemagglutination inhibition test (HI) (OIE, 2015) using homologous Avian Influenza virus (A/Chicken/Egypt/Q1995D/2010(H5N1) antigen for the local prepared vaccine, (A/Turkey/Egypt/14385/2014) (H5N1) antigen for the imported vaccine. by using standard 4 HAU of the antigen.

Statistics:

The results represent the mean with standard error of at least triplicate determinations (n=3). Statistical significance was determined by two- way analysis of variance (ANOVA) with LSD post hoc test using statistical software programme SPSS (version 21.0).

RESULTS:

Waning of Maternal immunity of non-vaccinated broilers chickens acquired from vaccinated parents:

The results of HI test to determine the maternal immunity are represented in Figure (1). There were high to moderate levels of maternal antibodies against AI (H5N1) on the 1st and 5th day of age vaccinated chicks and gradually decreased starting from 7 days and disappeared at 28 days of age.

Humoral immune response to the H5N1 vaccines

Results of HI test in the first week post vaccination revealed statistically differences in titers which were high in groups 1 and 4 compared to group 5. HI titers were low in groups 2, 3 and 6. However, at the 2nd week significant higher titers were recorded in groups 3, 4 and 6 in comparison with groups 1,4 and 5. By the 3rd week significant higher antibody titers were in groups 3 and 6. Significant high titers were in group 6 compared to others in groups 2, 3 and 5 whereas groups 1 and 4 demonstrated zero titers at 4 weeks post vaccination

Table (1). Serum antibody response following vaccination with local and imported inactivated AIV (H5N1) vaccines containing 300 HAU in chicken groups at different ages.

Groups	Days post vaccination			
	Virus titer (Log2)			
	1st week	**2nd week**	**3rd week**	**4th week**
Group 1	4.3 ± 0.5^{a}	1.6 ± 1.15^{a}	0.3 ± 0.5^{a}	0^{a}
Group 2	1.4 ± 0.5^{b}	1.2 ± 0.44^{a}	1.6 ± 0.9^{ac}	2.2 ± 0.8^{b}
Group 3	1.2 ± 0.44^{b}	3.8 ± 0.44^{b}	5.8 ± 0.83^{b}	5.6 ± 0.54^{c}
Group 4	3.6 ± 0.6^{a}	3.0 ± 1.0^{b}	0.6 ± 0.57^{a}	0^{a}
Group 5	2.2 ± 0.44^{c}	1.8 ± 0.4^{a}	2.2 ± 0.8^{c}	2.4 ± 1.1^{b}
Group 6	1.2 ± 0.4^{b}	3.8 ± 0.8^{b}	5.4 ± 1.8^{b}	7.0 ± 1.0^{d}

Means with different superscript letters (a, b, c, d) within the same column are significantly different at P value < 0.05 between chicken groups. Group1: vaccinated with local vaccine at 1day, Group2: vaccinated with local vaccine at 5 days, Group 3: vaccinated with local vaccineat10days,Group4: vaccinated with imported vaccine at 1day, Group5: vaccinated with imported vaccine at 5days, Group 6: vaccinated with imported vaccine at 10 days.

Protection % against challenge with HPAIV

The characteristic clinical signs for HPAI observed 3 days post challenge with mortalities occurred in different challenged groups. Sick birds displayed cyanosis of comb and wattle, echymosis on the shanks and feet, facial edema , greenish diahrria and nervous signs including torticollis and tremors. For vaccinated birds the protection % in the vaccinated groups with local vaccine was 0%, 14%, and 80% in groups 1, 2, and 3; respectively. However, protection % in the vaccinated groups with imported vaccine was 0%, 14%, and 86% in groups 4, 5, and 6; respectively.

Virus shedding post challenge

Virus shedding titers could be detected by both rRT-PCR and challenge virus re-isolation in ECE for tracheal swabs on days 3, 5, 7, and 10 post challenge. There were a statistical significant difference among groups, in the 3rd day results revealed a higher rate of virus shed in groups2, 3, and 7 , the virus shedding decreased in group 4, and 5, lower titers recorded in group 6. High titers of challenge virus could be detected from tracheal swabs in the SPF ECE, the results were 100% in groups1, 2, 3, 4, 5, and 7, while it was 60% in group 6. In the 5th day post

challenge, there were higher virus shedding titer in groups 1, 2, 3, 4, and 5, lower shedding titers detected in group 6. However, it found by virus isolation the results were 100% in groups1, 2, 4, and 5, 60% in group 3, and reduced in group 6 recording 20%. By day 7, the rRT-PCR results were significantly different with high titer in groups 1, and 4, reduced in groups 2, 3, and 5 with no shedding in group 6. Results of virus re-isolation were 100% in groups 1, 2, 4, and 5, 60% in group 4, and 0% in group 6. At the 10^{th} day post challenge, Significant high titers by rRT-PCR were determined in groups 1,2, 4 and 5, reduced in group 3, with no shedding in group 6. On the other hand, virus isolation results were 100% in groups 1, 2, and 4, (80%) in group 5, and 0% in groups 3, and 6.(Table 2).

Table 2: Results of rRT- PCR and virus isolation in SPF ECE for tracheal swabs collected from chickens vaccinated with local or imported Inactivated AIV (H5N1) vaccines containing 300HAU.

Groups[A]	Days post challenge							
	3rd		5th		7th		10th	
	Virus titer (Log10)							
	rRT-PCR	Isolation	rRT-PCR	Isolation	rRT-PCR	Isolation	rRT-PCR	Isolation
Group 1	4.6 ± 0.8^{b}	100%	4.1 ± 0.8^{b}	100%	4.8 ± 0.4^{b}	100%	4.4 ± 0.4^{b}	100%
Group 2	3.9 ± 0.5^{bd}	100%	3.8 ± 0.6^{b}	100%	3.9 ± 0.8^{bc}	100%	3.5 ± 0.3^{b}	100%
Group 3	3.5 ± 0.5^{bd}	100%	3.6 ± 0.7^{b}	60%	3.4 ± 0.5^{c}	60%	2.1 ± 0.6^{c}	0%
Group 4	4.2 ± 0.7^{b}	100%	4.5 ± 0.3^{b}	100%	4.6 ± 0.8^{b}	100%	4.2 ± 0.7^{b}	100%
Group 5	3.6 ± 0.5^{bc}	100%	3.7 ± 0.5^{b}	100%	3.8 ± 0.7^{bc}	100%	3.6 ± 1.1^{b}	80%
Group 6	2.7 ± 0.7^{C}	60%	2.4 ± 0.9^{c}	20%	0^{a}	0%	0^{a}	0%
Group 7	4.5 ± 0.9^{d}	100%	NS	NS	NS	NS	NS	NS
Group 8	0^{a}	0^{a}	0^{a}	0^{b}	0^{a}	0^{a}	0^{a}	0^{a}

Means with different superscript letters (a, b, c, d) within the same column are significantly different at P value < 0.05 between chicken groups. Group1: vaccinated with local vaccine at 1day, Group2: vaccinated with local vaccine at 5days, Group 3: vaccinated with local vaccine at10 days, Group4: vaccinated with imported vaccine at 1day, Group5: vaccinated with imported vaccine at 5days, Group 6: vaccinated with imported vaccine at 10 days. Group 7: positive challenge control. Group 8: negative non-vaccinated control. NS: non survival.

For cloacal swabs Virus shedding titers also could be detected by both rRT-PCR and challenge virus re-isolation in ECE. There were a statistical significant difference among groups detected by rtRT-PCR in the 3rd day results detected a higher rate of virus shed in groups1, 2, 4, and 7 , lower virus shedding titers were in groups 3, 5, and 6. On the other hand virus isolation results were 100% in groups 1, 2, 3, 4, and 5, group 6 was 80%. Testing of swabs collected at 5th days revealed significant higher virus shed in groups 1, 2,3, 4, and 5, the virus shedding was lower in group 6. Virus isolation from such swabs was 100% in groups 1, 2, 4, and 5, in groups 3, 6 was 80%, 20%, respectively. At 7th days post challenge, significant high virus shedding was detected in groups1,2,4, and 5, low titers in group3 , while there was no shedding in group 6. The virus isolation recorded 100% in groups 1,2,4, and 5, 60% in group 3, and negative result in group 6. Testing of swabs collected at 10th day post challenge by rtRT-PCR revealed significant high virus shed in groups1, 2, 4, and 5, low titer in group 3, no shedding in group 6 and the virus isolation results was 100% in groups 1, 2, 4, and 5, for both groups 3, and 6 there was 0% (Table 3) .

Table 3: Results of rRT- PCR and virus isolation in SPF ECE for cloacal swabs collected from chickens vaccinated with local or imported Inactivated AIV (H5N1) vaccines containing 300HAU.

Groups	Days post challenge							
	3rd		5th		7th		10th	
	Virus titer (Log10)							
	rRT-PCR	Isolation	rRT-PCR	Isolation	rRT-PCR	Isolation	rRT-PCR	Isolation
Group 1	4.6±0.3[ac]	100%	4.7±0.3 [a]	100%	4.8±0.2 [a]	100%	4.9±0.2 [a]	100%
Group 2	4.1±0.3 [ac]	100%	4.5±0.6 [a]	100%	4.7±0.3 [a]	100%	4.6±0.3 [ac]	100%
Group 3	3.9±0.9 [ab]	100%	3.7±0.6 [ab]	80%	3.8±0.3[b]	60%	2.3±0.6[b]	0%
Group 4	4.3±0.8 [ac]	100%	4.7±0.8 [a]	100%	4.9±0.6 [a]	100%	4.4±0.3 [ac]	100%
Group 5	3.9±0.9 [a]	100%	3.8±0.9 [ab]	100%	4.5±0.6 [a]	100%	4.2±0.3[c]	100%
Group 6	3.9±0.9 [a]	80%	3.2±0.7[b]	20%	0[c]	0%	0[d]	0%
Group 7	5.1±0.2[c]	NS	NS	NS	NS	NS	NS	NS
Group 8	0[d]	0[a]	0[c]	0[b]	0[c]	0[a]	0[d]	0[a]

Means with different superscript letters (a, b, c, d) within the same column are significantly different at P value < 0.05 between chicken groups. Group 1: vaccinated with local vaccine at 1 day, Group 2: vaccinated with local vaccine at 5days, Group 3: vaccinated with local vaccine at 10 days, Group4: vaccinated with imported vaccine at 1day, Group 5: vaccinated with imported vaccine at 5 days, Group 6: vaccinated with imported vaccine at 10 days. Group 7: positive challenge control. Group 8: negative non-vaccinated control. NS: non survival.

DISCUSSION

In this study, the efficacy of inactivated H5N1 AI vaccine with antigen content 300HAU formulated using the Egyptian strain was investigated and evaluated in commercial broiler chicken. Our results suggested that the ideal age for broiler chicken's vaccination is 10-days of age. Chickens with moderate or low maternal HI titers showed unsatisfactory immune response (HI titers) when vaccinated at 1 or 5-days age with H5N1 commercial inactivated oil-emulsion vaccines as mentioned in table 1, but when vaccinated at 10-days of age, they gave satisfactory immune responses as summarized in table 1. Previous studies have shown that the vaccines applied at 10 days of age in broiler gave satisfactory immune responses 4 weeks post vaccination (Ka-Oud *et al.,* 2008). And adequate HI titers (Sultan and Hussien, 2008). The different levels of immune responses are due to different antigenic factors including, antigenic quality and contents as well as the adjuvant composition (Cristalli *et al.,* 2007). Although, the immunogenicity of vaccines is correlated to antigen mass, its formulation and the age of vaccination are the key factor for success in induction of immune response (Trani *et al.,* 2003). In the present study, all non-vaccinated, challenged control chickens were dead 3 days post challenge. On the other side, the protection % was 0% for the broiler chicks vaccinated at one day-old with different H5N1 AI vaccines. Meanwhile, broiler chickens vaccinated at 5 day-olds with H5N1 AI vaccines showed low protection percentage (14-20%). However, the protection % for vaccinated chickens at 10 days with H5N1 AI vaccines were ranged from 80-86%. In challenged chickens the most pathognomonic signs in unvaccinated chicks, 1 and 5 days vaccinated chicks showed characteristic lesions of HPAI including cyanosis of comb and wattle, ecchymosis on the shanks and feet, facial edema, greenish diahrria and nervous signs (Swayne *et al.,* 2008). The trachea filled with mucoid exudates and the lung showed congestion and hemorrhages. Petechiae were noted throughout the abdominal fat, on the serosal surfaces especially on the peritoneum (Naeem *et al.,* 2007). The kidneys were congested and sometimes plugged with urate deposits (Capua *et al.,* 2002). The morbidity and mortality can be seen in some chickens with HI antibody titers of up to 2^3 after challenge with a high dose of HPAIV H5N1. Since an antibody titer 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 (Maas *et al.,* 2011). Virus shedding was determined in this study with rRT-PCR which were showed in tables 3 and 4 for 10 day old vaccinated chicken for imported vaccine positive virus shedding 3 and 5 days post challenge and for local prepared 300HAU vaccine positive virus shedding 3, 5, 7 and 10 days post challenge. In this study, the induction of antibodies after AI vaccination was markedly inhibited even by low maternal antibody titers. 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). The antigen content is playing an important role in vaccine protection and reduction of virus shedding. HI antibody titer and virus shedding in chickens is obviously arguable (Swayne *et al.,* 2015) because it depends on several factors, including vaccine antigen content, vaccine preparation, age of chickens at vaccination, and time between vaccination and challenge (Swayne *et al.,* 2015). It has been proposed that for effective vaccination strategy a vaccine strain should has antigenic relationship to the circulating field virus (Wood *et al.,* 1985). At least 300 HAU/dose is needed to induce better HI antibody response in broiler chickens. The importance of the vaccine antigen dose optimization is not simply for clinical protection but also extends to the prevention of virus circulation (Kilany *et al.,* 2016). Indeed, to achieve early protection especially in the first week of age, the use of vector vaccines may be of high contribution and even if applied in a prime-boost strategy combined with inactivated vaccines. It was previously reported that the use of live vector vaccines may be considered in chickens with maternal derived antibodies, since it has been demonstrated that live fowl pox vectored H5 vaccine efficacy was not inhibited by maternal antibodies (Bublot *et al.,* 2006).

In conclusion, the study reports the interfering effect of maternal antibodies to AIV vaccination at 1 and 5 days with variable impact on the protection of chicks against challenge with HPAI H5N1. In addition, the study highlights the importance of mass antigenic content rather than vaccine seed virus similarity to the challenge virus and the importance of such content on virus shedding. The study proposes the need of H5 vaccine with antigenic content more than 300 HAU to achieve the maximum reduction of virus shedding in case of field infection and exposure to wild H5N1 HPAI virus.

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