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Detection of Avian Influenza Anti-H5 Maternally-derived Antibodies and Its Impact on Antibody-mediated Responses in Chickens after *In Vivo* Administration of Inactivated H5N9 Vaccine

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ABSTRACT

In the current study, two experiments were performed to ascertain the existence of avian influenza H5 maternally-derived antibodies (MDA) in chickens and evaluate their effects on the humoral immune responses of chickens vaccinated with a commercial oil-emulsion inactivated avian influenza H5N9 vaccine. A total of 120 one-day-old ISA brown chicks were sourced from three different commercial hatcheries (n = 40 per hatchery) in Nigeria and used for this study. For the second experiment, ten chicks were randomly collected from each hatchery and grouped into A0, B0, and C0 at one day old, and one ml of blood was collected from five randomly selected chicks via the heart or brachial vein at 1, 7, 14, 21, 28, 35, and 42 days of age for the assessment of avian influenza H5 MDA. For the second experiment, 2 ml of blood was collected from the heart or brachial vein of 3 randomly selected chicks from each subgroup at 14, 21, 28, 35, and 42 days of age for evaluation of the interaction of MDA with anti-avian influenza vaccinal antibodies when different doses of the H5 antigen was administered via either IM or SC routes at 14 and 28 days of age. Sera were analyzed using ProFlok® AIV ELISA kit. This study detected AIV H5 MDA in all chicks sampled, with total decay times of 22.3, 27.3, and 26 and mean half-life ($t_{1/2}$) of 2.5 ± 0.4, 3 ± 0.6, and 2.9 ± 0.4 days for chicks from hatcheries A, B, and C. The obtained results of the second experiment showed that at 21 days of age, the mean antibody titer levels of chicks from A1, B1, and C1 were respectively 57.7 ± 49.9, 260.7 ± 124.8, and 2205 ± 409.1 when the antigen was administered IM and the reported values for SC administration were respectively 53.3 ± 36, 646.3 ± 237.9 and 2,444.3 ± 1,110.6. This means that variable MDA titers interfered with the humoral immune responses of the chick's post-vaccination. Chicks may, therefore, be vaccinated against AIV H5 subtypes between day 14 and 21 of age, preferable via the SC route to avoid significant interference by AIV H5 MDA.

Keywords: Avian influenza virus, Chicks, Dose, Hatcheries, Maternally-derived antibodies, Route, Vaccine

INTRODUCTION

Influenza viruses (IVs), like most RNA viruses, are genetically labile and have been classified into types A, B, or C, with type A being the most important in avian species (de Geus et al., 2012). Influenza A viruses (IAVs) are further divided into subtypes based on the nature of their surface glycoproteins among which Haemagglutinin (HA) and Neuraminidase (NA) are surface antigenic proteins that play a major role in the host humoral immune response against these viruses (Chiapponi et al., 2016), and are used in the nomenclature of influenza viruses. At present, 16 haemagglutinins (H1 to H16), and 9 neuraminidases (N1 to N9) give rise to the total of 198

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existing combinations of Influenza A subtypes (Tong et al., 2013; Wu et al., 2014), but only H3, H4, H5, H6, H7, H9, and H10 influenza A subtypes have been isolated in domestic birds (Cui et al., 2016; Lee et al., 2017). Influenza A viruses (IAV) are genetically diverse and unstable viruses due to their segmented genome, and they are prone to progressive mutation processes such as antigenic drift and shift (Yoo et al., 2018). Avian Influenza virus (AIV), a member of IAV, has continued to cause morbidity and mortality in poultry species worldwide. Increased mortality is strongly related to infection with highly pathogenic influenza A viruses (HPAIVs), characterized by mortality in gallinaceous poultry (Alexander, 2007). Although the innate immune response is the first line of defense against viruses, the adaptive immune response is ultimately responsible for viral clearance and protection against subsequent infections. Adaptive immunity is also very important to provide memory against subsequent infection (Waffarn and Baumgarth, 2011). Neutralizing antibodies from B cells is a key component in anti-influenza immunity, and anti-HA-specific antibodies are often used as correlates of influenza A immunity (Waffarn and Baumgarth, 2011).

The fact that maternally derived antibodies (MDA) confirm the transfer of MDA from vaccinated parents to offspring was stated by many researchers (Hamal et al., 2006; Gharaibeh et al., 2008). Maternal antibodies are immunoglobulins transferred from vaccinated or naturally infected breeder hens to the progeny through the egg, which provide passive immunity to progeny and protect them against infectious agents due to their immature immune system (Mondal and Naqi, 2001; Hamal et al., 2006). In addition, MDAs reduce the growth-suppressive costs of an innate immune response toward pathogens during the early development of the immune system (Soler et al., 2003; Brommer, 2004). However, this passive immunity has a relatively short duration, reaching its peak at 3 to 4 days post-hatch, and then gradually decreases to undetectable levels at 2 or 3 weeks of age (Hamal et al., 2006). This rapid decrease in the MDA titer makes chickens vulnerable to infectious diseases, especially during 2 weeks post-hatch.

Globally, AI vaccines are used in integrated control strategies to protect poultry against HPAI, such as H5N1. Vaccination decreases the prevalence of disease and reduces viral shedding among infected poultry farms (Swayne and Kapczynski, 2008). Also, vaccination against HPAI has shown decreased rates of environmental contamination, especially where enforcement of biosecurity is impracticable (Swayne and Kapczynski, 2008). In different countries, avian influenza (AI) vaccines may either be used routinely to protect poultry flocks, as an adjunct to existing control measures, or to protect valuable species, such as zoo birds from highly virulent viruses, including H5N1 (Capua and Marangon, 2006; White, 2013). However, most commercial vaccines rely on the generation of neutralizing antibodies against HA. However, the inability of the neutralizing antibodies to cross-react with heterotypic viruses or even viruses with variants of the same HA subtype limits the efficacy of such vaccines in providing broad-spectrum protection.

Several studies have shown that high levels of MDA could mask specific antigens in the offspring, thereby preventing B-cell responses (Elazab et al., 2010; Merrill and Grindstaff, 2014). This blocking effect could negatively affect the short-term immunological response of the offspring (Staszewski et al., 2007; Elazab et al., 2010) as well as the offspring's ability to mount sufficient humoral immune responses as the offspring ages (Carlier and Truyens, 1995). Maternally-derived antibodies could interfere with the successful vaccination of young animals because of the ability of MDAs to neutralize, at least partially, the vaccine's virus and increase the clearance of the vaccine antigens, thereby preventing the optimal exposure to the immune system (Maas et al., 2011; Abdelwhab et al., 2012; Poetri et al., 2014). Genetic selection can affect the quantity and quality of MDA transfer, as well as how long the MDAs could decay in the progeny (Grindstaff et al., 2003). There is also evidence indicating that MDAs decrease the efficacy of the killed vaccine against AIV (Maas et al., 2011; Abdelwhab et al., 2012). Therefore, the present study was designed to investigate the presence and possible impacts of avian influenza maternally-derived H5 antibodies on the outcome of vaccination with an inactivated AIV H5N9 vaccine in commercial chickens in Nigeria.

MATERIALS AND METHODS

Ethical approval

Ethical approval for this study was obtained from the Animal Care and Use for Research Committee of Ahmadu Bello University, Zaria (Approval number: ABUCAUC/2019/23).

Experimental animals

A total of 120 one-day-old ISA Brown chickens were purchased from three different major commercial hatcheries A, B, and C (n = 40 chicks per hatchery), respectively, through their retailing outlets within Kaduna

metropolis, and transported immediately to the Poultry Research Facility of the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. All the chickens were wing-banded with alphabetic and numeric tags for ease of identification.

Vaccine

An inactivated oil-emulsion avian influenza H5 vaccine (AVIFLU® H5, containing H5N9 subtype antigen and recommended for use in chickens at a dose of either 0.25 or 0.5 ml and administered via either subcutaneous or intramuscular routes) was purchased from Izovac, Italy, through their retailing agent in Nigeria and stored according to the manufacturer's instructions prior to usage.

Enzyme-linked immunosorbent assay

An enzyme-linked immunosorbent assay (ELISA) kit (ProFLOK®, Zoetis Inc., U.S.A) was used for the *in vitro* assessment of avian influenza H5 antibodies in sera of chickens according to the manufacturer's instructions.

Experimental design

Animal groupings

Immediately after purchase on the first day, 10 chicks per hatchery were randomly collected without replacement from the three commercial hatcheries (n = 30) to form groups A0, B0, and C0 for the assessment of maternally-derived AI H5 antibodies in the commercial chicks. Then the 90 commercial chicks (30 chicks per hatchery) remaining were divided on the first day of age into three groups of A, B, and C (n = 30 each) according to their sources, respectively. All the chicks were wing-banded with numeric ribbons for ease of identification and housed in clean and hygienic improvised cages (10 chicks per 60 cm × 55 cm cell) in the Poultry Research Unit of the Veterinary Teaching Hospital, Ahmadu Bello University, Zaria, Nigeria. The chicks in all the groups were granted access to potable drinking water and a commercial broiler's starter ration *ad libitum* throughout the experiment.

Treatment protocols

Hatchery A

The chicks in this group were subdivided into three subgroups of A1, A2, and A3 (n = 10 each) based on the dose of the AI H5N9 vaccine to be administered. Chicks in A1 were administered 0.2 ml of the commercial inactivated AI H5N9 vaccine via either the subcutaneous (at the nape of the neck, n = 5) and intramuscular routes (in the breast muscles) (n = 5) on days 14 and 28 of age. Chicks in A2 were administered 0.5 ml of the commercial inactivated AI H5N9 vaccine via either the subcutaneous (n = 5) and intramuscular routes (*n = 5*), respectively on days 14 and 28 of age. Chicks in A3 were administered 0.7 ml of the commercial inactivated AI H5N9 vaccine via either the subcutaneous (n = 5) and intramuscular routes (n = 5), respectively on days 14 and 28 of age.

Hatchery B

The chicks in this group were subdivided into three subgroups of B1, B2, and B3 (n = 10 each) based on the dose of the AI H5N9 vaccine to be administered. Chicks in B1 were administered 0.2 ml of the commercial inactivated AI H5N9 vaccine via either the subcutaneous (at the nape of the neck) (n = 5) and intramuscular routes (in the breast muscles) (n = 5), respectively on days 14 and 28 of age. Chicks in B2 were administered 0.5 ml of the commercial inactivated AI H5N9 vaccine via either the subcutaneous (n = 5) and intramuscular routes (n = 5), respectively on days 14 and 28 of age. Chicks in B3 were administered 0.7 ml of the commercial inactivated AI H5N9 vaccine via either the subcutaneous (n = 5) and intramuscular routes (n = 5), respectively on days 14 and 28 of age.

Hatchery C

The chicks in this group were subdivided into three subgroups of C1, C2, and C3 (n = 10 each) based on the dose of the AI H5N9 vaccine to be administered. Chicks in C1 were administered 0.2 ml of the commercial inactivated AI H5N9 vaccine via either the subcutaneous (at the nape of the neck) (n = 5) and intramuscular routes (on the breast muscle) (n = 5), respectively on days 14 and 28 of age. Chicks in C2 were administered 0.5 ml of the commercial inactivated AI H5N9 vaccine via either the subcutaneous (n = 5) and intramuscular routes (n = 5), respectively on days 14 and 28 of age. Chicks in C3 were administered 0.7 ml of the commercial inactivated AI H5N9 vaccine via either the subcutaneous (n = 5) and intramuscular routes (n = 5), respectively on days 14 and 28 of age.

Collection of samples

Assessment of Avian influenza maternally-derived antibodies and its decay pattern

For the serum assessment of the MDA to H5 AI vaccines in Nigeria and its decay pattern, one ml of blood was aseptically aspirated from the heart of each one-day-

old chick (n = 5 per hatchery) on arrival at the Poultry Research Facility at 1 day old. Two ml of blood was collected subsequently from each chick via the brachial vein at 7, 14, 21, 28, 35, and 42 days of age. The blood samples were collected using sterile hypodermic syringes into pre-labeled plain vacutainers. The tubes were then kept standing at room temperature for 24 hours for serum formation. Thereafter, serum from each tube was carefully aspirated using sterile pipettes into another set of one ml sterile, pre-labeled microcentrifuge tubes (Eppendorf®), and stored at -20˚C until assay for the AI MDA.

Evaluation of the humoral immune responses of pullet chicks to commercial inactivated avian influenza H5N9 vaccine

Two ml of blood was aseptically collected randomly via venipuncture of the brachial vein of 3 chicks in each subgroup (n = 3) using sterile 23G hypodermic needles and syringes on day 14 of age into plain vacutainers for serology. The tubes were also kept standing at room temperature for 24 hours for serum formation. Thereafter, serum from each tube was carefully aspirated using sterile pipettes into another set of 1 ml sterile, properly labeled microcentrifuge tubes (Eppendorf®), and stored also at -20˚C until assays for serum ant-AIV antibodies. The sampling procedure was repeated on days 21, 28, 35, and 42 of age.

Analysis of samples

Assessment of maternally-derived antibodies to avian influenza

The ELISA kit (ProFlok®) was used to assess the presence and decay pattern of AI maternally-derived antibodies in the chicks, as well as the anti-AI antibodies in serum samples post-vaccination with the AIV H5N9 inactivated vaccine. The ELISA Kit is a sandwich ELISA that could qualitatively and quantitatively assess the presence or absence of avian influenza H5 antibodies in avian serum, plasma, or other biological fluids, and was used according to the manufacturer's instructions. Briefly, all the reagents and samples were removed from the freezer and brought down to room temperature naturally for 30 minutes before starting the assay. The samples were completely thawed and thoroughly mixed prior to dilution. The serum samples were then diluted 50-fold (1:50) in sample dilution microplates and the diluted samples were allowed to equilibrate for 5 minutes before they were transferred to the ELISA microplates. The positive control wells, negative control wells, and sample wells in the ELISA microplate were set as appropriate. Then, 50 µl of the dilution buffer was added to each well in the ELISA microplates, and 50 µl each of the positive control and negative controls were then added to the positive control wells (A1, A3, and H11) and negative control wells (A2, H10, and H12). Thereafter, 50 µl of each sample dilution from the microplate was then transferred to the respective matching wells of the test microplate. The plates were then covered with an adhesive strip and incubated for 30 minutes at room temperature in a dark chamber. The content of each well in the test microplates was discarded by inverting and tapping the bottom of the plates. Then, 300 µl of the wash solution was then added to each test well and allowed to soak for 3 minutes. The contents were then again discarded by inverting and tapping the bottom of the plates. This wash procedure was repeated two more times before adding 100 µl of the conjugate solution to each test well and the plates were incubated for 30 minutes at room temperature. The plates were then washed again as earlier mentioned before adding 100 µl of the substrate to each test well. The plates were incubated again at room temperature for 15 minutes. Thereafter, 100 µl of the stop solution was then added to each test well to stop further reactions. The optical density (O.D) of each well on the plates was read at 450 nm wavelength using an ELISA reader (UNIEQUIP®) within 5 minutes of adding the Stop Solution.

Data analysis

Data were analyzed using the GraphPad Prism statistical software version 5.3 (Graph Pad Software, San Diego, California, USA). For the first experiment, the data were expressed as mean ± Standard errors of mean (SEM) and a two-way analysis of variance (ANOVA), followed by Bonferroni posttest were used to determine significant differences between the variables among all the sampled chicks. The mean MDA values obtained for all chicks of the three hatcheries were converted into Log base 2 for the estimation of the MDA half-life for the chicks in each hatchery. For the second experiment, the average antibody titer for each dose regime and route per hatchery were computed as mean anti-AI antibody titer per hatchery (irrespective of the dose or route of antigen administration), and also expressed as mean ± SEM, analyzed with ANOVA followed by Bonferroni posttest used to determine significant differences between the vaccine-induced antibody titers among the chicks from the three hatcheries. P values less than 0.05 were considered statistically significant for the study, and data were presented in tables and figures using Microsoft® excel version 13.

RESULTS

Detection of avian influenza maternally-derived antibodies in chicks

This study detected the presence of AI maternally-derived antibodies in all chicks sampled from the three different commercial hatcheries which were far above the detectable limits of 338 for the ELISA kit used at one day old (Table 1). There were highly statistically significant differences in the mean AI MDA titer levels between chicks from hatcheries C (2544.2 ± 244.6) and A (1107 ± 281.6), and C (1429.6 ± 471) and B (428.2 ± 173.3) at first ($p < 0.05$) and seven ($p < 0.05$) days of age. The mean AI MDA titer levels were however not statistically significantly different between the chicks from hatcheries A and B at 1, 7, 14, 21, and 28 days of age ($p > 0.05$) (Table 1).

Table 1. Presence of avian influenza H5 maternally-derived antibodies in ISA brown chickens from three different commercial hatcheries in Nigeria

Source of chickens / Age (days)	Hatchery A	Hatchery B	Hatchery C
	Maternally-derived antibody titers (Mean ± SEM)		
1	1107 ± 281.6[a]	1071.8 ± 155.9[b]	2544.2 ± 244.6[abc]
7	847.2 ± 238.4[a]	428.2 ± 173.3[b]	1429.6 ± 471.0[abc]
14	308.4 ± 234.4[a]	101 ± 48.1[a]	273.8 ± 28.8[a]
21	86 ± 44.1[a]	36 ± 18.6[a]	70.2 ± 35.8[a]
28	19.8 ± 19.8[a]	5 ± 3.9[a]	22 ± 14.3[a]

Mean ± SEM values in the same row with different superscripts are statistically significantly different at $p < 0.05$ according to the Bonferroni Posthoc test. SEM: Standard error of mean

Table 2. Kinetics of avian influenza H5 maternally-derived antibodies in ISA brown chickens from three different commercial hatcheries in Nigeria

Source of chicks						MDA depleted (%)
Hatchery A		Hatchery B		Hatchery C		
Mean MDA titer	Half-life (days)	Mean MDA titer	Half-life (days)	Mean MDA titer	Half-life (days)	
1107	0	1071.8	0	2544.2	0	0
553.5	4.4	535.9	5.4	1272.1	4.7	50
276.8	3.9	268	4.8	636.1	4.3	75
138.4	3.4	134	4.2	318	3.8	87.5
69.2	3	67	3.6	159	3.3	93.8
34.6	2.5	33.5	3.0	79.5	2.9	96.9
17.3	2.0	16.7	2.4	39.8	2.4	98.4
8.6	1.5	8.4	1.8	19.9	2	99.2
4.3	1.0	4.2	1.2	9.9	1.5	99.6
2.2	0.5	2.1	0.6	5	1.1	99.8
Mean half-life (days)	2.5 ± 0.4[a]		3.0 ± 0.5[ab]		2.9 ± 0.4[ac]	
CV (%)	53.9		54.8		43.2	
Total decay time (days)	22.3		27.3		26.0	100

Mean ± SEM values in the same row with different superscripts are statistically significantly different at $p < 0.05$ according to the Bonferroni Posthoc test. MDA: Maternally-derived antibodies, CV: Coefficient of variation, SEM: Standard error of mean

Decay pattern and half-life of the avian influenza anti-H5 maternally-derived antibodies in chicks from three different commercial hatcheries in Nigeria

The findings from this experiment showed that although there was no statistically significant difference in the regression coefficients of the mean AI MDA titer levels of chicks from hatcheries A, B, and C ($p > 0.05$), there were very strong negative correlations between the mean AI MDA titer levels and decay time for all chicks from hatcheries A ($r = -0.96$), B ($r = -0.88$) and C ($r = -0.91$), respectively (Table 2). Results from this experiment indicated that although the AI MDA titers for all chicks sampled from the three different commercial hatcheries persisted for 28 days (Table 2), there were statistically

significant differences in the mean half-life ($t_{1/2}$) of the MDA between the hatcheries ($p < 0.05$, Table 2). The results from this study showed also that although it took 17.2, 21.2, and 19 days for 95% of the MDA to decay for the chicks from hatcheries A, B, and C (equivalent to approximately 5 half-lives, Table 2), the total decay time was 22.3, 27.3, and 26 days for chicks from hatcheries A, B, and C, and the mean $t_{1/2}$ were 2.5 ± 0.4, 3 ± 0.6, and 2.9 ± 0.4 days for chicks from hatcheries A, B, and C with a coefficient of variations (CV) of 53.9%, 54.8%, and 43.2%, respectively (Table 2). Furthermore, the results showed that the MDA for the chicks from the three commercial hatcheries had a mean decay time of 25.2 ± 1.5 days, even after the administration of the first dose of the inactivated H5 avian influenza vaccine (Table 2).

Effects of maternally-derived avian influenza anti-H5 maternally-derived antibodies on the humoral immune response of ISA brown chicks administered a commercial inactivated avian influenza H5N9 vaccine

Although the differences between the mean AI H5 MDA and vaccine antibody titers for chicks from hatcheries A, B, and C, at 14 and 21 days of age, were not statistically significant ($p > 0.05$), the results from the present study showed that the mean maternally-derived H5 AI antibody titers were 308.4 ± 234.4, 101 ± 48.1 and 273.8 ± 28.8 as well as 86 ± 44.1, 36 ± 18.6 and 70.2 ± 35.8, respectively. Also, the mean AI H5 MDA titers for chicks from hatcheries A, B, and C were 136.7 ± 32.6, 113.6 ± 33.4, 213 ± 84.5 ($p > 0.05$) and 408.4 ± 124.7, 398.3 ± 66.8, 1580.7 ± 314.5 at 14 and 21 days of age (Figure 1).

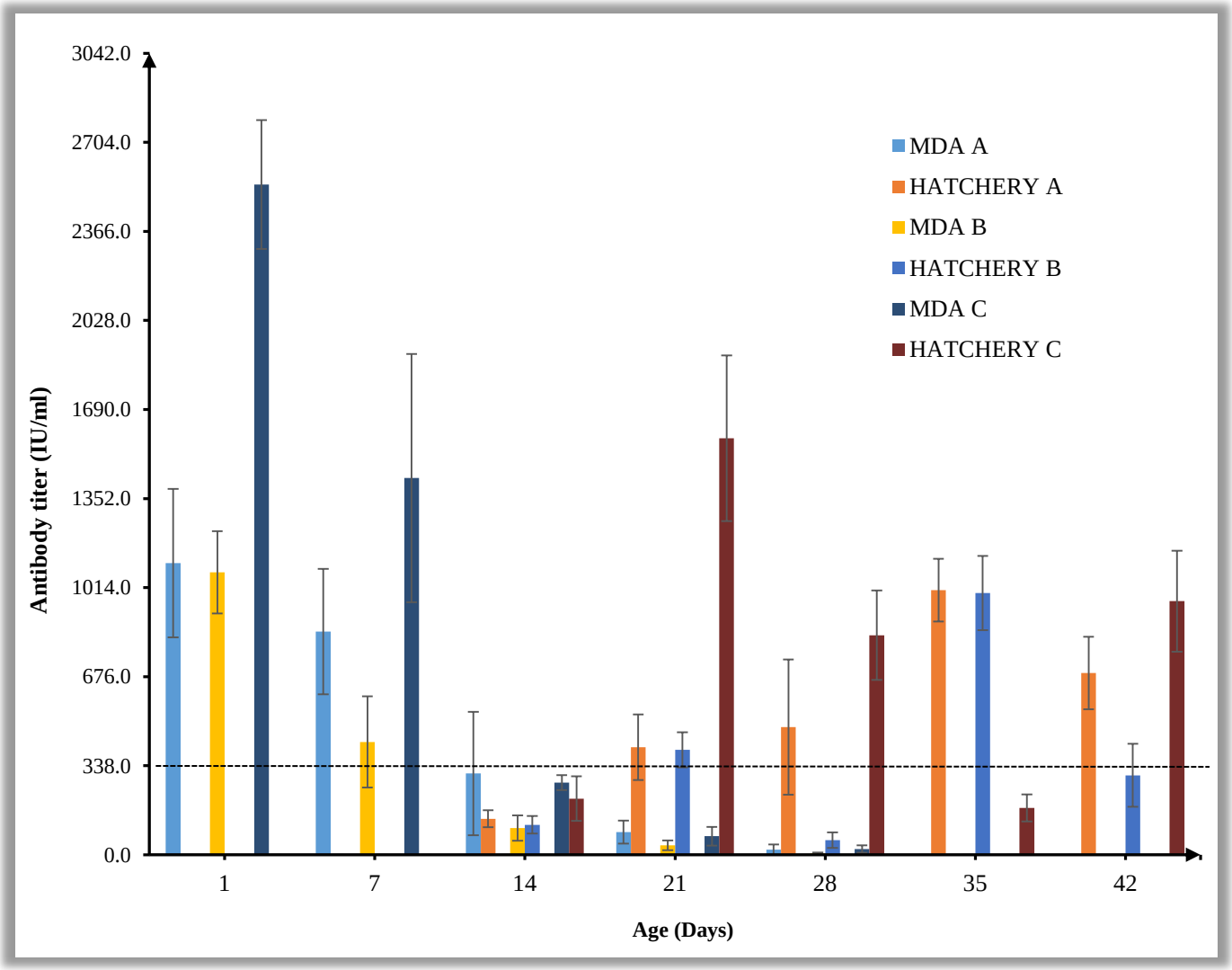

Figure 1. Effects of maternally-derived avian influenza H5 antibodies on inactivated avian influenza H5N9 vaccine in commercial chicks in Nigeria

DISCUSSION

Findings from the present study established the presence of maternally-derived AI H5 antibodies in all the one-day-old ISA brown chickens sampled from the three different commercial hatcheries in Nigeria. Although there is a government policy against the use of AI vaccines in the Nigerian commercial poultry industry, the detection of AI H5 MDAs from the current study could be attributed to the vaccination of breeder flocks with AI vaccines prior to the commencement of lay as most commercial hatcheries in Nigeria are high-capital ventures with little or no government interference. These findings are in tandem with the studies by Maas et al. (2011), Abdelwhab et al. (2012), and Kandeil et al. (2018) who also detected the presence of passively transferred AI antibodies in progeny chickens from vaccinated parent breeders.

Understanding maternal antibody decay and its impact on vaccine immunogenicity may provide guidance in determining vaccination schedules against some diseases in very young animals with persisting maternal antibodies. Although the findings from the present study showed a very strong negative correlation between the MDA titer levels in the chicks and age, there were however significant differences between the MDA titer levels in the chicks from hatchery C when compared to those from hatcheries A and B as evident by their coefficient of variations. The observed differences in MDA levels between the chicks from the three hatcheries could be due to the number of antibodies present in the sera of the hens, as well as the high amount of the MDA that was eventually transferred from the chicks as previously reported (Hamal et al., 2006; Grindstaff, 2010).

Although the MDAs in chicks could be depleted more rapidly under field conditions than in controlled settings such as in the present study, the observed variability in the MDA titers in the progeny chicks sampled in the present study could be attributed to the lack of uniformity of MDA titer levels in the one-day-old chicks from the three commercial hatcheries and by extension, the breeders from the parent flocks since breeder farms in Nigeria have varied medical and or operational regimes, as well as the different rates of growth and metabolism in the chicks sampled as previously reported (Hamal et al., 2006; Tarigan et al., 2018). The findings from the present study agree with previous studies on MDAs for other infectious viruses, such as avian influenza (Maas et al., 2011), infectious bursal disease (Abdu and Ibe, 2013), and Newcastle disease (Deka et al., 2020), which reported that the MDAs in chickens progressively decrease with increasing age.

The findings from the present study showed that although the MDA detected in all the chickens from the three commercial hatcheries persisted for 28 days, this temporal persistence of the MDA could be due to the level of maternal antibodies initially transferred into the egg yolk, and thus agrees with the findings from previous studies on MDA (Grindstaff, 2010), even though little is known about the potential role of other factors. However, studies in chickens have shown that the protection mediated by maternal antibodies is highly subtype- and strain-specific (Maas et al., 2011; Abdelwhab et al., 2012; Cardenas-Garcia et al., 2019) and that such MDA lack the ability to induce heterosubtypic responses that are often mediated by the mucosal and cell-mediated immune responses evoked by natural infection (Clements et al., 1986; Doherty and Kelso, 2008).

The attainment of population immunity is critical for the success of any vaccine-intervention program, and the achievement of flock-level immunity is commonly presented by the percentage coefficient of variation (CV) (Greenacre and Morishita, 2014). The presented study showed a coefficient of variations (CV) of 53.9%, 54.8%, and 43.2% for the chicks from hatcheries A, B, and C, respectively. The high CV obtained in this study provides evidence for considerable variation in antibody responses of the breeder hens from hatcheries A and B after vaccination, and our result agrees with the findings of Tarigan et al. (2018) who reported that the outcomes of field H5 N1 vaccination were highly variable and farm-related. Although the previous report has indicated a CV of $\leq$ 40% for vaccination against most poultry diseases (Greenacre and Morishita, 2014), the slight increases in CV obtained in the present study could be attributed to differences in intrinsic factors such as body weight gain and individual immune competence as well as extrinsic factors such as stocking density, underlying disease conditions, transportation stress which may differ between hatcheries. These assertions are in tandem with the findings of Tung et al. (2013) who stated that field conditions, which may be associated with environmental factors and farm management practices, immunization techniques, vaccine storage, vaccinator's skill, as well as other factors that vary across farms could determine the variability in flock immune response and antibody titers.

Although results showed that 95% of the MDA decayed over a period of 17.2, 21.2, and 19.0 days for the chicks from hatcheries A, B, and C (equivalent to

approximately 5 half-lives) respectively, the total decay time from this study was 22.3, 27.3, and 26 days for the chicks from hatcheries A, B, and C, with a mean MDA decay time 25.2 ± 1.5 days. The rate of depletion of MDA seen in the present study as evident in all the treated groups could be attributed to the usage of the yolk content as a source of energy. This rapid depletion of the MDA within the first few days of the chicks' lives as indicated by their mean MDA half-lives in the present study could be attributed to MDA catabolism in the process of growth and development (Garnier et al., 2012), and shows the inability of these MDAs to confer adequate protection against H5 subtypes of field AIVs circulating in Nigeria. This finding agrees with some previous studies in which the MDA was seen to last about 35 days (van der Lubbe et al., 2017), as well as 36 days for antibodies against pertussis toxin, 40 days for filamentous haemagglutinin in humans (Van Savage et al., 1990), 35 days for anti-diphtheria toxin antibodies (Barr et al., 1949), and 46 days for measles antibodies (Black et al., 1986). Also, the mean half-lives ($t_{1/2}$) obtained from this study were 2.5 ± 0.4, 3 ± 0.6, and 2.9 ± 0.4 days for chicks from hatcheries A, B, and C, respectively. These varied kinetics in the mean half-lives for all the hatcheries in this study could be due to the varied timing in the vaccination of parent breeders, the level of maternal antibodies transferred to progeny chickens, the genetic makeup of the chicks, and the growth rate of chicks.

The findings of the present study showed poor humoral immune buildups in the chicks from the three commercial hatcheries. This could be due to the interference of the antibody-mediated response by the AI H5 MDAs as evident by the duration of MDA depletion observed in the present study and agrees with findings from researchers who indicated that MDAs decrease the efficacy of inactivated vaccines against AIVs (Maas et al., 2011; Abdelwhab et al., 2012). The observed poor humoral immune response could also be attributed to the fact that MDAs generally bind to vaccine antigens and mask the epitopes from the B cells of the immunological naïve individuals, thereby dampening their immune responses, and preventing optimal exposure to the immune system as previously reported by Naqi et al. (1983) and van der Lubbe et al. (2017).

CONCLUSION

The present study was able to detect avian influenza H5 MDA from all the chicks sampled from the three different commercial hatcheries in Nigeria. Whereas this study has shown the existence of variability in the mean half-life of avian influenza MDA in chicks from different commercial hatcheries, the temporal persistence of the AI anti-H5 MDA of the chicks from the three different hatcheries was also highly variable and correlated negatively with the age of the chicks. Present findings showed variable interferences by the AI H5 MDA titers with the immune response of the chicks from all the hatcheries. Therefore, there is the need for the inclusion of strategies that differentiate infected from vaccinated animals (DIVA) in the national AIV surveillance programs, as well as a greater understanding of how seemingly minor changes in breeder management practices could affect the overall development and immune competencies of specific genetic lines of chickens.

DECLARATIONS

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Authors' contributions

Woziri AO, Abdu PA, Meseko CA, and Fasina FO conceptualized the experiments. Woziri AO, Abdu PA, and Adamu J designed the experiments. Woziri AO, Abdu PA, Nasir FI, and Abdulkarim K performed the experiments. Woziri AO, Abdu PA, and Babashani M analyzed the data. Woziri OA, Abdu PA, Meseko CA, and Fasina FO drafted the manuscript. All authors checked the statistical results and approved the final version of the manuscript for publication.

Competing interests

The authors declare that there is no conflict of interest in the outcome of this research work.

Ethical considerations

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by the authors before the submission. The final results of the statistical analysis have been checked and confirmed by all authors.

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