Field evaluation of avian encephalomyelitis maternal antibody transfer in chicken flocks in Southwest Nigeria

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Keywords

Poultry – Avian encephalomyelitis virus – Layer chicken – Broiler chicken – Hemagglutination test – Vaccination – Nigeria.

Summary

Avian encephalomyelitis (AE) is controlled by ensuring adequate level of maternal antibodies in chicks. Incessant outbreaks in chicken flocks in Southwest Nigeria in spite of vaccination of breeders prompted the assessment of AE virus antibody levels in breeder flocks and their progeny. Five prevaccinated chicken breeder flocks (A, B, C, D and E) were selected in Ibadan, Oyo State, and Mowe, Ogun State. Blood samples were collected from fifteen breeders from each flock, as well as their chicks at 1, 7 and 14 days of age and sera subjected to the passive hemagglutination test. Ranges of titers, modal titers, mean geometric titers (MGTs) and maternal antibody transfer rates were determined for each flock. MGTs ranged between 3.3 ± 0.09 and 4.8 ± 0.14 in breeder flocks, 0.8 ± 0.09 and 2.4 ± 0.21 in day-old chicks, and 2.8 ± 0.15 and 3.9 ± 0.06 in 7-day-old chicks. The highest values in all categories were observed in broiler flock D. MGTs ranged between 1.4 ± 0.18 and 2.3 ± 0.17 in 14-day-old chicks, and the highest value was found in broiler flock C. In general, significant ($p < 0.05$) declines in MGTs were recorded from breeder flocks to day-old chicks. Maternal antibody transfer rates were higher in broiler flocks C and D with 50 and 40.9%, respectively, whereas those of pullet flocks A, B and E were 24.2, 19.5 and 19.5%, respectively. Results showed that by six months post-vaccination, maternal antibody transfer was low especially in pullet flocks, resulting in low to undetectable antibody titers in day-old chicks. A modification of the vaccination schedule is therefore advised.

■ INTRODUCTION

Avian encephalomyelitis (AE) is an infectious viral disease of poultry, which occurs in young chickens, turkeys, pheasants, Japanese quails, pigeons, ducklings and partridges (4, 17, 19). It is characterized by clinical signs of central nervous system disorder, particularly ataxia and tremors of the head, neck and limbs from where the name epidemic tremor was derived. The clinical signs are usually accompanied by high morbidity and variable mortality. Older poultry can become infected but rarely develop clinical signs, except a drop in egg production in layers (6).

Avian encephalomyelitis virus (AEV) belongs to the Picornaviridae family and was temporarily classified as a *Hepatovirus* due to its relatedness to hepatitis A virus (6, 10). It has recently been re-classified as a *Tremovirus* (6) and only one serotype exists, i.e. AEV-1. All strains are enterotropic but some strains are more neurotropic than others, thus exhibiting varying levels of pathogenicity (6). The disease occurs worldwide including Nigeria (2), Zimbabwe (5) and Sudan (1). AE is a disease of economic concern to poultry farmers as it causes decrease in egg production in layers, decrease in hatchability, neurologic signs in chicks, and survivors are considered unprofitable (14).

Control is mainly by vaccination of breeders and layers with live or inactivated vaccine before the onset of laying to prevent vertical transmission of the virus and ensure transfer of maternal antibodies to progeny. Transfer of maternal antibodies from hens plays a crucial role in the protection of chicks against many poultry

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pathogens (16), in particular AE virus (20). Immunity develops in two to three weeks and is believed to be long lasting (18). However, incessant outbreaks of AE in poultry farms in Southwest Nigeria in spite of breeder vaccination remain a challenge. This study was therefore conducted to assess titers of AE antibodies post-vaccination in selected chicken breeder flocks and maternal antibody levels in their respective chicks. Maternal antibody transfer rates were also determined.

■ MATERIALS AND METHODS

Chicken flocks

Five chicken breeder flocks designated as flocks A, B, C, D and E, from two selected farms in Ibadan, Oyo State, and Mowe, Ogun State, in Nigeria, were used. Breeder flocks A, B and C were located in Ibadan whereas breeder flocks D and E were located in Mowe. Flocks A, B and E consisted of Isa Brown pullet breeders, and flocks C and D consisted of Anak broiler breeders. Parent stocks A, B, C and D were 36, 71, 36 and 45 weeks of age, respectively, and were vaccinated with AE live vaccine (Calnek 114, FATRO s.p. A-Ozzano Emilia {BO} – Italy) at 13 weeks of age, whereas parent stock E was 43 weeks of age and was vaccinated at 11 weeks. They were housed in naturally ventilated open-sided houses.

Twenty-five day-old chicks hatched from each flock were housed at a different location in an open-sided housing facility, in separate pens per flock for ease of monitoring. The chicks hatched from the batch of eggs laid on the same day the parent stocks were sampled for blood. They were fed with broiler chick starter (UAC Vital Feed®) and multivitamins (Vitalyte®) were administered till two weeks of age.

Blood collection

Fifteen chickens were randomly selected from each breeder flock and were sampled for blood (3 ml) via the jugular vein into plain vacutainer tubes. Blood samples were left slanted on the bench at room temperature and allowed to clot. Serum samples were harvested into eppendorf tubes, stored at -20°C and tested for the presence of AE antibodies within one week of collection. Blood samples were also collected from twelve of the twenty-five chicks from each flock at one-day-old terminally (intracardially), as well as at 7 and 14 days of age by jugular venipuncture.

Passive hemagglutination test

Serum samples were subjected to passive hemagglutination assay as described by Penner and Hennessy (15). Known positive antiserum was developed by inoculating two adult chickens from a disease-free flock belonging to the Department of Veterinary Medicine (which tested negative to AE virus antibody), with diluted AE vaccine (Calnek, Italy) equivalent to 10 doses, intramuscularly. The procedure was repeated twice within three weeks after which the chickens were sampled for serum, ten days after the second inoculation.

A suspension of turkey red blood cells (RBC) was harvested and washed three times in phosphate buffered saline (PBS). It was incubated with equal volume of 3% formalin in PBS for 20 h at 37°C (i.e. formalinization). Excess formalin was removed by repeated washing in double distilled water. The suspension of formalinized RBC was incubated with a solution of diluted tannic acid in PBS, pH 6.4, for 30 min at 56°C and subsequently washed in PBS. AEV vaccine diluted 1:10 in PBS was mixed with an equal volume of RBC suspension and incubated for 2 h at 37°C (sensitization). The suspension of sensitized RBC was centrifuged, washed three times in PBS, and RBC were re-suspended in PBS to a final concentration of 1%.

Fifty microliters of PBS were dispensed into each well of the microtiter plate, then 50 μl of sera were dispensed into the first well in each row of wells. A double-fold serial dilution was carried out in the rows till the eleventh well from which 50 μl were discarded. Fifty microliters of 1% AE antigen sensitized RBC suspension were then added into each well. The contents of the microtiter plate were mixed gently on a microshaker and allowed to incubate at room temperature from 40 min to 1 h. The last well showing agglutination of red blood cells was recorded as the AE antibody titer of the respective serum sample.

Analysis

Range of antibody titers, modal titer (i.e. most frequently occurring titer) and mean geometric titer (MGT) of each flock of chickens were determined and recorded. Rates of maternal antibody transfer from parent stocks to chicks were also determined by expressing the mean antibody titers in chicks as percentage of the mean titer of the respective breeder flock. MGTs were compared between flocks at each sampling time and within flocks using the analysis of variance and Duncan's multiple range test.

■ RESULTS

Ranges of antibody titers, modal titers and mean geometric titers of flocks A, B, C, D and E are presented in Table I. In the parent stocks, modal titers were 8 in flock A, and 16 in both flocks B and E, and they were 16 and 32 simultaneously within each group in flocks C and D. MGTs of the five parent stocks ranged between 3.3 and 4.8. In the day-old chick flocks, modal titers were 2, 0, 4, 8 and 2, in flocks A, B, C, D and E, respectively, and their MGTs ranged between 0.8 and 2.4. In the 7-day-old chick flocks, the modal titer was 8 in flocks A, B, C and E, and 16 in flock D. MGTs ranged between 2.8 and 3.9 at this age. In 14-day-old chick flocks, modal titers were 8, 8, 4 and 8 in flocks A, B, C and D, respectively, and 2 and 4 simultaneously in flock E; MGTs ranged between 1.4 and 2.3.

Significant declines ($p < 0.05$) in AE antibody titers, i.e. MGTs, were observed from parent stocks to day-old chicks (Figure 1). This was followed by significant ($p < 0.05$) increases at 7 days and significant ($p < 0.05$) declines at 14 days. Rates of maternal antibody transfer from parent stocks to day-old chicks were 24.2, 19.5, 40.9, 50.0 and 19.5% in flocks A, B, C, D and E, respectively.

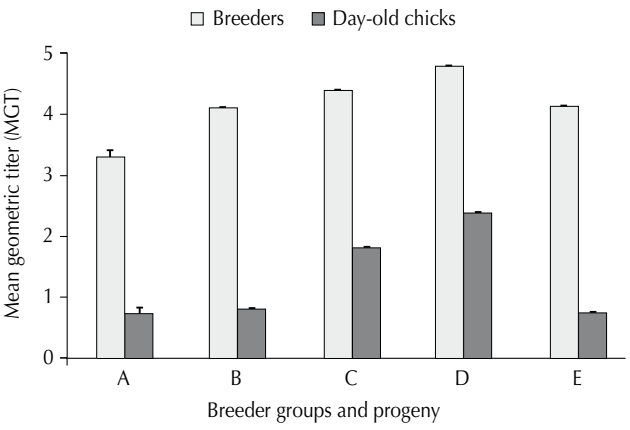

Figure 1: *Avian encephalomyelitis virus maternal antibody transfer in selected breeder flocks in Southwest Nigeria.*

Table I

Avian encephalomyelitis virus antibody passive hemagglutination titers in breeder flocks and progeny

Flock	Class	Range of titers	Modal titer	Mean geometric titer	Rate of maternal antibody transfer (%)
A	Parent stock	8-16	8	3.3 ± 0.09^{a}	24.2
	Day old	0-2	2	0.8 ± 0.09^{c}	
	7 days old	0-16	8	2.9 ± 0.23^{a}	
	14 days old	0-8	8	2.2 ± 0.19^{b}	
B	Parent stock	8-32	16	4.1 ± 0.11^{a}	19.5
	Day old	0-8	0	0.8 ± 0.19^{d}	
	7 days old	4-16	8	2.9 ± 0.16^{b}	
	14 days old	0-8	8	1.8 ± 0.26^{c}	
C	Parent stock	8-64	32,16	4.4 ± 0.15^{a}	40.9
	Day old	2-4	4	1.8 ± 0.08^{c}	
	7 days old	4-16	8	2.8 ± 0.15^{b}	
	14 days old	0-8	4	2.3 ± 0.17^{c}	
D	Parent stock	16-64	32,16	4.8 ± 0.14^{a}	50.0
	Day old	0-8	8	2.4 ± 0.21^{c}	
	7 days old	8-16	16	3.9 ± 0.06^{b}	
	14 days old	0-8	8	2.0 ± 0.23^{c}	
E	Parent stock	8-32	16	4.1 ± 0.12^{a}	19.5
	Day-old	0-2	2	0.8 ± 0.09^{c}	
	7 days old	2-16	8	3.0 ± 0.17^{b}	
	14 days old	0-8	2,4	1.4 ± 0.18^{c}	

Values with different superscripts are significantly different ($p < 0.05$)

■ DISCUSSION

Chicks depend on maternal antibodies as the main source of immune protection until they become immunocompetent. Our objective was to evaluate the capacity of chicks from vaccinated parent stocks to withstand challenges of avian encephalomyelitis outbreaks in poultry farms in Southwest Nigeria. As a first step, we assessed the rate of maternal antibody transfer from AE vaccinated hens to their progeny. There was a decrease in modal titers from breeder stocks (8-32) to day-old chicks (0-8). Also, MGTs reduced significantly ($p < 0.05$) from range 3.3–4.8 in breeder stocks to 0.75–2.41 in day-old chicks with transfer rates ranging from 19.5 to 50%. This decrease in antibody levels from parent stocks to day-old chicks is a regular occurrence in poultry health management (7). Hamal et al. (8) reported a dam-to-chick antibody transfer of approximately 30%. In our study, transfer rates of 40.9 and 50% were obtained for the two broiler flocks C and D, respectively. By contrast, it was 24.2, 19.5 and 19.5% in the three pullet flocks A, B and E, respectively. Sharma (16) evidenced a relation between high maternal antibody transfer efficiency and high antibody level in parent stock. Thus, the two broiler parent flocks C and D, which had the highest antibody titers in this study, i.e. 4.4 ± 0.15 and 4.8 ± 0.14, respectively, also had the highest maternal antibody transfer rates compared with the pullet parent flocks. This finding also concurred with Nemeth and Bowen (11) who reported high West Nile virus maternal antibody titers and extended decay period in chicks whose dams had high titers.

Vaccination programs for the control of most viral diseases of chickens such as Newcastle disease and infectious bursal disease usually comprise one or two live vaccines administered early in life, as well as an inactivated (killed) vaccine administered prior to lay in order to ensure transfer of high level maternal antibody to progeny (12, 13). However, the breeder flocks investigated in this study were vaccinated only once with a live AE vaccine at either 11 or 13 weeks of age as recommended by the manufacturer and which is the usual practice in the industry (6). This schedule contrasts with multiple vaccinations used for the control of most diseases of poultry (6). It is our belief that the administration of a single live vaccine to these breeder flocks was responsible for the generally low antibody levels in parents with subsequent low to undetectable levels in day-old chicks.

The absence of detectable antibodies observed in some day-old chicks in most of the flocks is undesirable at this age as it may provide a portal of entry of infection into the flock. The higher maternal antibody titers, reflected by MGTs recorded at 7-day-old compared with day-old and 14-day-old titers, are believed to result from further release of immunoglobulins (IgY) from yolk sac into chick immediately post hatching. According to Vegad (18), the level of maternal antibodies in circulation of newly hatched chicks peaks at about 2-3 days of age and decreases thereafter to undetectable levels by 2-5 weeks of age.

The passive hemagglutination test used in this study was declared to be more sensitive than the immunodiffusion test which can detect antibodies as early as four days post-inoculation of AE virus (3). Thus, the absence of detectable antibodies in the serum of a large proportion of day-old chicks, which indicates inefficient transfer of maternal antibodies as a result of low levels in parent stocks, highlights the need to revise the vaccination schedule.

■ CONCLUSION

This study showed that by six months post-vaccination of breeders, AEV antibodies were low resulting in the inefficient transfer of maternal antibodies to chicks especially in pullet breeders. The administration of a secondary vaccine is therefore advised to boost the primary immune response. This should be an inactivated or killed vaccine administered prior to laying in order to prevent vertical transmission of the virus via eggs and decrease in egg production, which could occur with the use of live vaccines after the onset of lay.

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Résumé

Oladele O.A., Onwuka C.O. Evaluation sur le terrain du transfert des anticorps maternels contre l'encéphalomyélite aviaire dans des élevages de poulets du sud-ouest du Nigeria

L'encéphalomyélite aviaire (AE) est contrôlée par l'apport d'un niveau suffisant d'anticorps maternels chez les poussins. Des épidémies persistantes, malgré la vaccination des reproducteurs dans les élevages de poulets du sud-ouest du Nigeria, ont incité les auteurs à évaluer les taux d'anticorps contre le virus AE dans des troupeaux de reproducteurs et de leur progéniture. Cinq troupeaux de reproducteurs de poulets prévaccinés (troupeaux A, B, C, D et E) ont été sélectionnés à Ibadan, Etat d'Oyo, et Mowe, Etat d'Ogun. Des échantillons sanguins ont été prélevés sur quinze reproducteurs de chaque troupeau ainsi que sur leurs poussins âgés de 1, 7 et 14 jours, et les sérums ont été soumis à l'épreuve d'hémagglutination passive. La variation des titres, la moyenne géométrique des titres (MGT) et le taux de transfert des anticorps maternels ont été déterminés pour chaque troupeau. La MGT a varié entre 3,3 ± 0,09 et 4,8 ± 0,14 dans les troupeaux de reproducteurs, 0,8 ± 0,09 et 2,4 ± 0,21 chez les poussins âgés d'un jour, et 2,8 ± 0,15 et 3,9 ± 0,06 chez les poussins âgés de 7 jours. Les valeurs les plus élevées toutes catégories confondues ont été observées dans le troupeau de poulets de chair D. La MGT a varié entre 1,4 ± 0,18 et 2,3 ± 0,17 chez les 14 poussins âgés de 14 jours, et la valeur la plus élevée a été obtenue dans le troupeau de poulets de chair C. Dans l'ensemble, la MGT des poussins d'un jour a baissé significativement ($p < 0,05$) par rapport à celle des reproducteurs. Les taux de transfert des anticorps maternels ont été plus élevés dans les troupeaux de poulets de chair C et D avec respectivement 50 et 40,9 p. 100, alors que ceux des troupeaux de poulettes A, B et E ont été respectivement de 24,2, 19,5 et 19,5 p. 100. Cette étude a montré que le transfert des anticorps maternels était faible six mois après la vaccination, en particulier dans les troupeaux de poulettes, se traduisant par des titres d'anticorps faibles ou indétectables chez les poussins âgés d'un jour. Une modification du calendrier de vaccination est donc recommandée.

Mots-clés : Volaille – Virus encéphalomyélite aviaire – Poule pondeuse – Poulet de chair – Epreuve d'hémagglutination – Vaccination – Nigeria.

Resumen

Oladele O.A., Onwuka C.O. Evaluación de campo de la transferencia de anticuerpos maternos contra la encefalomielitis aviar en parvadas de pollos en el suroeste de Nigeria

La encefalomielitis aviar (AE) se controla asegurando un nivel adecuado de anticuerpos maternos en pollitos. Brotes constantes, en parvadas de pollos en el suroeste de Nigeria, a pesar de la vacunación de los reproductores, impulsó la evaluación de los niveles de anticuerpos contra el virus AE en parvadas de reproductores y sus progenituras. Se seleccionaron cinco parvadas de pollos reproductores pre vacunados (grupos A, B, C, D y E) en Ibadan, estado de Oyo, Mowe y estado de Ogun. Se sangraron quince reproductores de cada grupo, así como sus pollitos, de 1, 7 y 14 días de edad, cuyos sueros se sometieron al test de hemoaglutinación pasiva. Se determinaron los rangos de titulación, promedios de las titulaciones geométricas (MGTs) y tasas de transferencia de los anticuerpos maternos para cada grupo. Los MGTs variaron entre 3,3 ± 0,09 y 4,8 ± 0,14 en parvadas de reproductores, 0,8 ± 0,09 y 2,4 ± 0,21 en pollitos de un día de edad y 2,8 ± 0,15 y 3,9 ± 0,06 en pollitos de 7 días de edad. Los valores más elevados de todas las categorías se observaron en el grupo D de reproductores. Los MGT variaron entre 1,4 ± 0,18 y 2,3 ± 0,17 en pollitos de 14 días de edad y el valor más elevado se encontró en el grupo C de pollos de engorde. En general, se registraron disminuciones significativas ($p<0,05$) en MGTs en parvadas de reproductores a pollitos de un día de edad. Las tasas de transferencia de anticuerpos maternos fueron más elevadas en los grupos C y D de pollos de engorde, con 50 y 40,9%, respectivamente, mientras que los grupos de pollitas A, B y E fueron 24,2, 19,5 y 19,5%, respectivamente. El presente estudio mostró que a los seis meses post vacunación, la transferencia de anticuerpos maternos fue baja, especialmente en las parvadas de pollitas, resultando en titulaciones de anticuerpos bajas o no detectables en pollitos de un día de edad. Por lo tanto se recomienda una modificación de los esquemas de vacunación.

Palabras clave: Ave de corral – Virus encéfalomielitis aviar – Gallina ponedora – Pollo de engorde – Prueba de hemaglutinación – Vacunación – Nigería.