



## Maternal antibody titre as a monitoring tool for vaccination against infectious bursal disease

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### Abstract

The study was designed to investigate the presence and level of maternally derived antibodies (MDA) in broiler and pullet chicks to determine the right days and time of vaccination against Infectious bursal disease (IBD). A total of 150 day old broiler chicks and pullets chicks were obtained from reputable breeder farmers and the birds divided into 5 groups (A, B, C, D and E) comprising 30 chicks per group. Coefficient of variation (CV), central vaccination time (CVT), and vaccination days (VD) from mean MDA (passive immunity chicks derived from parents' stock) ELISA titres were also determined using Hipra calculator (a device online for determining vaccination day and time from mean ELISA titers). One milliliter of blood sample was collected from each broiler and pullet chick from all the groups to obtain sera. The antibody titre level of broiler chicks ranged from  $1,962 \pm 438$  to  $4,363 \pm 974$  while that of pullets ranged from  $2,111 \pm 471$  to  $4,526 \pm 1011$ . The highest CV was 46% for broiler chicks from farm A and the highest CV for pullets was 42.5% from another breeder farm D. The CVT for broilers to be vaccinated with mild IBD vaccine was 19 days, it was 18 days for intermediate vaccine and for intermediate plus vaccines was 12 days. The CVT was 33 days for pullets to be vaccinated with mild vaccine, 31 days for intermediate vaccine, while with intermediate plus vaccines it was 20 days. While the vaccination days for broiler was 7 and 17 days and it was 10 and 25 days for pullets. From the study the presence and level of MDA in day old chicks has been established and was above 1000 ELISA titre in chicks from all the five breeder farms.

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### Introduction

Infectious bursal disease (IBD) or Gumboro disease is an infectious, acute, mild or subclinical viral disease of young chicks characterized by trembling, in coordination, inflammation followed by necrosis and atrophy of the bursa of Fabricius and immunosuppression (Abdu, 2007). It is caused by a double stranded RNA virus belonging to the genus *Avibirnavirus* under the family *Birnaviridae*,

(Palmquist *et al.*, 2006). IBD has been a persisting problem for the commercial chicken industry since its discovery in Gumboro, Delaware in the late 1950s (Giambrone, 2008). In the 1960's and 70's, the highly contagious viral disease mainly appeared in the clinical form, affecting chickens between 2 and 4 weeks of age (Giambrone, 2008). The disease was particularly important due to high mortalities,

lowered productivity among infected chicks and immune depression to other infections and poor response to vaccination (Durojaiye & Adene, 1989). Despite the fact that vaccination against IBD has been introduced into Nigeria since its recognition in 1969, the disease has remained a major threat to the Nigerian poultry industry (Durojaiye & Adene, 1989). Post-vaccination IBD outbreaks continue to occur in many poultry farms and have thereby undermined the confidence of poultry farmers (Durojaiye & Adene, 1989). Economic losses are incurred as a result of the high mortality rate and a predisposition to secondary infection (Muller *et al.*, 2012). It has been observed, for instance, that Newcastle disease outbreaks occur more commonly in flocks that recovered from IBD (Durojaiye & Adene, 1989). Since MDA declines steadily after hatch and is absent by three or four weeks of age, it cannot be depended on to protect against clinical IBD (Naqi *et al.*, 1983). The type of vaccine to use and programme to follow will depend on the virulence of the field IBDV and level of MDA in birds (Giambrone, 2008). Neutralization of IBDV by MDA present at the time of vaccination was reported (Abdu, 1997). High level of MDA can neutralise IBDV during early vaccination, therefore MDA may be most probable cause of vaccination failures (Abdu, 1997). The failure to control IBD in Nigeria inspite of vaccination necessitates a reappraisal of level of maternal antibodies in commercial chicks. The present study aimed at determining the level of maternal antibodies to infectious bursal disease in five breeder farms in Nigeria as vaccination strategies for commercial farms.

## Materials and Methods

### *Experimental birds*

One hundred and fifty day old broiler and pullet chicks were purchased from five breeder farms in Ibadan. Thirty chicks were purchased from each breeder farm for the broilers and pullets. The breeder farms were designated A - E.

### *Sampling*

One milliliter of blood was collected from the heart of each chick at day old from five breeder farms designated as A , B, C, D and E as shown in table 1 . The blood was emptied into sterile plain tubes and left in horizontal position for an hour at room temperature, then left for another hour at 4<sup>o</sup> C. The serum samples were carefully separated in clean test tubes and stored at -4<sup>o</sup> C till used.

### *Enzyme Linked Immunosorbent Assay*

The enzyme linked immunosorbent assay (ELISA) technique was carried out according to the methods described by IDEXX Laboratories Incorporation, USA. The reagents in the ELISA kit were adjusted to room temperature (18-25<sup>o</sup> c) prior to the test.

The test sample was diluted to five hundred folds (1:500) with sample diluents prior to the assay. One hundred microlitres of the diluted sample were then dispensed into each well of the plate, this was followed by 100µl undiluted negative control into wells A1 and A2, 100 µL of undiluted positive control were dispensed into wells A3 and A4. The plate was incubated for 30 minutes at room temperature. Each well was washed with approximately 350µl of wash buffer, 3-5 times. Goat anti-chicken peroxidase (100 µl) as conjugate was dispensed into each well and incubated for 30 minutes at room temperature. After incubation the liquid contents were aspirated into a waste reservoir and each well was washed with about 350 µl of wash buffer 3-5 times and then the water was aspirated completely.

Tetramethylbenzidine solution (100 µl) was dispensed into each well and then incubated for 15 minutes at room temperature. Finally, 100 µl of stop solution was dispensed into each well to stop the reaction. The absorbance values at 650 nm were measured and recorded. Infectious bursal disease antibody titre was calculated automatically, using the methods of Blankfard & Silk (1989).

### *Statistical analysis*

Data collected were analysed, using Statistical Package for Social Science (SPSS) version 17.0. Mean antibody titre and coefficient of variation were analysed using analysis of variance of both broiler and pullet chicks. Tukey's test compared the means across the groups, value of  $P \leq 0.05$  was considered significant.

## Results

The result shows the presence of maternally derived antibodies (MDA) in chicks from all the breeder farms. The result of the present study also shows that the MDA were above the minimum protective level of 1000 ELISA titre (Figure 1). The mean ELISA titre for broilers were 1,962 ± 438, 2886 ± 644, 2777 ± 507, 4303 ± 974, 3079 ± 687 for breeder farms A ,B, C ,D and E, respectively, while the mean ELISA titre for pullets were 2567 ±573, 3,961 ±885, 4, 106 ± 1011, 2111 ± 472 and 4526 ± 1011. For

**Table 1:** Experimental design for bleeding at day old of chicks from five breeder farms for maternally derived antibodies determination

Groups	A	B	C	D	E
NO of chicks	30	30	30	30	30
Age of bleeding. (days)	1	1	1	1	1

**Table 2:** Central vaccination time (in days) for broilers and pullets

Breeder Farm	Type of Vaccine					
	Mild		Intermediate		Intermediate plus	
	Type of Chicken					
	Broilers	Pullets	Broilers	Pullets	Broilers	Pullets
A	17	32	16	29	10	18
B	19	35	18	33	12	22
C	19	35	18	33	12	22
D	21	30	20	28	14	17
E	21	35	20	33	14	22

**Table 3:** Vaccination days for Broilers and Pullets

Breeder Farm	Type of Vaccine					
	Mild		Intermediate		Intermediate plus	
	Type of Chicken					
	Broilers	Pullets	Broilers	Pullets	Broilers	Pullets
A	14 & 20	29 & 35	13 & 19	15 & 21	7 & 13	15 & 21
B	16 & 22	32 & 38	15 & 21	30 & 36	9 & 15	19 & 25
C	16 & 22	32 & 38	15 & 21	25 & 31	9 & 15	19 & 25
D	18 & 24	35 & 41	17 & 23	25 & 31	11 & 17	14 & 20
E	18 & 24	32 & 38	17 & 23	30 & 36	11 & 17	19 & 25

breeder farms A, B, C, D and E, respectively (Figure 1). The coefficient of variation for broilers from five breeder farms were 35.0%, 32.0%, 44.5%, 23.3% and 46.0% for breeder farm A, B, C, D and E respectively. For pullets, the coefficient of variation for breeder farms were 42.5%, 29.2%, 20.5%, 38.0% and 23.9% for breeder farm A, B, C, D and E, respectively (Figure 2). The central vaccination time for broilers for breeder farm A for mild vaccine was 17 days, intermediate was 16 days and intermediate plus 10 days, while chicks from breeder farm B has 19, 18 and 12 days for mid, intermediate and intermediate plus respectively. For breeder farm C was 19, 18 and 12 days for mild, intermediate and intermediate plus respectively. It was 21, 20 and 14 day for mild, intermediate and intermediate plus respectively for breeder farm D. However, for breeder farm E, it was, 20 and 14 days for mild, intermediate and intermediate plus vaccine (Table 2). The central vaccination time for pullet for breeder farm A for mild vaccine was 32 day, intermediate was 29 days and 18 days for intermediate plus. Chicks from breeder farm B had 35, 33 and 22 days for mid, intermediate and

intermediate plus respectively. For breeder farm C was 35, 33 and 22 days for mild, intermediate and intermediate plus, respectively. It was 30, 28 and 17 days for mild, intermediate and intermediate plus respectively for breeder farm D. Breeder farm E was, 35, 33 and 22 days for mild, intermediate and intermediate plus vaccine (Table 3). Central vaccination days for pullets using hipra calculator with mild vaccine was 29 days earliest and latest 38 days, with intermediate vaccine the earliest day to vaccinate broiler 112 was 15 days, the latest was 36 days. For intermediate plus vaccines the earliest was 10 days and the latest was 25 days as shown in the Table (Table 3).

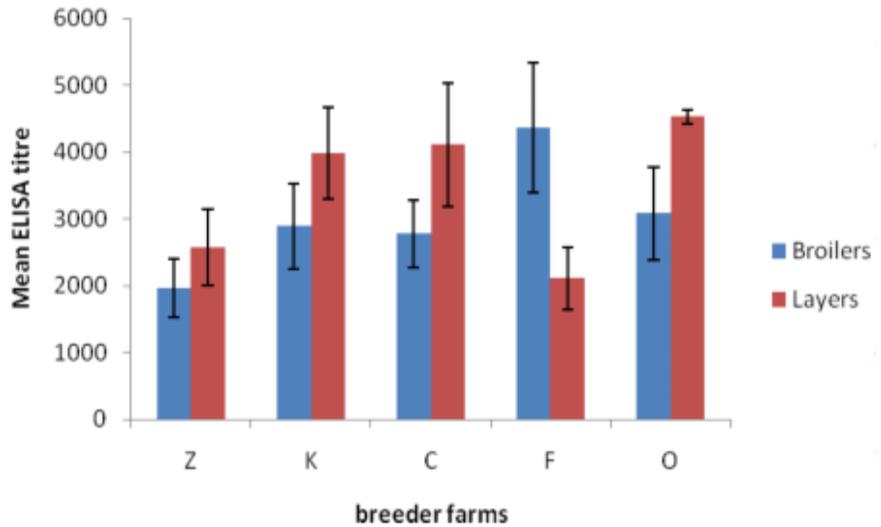
**Discussion**

The results indicated the presence of MDA in chicks from all the breeder farms; this implies that the chicks would be protected against early infection by infectious bursal disease virus. This was in line with the work of previous researchers (Omar *et al.*, 2015), but not in agreement with the work of Abdu & Audu (1987) who used agar gel immune-diffusion test while ELISA were used in this study.

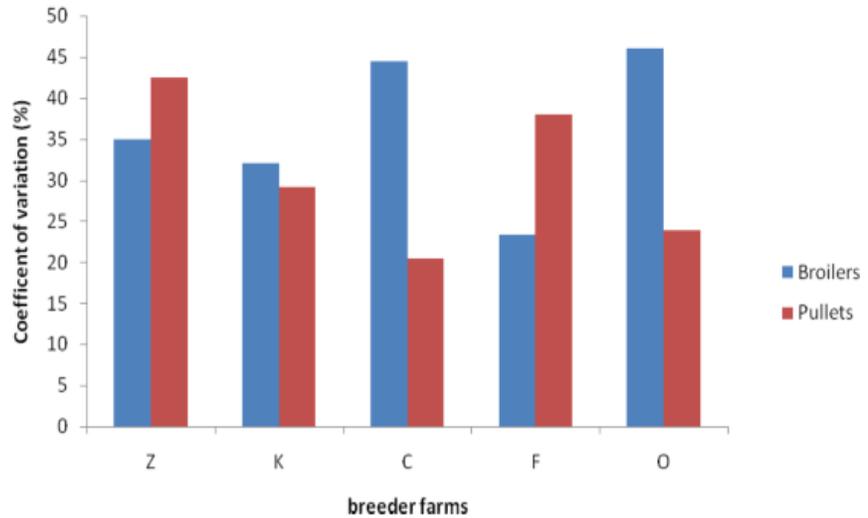
Based on the result of the present study, the MDA ELISA titre in all the chicks was above 1000, which was above the minimum, protective level. This implies that the chicks may be protected against field IBD virus and this is in line with the work of Zaheer & Saeed (2003) where the chicks that had MDA were protected against IBD. Based on this study, both broiler and pullet chicks from breeder farms in Nigeria need not to be vaccinated before ten days of age as they have protective levels of MDA at day old (Omar *et al.*, 2015). However, it is recommended that commercial poultry farmers need to vaccinate after 14 to 31 days of age, depending on the source and the type of bird with intermediate or intermediate plus to avoid neutralization of the vaccine virus by MDA. Also, based on this study the vaccination interval should not be too long as practiced by our commercial poultry farmers. The result of this study also indicates that vaccination interval for commercial broiler and pullets chicks should be six days and the MDA levels of the chicks from all the breeder farms were at different levels.

Therefore, the vaccination schedule should also differ, but in Nigeria regardless of the level of MDA, source of chicks and type of chicks, farmers use the same vaccination schedules that accounts for the high incidence of Gumboro disease outbreaks. This data further suggested that the level of passively transferred MDA should be tested while implementing vaccination regime as suggested by Sarachai *et al.* (2010). If the levels of MDA are high enough within the first few days of hatching, any active vaccine may not be as effective; infact will be neutralized by the circulating MDAs (Omar *et al.*, 2015). The success of any vaccination programme is related to the level of MDA in chickens. The MDA in

chicken can impede the virus in the vaccine, thus, reducing the ability of the virus in the vaccine to stimulate the chicken immune system (Chansiripornchai & Wanasawaeng, 2009). According to Al-Natour *et al.* (2004) divided the ELISA titre of the MDA of day old chicken into 3 levels, the low level >3,000 , intermediate (3,000-5,000) and high >6,000. In this study, the ELISA titre level for both broiler and pullet chicks were between 2000 and 4500. The implication of this is that different MDA levels require different vaccine strains, high MDA means MDA will neutralize the vaccine, thus diminishing response to the vaccine (Omar *et al.*, 2015). The implication of the coefficient of variation



**Figure 1:** Graphical representation of mean ELISA titre for broiler and pullet from five breeder farms



**Figure 2:** Coefficient of variation for MDA in broiler and layer from five breeder farms

of above 30% in 90% of the breeder farms of broiler chicks and about 80% in pullet chicks is alarming as this signifies poor uniformity of response to vaccination, the causes of this variation are numerous and include the type of vaccines, mixing of eggs from different breeder flocks, difference in resorption speed of yolk sac and difference in mean MDA titre between individual breeders (Pooja *et al.*, 2017). The CV above 30% signifies that the chicks would require two vaccinations and such chicks may be susceptible to early outbreaks of IBD before ten days of age. Therefore develop bursal atrophy and immunosuppression (Giambone, 1995). The lack of uniformity in MDA in broiler and pullets chicks indicated that the vaccination programmes of the parent stock need improvement. All flock with percentage CV of more than 30% would have a significant number of birds susceptible to IBDV infection before 10 days of age and develop bursal atrophy and immunosuppression (Giambone, 1995). There is the need for biosecurity and vaccination for the effective control of IBD (Chansiripornchai & Sasipreeyajan, 2009). Vaccination days based on Hipra calculators is most appropriate to vaccinate broiler with intermediate vaccines from 14 to 17 or 20 days for broilers but for pullets 21 or, 30 to 36 days is recommended and is in line with the work of Sarachai *et al.* (2010), who found no antibody titre at day 30<sup>th</sup>, but this was in contrast to Al-Mayah & Al-Mayah (2013) who found antibody titre at day 20 but the vaccination time was much earlier with intermediate plus for broilers and 16 pullets 13 to 17 days and 10 to 25 days respectively, this is because intermediate plus vaccines has the ability to break through MDA as such offer protection at an early age (Zaheer & Saeed, 2003). In this study vaccination interval for calculated and Hipra calculator was six days in contrast to the seven to fourteen days interval as practiced by most commercial poultry farmers in Nigeria. It is concluded that both broiler and pullet chicks from breeder farms in Nigeria have protective levels of MDA at day old. Coefficient of variation of the chicks was above 30%, the vaccination day for broilers is earlier than pullets. Broilers are to be vaccinated between 7 and 17 days while pullets between 10 and 25 days and the vaccination days also depend on the type of vaccine. Therefore, it is recommended that commercial poultry farmers need not to vaccinate chicks at less than 7 d days of age, vaccination day should depend on the source, type of chicks and the

level of MDA, as level of MDA for breeder farms differs. Broilers should be vaccinated earlier than pullets. Chicks can be vaccinated with intermediate plus at an early age (10 days) while intermediate and mild at a later age above 14 days. The use of same vaccines and vaccination schedule for both pullets and broilers should be discouraged, and the interval of vaccinations should be six days.

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