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https://dx.doi.org/10.4314/nvj.v44i3.3 **ORIGINAL ARTICLE**

Evaluation Of Infectious Bursal Disease Virus Maternally Derived Antibody Decay Rate In Day Old Broilers From Different Hatcheries In Nigeria

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ABSTRACT

Infectious Bursal disease (IBD) is an immunosuppressive disease of young chicks, which is responsible for major economic losses in the poultry industry worldwide, particularly for the last decades. This study utilized a cross-sectional approach to evaluate Infectious bursal disease virus (IBDV) maternally derived antibody decay rate in day old chicks (D.O.C) obtained from different hatcheries in Nigeria. Of the 450 serum samples collected through random selection with replacement method, (i.e 90 samples from the 5 hatcheries designated as farms A – E), it was observed that farm A at day 1 had mean Elisa titre of (4213 ± 366.66), farm B (2178.225 ± 292.477), farm C (1629.699 ± 229.2197), farm D (3452.609 ± 403.64.6469) and farm E (1651.789 ± 201.6811), these values were far above the protective (positive) level of 875, recommended by IDvet manual with (S/P ratios of 0.350). This level was maintained, although, with minimal decays, up to day 7 for farms A, D and E but farm B and C did not exceed day 6 and day 5 respectively. Only farm D presented protective (positive) value that lasted to day 13. The best fit for this decay rate was calculated to be Y = -2263In(x) + 3714 (week) with R^2 = (0.903). From these studies we could deduce that these farms were truly vaccinated or immunized as expected and they presented different antibody titres with variable decay rates. It would have been a good idea to recommend that same vaccination schedule should not be applied indiscriminately to broilers from these farms like other researchers have, but this would rather be to the detriment of many farmers who may not be privileged to access the detailed information on which schedule is meant for which farm .We therefor recommend that broilers from these hatcheries be vaccinated between day 7 to day 10 post hatch and a booster dose (if necessary) should be administered a week after, with an intermediate plus strain vaccine type that has the capacity to penetrate through the MDA and induce humoral immunity in the chicks.

Keywords: infectious bursal disease, maternally, derived, antibody, day old chick

INTRODUCTION

Infectious bursal disease (IBD) is an immunosuppressive disease of young chickens. It is responsible for major economic losses in the poultry industry worldwide, (Mahgoiub, 2012). Gumboro disease was discovered by Cosgrove (1962) in Gumboro city in Delaware, USA. In Nigeria, the disease was first described in 1973 (Ojo et al., 1973), and confirmed by Onunkwo (1975). The disease caused by infectious bursal disease virus (IBDV), has continued to plague the poultry industry worldwide. IBD is an acute, highly contagious viral disease of poultry affecting mainly chickens aged 3-6 weeks, causing severe immunosuppression in infected birds below 3 weeks of age (Chowdhury, 2015). However, evidences of outbreaks in older chickens have been documented (Okoye and Uzoukwu, 1981; Gary and Richard, 2015). The organ in chickens targeted by IBDV is the bursa of Fabricius where it causes a depletion of the B lymphocytes (Silva et al., 2016). It is where B-lymphocytes (cells of the humoral immune system) are programmed to produce specific antibodies in response to the disease and also to vaccine virus in birds. If the IBD Virus damages the BF in young chickens, it will destroy the immature B lymphocytes, causing lymphoid depletion of the bursa. The BF will then not be capable of programming sufficient number of lymphocytes and the chicken becomes immunosuppressed (not capable of protecting itself against any disease agent) (Zachar et al., 2016).

The reason for vaccination of breeder hens prior to laying of eggs is to produce maternal immunity that is passed on to the chick for IBD control, IBDV vaccines have been reviewed recently (Müller et al., 2012). Types of vaccines: Four major types of vaccines are available for the control of IBD, these are: i) live attenuated vaccines; ii) immune-complex vaccines; iii) live recombinant vectored vaccines expressing IBDV antigens; and iv) inactivated oil-emulsion adjuvanted vaccines.

To date, IBD vaccines have been made with serotype 1 IBDV only, although a serotype 2 virus has been detected in poultry. The serotype 2 virus has not been associated with disease, but its presence will stimulate antibodies. Serotype 2 antibodies do not confer protection against serotype 1 infection, neither do they interfere with the response to type 1 vaccine. There have been numerous descriptions of antigenic variants of serotype 1 virus (Rosenberger & Cloud, 1986). Cross-protection studies have shown that inactivated vaccines prepared from ‘classical’ serotype 1 virus require a high antigenic content to provide good protection against some of these variants. IBD vaccines that contain both classical and variant IBD serotype 1 viruses are now licensed. vvIBDV strains with limited antigenic changes as compared with ‘classical’ serotype 1 viruses have emerged since 1986. Active immunisation with a ‘classical’ serotype 1 virus or vaccine provides a good protection against the vvIBDVs, however the latter viruses are less susceptible to neutralisation by MDA than ‘classical’ pathogenic viruses (Van den Berg & Meulemans, 1991).

To make an immune complex IBD vaccines a live infectious IBDV vaccine virus is blended with IBDVs specific antibodies. Such vaccines may be administered in the hatchery by in-ovo injection at 18 days of incubation. The eggs go on to hatch and

the vaccine virus is supposedly released when the chicks are about 7–14 days of age. In this way, the problem of maternally derived IBD antibody is overcome and the chicks are effectively immunized (Haddad et al., 1997). The immune complex vaccine can also be injected subcutaneously at 1-day old in the hatchery (Ivan et al., 2005).

Inactivated IBD vaccines are mostly used to produce high, long-lasting and uniform levels of antibodies in breeding hens that have previously been primed by live vaccine or by natural exposure to field virus during rearing (Müller et al., 2001). The usual program is to administer the live vaccine at about 8 weeks of age. This is followed by the inactivated vaccine at 16–20 weeks of age. Occasionally, inactivated vaccines may be used in program combining inactivated and live vaccines, in young valuable birds with high MDA levels reared in areas with high risk of exposure to virulent IBDV. The inactivated vaccine is manufactured as a water-in-oil emulsion, and has to be injected into each bird. The preferred routes are intramuscular into the leg muscle, avoiding proximity to joints, tendons or major blood vessels or the subcutaneous route. A multidose syringe may be used. All equipment should be cleaned and sterilized between flocks, and vaccination teams should exercise strict hygiene when going from one flock to another. Vaccine should be stored at between 2°C and 8°C. It should not be frozen or exposed to bright light or high temperature.

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., 2001). 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 program 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 neutralize IBDV during early vaccination, therefore MDA may be most probable cause of vaccination failures (Abdu, 1997). The failure to control IBD in Nigeria irrespective of vaccination necessitates a reappraisal of level of maternal antibodies in commercial chicks. The present study aimed at determining the initial antibody titres and the decay rate of the IBDV maternal antibodies in chicks in order to determine when best to vaccinate against IBD and consequently provide a provisional vaccination schedule for IBD in Nigeria.

MATERIALS AND METHODS

Experimental Design:

This study utilized a cross-sectional approach to evaluate infectious bursal disease virus (IBDV) maternally derived antibody (MDA) decay rate in day old chicks obtained from different hatcheries in Nigeria.

Sampling Site

Five major hatcheries in Nigeria were identified, four hatcheries from south western part of Nigeria (AGRITED, CHI, SAYED and ZARTECH) and the only major hatchery from Kaduna state (OLAM).

Sampling Technique:

1. Judgmental sampling technique was used.
2. Five of the leading hatcheries in Nigeria were selected based on their perceived profiles (Credibility, availability and accessibility)
3. The hatcheries were designated as farm A – E
4. A total of 500-day old broiler chicks were purchased from these hatcheries (i.e 100 chicks per hatchery).
5. The brooding house was prepared following standard brooding procedures and demarcated according to groups (farm A – E).
6. Feed and water was given ad-libitum; no vaccine was administered but glucose, anticoccidia and antibiotics were used at interval when there were need
7. 15 birds were selected using random sampling and replacement method from each group and bled for serum at designated days, which were (day 1,7,10,14,17, and 21) respectively.

Sample Collection and processing

1. Blood samples were collected using Disposable insulin syringes (1ml x 29G x 12'') at day 1 to day 7 and subsequently 2ml 23G were used as the chicks matured , following proper restraint and disinfection
2. 0.5 – 0.8mls of blood was collected by cardinal puncture of each bird through the thoracic inlet from day 1to day 7 while wing vein was used from day 10 to 21 day.
3. The blood was allowed to settle, serum separated into cryo vials and stored in a deep freezer (-20^0C) until required.
4. A total of 450 serum samples were collected (90 samples from each farm)
5. Indirect ELISA test was used for the sample Processing

Sample Analysis

1. The test was carried out by the use of ELISA Kit purchased from ID vet
2. It is a quantitative test for the detection of IBDV-specific antibodies in chicken

Testing Procedure

- All reagents was allowed to come to room temperature (21^oC ± 5^oC) and homogenized by inversion or vortexing
- The negative and positive controls were supplied ready-to-use.
- Samples, however, was tested at a final dilution of 1:500 in Dilution Buffer 14 (1:50 pre-dilution, followed by 1:10 dilution in the microplate).

1. In a pre-dilution plate, wells A1, B1, C1 and D1 were set aside as negative and positive controls respectively

- 5 µl of each sample to be tested was added to each well except the positive and negative controls

- 245 µl of Dilution Buffer 14 was added to all wells EXCEPT the control wells A1, B1, C1 and D1.

Note: The recommended order of deposit was respected as directed and that enabled visually control addition of sample to each well.

2. In the ELISA micro-plate, was added:

- 100 µl of the Negative Control to wells A1 and B1.
- 100 µl of the Positive Control to wells C1 and D1.
- 90 µl of Dilution Buffer 14 to as many wells as there are samples to be tested (will not add to control wells A1, B1, C1 and D1),
- 10 µl of the pre-diluted samples as prepared above

3. The plate was covered and incubated for 30 min ± 3 min at 21°C (± 5°C).
4. Conjugate was prepared at 1x by diluting the Concentrated conjugate 10x to 1:10 in Dilution Buffer 3.

5. Wells were emptied, Washed 3 times with approximately 300 µl of the Wash Solution 1x.I avoided drying of the wells between washes as recommended.

6. 100 µl of the Conjugate 1x was added to each well
7. Plates were covered and incubated for 30 min ±2 min at 21°C (±5°C) in the dark cupboard
8. Each well was washed 3 times with approximately 300 µl of the Wash Solution 1x.I avoided drying of the wells between washes as much as necessary .
9. 100 µl of the Substrate Solution was added to each well.
10. Each Plates was covered and incubated 15 min ±2 min at 21°C (±5°C) in the dark.
11. 100 µl of the Stop Solution was added to each well in order to stop the reaction. The stop solution was added in the same order as in step N°9.
12. The plates were placed in ELISA reader and OD readings of the test was taken at 450nm wave length.

Validation of Expected Results

- The test results was valid because:

- The mean O.D value of the positive control (ODpc) was greater than 0.25.

  ODpc>0.25

- The ratio of the mean value of the Positive and Negative Controls (OD_{pc} and OD_{nc}) was also greater than 3.

  ODPC/ODNC>3

Interpretation of Expected Results

For each sample, Sample positive (S/P) ratio and antibody titer was calculated as follows:

1. S/P ratio

$$S/P = \frac{\text{OD sample} - \text{ODNC}}{\text{ODPC} - \text{ODNC}}$$

2. Antibody titer

$$\text{Log}_{10}\,(\text{titer}) = 0.97 \times \log_{10}\,(S/P) + 3.449$$

$$\text{titer} = 10^{\log 10}\,(\text{titer})$$

Result was interpreted as follows:

S/P value	ELISA Antibody titer	IBD immune Status
S/P ≤ 0.3	Titer ≤ 875	Negative
S/P > 0.3	Titer > 875	Positive

Statistical analysis: Statistical analysis of variance (ANOVA) test was used and correlation and linear regression analysis were performed using Microsoft excel program.

RESULTS

The result of a total of 450 serum samples obtained from 5 hatcheries and analyzed is presented in tables 1-5 and fig. 1-5

The mean Elisa titers in the chicks at day 1 for farm A was (4213 ± 366.66), farm B (2178.225 ± 292.477), farm C was (1629.699 ± 229.2197) ,farm D was (3452.609 ± 403.6469) and farm E was (1651.789 ± 201.6811).These were above protective (positive) value of 875 as recommended by IDvet with (S/P ratios of 0.350) . This level was maintained up to day 7 for farm A, D and E while farm B and C lasted only to day 6 and 5 respectively. Only farm D had immunity that lasted to day 13 of the experiment. The line of best fit to this decay rate of the MDA to IBD was calculated to be Y = -2263In(x) + 3714.6 (week) with correlation coefficient (R^2) = 0.903. Table 6 and fig. 6 & 7 show a combined result from the 5 hatcheries.

Mean ELISA Titre of the Chicks

Mean ELISA Titre of Farm A

Days	Titre	SEM
1	4213	366.66
7	1738	280.49
10	468	51.64
14	743	92.20
17	150	39.27
21	84	26.99

Table: 1 Mean ELISA Titre of Farm A

Mean ELISA Titre of Farm B

Days	Titre	SEM
1	2178.225	292.477
7	555.4813	74.34956
10	949.7991	139.638
14	301.4941	36.26277
17	349.118	55.54271
21	420.358	162.9277

Table 2: Mean ELISA Titre of Farm B

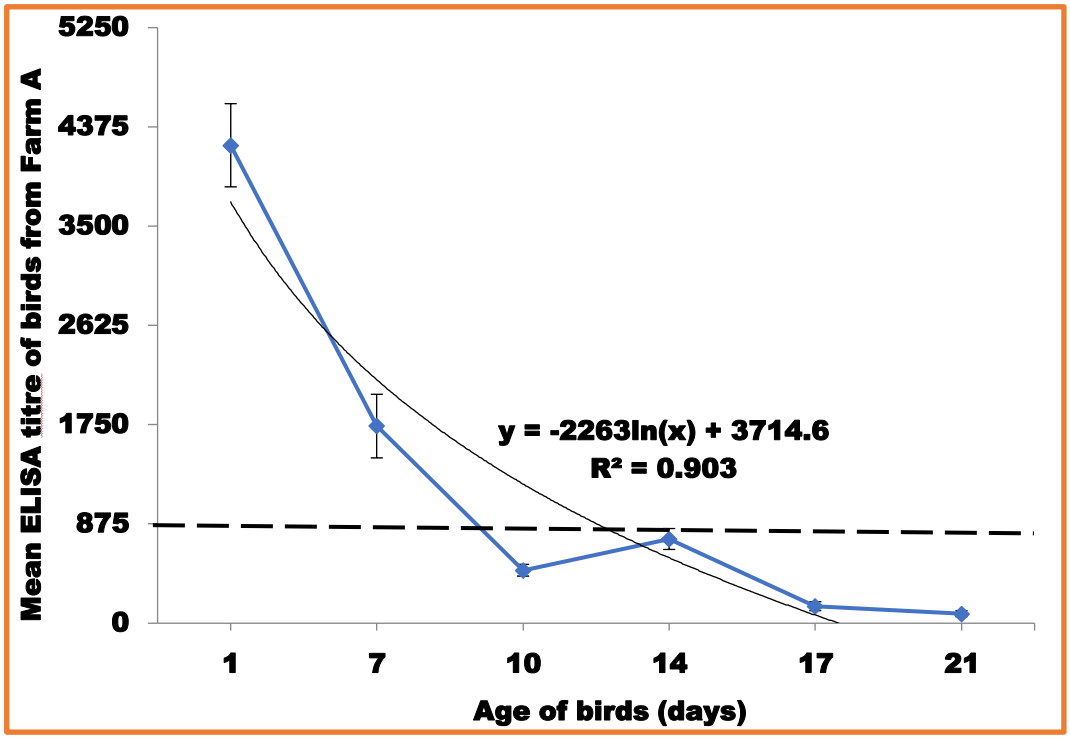

Fig. 1. A Graph Representing Mean ELISA titre of birds from farm A

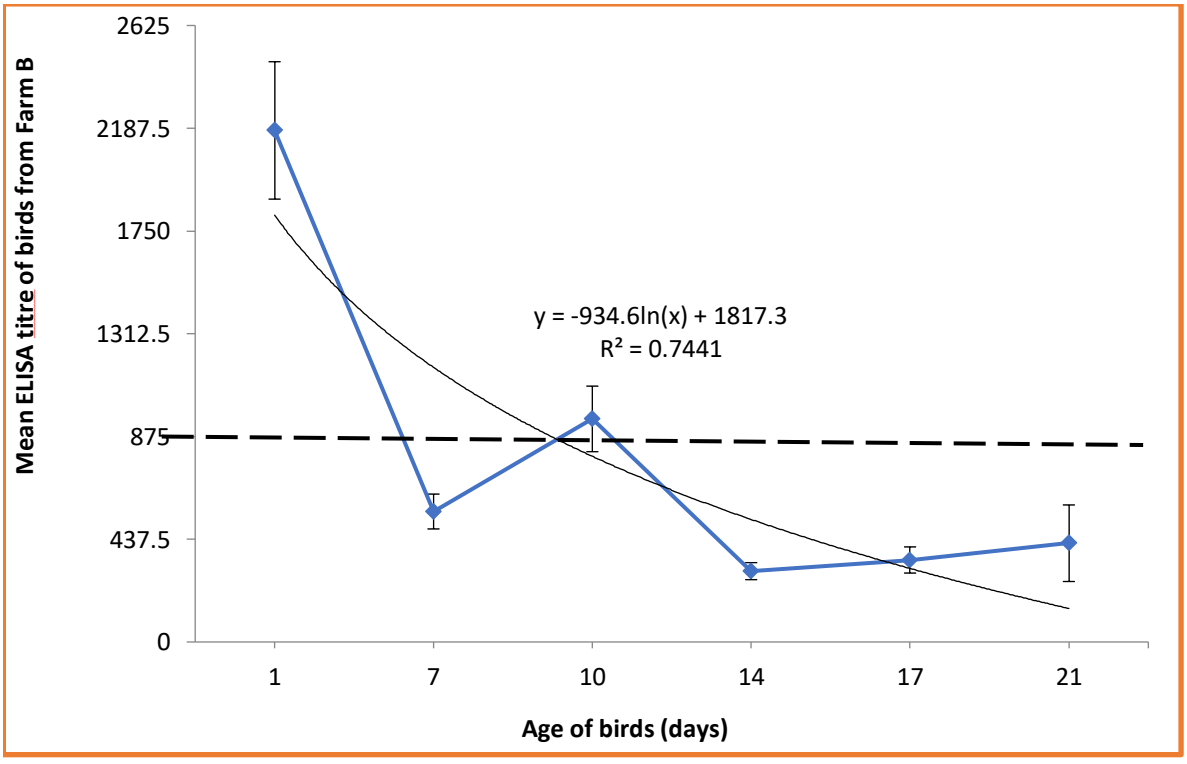

Fig. 2. A Graph Representing Mean ELISA titre of birds from farm B

Mean ELISA Titer of Farm C

Days	Titre	SEM
1	1629.699	229.2197
7	423.3665	29.01446
10	569.4302	46.18224
14	639.2332	62.46609
17	107.2621	18.23843
21	483.7057	104.6214

Table 3: Mean ELISA Titre of Farm C

Mean ELISA Titer of Farm D

Days	Titre	SEM
1	3452.609	403.6469
7	1596.802	266.493
10	1239.473	355.3867
14	612.0035	108.866
17	378.4942	76.54972
21	168.3869	16.47786

Table 4: Mean ELISA Titer of Farm D

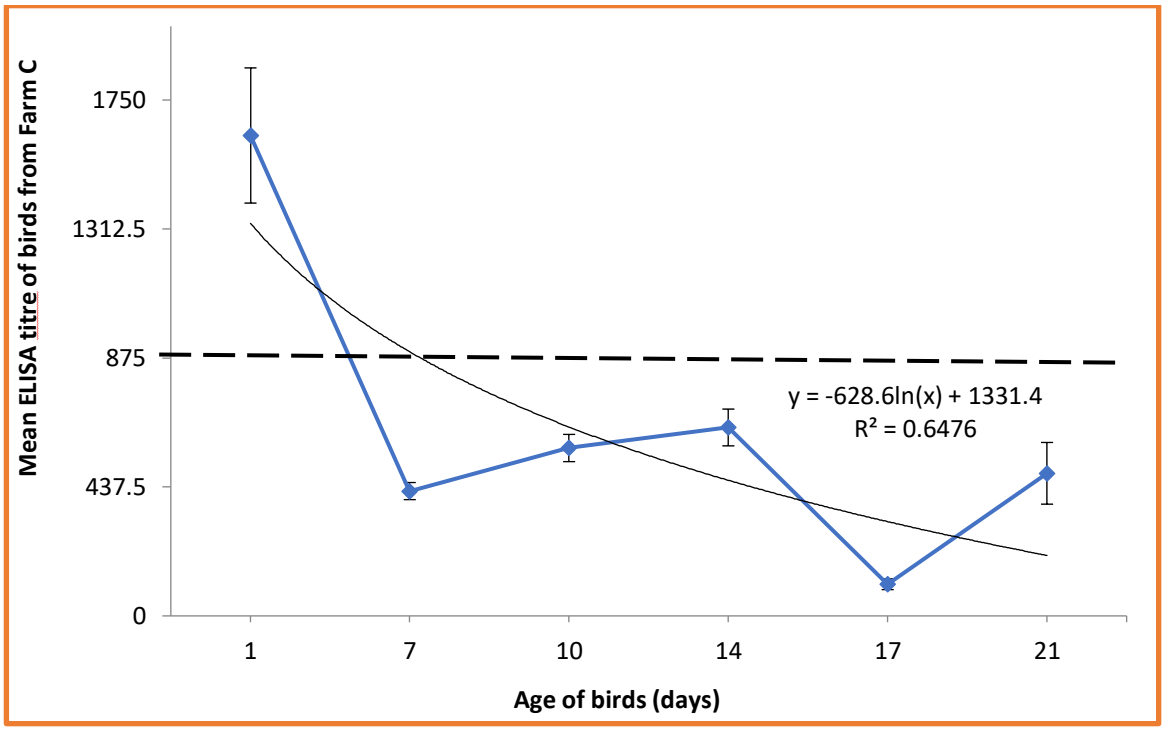

Fig. 3. A Graph Representing Mean ELISA titre of birds from farm C

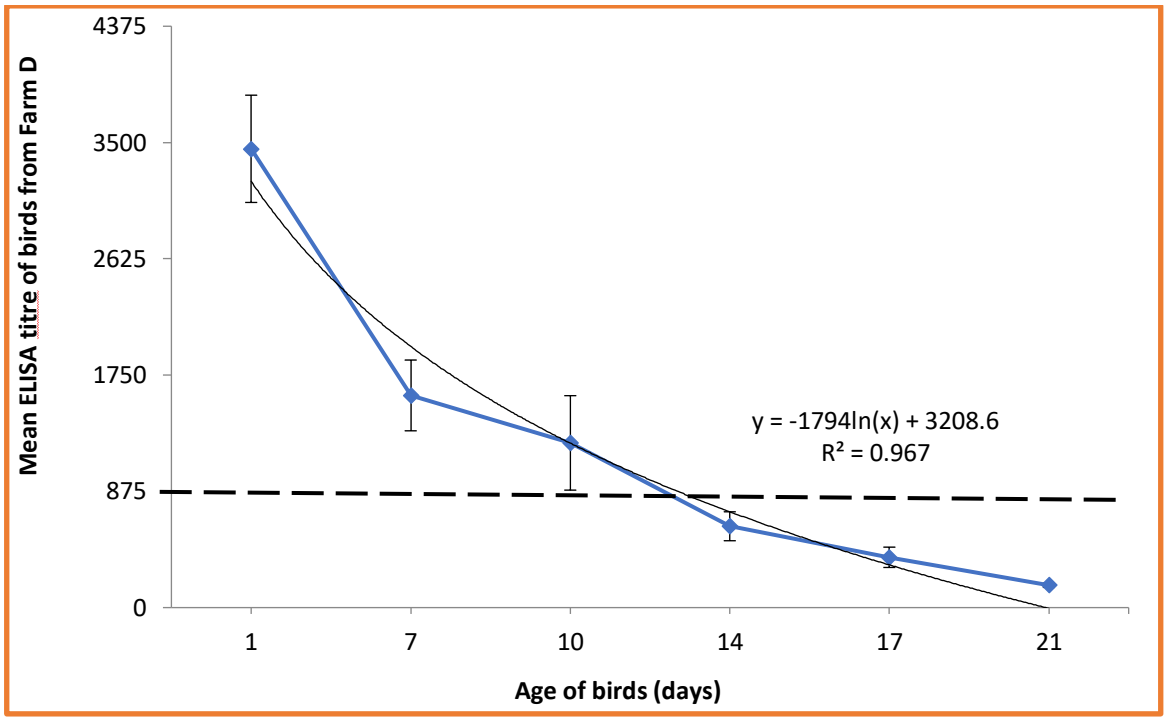

Fig. 4. A Graph Representing Mean ELISA titre of birds from farm D

Table V Mean ELISA Titer of Farm E

Days	Titre	SEM
1	1651.789	210.0811
7	1244.593	200.4117
10	326.8203	84.27254
14	276.4752	80.44611
17	456.073	58.30642
21	273.4661	32.23788

Table 5: Mean ELISA Titer of Farm E

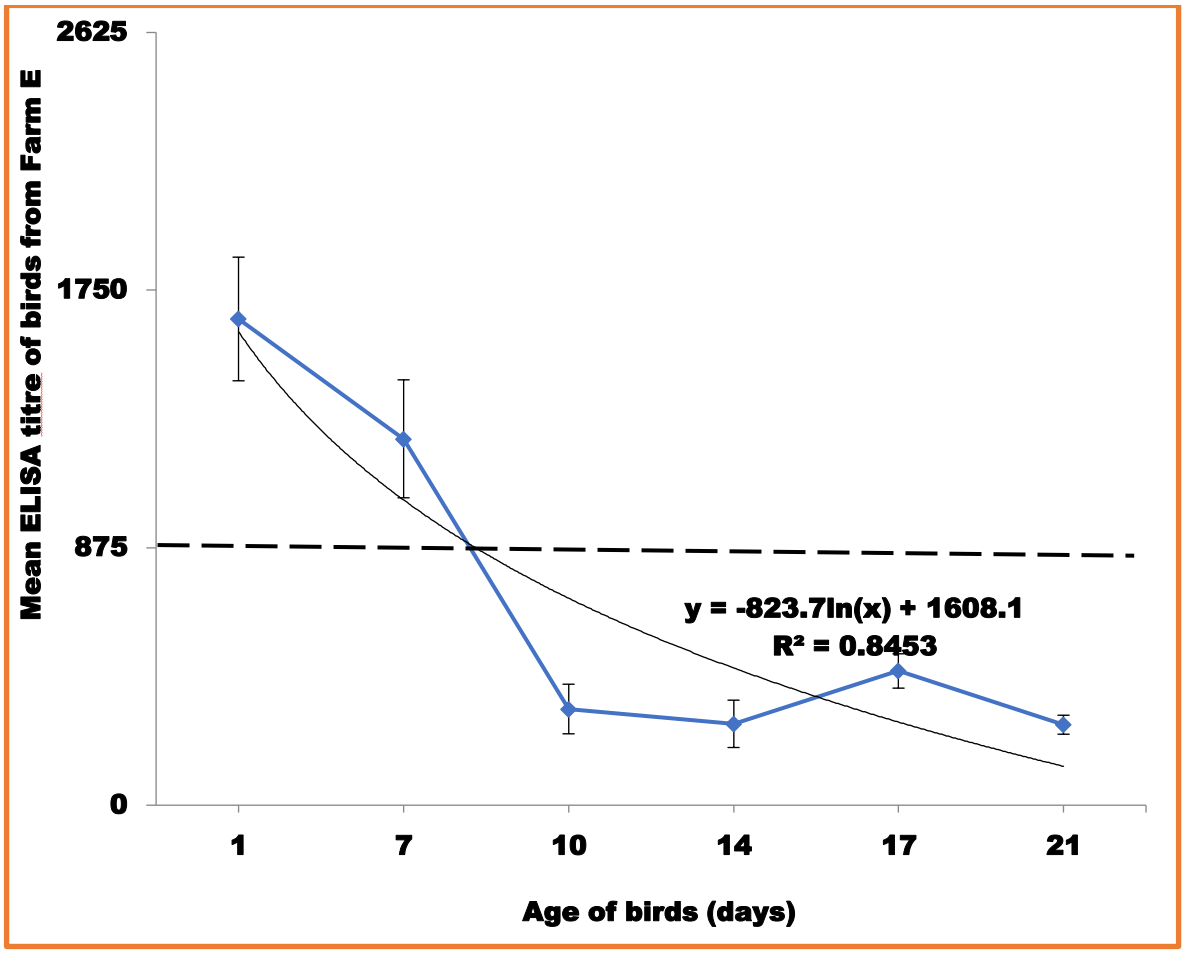

Fig. 5. A Graph Representing Mean ELISA titre of birds from farm E

COMBINED Mean ELISA Titer of Farm A - E

Days	Farm A	Farm B	Farm C	Farm D	Farm E
1	4213	2178.225	1629.699	3452.609	1651.789
7	1738	555.4813	423.3665	1596.802	1244.593
10	468	949.7991	569.4302	1239.473	326.8203
14	743	301.4941	639.2332	612.0035	276.4752
17	150	349.118	107.2621	378.4942	456.073
21	84	420.358	483.7057	168.3869	273.4661
	SEM	SEM	SEM	SEM	SEM
1	366.66	292.477	229.2197	403.6469	210.0811
7	280.49	74.34956	29.01446	266.493	200.4117
10	51.64	139.638	46.18224	355.3867	84.27254
14	92.20	36.26277	62.46609	108.866	80.44611
17	39.27	55.54271	18.23843	76.54972	58.30642
21	26.99	162.9277	104.6214	16.47786	32.23788

Table 6: Combined Mean ELISA Titre of Farm A – E

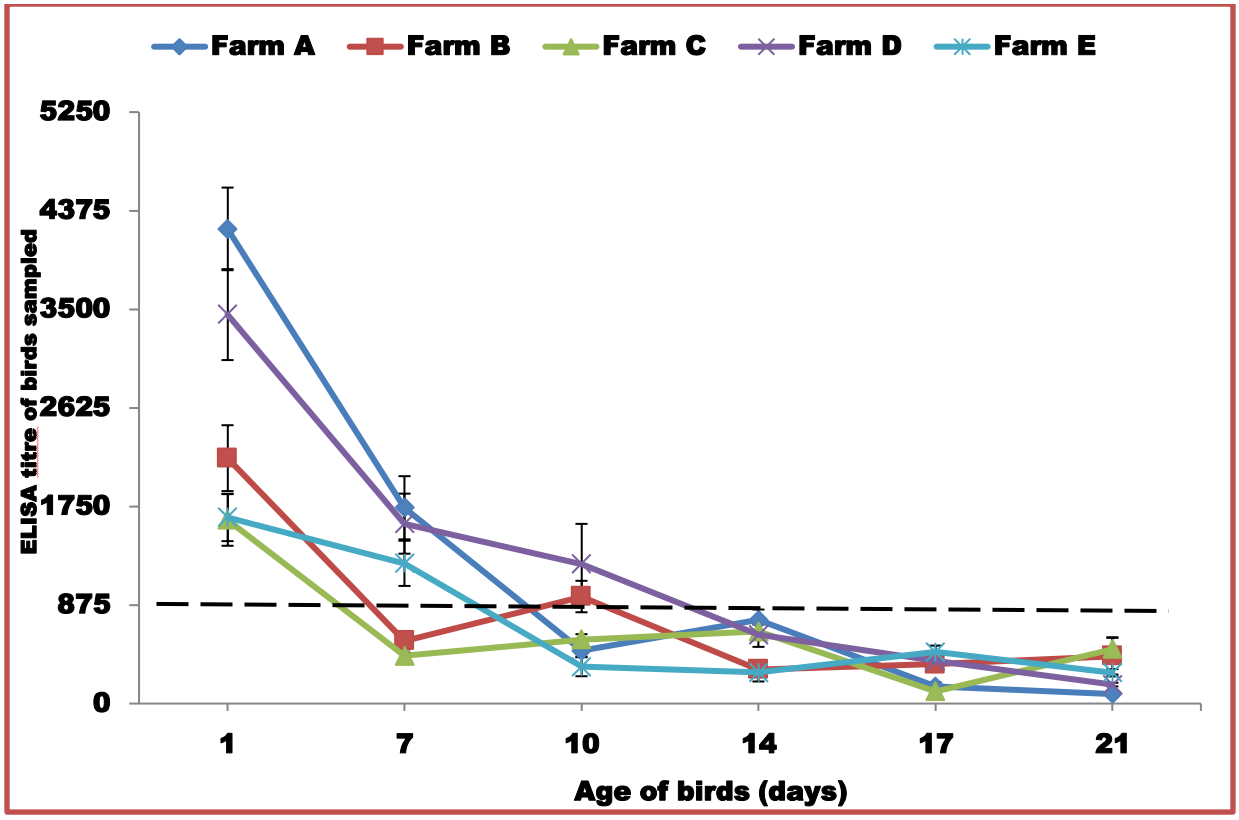

Fig. 6. A Graph Representing Combined Mean ELISA titre of birds for farm A - E

Combined Bar Chart Representing the 5 hatcheries and their Decay Rate

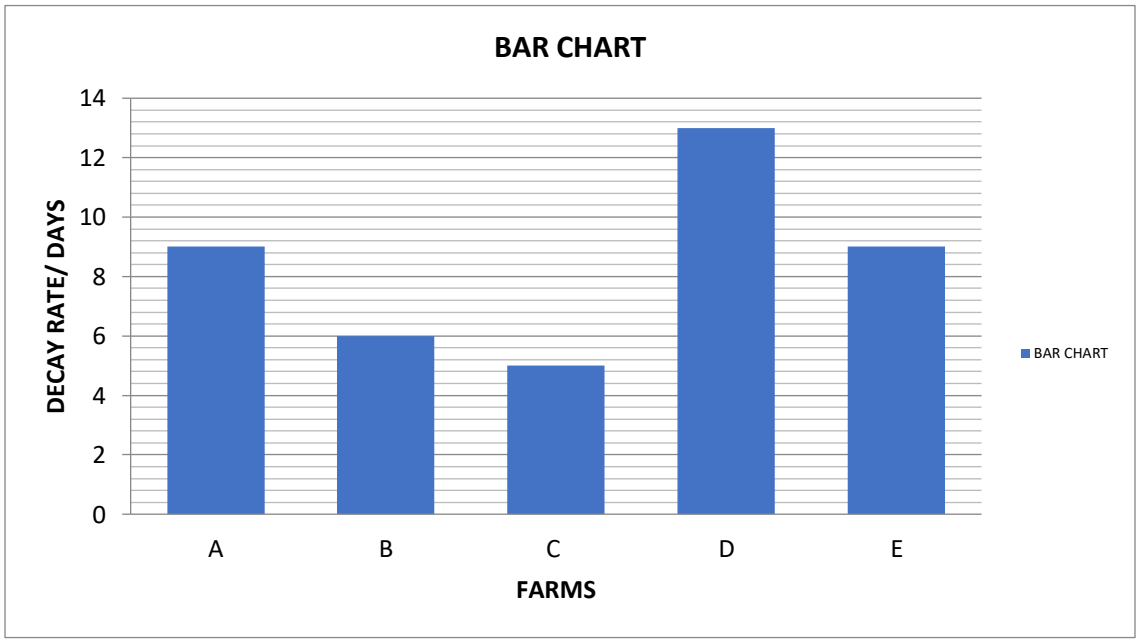

Fig. 7. A Combined Bar Chart Representing the 5 hatcheries and their Decay Rate

DISCUSSION

This study utilized a cross- sectional approach to evaluate infectious bursal disease virus (IBDV) maternally derived antibody (MDA) decay rate in D.O.C obtained from different hatcheries in Nigeria.

The vaccination of breeders and their chicks had been intended especially for IBD which was discovered for the first time by (Cosgrove in 1962), hygienic measures still ineffective to prevent the disease (Benton et al., 1967). So, vaccination is the most effective measures to control IBD with this in mind, the importance of maternal immunity has appeared to have a great role in protection of young chicks against early infection and immunosuppression, which may interfere with vaccination procedures (Chettle and Wyeth, 1994). The level of MDA was above the protective (positive) value of 875 for farms A – E for the 1st day of life with (S/P ratios 0.350), which shows that the breeders were truly vaccinated against IBDV and MDA was transferred to the chicks.

This level of MDA were maintained up to day 7 of the experiment for farm A,D and E respectively while farm B and C did not exceed day 6 and 5 of the experiment. Only farm D had protective immunity up to day 13. And the best fit for this decay rate was calculated at $Y = 2263\ln(x) + 3714$ (weeks) with $R^2 = 0.903$.

These substantial differences could be ascribed to the amount of antibodies transferred from the breeder hen to the chicks through the egg (Hamal et al.2008,).It was concluded that the MDA depends on the quality of the egg yolk (Rao et al.,1987).It was also noticed that the half-life of MDA to IBDV is between 3 and 5 days (Lukert and Saif,1997). Similarly, other studies have reported that the half-life of MDA to IBD in chicks was 3.46 days (Saijo & Higashihara 1998) and decreased every 4 days (Gardin 1994). Others reported that the rate of decline was by about half every 5 days (Alam et al. 2002; Shreshtha et al. 2003) and between 4 and 5 days (Sheku 2013). In newly hatched layer-type chicks, MDA exhibits a linear or curvilinear decline with a mean half-life of 5 to 6 days (Miiller et al. 2012). Fahey, Crooks & Frazer (1987) reported a half-life of 6 – 7 days for IBDV – Specific MDA. It is generally thought that the half-life of MDA in broiler lines is much shorter, approximately 3 days (Block et al., 2007). Date from this study revealed that the decrease of MDA to IBDV is variable during the growing period.

This divergence may be explained by the influence of the half-life of MDA on the vaccine type, its time of application in hens (Alam et al. 2002) and probably the immune status of the hen (Kouwen hoven & Van den Bos ,1992). Moreover, whilst the antibody titres may not vary greatly amongst hens in a single flock of similar age, the offspring of different vaccinated flocks may show different IBDV MDA titres. Under field conditions however, the decay pattern of IBDV-Specific MDA proved to be more complex, as it depends largely on initial antibody levels, which may vary between batches and also within a batch, making it difficult to predict the optimal time for vaccination (De wit 1998). The discrepancy most probably reflects the use of different types of vaccine and vaccination schedules (Alam et al. 2002).It was concluded that intermediate plus vaccines induced higher antibody titres than other vaccines(AI Mayal,2013) although some intermediate vaccines induced similar titres (Amer et al. 2007).

Therefore, if these chicks were to be vaccinated early in life, the vaccine may fail to stimulate immune response in the chicks because maternal antibody will react with the live vaccine virus and becomes neutralized or interferes with MDA (Zhuo et al. 1998).

From the result of this study, it was shown that the level of MDA from farms A – E varied due to the already explained reasons above. It would have been fair to advise that the five hatcheries will be presented with different vaccination schedules based on the results obtained (De Wit, 1998).But this would be detrimental to most farmers that may not have access to the detailed information on when to vaccinate the various farms ,therefore, we recommend that these broilers should be vaccinated at day 7 to day 10 post hatch and a booster dose (if necessary) a week after with an intermediate plus strain vaccine type due to its ability to penetrate MDA and induce humoral immunity in the chicks unlike other vaccine types.

CONCLUSION AND RECOMMENDATIONS

Conclusion

This research work has provided the following important information on the hatcheries in Nigeria.

1. That the leading hatcheries in Nigeria do vaccinate their breeder hens as expected and that there is transfer of MDA to the chicks.
2. That the antibody titres transferred to the chicks varied in the chicks within and between farms.
3. That the decay rates of the antibodies in the chicks varied overtime between and within farms and only farm D had immunity up to day 13 of the experiment.

Recommendation(s)

Based on the findings of this study, the following recommendations were made:

1. That the chicks from these hatcheries should be vaccinated between day 7 to day 10 post hatch and a booster dose (if necessary) a week after.
2. Based on the foregoing, an intermediate plus strain vaccine type may be most recommended for primary and booster doses.
3. Similar work should be carried out in pullets from same farms to generate a comparative indices.

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