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Immunomodulatory Effects of Neem (*Azadirachta indica*) Leaf Aqueous Extracts in Cockerels Vaccinated and Experimentally Infected with Infectious Bursal Disease Virus

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Abstract

The aim of the present work was to study the immunomodulatory potentials of aqueous extract of Neem (*Azadirachta indica*) leaf in cockerels vaccinated and/ or infected with infectious bursal disease virus (IBDV). Four hundred and eighty (480) day old cockerels were used and allocated into 8 groups. The birds were grouped as vaccinated/ unvaccinated, challenged/ unchallenged, neem leaf treated/ untreated groups. The IBD vaccines (intermediate plus strain) were given at 14 and 28 days of age while the experimental infection using very virulent IBD virus (vvIBDV) was inoculated at 35 days of age and the extracts were given from day old to 6 week old.

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Serum samples were collected on first day and on weekly intervals while post challenge, onset of the disease, clinical signs and mortality rate were recorded. The results obtained showed higher antibody titre, faster seroconversion, mild clinical sign and very low mortality in the neem leaf treated groups. These results indicated that the neem leaf aqueous extract has immunomodulatory potentials by increasing the antibody titre post vaccination and the ability to prevent mortality.

Keywords: Infectious bursal disease; neem leaf aqueous extracts; immunomodulation; vaccine; challenge; antibody; cockerels.

1. Introduction

Infectious bursal disease (IBD) also known as gumboro disease and infectious bursitis is an acute, highly contagious viral disease of young (immature) chickens (three to five weeks old) caused by the infectious bursal disease virus belonging to the genus *Avibrinavirus* (*birinavirus)* an RNA virus of the family *Birinaviridae* [1]. The virus is a non-enveloped icosahedral, bisegmented double stranded (dsRNA) virus with a diameter of about 55-60 nm [2].The disease was first discovered in 1957 in Gumboro county, Delaware, USA by Cosgrove as a result, it is often referred to as Gumboro [3]. Since its first appearance in 1962 in Gumboro county USA, IBD has been reported in the poultry industries and in concentrated poultry production areas all over the world [2,1] causing devastating outbreaks [4,5].

The disease is particularly important due to high mortalities, lowered productivity among infected chickens [6]. It is of high economic importance in Nigeria as it results in tremendous loss to poultry farmers in terms of mortality and immunosupression [7]. Outbreak of IBD has been reported in IBD vaccinated flocks [8]. Suboptimal humoral immune response to IBD vaccination has been observed in birds [9, 10].

Research has therefore been targeted at improving the immunogenicity of some vaccines by using antioxidants such as Vitamin C [11]. Reports on their effectiveness or otherwise are conflicting and therefore inconclusive, possibly due to difference in the virulence of the virus strains or weather conditions [12].

Researches into natural products for solving health problems have been encouraged by the World Health Organisation [13] and Food and Agricultural Organisation [14].

In ancient immemorial period Neem has been used as a disincentive agent against highly contagious smallpox and other infectious diseases and was also regarded to defend against evil spirits from time [15]. In the Indus civilization, the use of Neem tree is as old as 4,500 years during the period of Harappa culture (one among the great civilisation in the world). Centre for Traditional Medicine and Research (CTMR), Chennai, India, revealed the medicinal uses of different parts viz, fruits, seeds, leaves, roots, bark etc., of neem trees. It explains use of neem flower against bile disorders, neem leaves to prevent and treat ulcers and neem bark to brawl against paralysis and CNS disorders [16]. Old evidences obtained from two great civilisations Harappa and Mohenjo-Daro of ancient world also witnessed that A. indica was the prominent herb of therapeutic importance at that time not only in Indian context but in world as well. According to epic of Mahabharata, Nakul and Sahadeva used Neem oils for treatment of wounds in horses and elephants [16].

2. Materials and Methods

2.1 Study Area

The study was conducted in Sokoto, in the Poultry pen of the Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto. Sokoto State is geographically located to the North Western part of Nigeria between the longitudes 4^0 8'E and 6^0 54'E, and latitudes 12^0 and 13^0 58N [17]. The state falls within two vegetation zones: the Sudan Savannah and Northern Guinea Savannah. The climate is characterized by altering dry and wet seasons with short cold and dry period of harmattan usually accompanied by dust-laden winds and fogs which start from October and last through February. The duration and intensity of annual rain fall ranges from 60-160 days and 635-1000mm (occurring between May to October) respectively. The mean monthly temperature is generally 20-46^0 C, relative humidity ranges from 12-17% with the highest occurring in August (NPC, 2006).

2.2 Experimental Birds

Four hundred and eighty (480) day old cockerels for the study which were purchased from a commercial hatchery (Farm support^R) in Ibadan were used. The experimental birds were raised for 8 weeks.

2.3 Housing and Feeding

The birds were managed on deep litter system in cleaned formalin- potassium fumigated pens. One 200 watt electric bulb was fixed to provide warmth to the birds for each of the groups with additional source of heat around the pens. The pen temperature was maintained at 33 to 35^0C for 1 to 3 weeks old and 28 to 29^0C for the remaining 3 to 8 weeks old. They were fed on commercial feed; chick mash (Animal care^R) for the 8 weeks but the treated water was given from day old to 6 weeks. The feed and water were provided *ad libitum*.

2.4 Biosecurity Measures

Strict biosecurity measures were taken for all the groups. Moreover, the challenged groups were separated into a different pen entirely, employing 2 well trained poultry attendants to routinely feed the birds, disinfect themselves, watering and feeding utensils as well as the environment in order to prevent the spread of microorganisms into or outside the poultry pen. Each of the attendants was responsible for particular groups of birds (unchallenged: groups A to D and challenged: groups E to H). Groups A to D were raised in the main Faculty of Veterinary Medicine poultry pen while groups E to H within a separate pen. Complete personal protective wears were used during vaccination and experimental infection which were incinerated immediately after used. At the entrance of each pen, footbath was provided containing 2% formalin and shoes were provided and hanged just at the interior side of the pens door for use specifically within the pens. At the end of the experiment, the whole pens were fumigated with formalin- potassium composition while the litter was removed and incinerated.

2.5 Preparation and Extraction of Neem (Azadirachta indica) leaf Aqueous Extracts

Mature green neem leaves were used for the experiment. The leaves were obtained from Shehu Kangiwa Square and a botanist from UDUS herbarium professionally identified the Neem and labelled it (UDUH/ANS/0004). The leaves were rinsed in distilled water, air dried and pulverized. The extract was prepared following the procedure reported by [18].

The concentration of the aqueous Neem leaf extract used was 3.0mg/ml which was administered through drinking water for 6 weeks. The choice of the concentration was based on the earlier preliminary work done on the safety margin of the extract.

2.6 Vaccine

A live IBD (intermediate plus strain) vaccine containing $\geq 10^3$ EID_{50}/ml was used for the study which was sourced from an Agro- Veterinary Company in Kaduna, Nigeria. The vaccination was carried out at 2 and 4 weeks of age via oral route.

2.7 Challenge IBD Virus

At day 35 the birds in groups E, F, G and H were challenged orally with 0.1ml of a live vvIBD virus containing $10^{9.76}$CID/ml.

2.8 Experimental Design

2.8.1 Grouping

The 480 birds were assigned randomly into eight groups (A to H) with 60 birds per group. Group A was the negative control group and therefore, neither receive the aqueous extracts nor were they vaccinated against IBD. Group B was the positive IBD- vaccinated control group, thus, did not receive the aqueous extracts but was vaccinated against IBD. Group C, D, G and H were treated with 3.0mg/ml of Neem leaf aqueous extracts in drinking water from day old to 6 weeks. Group C, F and G were vaccinated against IBD while D, E and H were not vaccinated against the IBD. Birds in group E, F, G and H were challenged with vvIBD virus while those in group A, B, C and D remained unchallenged.

2.8.2 Vaccine and Vaccination

Birds from groups B, C, F and G were vaccinated against IBD at 14 and 28 days of age with IBD intermediate plus vaccine containing $\geq 10^3$ EID_{50}/ml, 0.2ml/bird via oral route while groups A, D, E and H remained IBD-unvaccinated.

2.8.3 Experimental Infection

Birds from group E, F, G and H were experimentally challenged orally with 0.2ml of vvIBD virus at 35 day of age (5 week old) while A, B, C and D were not challenged.

Table 1: Experimental Design

Group	IBD Vaccination Status	Experimental Challenge with vvIBDV	Neem Extract Treatment
A	Unvaccinated	Unchallenged	No Extracts given
B	Vaccinated	Unchallenged	No Extracts given
C	Vaccinated	Unchallenged	3.0mg/ml Extract
D	Unvaccinated	Unchallenged	3.0mg/ml Extract
E	Unvaccinated	Challenged	No Extracts given
F	Vaccinated	Challenged	No Extracts given
G	Vaccinated	Challenged	3.0mg/ml Extract
H	Unvaccinated	Challenged	3.0mg/ml Extract

2.9 Clinical Signs

Clinical signs exhibited by the birds following experimental challenge were recorded including the onset, course and mortality rate.

2.10 Blood Collection

At day old and weekly interval, blood samples from five birds in each group were collected randomly for serology. Using 21 gauge needle and 2 ml syringe blood was collected directly from the heart and for the day old chicks, by salvaging the birds in which 1.5ml was transferred into a plain test tube. The plain test tubes containing the blood samples were then allowed to stand for two hours and then centrifuged at 10062×10^{6}g for 5 minutes and the sera harvested and stored at -20^{0}c.

2.11 Antibody titre Determination for IBD

Antibody titre against IBD was determined using a standard commercial ELISA kit (KrishgenBioSystem), USA.

2.11.1 Enzyme-linked immunosorbent assay for IBD

All the sera were tested for antibodies against IBD using a standard commercial ELISA kit which was obtained from KrishgenBioSystem, USA. The procedure for the test was carried out according to the manufacturer's instructions. Briefly, the antigen coated plates and the ELISA kit reagents were adjusted at room temperature prior to the test. The test sample was diluted to 500 folds (1: 500) with sample diluents prior to the assay. A 100µl of diluted sample was then put into each well of the plate. This was followed by 100µl of undiluted

negative control into the well A1 and A2, 100µl of undiluted positive control into well A3 and A4. The plate was incubated for 30 minutes at room temperature. Each well was then washed with approximately 350µl of distilled water 3 times. Goat antichicken conjugate (100µl) was dispensed into each well.

The plate was then incubated at room temperature for 30 minutes, followed by washing each well with 350µl of distilled water 3 times.

Tetramethylbenzidine (TMB) solution (100µl) was dispensed into each well. The plate was then incubated at room temperature for 15 minutes. Finally, 100µl of stop solution was dispensed into each well to stop the reaction. The absorbance values were measured and recorded at 650nm.

IBD antibody titre was then calculated.

2.12 Statistical Analysis

The obtained data from mortality were recorded, tabulated and presented in a form of graph where necessary.

The ELISA antibody (IBD) titres were presented in tables and their standard deviations calculated and further presented in a form of graph with error bars.

3. Results

3.1 Enzyme- Linked Immunosorbent Assay Antibody Titre against IBD

3.1.1 Infectious Bursal Disease Antibody for the Neem Leaf Treated and Untreated Cockerels

The result of this study shows that, the maternal derived antibody (MDA) in the experimental groups (A to D) at day old had 9581.20±290.97, 10461.6±156.6, 10163.44±209.09 and 10107.49±386.79 for group A, B, C and D respectively.

There was a general decrease of the MDA of the chicks in all the groups with age irrespective of whether they were treated or not as well as no significant difference observed. However, by 2 weeks old, only the treated groups (C and D) had a titre ≥ 1000.

At 3 weeks of age, group C had the highest antibody titre (1200.95±76.23) followed by group B (613.61±70.86), D (366.97±9.76) and A (273.75±66.33). By 4 weeks of age.

The following antibodies were detected: group C (2777.52±631.86), group B (1000.27±26.37), group D (169.64±7.91) and then group A (123.75±18.05).

At 5 weeks of age, the following antibodies were detected for the respective groups with group C having the highest titre (4057.52±209.42), followed by group B (1253.61±173.96), group D (113.97±7.58) and then group A (74.42±28.65). At 6 weeks old, group A had no detectable titre while group B, C and D had 2053.61±135.01,

4966.85 ±156.62 and 57.30±31.79 titres respectively. At 7 weeks of age, group A and D had undetectable titre while group B and C had 108.27±98.44 and 3870.18±844.79. Also at 8 weeks old, group A and D had undetectable titres while group B and C had 1159.94±104.15 and 3986.85±8351.42 respectively.

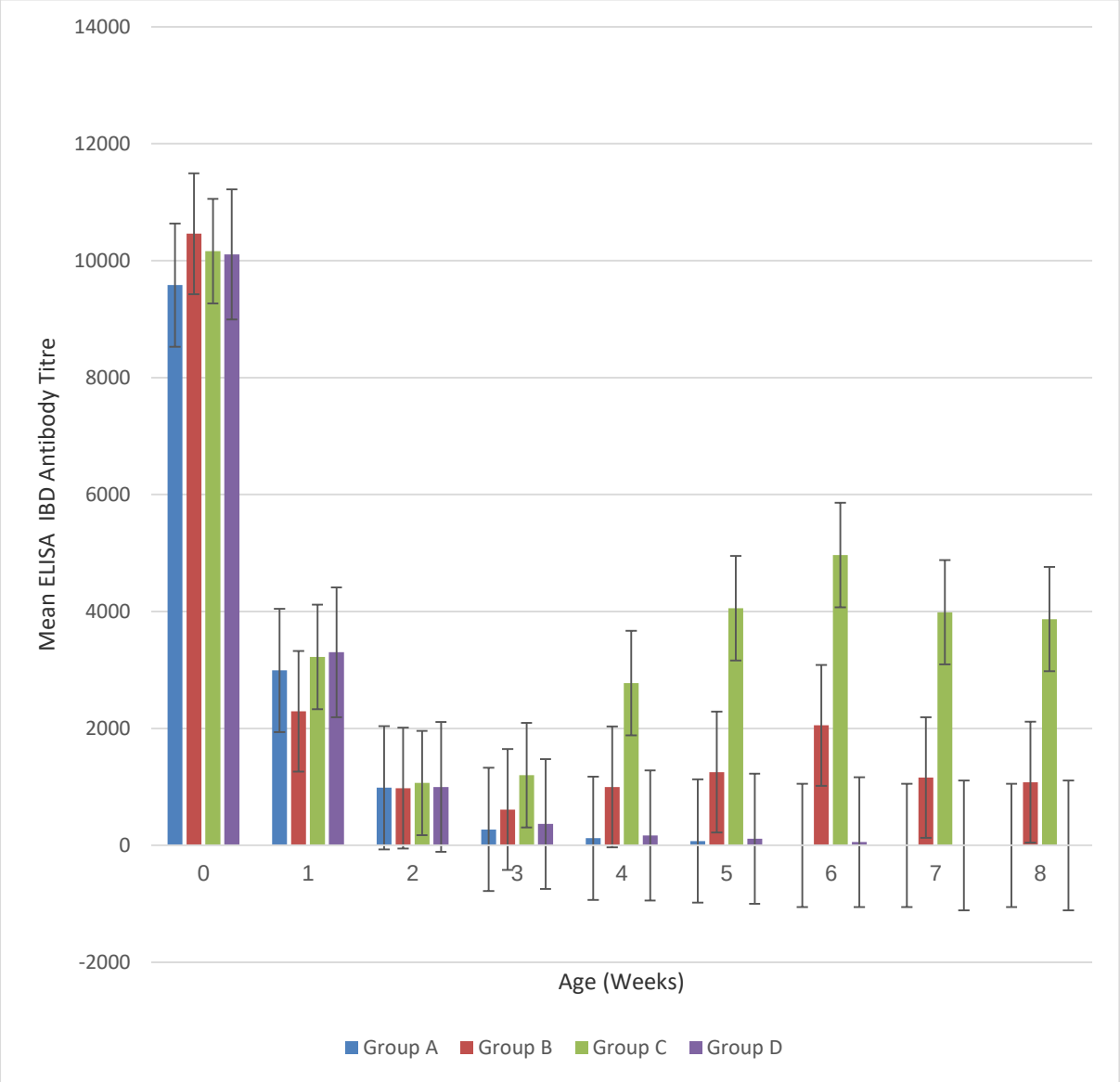

Figure 1: Infectious Bursal Disease Antibody Titre for the Neem Leaf Treated and Untreated Cockerels

3.1.2 Infectious Bursal Disease Antibody for the Vaccinated and Unvaccinated Cockerels

A week post primary vaccination (PPV) (3 weeks of age), group C had the highest antibody titre (1200.95±76.23) followed by group B (613.61±70.86), group D (366.97±9.76) and then group A (273.75±66.33).

Two weeks PPV (4 weeks of age), the following antibodies were detected: group C (2777.52±631.86) followed by group B (1000.27±26.37), group D (169.64±7.91) and then group A (123.75±18.05).

A week post booster vaccination (PBV) that was, at 5 weeks of age, the following antibodies were detected for the respective groups with group C having the highest titre (4057.52±209.42) followed by group B (1253.61±173.96), group D (113.97±7.58) and then group A (74.42±28.65).

Two weeks PBV (6 weeks old), group C had the highest titre (4966.85±152.62) followed by group B (2053.61±135.01) and then group D (57.30±31.79). However, group A had zero titre.

Three to four weeks PBV (7- 8 weeks old), the titres for both the group C and B decreases with age; however, group C had significantly higher antibody titre when compared to group B. Moreover, undetectable titre was also obtained in group A.

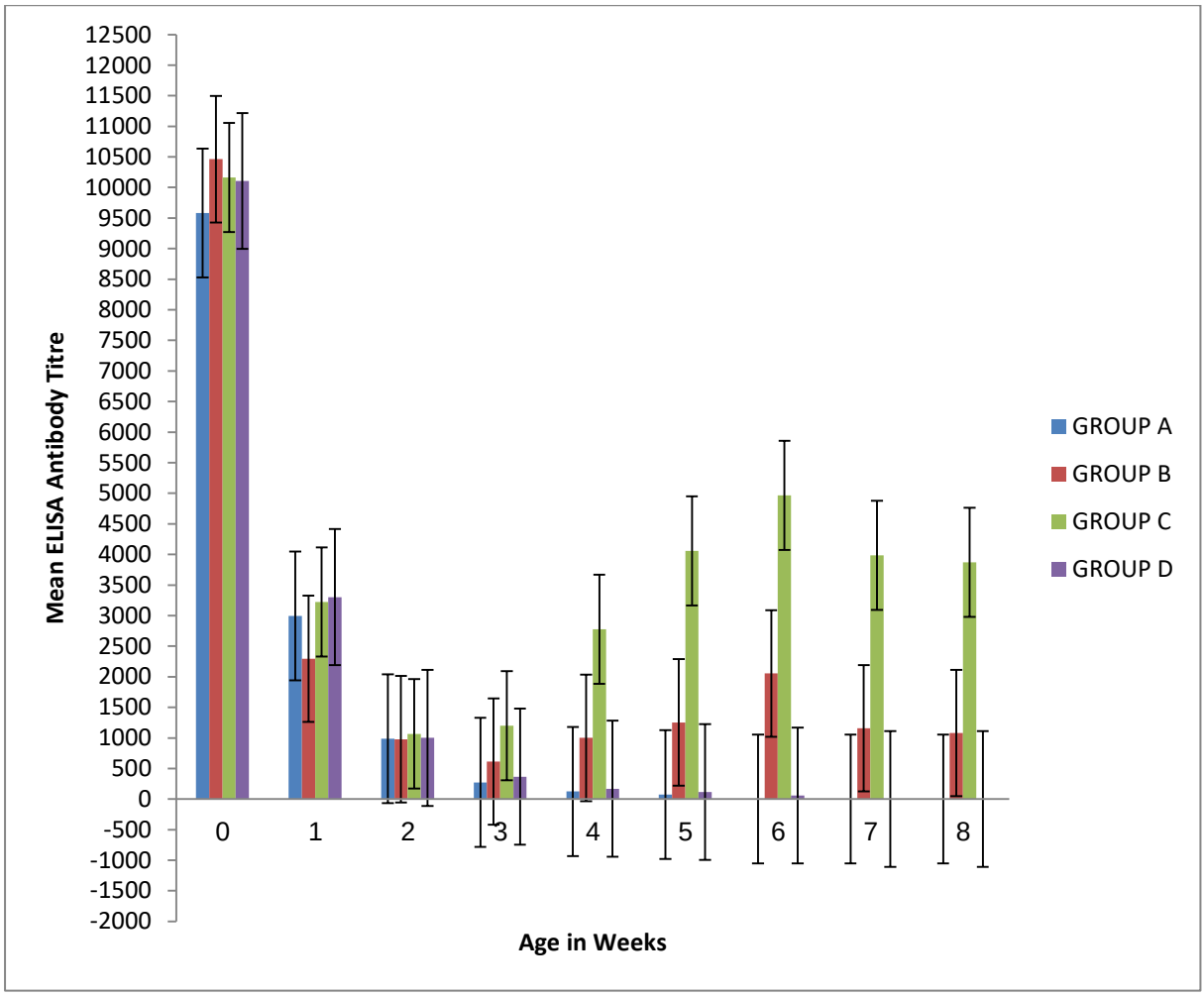

Figure 2: Infectious Bursal Disease Antibody Titre for the Vaccinated and Unvaccinated Cockerels

3.1.3 Infectious Bursal Disease Antibody Titre for the Challenged and Unchallenged Cockerels

The following antibody titres were obtained for the challenged groups (E, F, G and H) a week post challenge that was, at 6 weeks of age: 229.70±69.58 for group E, 1959.66±394.05 for group F, 6220.56±599.06 and 317.89±86.08 as compared to the unchallenged groups (A, B, C and D): Zero titre for group A, 2053.61±135.01

for group B, 4966.85±152.62 for group C and 57.30±31.79 for group D.

Two weeks post challenge that was at 7 weeks old, the challenged groups E, F, G and H had 669.70±101.92, 4726.32±561.71, 9587.61±1427.53 and 792.16±25.13 respectively as compared to the unchallenged groups (A, B, C and D) which had zero titre, 1159.94±104.15, 3986.85±8351.42 and zero titre respectively.

Three weeks post challenge that was at 8 weeks old, the challenged groups (E, F, G and H) had 5003.04±569.32, 7359.51±476.16, 11187.28±1096.69 and 5925.49±305.67 respectively when compared to the unchallenged groups (A, B, C and D) which had 0±0, 1080.27±98.44, 3870.18±844.79 and 0±0 titres respectively.

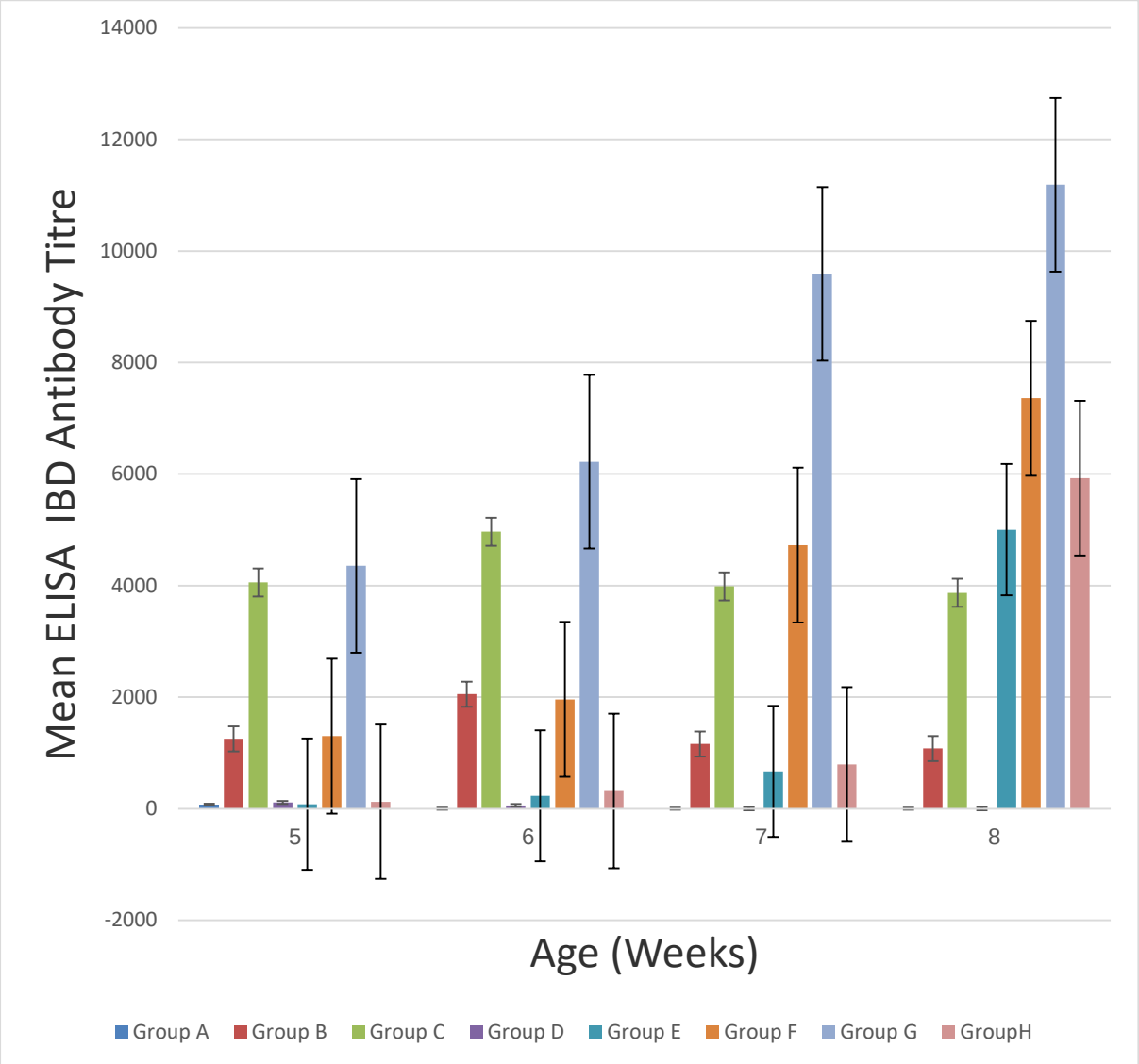

Figure 3: Infectious Bursal Disease Antibody Titre for the Challenged and Unchallenged Cockerels

3.2 Clinical Signs Post Challenge

Amongst the four challenged groups (E to H), the typical signs of IBD were observed in group E, F and H which

include: depression, ruffled feathers, creamy- whitish diarrhea, anorexia, recumbency and somnolence. At 36 hours post challenged, 2 birds in group E first showed sign of depression and 12 hours later (48 hrs PC) 15 chicks in the same group E showed depression and ruffled feathers while at the same time 8 chicks in group F Showed depression and ruffled feathers. At 60 hours PC, 25, 9, 1 and 19 birds in group E, F, G and H respectively became affected showing a typical dullness, depression, recumbency, ruffled feathers, somnolence, creamy- whitish diarrrhea and anorexia with the exception of the one bird that was affected in group G which only showed depression. The clinical signs and the number of birds affected reach peak level at 72 hours post challenged for group E, 84 hours post challenged for group F and 60 hours post challenged for group H while the symptoms disappeared completely at 132 hours post challenged (Figure 4).

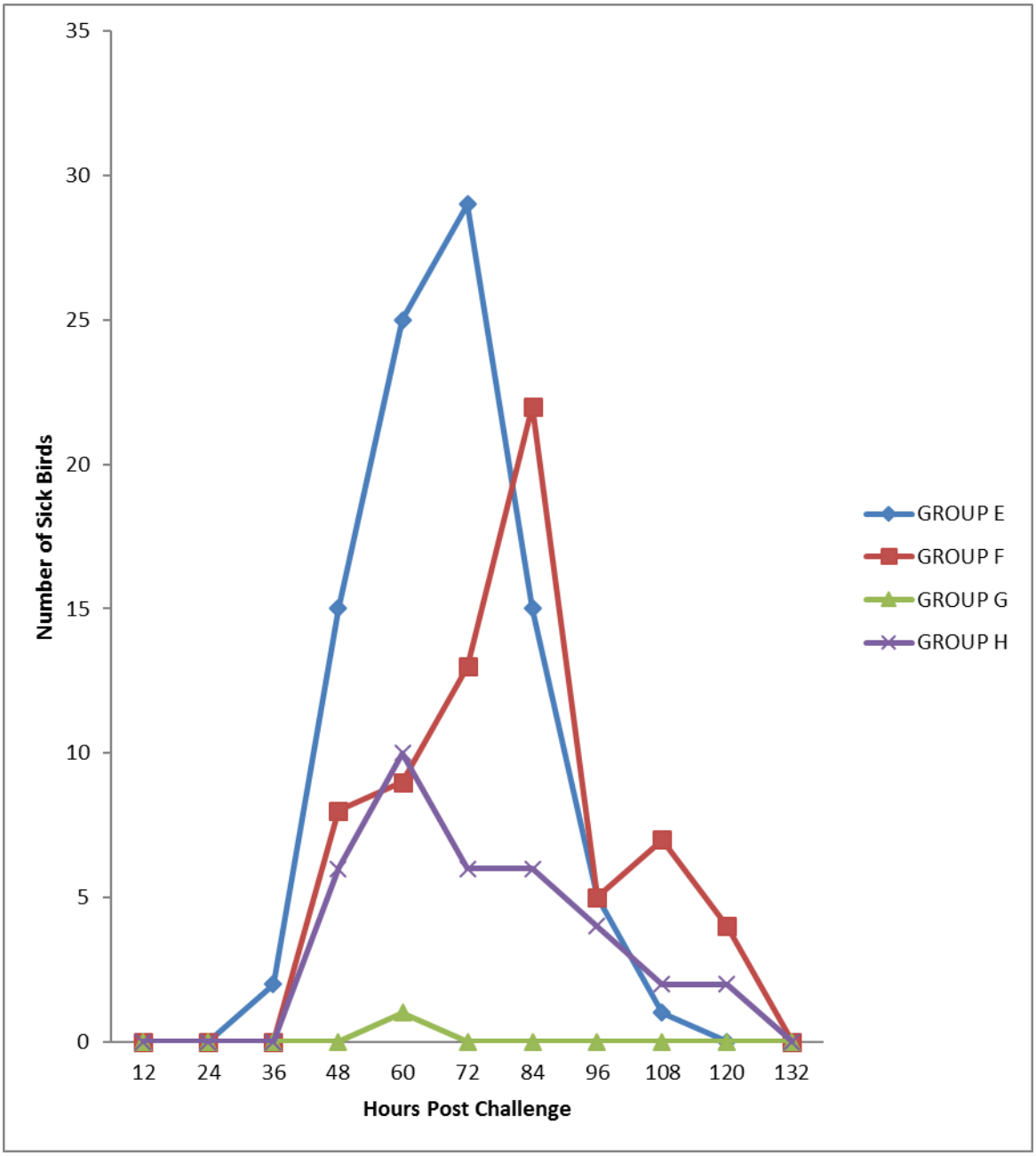

Figure 4: Disease Course Pattern in Chicks Treated with Neem leaf Aqueous Extract Vaccinated with IBD

Vaccine against IBD at 2 and 4 Weeks of Age and Challenged with vvIBD Virus at 5 Weeks of Age

KEY:

Group E: Untreated, unvaccinated and challenged (challenged control group)

Group F: Untreated, vaccinated and challenged

Group G: Treated, vaccinated and challenged Group H: Treated, unvaccinated and challenged

Table 2: Frequency (%) of Observed Clinical Signs Following Experimental Challenge of Five Weeks Old Chickens with vvIBDV

Clinical Signs	Group E	Group F	Group G	Group H
Depression	96	66.7	2.2	40.0
Dehydration	55.56	40.0	0	15.56
Whitis- Diarrhea	84.4	53.3	0	26.7
Inappetance	88.9	46.7	0	33.3
Swollen vent	33.3	20	0	20
Recumbency	60	37.8	0	17.8
Ruffled feather	97	64.4	2.2	33.3
Huddling	42.2	35.6	0	24.4
Vent pecking	17.8	11.1	0	11.1
Soiled vent	84.4	53.3	0	26.7
Anorexia	79.2	37.78	0	17.9
Trembling	17.8	2.2	0	46.7
Prostration	46.7	11.1	0	6.7
Shivering	72.4	35.5	0	10

3.3 Mortality Rate Post Challenge

Mortality due to IBD started 60 hours post challenged (PC) in group E with the death of 3 birds and 12 hours later (72 hours PC), 10, 1, 1 and 4 birds died in groups E, F, G and H respectively.

The mortality reached peak level at 84 hours PC in group E, 108 hours PC in group F and 72 hours PC in group H. The mortality attained its peak level at 84 hour PC in group E, 108 hours PC in group F and 72 hours PC in group H while in group G, only one bird died.

The mortality declined to 0 at 120 hours PC in group E, 132 hours PC in group F, 84 hours PC in group G and 108 hours PC in group H (Figure 5). The total mortality rates recorded were 82.2%, 42.2%, 2.2% and 15.5% for

groups E, F, G and H respectively (Table 3).

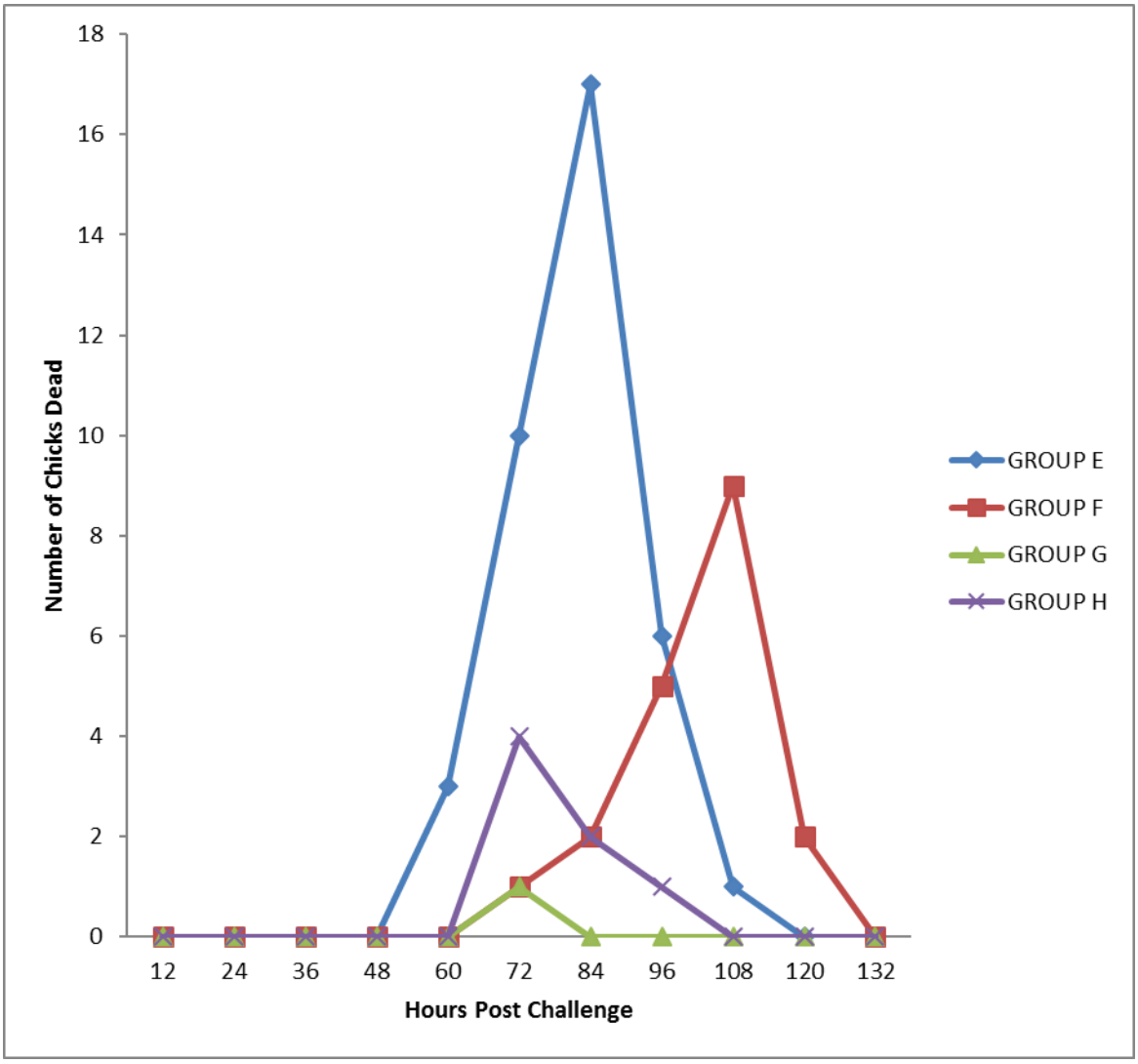

Figure 5: Mortality Pattern in Chicks Treated with Neem leaf Aqueous Extract, Vaccinated with IBD Vaccine against IBD at 2 and 4 Weeks of Age and Challenged with vvIBD Virus at 5 Weeks of Age

KEY:

Group E: Untreated, unvaccinated and challenged (challenged control group)

Group F: Untreated, vaccinated and challenged

Group G: Treated, vaccinated and challenged

Group H: Treated, unvaccinated and challenged

Table 3: Mortality Rate of Chickens Treated and Untreated with Neem Leaf Aqueous Extract Post vvIBDV Challenge

Treatment						
Group	Extract	Vac	Chal	Number Challenged	Number Dead	Mortality Rate (%)
E	-	-	+	45	37	82.2
F	-	+	+	45	19	42.2
G	+	+	+	45	1	2.2
H	+	-	+	45	7	15.5

KEY:

Group E: Untreated, unvaccinated and challenged (challenged control group)

Group F: Untreated, vaccinated and challenged

Group G: Treated, vaccinated and challenged

Group H: Treated, unvaccinated and challenged

Vac: Vaccinated

Chal: Challenged

4. Discussion

In this research, the immunomodulatory potentials of aqueous extract of Neem (*Azadirachta indica*) leaf in cockerels vaccinated and/ or infected with IBD virus (IBDV) was studied.

On the arrival day of the experimental birds at day old, the sera collected from the chicks revealed the presence of maternally derived antibodies (MDA) to IBDV and the MDA ELISA antibody titre level were all above 9,500 in all the groups and the titre decreased just below 1000 at 2 weeks of age in the Neem leaf untreated groups (group A and B) when compared to the Neem leaf treated groups (C and D) as shown in figure 1. The decay of MDA observed agrees with the finding of [19] who reported a drop in MDA levels at 14 days of age. Also, [20] and [21] reported a drop in MDA after 17 days of age. The presence of the MDA could be attributed to the passive transfer of antibodies from the parent stock to the chicks. This implies that, if the chicks were exposed to IBDV at less than 2 weeks of age they may not succumb to the disease and for the chicks on the Neem leaves extract, the level of protection may even extend beyond 2 weeks of age. The extended spectrum of protection of the MDA in the Neem leaves treated groups could be attributed to immunopotentiating effects of the extract. The overall MDA lasted for up to 6 weeks in the Neem leaf treated groups during this study and this

was contrary to the results of [22,23] who reported that by 4 weeks, all birds were devoid of MDA.

The increased antibody titre level in group C with subsequent decreased titre level observed in group A, B and D at 3 weeks of age could also be attributed to the Neem leaf aqueous extract administered in group C which might have potentiated the IBD vaccine administered at 2 weeks, even though there was no statistical difference when compared to the other 3 groups (A, B and D). Moreover, from 4 weeks up to the 6 weeks, a statistically significant increase in antibody titre followed by a steady decrease from the 6 weeks was observed in group C as compared to birds in group A, B and D which on the other hand had a progressive decrease in their antibody tire right from 3 weeks to undetected level at 6 weeks for group A and 7 weeks for group D but with an increase in the titre level in group B even though statistically lower than that of group C was seen from 4 weeks to 6 weeks followed by a decrease in the titre. The increase in antibody level observed in group C at 3 weeks as compared to those in group B (Untreated- vaccinated group) implies that the birds had a faster seroconversion rate. The higher seroconversion observed in the treated- vaccinated group (group C) could be due to the ability of the Neem leaves extract to immunostimulate the B- cells and subsequently the plasma cells to secret more antibodies despite the presence of MDA. It may also be possible that the Neem leaves extract ameliorates some of the bursal follicular lymphoid destructions and hence, more antibodies would be secreted.

At day old and 1 to 3 weeks of age, a progressive decay in MDA was observed between the vaccinated groups (B and C) and unvaccinated groups (A and D) with the exception of group C which had a slight increase at 3 weeks as shown in figure 2. At 2 weeks post primary vaccination (4 weeks old), the IBD antibody titre in the unvaccinated groups (A and D) continue to decrease with age but group D had higher titre as compared to group A but the difference was statistically insignificant. Conversely, the vaccinated groups (B and C) at 2 weeks post vaccination (4 weeks old) had an increase in the antibody titre and the titre continue to increase up to 6 weeks of age which was followed by a gradual decrease. Moreover, between the vaccinated groups, group C had a significantly higher antibody titre when compared to group B as shown by the error bars in figure 2.The decrease in antibody titre observed in group A and D was attributed to the fact that the birds have not been vaccinated against IBD at 2 and 4 weeks of age and probably have not been exposed to field IBD virus. While the increase in the antibody titre observed in group B and C was attributed to the IBD vaccine administered at 2 and 4 weeks old while the significant difference noticed between the treated groups (B and C) could be attributed to the Neem leaf extract that was administered to the birds in group C. This result further showed that, the administration of neem leaves aqueous extract might have potentiates the vaccinal effects on the B-lymphocytes which differentiate into memory cells and plasma cells. The plasma cells secrete antibodies while the memory cells have the ability to rapidly differentiate into antibody- producing plasma cells when they encounter the same antigen in another infection. This finding was compatible with those of previous reports that neem leaf extract given orally to mice enhanced the level of IgG and IgM [24]. Also, Neem leaves when used in immunosuppressed birds increased both the humoral and cell mediated immune responses, thus, preventing further infections [25]. Antibody titre to Newcastle disease antigen has been shown to be enhanced in broiler chickens that were treated with neem leaves aqueous extract [26]. The leaf preparation of neem has been used as an adjuvant to enhance the efficacy of poorly immunogenic B16 melanoma surface antigen vaccine [27, 28].

Figure 3 reveals the antibody titre obtained between the unchallenged (A, B, C and D) and challenged (E, F, G

and H) groups. At 6 weeks of age (one week post challenge), the antibody titre for group A (untreated- unvaccinated- unchallenged group) and group D (treated- unvaccinated- unchallenged group) declined to undetected level at 6 (one week post challenge) and 7 (two weeks post challenge) weeks of age respectively while group B (untreated- vaccinated- unchallenged) and C (treated- vaccinated- unchallenged) had an increase in the titre upto the 6 weeks of age followed by a gradual decrease in the titre at 7 to 8 weeks of age as clearly seen in figure 3 but the titre in group C was significantly higher than that of group B throughout. The titre in group E (untreated- unvaccinated- challenged) and H (treated- unvaccinated- challenged) increased at 6 weeks of age when compared to that in group A and D respectively and the titre progressively continue to increase upto 8 weeks. Also, the titre in group F (untreated- vaccinated- challenged) and G (treated- vaccinated- challenged) increases at 6 weeks to 8 weeks of age when compared to that in group B and C respectively which had an increase in the titre at 6 weeks but the titre decline progressively as clearly seen at 7 to 8 weeks of age (figure 3). The progressive decrease in antibody titre to zero level at 6 and 7 weeks in group A and D respectively could be attributed the lack of previous exposure to the IBD vaccines/ vvIBD virus as compared to the increase observed at 6 weeks and above in group E and H whom were challenged at 5 weeks old with vvIBDV despite the fact that they were not vaccinated against the IBD. The increase in the antibody titre seen at 6 weeks old which followed by a progressive decrease at 7 to 8 weeks old in group B and C may be linked to the IBD vaccine administered at 2 and 4 weeks old while the gradual decrease in the titre indicates that even the active vaccinal immunity decay with time. On the other hand, progressive increased antibody titre without decrease observed in group E, F, G and H was attributed to the vvIBD virus given at 5 weeks of age which possibly lead to the production of antibodies and the continued shedding of the virus through faeces probably maintained the viral load within the pens and hence, the observable progressive increase in the titre in all the challenged groups (E, F, G and H) as compared to the unchallenged groups (A, B, C and D), figure 3.

At 1 to 3 weeks post challenge, the lowest ELISA antibody titre detected in group E (challenged control group) was attributed to the lack of previous exposure to both the IBD vaccine and/ or field IBD virus. However, amongst the whole challenged groups (E, F, G and H), group G had the highest ELISA antibody titre and that has been attributed to the previous exposure to the acquired immunity through both primary and booster IBD vaccination as well as the administration of the neem leaves aqueous extract and the vvIBDV inoculation, followed by group F which were not given the extract but were vaccinated with the same IBD vaccine as well as challenged with vvIBDV, and group H which were given the extract but not vaccinated with the IBD vaccine but challenged with the vvIBDV.

The clinical signs observed in the challenged groups during this study were the same as reported by other researchers on IBD infections [29, 30]. The mortality rate of up to 82.2% recorded in the challenged control group (group E) was highly suggestive of vvIBDV infection.

In this study, the course of the disease pattern post challenged followed the usual course in vvIBD outbreak; reaching a peak between 2 to 3 days post infection and receded in a period of 5 to 7 days as also reported by some authors [31, 32]. The mortality rate recorded per group were 82.2%, 42.2%, 2.2% and 15.5% for group E (untreated- unvaccinated- challenged group), F (untreated- vaccinated- challenged group), G (treated- vaccinated- challenged group) and H (treated- unvaccinated- challenged group) respectively and the mortality

pattern. The low mortality recoded in the treated groups could be attributed to the antioxidant properties of vitamin C, D and E contained in the neem aqueous extract used during the experiment. These vitamins are able to protect immature lymphocytes from damage by free radicals due to oxidation, thus enhancing the immune response. Also contained in the extract include: Zn, Na, Mg, Ca, vitamin A, B2, B3 and B6 as observed. In small pox, neem has been found to be an effective antiseptic for the treatment. Neem extracts have been shown to possess potent antiviral properties against different viruses including herpes simplex virus type-1 infection [33, 34]. In vitro antiviral activity of leaf extract has been documented against Coxsackie virus B, Vaccinia virus, Variola virus, Chikungunia, dengue virus, poliovirus, measles viruses, fowl pox viruses, Gumboro viruses and Newcastle disease viruses [35, 36].

Conclusively, the MDA observed indicated the passive transfer of antibodies from the parent stock to the chicks. The overall MDA lasted for 5 to 6 weeks in the Neem leaf treated groups while for the untreated groups, it lasted for 4 to 5 weeks. Neem leaf aqueous extract enhances the humoral antibody titre against IBD in both the vaccinated and challenged groups. The extracts suppressed the clinical signs and prevent the mortality caused by the vvIBDV. Hence, we recommend that the Neem leaf aqueous extract should be given to birds pre and post vaccinations. Also, in the case of outbreaks, the extract can be used in our poultry industry as a therapeutic agent.

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