Journal of Applied Sciences

ISSN 1812-5654

science alert

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OPEN ACCESS Journal of Applied Sciences

ISSN 1812-5654
DOI: 10.3923/jas.2019.466.472

Research Article
Antibody Response to Newcastle Disease Vaccine of Cockerels Challenged with Virulent Infectious Bursal Disease Virus and Administered Some Complementary and Alternative Therapies

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Abstract

Background and Objective: This study evaluated the efficacy of some complementary and alternative therapies commonly used by farmers for the control of infectious bursal disease (IBD) in Nigeria. **Materials and Methods:** A total of 280 days old commercial chicks were assigned into 7 groups (NC, PC, RS, KHOS, AVS, GND and GND+KHOS) of 40 chicks each and housed on deep litter. Each group was identified by a group acronym, based on the type of treatment administered. Chicks in groups PC, RS, KHOS, AVS, GND and GND+KHOS were inoculated via intra ocular route at 35 days of age with a very virulent IBD virus (vvIBDV). Treatment was instituted two days post inoculation (dpi) at the onset of clinical signs of IBD. The chicks were also vaccinated orally with a live Newcastle Disease (ND) La Sota vaccine at 7 dpi. Chicks in PC group were challenged but not treated while those in NC group were neither challenged nor treated. Blood was collected from five chicks in each group via the wing vein at 35, 38, 42, 49 and 56 days of age to evaluate for IBD and ND antibody titre levels using agar gel precipitin test (AGPT), enzyme linked immunosorbent assay (ELISA) and haemagglutination inhibition (HI) test, respectively. **Results:** None of the chicks in all the groups had a precipitin antibodies before challenge at day 35 (0 dpi) and 3 dpi. At 7 dpi, 100, 80 and 20% of the chicks in groups AVS, GND and GND+KHOS, respectively had precipitin antibodies. A significant difference ($p<0.05$) was observed in the mean ELISA antibody titre of chicks in group RS and KHOS when compared with the other groups at day 0 (pre-infection). By day 7 dpi, significant increase ($p<0.05$) was observed in the mean ELISA IBD antibody titre level of chicks in groups RS, GND and GND+KHOS. Also, a significant increase was observed in the mean ELISA IBD antibody titre level of chicks in groups GND and GND+KHOS and groups RS and GND at day 14 and 21 dpi. There was an increase in the mean Log_2 HI titre level of chicks in the KHOS, AVS, GND and KHOS+GND groups at 7 days post vaccination (dpv) with ND La Sota vaccine. **Conclusion:** The GND, RS and AVS had positive effects on humoral immune response of cockerels challenged with vvIBDV. The KHOS and AVS ameliorated the negative effect of vvIBDV on humoral immune response to ND La Sota vaccine. The GND, RS, AVS and KHOS used in this study can be used as immune modulators. Farmers, veterinarians and other animal health workers are advised to adhere to the routine vaccination against IBD and strict bio-security.

Key words: Complementary, alternative, infectious bursal disease, newcastle disease, antibody titre

Citation: Balami Arhyel Gana, Pilarshimwi James Yidawe, Shallmizhili, Johnson, Etim Alex Bassey and Abdu Paul Ayuba, 2019. Antibody response to newcastle disease vaccine of cockerels challenged with virulent infectious bursal disease virus and administered some complementary and alternative therapies. J. Applied Sci., 19: 466-472.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Infectious bursal disease (IBD) has been considered as one of the important naturally occurring viral diseases of chickens and a cause of economic losses that threatens the poultry industry worldwide[1-3]. Infectious bursal disease has continued to be a major disease problem of commercial and rural chickens and constitute a major threat to poultry production in Nigeria[4,5]. The dreaded nature of IBD has rendered investment in poultry to be fearful and unrealistic to both organizations and individuals[6-9]. Despite rigorous vaccinations, outbreaks of IBD in commercial poultry have been reported to account for high loss in Nigeria[3,5,7,10-12] causing damage to the bursa of Fabricius (BF) ultimately prolonged immunosuppression of chickens which consequently leads to increased susceptibility to various bacterial and viral diseases and poor response to vaccines[9,13,14]. Complementary and alternative medicine (CAM) were defined by the National Centre for Complementary and Alternative Medicine (NCCAM) as "a group of diverse medical and health care systems, practices and products that are not presently considered to be part of conventional medicine[15]. Ernst *et al*.[16] also defined CAM as diagnosis, treatment and/or prevention which complements main-stream medicine by contributing to a common whole, satisfying a demand not met by orthodoxy or diversifying the conceptual framework of medicine. Complementary and alternative medicine and the numerous modalities it entails continue to be an increasingly popular option for individuals seeking relief from or prevention of a wide range of bodily complaints, ailments and illnesses[17-21].

Today in Nigeria, the dreaded nature of IBD, the economic losses the disease has caused and the poor immune response to various vaccinations the disease create on the birds[3,22] has led a lot of farmers to resort to the use of different complementary and alternative therapies in other to control the menace of the disease. This study was therefore; aimed at validating the efficacy of some of the complementary and alternative therapies used in the control of very virulent infectious bursal disease virus (vvIBDV) in Nigeria, with the objective of evaluating the humoral immune response in chickens to ND La Sota vaccine following challenged with vvIBDV.

MATERIALS AND METHODS

The study was carried out at the Poultry Research pens of the Faculty of Veterinary Medicine, University of Maiduguri, Borno state, Nigeria, between the months of May and June, 2018. The research was approved by the ethics committee of the University of Maiduguri and guidelines for the care and humane handling of animals were adhered to throughout the study. A total of 280 day old Isa brown cockerels were purchased from a hatchery located in Ibadan, Nigeria. The chicks were brood on deep litter in a house that was previously thoroughly cleaned and disinfected and wood shaving were used as litter material. The chicks were randomly assigned into 7 different groups of 40 chicks each corresponding to the type of treatment given and housed in separate compartment. A 100-watt bulb was provided in each of the compartment to supply light and heat during brooding with 2 feeders (4 feet long) and 2 plastic drinkers provided in each of the pen. Each group was identified by a group acronym (NC, PC, RS, KHOS, AVS, GND and GND+KHOS) based on the type of treatment administered. The chicks were brood for 5 weeks before inoculation with a vvIBD virus.

Feeds, feeding and feed analysis: The chicks were fed with a pelletized chick mash purchased from an accredited large distributor of a reputable commercial feed company. Proximate analysis of the feed was carried out in the Feed Analysis Laboratory of the Department of Animal Science, University of Maiduguri, to determine the level of metabolizable energy, crude protein, crude fibre, moisture, ash content and dry matter. Feed and water were provided *ad libitum* using galvanized feeders and plastic drinkers, respectively.

Challenge virus: Field IBDV was obtained from the Department of Veterinary Medicine, ABU, Zaria, Nigeria. The field IBDV was a very virulent strain obtained from previously vaccinated commercial layers that died of a natural outbreak of IBD. About 65% of commercial cockerels inoculated at 30 days of age with 50 µL of bursal suspension (v/w) in PBS (pH 7.4) died. About 1 mL of bursal suspension (v/w) in PBS (pH 7.4) contains $16\times10^{4.6}$ CID_{50} of IBDV.

Medicaments: The RS contain Vitamin K, ascorbic acid, Iodine and boric acid were obtained locally from accompany representative in Jos and administered at 1 mL per litre of drinking water for 5 days, 2 days post inoculation (dpi) with vvIBDV. The KHOS is a mixture of herbal extract from Aether Centre (Beiging) Biological Co., Ltd. and sourced from a company's sole representatives in Nigeria and administered at 2 mL per 1 L of drinking water for 5 days. The AVS contain sodium hypochloride and vitamin C which was sourced locally from a representative in Jos and administered at 1 mL per litre of drinking water for 5 days. The GND contain Sal-ammoniac,

Acidium boricum, Sodium 2 hydroxy benzoate, D-glucitol, L-ascorbic acid, L-2-amino-4-(methylthio) butyric acid, bromhexine HCL, *Astragalus polysaccharide* and sourced from a company's sole representatives in Nigeria. It was administered at 2 mL per 1 L of drinking water for 5 days. A combination of GND and KHOS was administered at 2 mL for each of the drugs per 1 L in drinking water for 5 days.

Experimental challenge: At 5 weeks of age, five identified chicks were bled and serum obtained for determination of the level of IBD maternal antibody titre level. At the same age, chicks in all the test groups except negative control were inoculated with 0.04 mL of vvIBDV inoculum equivalent to $16\times10^{4.6}$ CID_{50} via conjunctival instillation[23].

Group A chickens served as negative control (NC), which were not inoculated and not medicated. Group B chickens served as positive control (PC) and were inoculated with vvIBDV but no treatment was instituted. Group C chickens were inoculated with vvIBDV at day 35 of age and treated with RS starting from 2 dpi when the inoculated birds began to show clinical sign of IBD. Group D chickens were inoculated with vvIBDV at day 35 of age and treated with KHOS starting from 2 dpi when the inoculated birds began to show clinical sign of disease. Group E chickens were inoculated with vvIBDV at day 35 of age and treated with AVS starting from 2 dpi when the inoculated birds began to show clinical sign of disease. Group F chickens were inoculated with vvIBDV at day 35 of age and treated with GND starting from 2 dpi when the inoculated birds began to show clinical sign of disease. Group G chickens were inoculated with vvIBDV at day 35 of age and treated with GND in combination with KHOS starting from 2 dpi when the inoculated birds began to show clinical sign of disease.

Sample collection

Collection of blood for determination of antibodies to infectious bursal disease and newcastle disease: About 2 mL of blood was collected from already identified birds at 35, 38, 42, 49 and 56 days of age via the wing vein for determination of antibody titre levels to IBD and Newcastle disease (ND). Blood was collected using 5 mL syringe and a 23 gauge needle. The blood collected was poured into a screw capped container and allowed to stand on a table at room temperature to allow for serum formation. After 24 h, serum was decanted into a bijou bottle and stored at -20°C in a freezer until used for the detection of antibody against IBDV and NDV.

Enzyme-linked immunosorbent assay: All sera collected were tested for antibodies against IBD using a standard commercial ELISA kit which was purchased from IDEXX Laboratories Incorporation, USA. The procedure for the test was carried out according to the manufacturer's instructions.

Haemagglutination inhibition test

Preparation of chicken red blood cells (C-RBCs) for HI test: A total of 4 mL of blood was collected aseptically from ND antibody-negative chicken in a disposable syringe containing 1 mL of Acid Citrate Dextrose (ACD) as anticoagulant. Cells were washed three times in Phosphate buffered saline (PBS) (pH 7.2) by centrifuging[24] at 47.2 g for 5 min. One percent RBC (packed cell V/V) suspension was prepared by adding 99 mL of phosphate buffered saline (PBS) to 1 mL of washed RBC.

Determination of titre of newcastle disease antigen using haemagglutination test: Haemagglutination (HA) test was carried out according to the method described by OIE[23]. The HA titre was the highest dilution that caused agglutination of the RBCs. The titration was read to the highest dilution giving complete HA (no streaming of RBCs). This represents 1 HA Unit (HAU). The titre of the antigen was determined as: 1:256, i.e., 8.0 $\log_2$ or 4HA units and this were used in the haemagglutination inhibition test.

Determination of newcastle disease virus antibody levels using the haemagglutination inhibition test: The HI test was carried out as described by OIE[23]. The HI titre was considered to be the highest dilution of serum causing complete inhibition of 4 units of virus (4 HAU). The HI titre of each serum sample was determined and expressed in $\log_2$.

Data analysis: Using Blankfard and Silk software, data obtained from ELISA mean optical density values were expressed as means (±standard deviation). The ELISA antibody titre levels were reduced to means and standard deviation. They were further subjected to one way analysis of variance (ANOVA) followed by tukeys *post-hoc* test for multiple comparison. The HI titres obtained were expressed in $\log_2$. Values of $p<0.05$ were considered significant using Statistical Package for Social Science (SPSS) version 20 for windows.

RESULTS

Humoral immune response of birds challenged with vvIBDV following administration of some complementary and alternative therapies: None of the chicks in all the groups had precipitin antibodies before challenge at day 35 (0 dpi)

and 3 dpi. Precipitin antibodies were also not detected in chicks in group NC, PC and RS at 3 and 7 dpi. However, at 7 dpi, 100, 80 and 20% of the chicks in groups AVS, GND and GND+KHOS, respectively had precipitin antibodies. They were detected in chicks in groups GND (100%) and GND+KHOS (40%) at 14 dpi. By 21 dpi, precipitin antibodies were detected in chicks in groups RS (50%), KHOS (100%), AVS (100%), GND (100%) and GND+KHOS (100%) (Table 1).

There was no significant difference observed in the mean ELISA IBD antibody titre between chicks of groups NC and PC throughout the study period. However, a significant difference was observed in the mean ELISA antibody titre of chicks in group RS (368.60±132.82) and KHOS (337.80±8 6.93) when compared with AVS (166.60±57.26), GND (166.40±23.04) and KHOS +GND (155.20±43.36) at day 0 pi (pre-infection). No significant difference was observed in the mean ELISA IBD antibody titre of all the groups at day 3 PI. By day 7 PI, significant increase was observed in the mean ELISA IBD antibody titre of chicks in groups RS (987.50±604.82), GND (1,331.00±339.83) and GND+KHOS (1,360.00±126.23). Again, significant increase was observed in the mean ELISA IBD antibody titre of chicks in groups GND (2,624.60±852.06, 2,326.00±1050.08) and GND+KHOS (2,200.00±1571.39, 1,110.60±41.99) and between groups RS (1,435.50±520.62, 1,922.75±1044.90) and GND (2,624.60±852.06, 2,326.00±1050.08) at day 14 and 21 PI (Table 2).

Humoral immune response to ND La sota vaccine of birds challenged with vvIBDV following administration of some complementary and alternative therapies: The results of the humoral immune response of ND La Sota vaccination after challenge with vvIBDV showed an increase in the mean HI antibody titre of chicks in the KHOS, AVS, GND and KHOS+GND groups at 7 days post vaccination (dpv) with ND La Sota vaccine. While the mean HI antibody titre was maintained in the KHOS and AVS groups at 14 dpv, it was however, lower in the GND and KHOS+GND groups at 14 dpv (Fig. 1).

DISCUSSION

The absence of precipitin antibodies to IBD in all groups on 0 dpi (35 days of age) and 3 dpi is possibly due to low sensitivity of AGPT[25]. However, low antibody titre against IBDV was detected at 0 and 3 dpi using ELISA. As the chicks were not vaccinated against IBDV, they became susceptible to the challenge with vvIBDV at 35 days of age. Though

Table 1: Distribution of precipitin antibodies in cockerels challenged with a vvIBDV and administered some complementary and alternative therapies

	Day before inoculation				Days post inoculation					
	0		3		7		14		21	
Groups	No. of tested	No. of positive (%)	No. of tested	No. of positive (%)	No. of tested	No. of positive (%)	No. of tested	No. of positive (%)	No. of tested	No. of positive (%)
NC	5	0	5	0	5	0	5	0	5	0
PC	5	0	2	0	1	0	1	0	1	0
RS	5	0	4	0	4	0	4	0	4	2 (50)
KHOS	5	0	1	0	1	0	1	0	1	1 (100)
AVS	5	0	3	0	2	5 (100)	2	0	2	2 (100)
GND	5	0	5	0	5	4 (80)	5	5 (100)	5	5 (100)
KHOS +GND	5	0	5	0	5	1 (20)	5	2 (40)	5	5 (100)

NC: Negative control, PC: Positive control, RS: Royal solution, KHOS: Kings herbs oral solution, AVS: Anti-viral solution, GND: Gumbo ND, KHOS+GND: Kings herbs oral solution+Gumbo ND

Table 2: Mean ELISA antibody titre levels of cockerels challenged with a vvIBDV and administrated some complementary and alternative therapies

	Day of inoculation		Days post inoculation (age in days)		
Groups	(35) 0	(38) 3	(42) 7	(49) 14	(56) 21
NC	112.40±69.97^{a}	404.00±447.67^{a}	243.80±232.69^{a}	161.00±158.92^{a}	94.60±49.91^{a}
PC	158.20±106.60^{a}	170.00±9.13^{a}	164.00±0.00^{a}	1,008.00±0.00^{a}	662.00±0.00^{a}
RS	368.60±132.82^{b}	326.75±20.52^{a}	987.50±604.82^{b}	1,435.50±520.62^{a}	1,922.75±1044.90^{b,a}
KHOS	337.80±86.93^{b}	168.25±54.58^{a}	156.00±0.00^{a}	689.00±0.00^{a}	118.00±0.00^{a}
AVS	166.60±57.26^{a}	238.00±90.11^{a}	156.67±104.50^{a}	1,994.67±833.69^{a}	1,480.67±107.97^{a}
GND	166.40±23.04^{a}	111.60±32.48^{a}	1,331.00±339.83^{b}	2,624.60±852.06^{b}	2,326.00±1050.08^{b,a}
KHOS+GND	155.20±43.36^{a}	90.60±37.77^{a}	1,360.00±126.23^{b}	2,200.00±1571.39^{b}	1,110.60±41.99^{a}

Means±standard deviation with different superscripts alphabets^{a,b}, on same column differ significantly with the control ($p<0.05$)

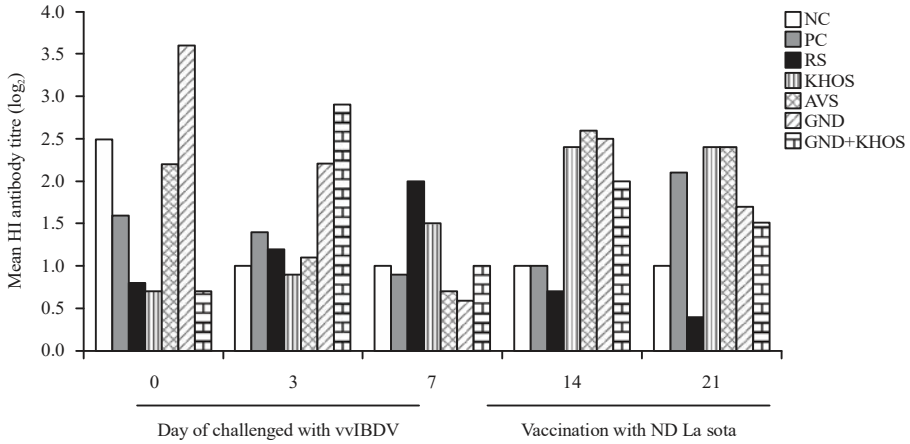

Fig. 1: Mean haemagglutination inhibition (Log_2) antibody response to ND La Sota vaccine in chicks challenged with vvIBDV at 35 days of age and vaccinated 7 dpi following administration of some complementary and alternative therapies
NC: Negative control, PC: Positive control, RS: Royal solution, KHOS: Kings-Herbs oral solution, AVS: Anti-viral solution, GND: Gumbo ND, GND+KHOS: Gumbo ND+kings herbs oral solution

IBDV ELISA antibodies (presumed to be MDAs) were detected in this study up to day 35 of age, in previous studies, they were absent in chicks at 14 days[26], 17 days[27], 21 days[28-30] and 28 days of age[31]. The significant difference in the IBD ELISA antibody titre level between chicks in group RS and KHOS at 35 days of age (0 dpi), may be due to differences in the rate of decay of MDAs. The rates of MDA decay are usually rapid in fast growing chicks than in chicks that grow slowly[31]. Group RS and KHOS probably had chicks that were fast growers than those of the other groups. However, by 14 dpi, the IBD ELISA antibody titres increased significantly in all challenged groups. This is because viral replication in the bursa of Fabricius is self-limiting and when the acute phase of IBD sub-sides, the birds usually recover from the pathogenic effect of the virus and the bursal follicles become repopulated with B cells and immune competence is re-established[32].

The significant increase in the IBD ELISA antibody titre levels observed in the KHOS and KHOS in combination with GND treated groups at 14 dpi suggest that the two drugs ameliorates the immunosuppressive effect of the vvIBDV, hence a better immune response was observed when compared to the other treated groups. Though the active ingredients (herbs) in KHOS were not stated by the manufacturers, GND contains among other ingredients, *Astragalus polysaccharide*, which have been reported to have antiviral and anti-inflammatory properties[33].

The result of the humoral immune response to ND La Sota vaccine showed that all the vvIBDV challenged groups had a HI antibody titre of between Log 2^5 and Log 2^9 from 7 dpv, with the exception of chicks in the PC group. Haemagglutination inhibition antibody titre levels of $\geq 2^3$[24,34,35] $\geq$Log 2^4[36-38] and $\geq 2^7$ have been considered as protective[39]. Although chickens that suffer from infectious bursal disease virus infection have been reported to response poorly to vaccination against other diseases[40-43] due to the damage the IBDV cause to the bursa of Fabricius, it was however observed, in this study, that, despite challenge with a vvIBDV, the chicks in the various treated groups (KHOS, AVS, GND and GND+KHOS) especially those treated with KHOS and AVS and vaccinated with ND La Sota vaccine were able to seroconvert with protective ND antibody titre levels from 7 dpv. This higher HI titre observed could probably either be as a result of the response of the cell mediated immunity (though not measured in this study) which has been reported to play an important role in the development of protection following vaccination against NDV[44-48] or it could be attributed to some of the active ingredients such as boric acid and *Astragalus polysaccharides* that is contained in some of the drugs. While boric acid has been reported to have some antioxidant properties, Astragalus essentially regulates the body's immune responses[49] and polysaccharides are known to have antimicrobial, antiviral and anti-inflammatory capabilities, among other health benefits[33] and this may imply that some of the drugs used in this study especially GND, RS and AVS were able to ameliorates the immune suppressive effect of vvIBDV on the humoral immune response to ND La Sota vaccine.

CONCLUSION AND RECOMMENDATIONS

The GND, RS and AVS had positive effects on the humoral immune response of cockerels challenged with vvIBDV. The

KHOS and AVS ameliorated the negative effects of vvIBDV on humoral immune response to ND La Sota vaccine following challenge.

Complementary and alternative drugs such as GND, RS, AVS and KHOS evaluated in this study can be used as immune modulators. Farmers, veterinarians and other animal health workers are advised to adhere to routine vaccination against IBD and strict bio-security.

SIGNIFICANCE STATEMENT

This study discovered the efficacy of GND, RS and AVS on humoral immune response of cockerels challenged with vvIBDV and also shows that KHOS and AVS ameliorates the negative effect of vvIBDV on humoral immune response to ND La Sota vaccine. These drugs can be beneficial to be used as immune modulators.

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