ISSN 2714-4410
J. Anim. Prod. Res. (2019) 31(2):202-208

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EVALUATING DIFFERENT ROUTE(S) OF ADMINISTERING BURSA B2K VACCINE IN THE CONTROL OF INFECTIOUS BURSAL DISEASE OUTBREAKS

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ABSTRACT

Out breaks of infectious bursal disease (IBD) despite vaccinations, necessitated this study. 150 day old cockerels were used for this study. The cockerels were divided into five groups of 30 chicks each (based on the routes) and vaccinated at day 7 and 17. Group A, (oral and intra ocular,) B (Oral and intra muscular routes) C (oral and oral routes). D (positive) control and E (negative control). Blood sample was collected pre and post vaccinations at day 0, 7, 14, 17, and 31 days. Sera were analysed using enzyme-linked immunosorbent assay technique, gross lesions were scored, morbidity and mortalities were expressed as percentages. The antibody titre pre vaccination at days 0 and 7 for group A, B, C, D and E were; 3413.2 ± 23, 1313.8 ± 28; 2261.9 ± 79, 920 ± 24; 3290.8 ± 0.2, 1184.9 ± 66; 1835.6 ± 84, 1565.2 ± 50; 1965.9 ± 45, 500.0 ± 43 respectively. while the antibody titre post vaccination at day 14 , 17 and 31 for group A, B, C, D and E were; 475.2 ± 82, 251.5 ± 33, 4041.3 ± 30; 1238.3 ± 36, 376.7 ± 20, 220.1 ±17; 318.9 ±31, 218.1 ± 19, 3904.7 ± 10; 219.2 ± 46, 185.1 ± 13, 3591 ± 43; 97.7 ± 90, 47.7 ± 71, 4391.1 ±74 respectively. The morbidity and mortality were, 0% for group D while 100% and 30% group E. Lesion on the thigh muscle was moderately severe for group B and E, while *bursa* of *fabricius* was moderately severe for group D, kidney was severe in group E. Lesions were mild on second challenge. Hence, it can be concluded that intra ocular and conventional oral route was the best route of IBD vaccine administration.

Keywords: IBD, bursa B2K vaccine, routes of administration, cockerels, Kaduna metropolis

INTRODUCTION

Infectious bursa disease is a highly contagious disease of young chickens caused by Infectious bursa disease Virus characterized by immune suppression and mortality generally at 3 to 6 weeks of age. (Caston, 2008). It is commercially important to poultry industry worldwide due to increased susceptibility to other disease and negative interference with effective vaccination. Although turkeys, ducks, guinea fowl and ostriches may be affected, clinical disease occur solely in chickens. (Lim *et al.*, 2003). This can cause secondary problems due to the effect of the virus on the *bursa* of *fabricius*. IBD virus causes lymphoid depletion of the *bursa* and it occurs in the first 2 weeks of life, significant depression of the hormonal antibody response may result. (Wang *et al.*, 2009). Antibodies are sometimes found in other avian species, but no signs of infection are seen. Gumboro disease occurs in the poultry rich southern States of Nigeria with 90 - 140 outbreak involving 0.5 million chicks annually (Hassan, 2014). The disease causes chick mortality up to 30% in addition to poor feed conversion and immune suppression against vaccination with Newcastle disease, fowl pox, fowl typhoid. Vaccination with mesogenic komorov strain of Newcastle disease virus is known to cause serious recrudescence because of failure of primary vaccination undertaken at a day-old age with a tentogenic vaccine in

presence of maternal antibody and inter-current infection with Gumboro disease. The disease is commonly observed in broilers between the third and sixth week of growth (Muller *et al*., 2012) and in egg producing birds (pullets). In susceptible flocks, the disease emerges abruptly with high morbidity (up to 100%) and with average mortality of 50- 60%. The disease causes large economic losses in intensive poultry production (Van der Sluis, 1999). The emergence of highly virulent types of gumboro disease became a significant problem for European poultry industry and cause great economic losses (Van der Berg, 2000). Some virus types caused flock mortality of over 90%. Today, the disease is widespread all over the world which gives it a great socio-economic importance. In addition to the direct losses from mortality, the disease also causes indirect losses by immune-suppression and subsequent outbursts of other diseases such as coccidiosis, salmonellosis, colibacillosis, Marek's disease (Birghan *et al.,* 2000).

There is an increase in production of commercial chicken in Nigeria and serious loss due to outbreak of gumboro disease (Hassan, 2014). Despite vaccination against infectious bursa disease through oral route, farmers are still face up with problem of vaccination failure. This has discouraged farmers which leads to serious economic loses and has affected the protein intake of humans (Hassan, 2014). Number of affected birds in the flock is variable and can approach 100% mortality. (Caston, 2008). There is the need to find the most effective route of gumboro vaccination.

MATERIALS AND METHODS

The experiment was conducted at the livestock farm of College of Agriculture and Animal Science Mando, Kaduna, located in the Sourthern Guinea Savannah Zone of Nigeria on latitude 11.3^0N and Longitude 60.10^0 E. The mean annual temperature was 34^oC with the hottest month, April at 40^oC and the coldest period was 13.2^oC between July and August. Rainfall lasted for 150 to 200 days and dry season occurred from late October to early April (GPS, 2015).

The pen was swept, cleaned with detergent and fumigated with 15% formaldehyde, glutarldehyde and didecyl dimethyl Ammonium chloride. Seven days prior to arrival of chicks, wood shaving were used as bedding and were replaced with new ones on weekly basis. Adequate biosecurity measures were then taken to reduce external influence. The chicks were sourced from a reputable hatchery in Ilorin Kwara state of Nigeria. The parent stocks are brown which were vaccinated every six month with IBDVK (Gumboro oil base inactivated vaccine).

150 day old cockerels were randomly distributed into five experimental groups consisting of 30 birds each. Group A were vaccinated at 7 day through oral and at day 17 intraocular route, group B were vaccinated at 7 day through oral route and at day 17 intramuscular route, group C were vaccinated through oral route at 7 day and oral route at day 17, group D served as positive control and were not vaccinated and were not challenged. Group E serves as negative control; they were not vaccinated, but were challenged. One milliliter of blood was collected from the heart of each chick at day old which served as bench mark using an insulin syringe. The blood was emptied into a clean dry test tube and was left to clear. The serum sample separated in a clean test tube which was labeled according to the group and stored in a freezer till used.

CHALLENGE PROCEDURE

The virus used to challenge the birds was obtained from faculty of Veterinary Medicine, Ahmadu Bello University Teaching Hospital. The experimental pen use to challenge was in different location to avoid infection. 10 birds were randomly selected from the various groups. They were inoculated with 0.2mls of

the virus using 5mls syringe, a drop was administered into the eye and mouth respectively to all the groups. Three days after the challenge, clinical signs, mortality and morbidity rates, were observed and recorded for each group. The challenge was done twice, the first was on 21st day (3 weeks) three days after, and postmortem was performed on each group using 3 birds. Lesions observed are: the bursa of fabricious, thigh, breast muscle, proventiculus, spleen and kidney. Second challenge was done on 31st day (4 weeks) and gross lesion was observed. BURSA B2K invasive intermediate strain, manufactured by INDOVAX private limited company siswala hissar, Haryana India. Batch number: GP115 were used for the research. Gumboro kit solid phase antigen was used to detect specific anti body against IBD.

PROTOCOL ON ANTIBODY CAPTURE ELISA (THE IDEXX MODEL)

1ul of serum sample was added to 500ul of diluents in dilution tubes and was mixed thoroughly. Out of the mixture, 100ul was dispensed into antigen-coated wells of the mixture plate. Controls of negative and positive was dispense at 100ul in their respective wells without prior dilution. It was incubated for 30 minutes at room temperature. The content of the well was discarded and washed with distilled water and phosphate buffer saline four times. 100ul of conjugate was added and incubated for 30 minutes at room temperature, the content was discarded and washed four times and 100ul of *tetramethlbenzidine* substrate was added and incubated at room temperature for 15 minutes to enhance colour development. 100ul of stop solution was added and read at 650nm. Finally, the result was recorded using on a prepared template using Idexx software.

RESULTS AND DISCUSSIONS

The mortality and morbidity rate from 21-24 day after first challenge is presented in Table 1. There was no mortality in all the groups except group E which had 30% mortality. Result also revealed that group A and B has the least morbidity and mortality rate compare to group C which has 80% morbidity and group E with 100% morbidity. However, there was no mortality and morbidity in group D.

Table 2: The gross lesions three days after first challenge and three days after second challenge. Three days following first challenge, the chick shows moderate haemorrhage on the breast muscle, inflammation and haemorrhage of bursa was moderate while thigh muscle was moderately severe and the kidney was mildly inflamed. Haemorrhage of thigh muscle and inflammation of kidney were moderate while haemorrhage of breast muscle was mild and the bursa was moderately severe. Also, haemorrhage of thigh muscle and breast muscle was mild while inflammation and haemorrhage of bursa was moderately severe and the kidney was normally inflamed. Haemorrhage of breast muscle and inflammation of bursa was moderate while haemorrhage of thigh muscle was moderately severe and the kidney was severely inflamed. However, haemorrhage of breast muscle, thigh muscle, inflammation of kidney and inflammation of bursa of fabricius are all mild following three days after second challenge.

Table 1: Mortality and morbidity rate from 21-24 day after first challenge

Group	Mortality	Percentage (%)	Morbidity	Percentage (%)
A	Nill	0	3/10	30
	Nill	0	Nill	0
	Nill	0	Nill	0
B	Nill	0	2/10	20
	Nill	0	3/10	30
	Nill	0	3/10	30
C	Nill	0	1/10	10
	Nill	0	2/10	20
	Nill	0	1/10	10
D	Nill	0	Nill	0
	Nill	0	Nill	0
	Nill	0	Nill	0
E	Nill	0	2/10	20
	Nill	0	8/10	80
	3/10	30%	Nill	

Table 2: Gross Lesions Three Days After First Challenge And Three Days After Second Challenge

Groups	Haem of breast muscle	Haem of thigh muscle	Inflammed and Haem bursa	Inflammed kidney
Gross lesion 3 days after first challenge				
A	++	++	+	++
B	++	+++	++	+
C	+	++	+++	++
D	+	+	+++	
E	++	+++	++	++++
Gross lesions 3 days after second challenge				
A	+	+	+	+
B	+	+	+	+
C	+	+	+	+
D	+	+	+	+
E	+	+	+	+

Normal (0), Mild (+), Moderate (++), Moderately Severe (+++) And Severe (++++)

From the result of Elisa test (Table 3), group **A** at day 1 shows high value of anti-body titre (3413.2 ± 31) but later drop to 1313.8±92 at day 7 pre-vaccination. After vaccination, at day14 the anti-body titre further drop because the vaccine has swiped away the maternal anti body, and at day 17 before vaccination, the antibody decreased to 251.5 ± 83, the after vaccination at day31. Although, the maternal anti-body shows a remarkable improvement with 4041.3±73. In group B, the benchmark was 2261.9 ± 07, but later drops to 920.9±42 at day 7. The anti- body increase to 1238.3±93 at day 14 and decrease at day 17 to 376.7±82, after vaccination through intra muscular route, the anti- body titre further drops to 220.1 ± 31 at day 31. From the result of group C, the benchmark was 3290±8102, its drops to 1184.9±06 at day 7, it further dropped to 318.9±43 at day 14 and 218.1 ± 90 at day 17. There was an improvement at day 31 which was 3904.7 ± 71 at day 31. Group D which serves as the positive control was not vaccinated and challenged. The benchmark for the group was 1835.6 ±58 which drops to 1565.2 ± 45 at day 7 and 219.2 ± 04 at day 14, which further drops to185 ± 1315 at day 17 and had a remarkable improvement of 3591.7 ± 94 at day 31. From group E, the benchmark was 1965.9 ± 04, it drops to 500.0 ± 36 at day 7 and 97.7 ± 19 at day 14. There was a remarkable improvement of 4391.1 ± 57 at day 31.

Table 3: Maternally derived anti-body titre level pre and post vaccination

Day (s)	Group	Group B	Group C	Group D	Group E
1	3413.2±23	2261.9±79	3290.8±02	1835.6±84	1965.9±45
7	1313.8±28	920.9±24	1184.9±66	1565.2±50	500.0±43
14	475.2±82	1238.3±36	318.9±31	219.2±46	97.7±90
17	251.5±33	376.7±20	218.1±19	185.1±13	47.7±71
31	4041.3±30	220.1±17	3904.7±10	3591.±43	4391.1±74

DISCUSSION

The maternally derived antibodies (MDAs) at day 1 was high for all the groups, this has shown that the parent stocks were well vaccinated and have passively transfer the required immunity to their off spring, and therefore chicks will be protected at young age and this agree with the work of Hassan *et al.*, (2018). At day 7, the MDAs decreased linearly in all the groups this is also in line with the work of Hassan (2014) and Hassan *et al*., (2018). Post vaccination titre was sero negative in all the 3 groups at 14 and 17 days, because MDAs had neutrilised the vaccinal viruses, similar to the work of Kembi *et al*., (1995), but because of presences of precipitin antibodies in the birds in all the groups, upon vaccination, the antibody titres increased at 31 days due to sero conversion except group B oral / intramuscular routes that remain sero negative, IBD vaccines are more potent through the oral route (Barbiker and Tawge, (2008) and Hassan *et al*., (2016).

The mortality was zero for all the groups except the negative control groups which shows that the routes were good and the vaccine was potent. This agrees with the work of El-Mahdy *et al*., (2013). The morbidly was highest for group vaccinated through oral and intramuscular routes and the negative, similar to the work of (Babiker and Tawfeq, 2008). Gross lesions was milder for group A (oral and intraocular routes) and C (Oral and Oral routes), similar to the work of Hassan *et al*.,

(2016). Therefore, the best routes of infectious bursal disease vaccine administration is oral and intraocular route at 7, 14 and 21 days , or oral and oral routes at 7, 14 and 21 days. Group A gave the highest antibody titre, follow by group C and D, it can be deduce that oral/intra ocular route is the best route of Gumboro vaccine administration follow by oral/ oral route because of the high anti-body titre recorded despite the fact that there were challenged. In group D, the reason why it has a remarkable anti-body titre was due to cross protection from the vaccinated chick to group D which serves as positive control.

However, group B gave a very poor anti-body titre because intra- muscular route of Gumboro vaccine administration didn't give the required protection needed. The results of the antibody titre, agrees with the reports of El-Mahdy *et al*., (2013) and Babiker and Tawfeq, (2008). Group E which serves as negative control were not vaccinated and were challenged. The antibody keeps dropping with high record of mortality and morbidity, after recovery, the antibody titre shows a solid immunity which is expected of gumboro disease after recovery thereby giving a permanent immunity against the virus. It further explains that gumboro disease cannot occur twice in the life-span of bird (Hassan *et al*., 2016)

CONCLUSION

From the result obtained, it can be concluded that intra-ocular and conventional oral route of gumboro vaccine administration might be the best and reliable route of the vaccine administration. It is recommended that farmers should administer either intra-ocular or oral route in cockerel using BURSA B2K invasive intermediate strain.

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