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IMMUNE RESPONSE OF BROILER CHICKS TO LOCAL IBD VACCINE USING DIFFERENT ROUTES OF ADMINISTRATION.

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Four groups of ten-day-old broilers each were vaccinated at 7 and 14 days post hatch (PH) against infectious bursal disease IBD, using the local IBD vaccine (VOM & Nigeria). The vaccine was administered using different routes; intramuscular, subcutaneous and oral. 10- day old broiler was randomly sampled from a group of 40 for the presence of IBD antibodies using qualitative and quantitative agar gel precipitation test (AGPT). The maternal antibodies in the chicks were variable, low and waned completely 12 days post hatch (PH). The group that received subcutaneous route of vaccination (sc/sc) gave consistently higher antibody titers than the oral and intramuscular route in post vaccination days. All the groups including the unvaccinated control were challenged 16 days post vaccination with a field strain. All the routes were appreciably protective (90%) against the field strain with the unvaccinated control group recording 30% mortality. However, subcutaneous route had a complete protection (100%). The gross and microscopic lesions seen in the study were consistent with IBDV. This study has shown that the maternal antibodies in the broiler chicks were low, variable and waned completely by 12 days post hatch. The subcutaneous route of vaccination achieved better response and protection when given at day 7 and day 14-post hatch.

INTRODUCTION

Cosgrove first described infectious bursal disease IBD of chickens (1) and it is caused by a birnavirus (2). Although, efforts on its control have been unrelenting, "SD infection has been reported in vaccinated flocks (3), maternally immune chicks (4) and adult flock of 20 weeks (5). There is also the emergence of very virulent strain of IBDV (6) that has been found to be highly pathogenic. Hence it is evident that many factors are militating against success fill vaccination with IRD live vaccines in the field apart from poor and improper handling of the vaccines.

Most of the IBD vaccines are administered intraocularly or orally except for the Vero cells adapted "SD vaccine that was given parenterally (7). Komine et al (8) also showed the efficacy of the subcutaneous route of administration in specific pathogen free (SPF) young chicken and those with maternally derived antibodies. In this report we studied the effect of routes of vaccine administration on the immune response of broilers chicks to our local "SD vaccine.

MATERIALS AND METHODS

(a) Chicks.

A flock of 50-day-old avian breed of broiler chicks was obtained from a local hatchery. The breeders were vaccinated against IBD and boosted at 16 week of age with an IBD oil emulsion vaccine. They were raised from day old until termination of the experiment at the poultry experimental unit of department of veterinary medicine University of Ibadan

(b) Vaccines

The local IBD vaccine produced at the Nigerian Veterinary Research Institute verb (NVRI) Vom was used. The vaccine was constituted with sterile physiological saline by Dissolving a vial in 4Omls and 0.2mls given using different route of administration (oral subcutaneous and intrarnuscular routes).

(c) Field Virus

A 20% suspension in phosphate buffered saline (PBS) of bursae from birds that died in a recent confirmed outbreak of IBD was used. The homogenate was centrifuged at 5OOpgm for 10 minutes and the supernatant harvested and stored at 4°C after the addition of procaine penicillin to prevent bacterial growth. This was tested using Agar gel precipitation test as described by Durojaiye et al. (9).

Experiments Two experiments were performed.

(a) Qualification of material antibody (MA) levels in chicks: -

Sera were collected through the jugular vein from 40 birds randomly selected at day 1 3, 7; and 12 post hatch, 10 chicks were sampled on each occasion and monitored for the presence of IBD antibodies by qualitative and quantitative AGPT after inactivation at 56%.

(b) Vaccination/challenge

40 chicks were divided into 4 groups of 10 each and placed in separate isolation units. Vaccination was carried out on days 7 and 14 using different routes of application as on Table I. Sixteen days after the last vaccination (30 days post hatch, PH) all the birds were challenged using the homogenate of infected bursa of birds from the recently confirmed Field outbreak. The intraocular route was used as described by Adene et al (10). All birds in each of the groups were observed for clinical signs and mortality rates recorded alongside with the presented signs. Chicks that died during the course of the experiment were

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necropsied and tissues were fixed in 10% buffered formalin and processed routinely for histopathology. Section 5u thick were cut, stained with haematoxylin and eosin (H & E) and examined under the light microscope. The various groups were bled weekly for 7 weeks post hatch. Serum samples were collected and tested for the presence of HID antibodies using qualitative and quantitative AGPT as described by Cullen and Wyeth (11)

Statistical Analysis

The data were analyzed by standard Anova procedure and Duncan's multiple - range a Test (a = 0.05)

RESULTS

Antibody Profiles

Table 2 shows that at day *1, 5* out of 10 chicks sample showed the presence of MA with antibody titer varying from 4 to 32 GMT (Geometrical mean titer) of 8. At day 3, 50% that is, S out of 10 chicks were positive for M& The antibody flue varied from 2 4 Showing decay in the MA3 days post hatch and the GMT was 2.

Seven days post bath, there was a further decay of the MA. Only 10% of the chicks had IBD antibody titer. The GMT had reduced to 0.5 No maternal antibodies were detected on day12 post hatch.

The antibody response of Group B (SC/SC) was higher than those of other groups at the post vaccination days, while ant of group A (oral/oral) was same as that of Group C (IM/IM) route 14 days post vaccination. After challenge, there was a steady increase in antibody response in Group B (SC/SC) while Group A (Oral/Oral and G (IM/IM) showed a decrease 12 days post challenge.

At day l9 post challenge, the values 52 and 59.7 for the subcutaneous and intramuscular routes were significant (P<0.05).

(1) **Clinical Signs**

Morbidity was highest in the control group After the challenge infection and clinical signs were observed only in group A, B and C shows in tables 3 and 4. Table 5 shows that the Group that received subcutaneous route of vaccination at day 7 and 14, Group B had 100% protection while groups that received only oral and intramuscular routes of vaccination had 90% protection with 10% mortality. The control group lost 30% of its chickens. At post mortem examination the carcasses were well-fleshed and showed eccymotic haemorrhages on the leg muscle and the proventriculus - gizzard junction. The lung' were slightly congested while the kidney in the dehydrated carcass was slightly swollen. The bursa was markedly swollen, haemorrhagic and contained some caseous material on incision. Proventicular haemorrhagic and petechial haemorrhages on the duodenum and part of the jejunum were observed in the control birds.

Insert Table 5 and figure 1

The histopathological findings included muscular and proventricular haemorrhages with marked amounts of protein casts in the renal tubules. Although there were no gross hepatic lesions, there was fatty degeneration of hepatocytes with lymphocytic infiltration around some portal veins. The splenic lesions were that of marked heterophilic infiltration with some follicles showing lymphocytic depletion. The bursa showed oedema in the interfollicular spaces with most follicles showing lymphatic depletion and necrosis.

COMPARISON OF ORAL ROUTES TO PARENTERAL ROUTES OF ADMINISTRATION

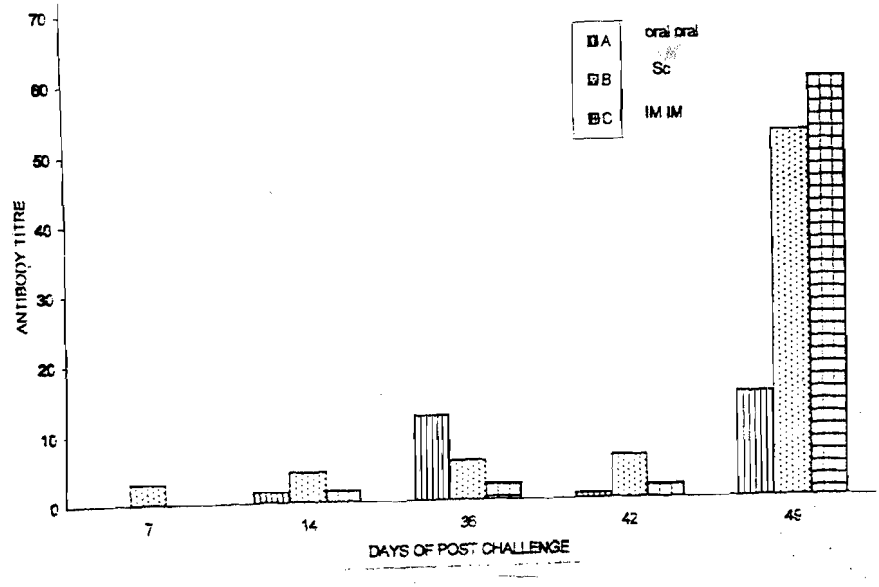

Table 1: **Vaccination groups and routes of administration at different ages.**

Group	Day 7	Day 14
A	Oral	Oral
B	S/C	S/C (subcutaneous)
C	I/M	I/M intramuscular)
D (control)	Distil water	Distil water

Table 2: **Material Antibody Levels of Chicks**

Group	1	3	7	12
A	0	0	2	0
B	4	0	0	0
C	0	0	0	0
D	0	0	0	0
E	0	0	0	0
F	8	4	0	0
G	0	2	0	0
H	16	2	0	0
I	32	4	0	0
J	16	4	0	0
GMT	8.0	2.0	0.5	0
Percentage Positivity	50	50	10	0

Table 3: **Morbidity**

Group	Route	No. In group	No. In group	No. In group
A	Oral/Oral	10	1	10
B	SC/SC	10	None	0
C	I/M I/M	10	1	10
D	Control	10	5	50

Table 4; **Clinical Signs**

Clinical signs	A	C	D
	Oral Oral	I/M I/M	Control
Ruffled feathers	1/10	1/10	5/10
Depression	1/10	1/10	5/10
Greenish diarrhoen	0/10	0/10	3/10
Weight loss or Emaciation	1/10	1/10	3/10
Prostration	0/10	0/10	3/10
Death	1/10	1/10	3/10

Table 5: **Mortality and level of protection against clinical IBD**

Group	Route	Days PC	No Dead	%	Protection %
A	Oral/Oral	2	1	10	10
B	SC/SC	0	0	0	0
C	IM/IM	3	1	10	10
D	Control	6-7	3	30	30

DISCUSSION:

The results of the MA assay showed that antibodies in the broiler chicks waned completely 12 days post hatch. This observation is in arrangement with the report of HOMER et al. (12). This observation is interesting especially when the parent stocks from which the chicks were derived received oil emulsion boosters at 18th week of age before the onset of lay. However, other reports indicated that the MA disappeared at 3-5 weeks post hatch (13), while some workers reported 4 days (14) and 7days post hatch (10).

The GMT at hatch however, was low while individual antibody titer varied between 2-32. The variation is similar to that earlier reported by Winterfield et al, (15), and different front that reported by Vielitz (16) who stated that high and uniform level of MA were observed when oil based vaccines were used as booster in breeders. The difference in MA in chicks showed the variation in seroconversion in the parent stock since it has been reported by Lucio and Hitchner (17), that there was a direct correlation between the antibody titre of the dam and the MA of the chick.

In this Study, only half of the chicks had MA on day 1 PH and the levels were low. Again, this observation agrees with that of Adene et al; (10) who reported low MA levels in chicks horn some of the major commercial hatcheries in Nigeria. This has been attributed to the fact that the parent stocks are rarely given consistent booster doses. Thus, early vaccination or if possible double vaccination in the first 21 days post hatch of such chicks was recommended (10). Precipitating antibodies can be detected early, 14- 25 days (post-*vaccination as* reported by some workers (18). In this experiment, it was detected within 7-14 days post vaccination. This is earlier than reported, and May be associated with the vaccination, which was done twice, at days 7 and 14-post hatch as against single vaccination reported by all the workers mentioned above. In this case, the first vaccination served as primer to the antibody producing cells while the other served as a booster especially when there was no Interference or mopping up of vaccine virus by MA as previously reported by WOOD et al (4), Winterfield and Thatchkel, (19)

The NVRI vaccine has been reported to induce antibody levels that withstood challenge infections when given orally and intramuscularly (20) The results obtained in this study where various vaccination (oral, subcutaneous and intramuscular) routes were used showed that the double subcutaneous route induced more antibodies response than the other route. The parental routes also induce more response than the oral routes at day 49-post hatch. This observation agrees with the reports of some previous workers (8,21).

The antibody response to live vaccines has been reported to correlate with the degree of protection (22). In this study however, the subcutaneous route was found to be more consistent in antibody response and also more protective than the oral and intramuscular routes. The enhanced antibody response observed in chicks vaccinated by the parental route may be associated with the fact that the antibody producing cells were exposed to the vaccine virus earlier than those of chicks vaccinated using the oral routes (23). In the same vein, the enhanced and consistent response in antibody production by the chicks that received subcutaneous vaccination could be explained by the fact that the vaccine virus was slowly release into the blood stream hence the irnmunopotentiating effect (23).

There is direct correlation between the antibody titer and resistance to IBDV challenge (17). The low morbidity rate found in this study (10 30%) when compared to 44-100% earlier reported by Onunkwo, (25) may be associated with the type of bird used and the presence of antibody in the birds (26). Broilers have been found to have more natural resistance to IBD than other types of chickens (19).

The clinical signs seen which included depression ruffled feathers, greenish diarrhoea, weight loss, prostration and death were more pronounced in the control group than the other groups. Similar clinical sign have been described by earlier workers (25). However, vent pecking and trembling reported by Cosgrove (1) and Hitchner (27) were not observed in this study and other studies so far in Nigeria (28). The mortalities reported in this study were low in vaccinated flocks (10%). This observation is also in agreement with the report of Awolaja and Adene (29). In the control chickens, a mortality of 30% was not too different from the 43% previously reported in exotic chicken (3O). In IBD Mortality do occur on day 3 PI (27),

however in group A which was given oral/oral vaccination, the mortality occurred 2 days; PI corresponding with the timing of marked lymphocytic destruction reported by Resenberger (31). A bird from this group (oral/oral) also showed acute splenitis. Mortality occurred on day 3 in Group C (I/M I/M) and stopped on day 7 in the control group. These are in agreement with the incubation periods and mortality pattern of the disease (32).

However, to further enhance the find of the study, there is need to compare the results obtained through quantitative AGPT and ELISA, which has been known to be sensitive (33). Also there is a need to determine the titer of the vaccine and the challenge virus to check for the reason behind the low titers obtained during post vaccination days. Other works are being carried out in this area.

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FORTHCOMING CONFERENCES

THEME: HIV/AIDS and Sexually Transmitted Diseases

1. Association of Pathologists of Nigeria (ASSOPON) Annual Conference and General Meeting Jos University Teaching Hospital Jos, Plateau State, Nigeria. 24th to 26th July, 2002.
2. National Postgraduate Medical College of Nigeria All-fellows Congress Aminu Kano Teaching Hospital, kano, Nigeria. August 13th - 15th, 2002.
3. National Association of Resident Doctors (NARD) of Nigeria Annual Conference and General Meeting, University College Hospital, Ibadan, Nigeria. September, 2002.