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With 5 tables in the text

Immunosuppressive Effect of Infectious Bursal Disease (IBD) Vaccine Strains in use in The Sudan

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Summary

The immunosuppression exerted by administration of three IBD live vaccines was studied in two and three weeks old chicks. Strain E caused marked bursal regression at both ages; strain G did the same in two-weeks-old chicks only, while strain B had no bursal involvement. The lowest HI antibodies titre to ND vaccination was reported in two weeks old chicks when strain E was used, while the highest titre was obtained when strain B was used at the same age. S train G produced, however, a moderate response at both ages.

Introduction

Immunosuppression (IS) is a state of temporary or permanent general dysfunction of the immune response resulting from an insult to the immune system and leading to increased susceptibility to diseases. There are four criteria for evaluating IS (Dohm and Saif, 1984). Briefly, they are morphometric changes in the lymphoid tissues, changes in the immunoglobulins concentrations and complement level, changes in the functional activities of the immune system, interference with vaccination and/or exacerbation of other diseases. Evidences for incidence of two or more of these criteria should be provided to express the status of immunosuppression.

Infectious bursal disease (IBD) virus is a known immuno-suppressive agent as sub-clinically affected birds respond poorly to vaccination; they are much less resistant to field challenge and vulnerable to persistent respiratory diseases which are usually complicated by secondary bacterial infections (Sharma *et al*., 1989; Rosenberger, 1994; Rosenberger and Gelb, 1977). Transient IS caused by virulent, less pathogenic and/or biological variants of IBD virus was reported. Several factors that may explain the immunosuppressive effects of IBD virus have been suggested (Hirai *et al*., 1979; 1981). In general, any IBD vaccine strain should fulfill the required standards stated by Thornton (1976) regarding virulence and potency.

This work was designed to assess the passive immunocompromizing effect of three IBD vaccine strains which are in use in the Sudan. Two criteria were adopted; the morphometric effect expressed by bursa/body weight (B/Bwt) ratio and humoral immune response of birds vaccinated against Newcastle disease (ND).

Materials and Methods

Experimental chicks:

Two batches of two and three-week-old chicks, each consisting of 120 birds were used. They were obtained from a commercial hatchery as one-day-old chicks.

Vaccines:

Three IBD live vaccine strains; B, G (which are intermediate strains) and E. (intermediate-plus or hot strain) were used in the experiments. They were supplied as lyophilized vaccines. The ND vaccine used was Komarov strain, which is locally produced at the Central Veterinary Research Laboratories, Khartoum, Sudan.

Sera:

Serum samples were taken from birds at one-day-old and at weekly intervals till the end of the experiments. They were tested to detect maternal antibodies and response to ND vaccination.

Vaccination:

1- Administration of IBD vaccines:

Two vials from each IBD vaccine were reconstituted, pooled and used as recommended by the manufacturer. They were administered through drinking water. Strain B was used in a dose of 10^4 $CCID_{50}$, G in 10^3 $CCID_{50}$ and E in 10^2 $CCID_{50}$. From each batch

of chicks, 30 birds were used per a vaccinal strain (B, E and G groups) whereas 20 birds were left as unvaccinated control group.

2- Vaccination against ND:

Two vials of ND vaccine were pooled after reconstitution and one dose per bird was administered intranasally to 10 birds from each group. ND vaccination was carried out one week after IBD vaccine administration.

Sampling of bursae:

At the 3rd and the 9th day following IBD vaccine administration five birds from each group were killed and immediately weighed before their bursae were carefully removed and weighed separately.

Haemagglutination inhibition test:

Titration of antibodies to ND vaccine was carried by the beta procedure of haemagglutination inhibition (HI) test. Four HA units (at the dilution 2^{-6}) of ND virus Komarov strain were used as an antigen. The test was carried out in a microtitre plate using 0.025 ml of reagents, i.e 0.025 ml saline diluent, 0.025 ml serum sample, 0.025 ml antigen and 0.025 ml of 1% washed chicken RBCs (OIE, 1996).

Results

Maternal antibodies against ND virus showed mean $\log_2$ titres of 3.98 at day one, 3.05 at day seven, 2.82 at day 14 and 2.13 at day 21 of age and geometric mean titres (GMT) of 16.0, 8.6, 7.0 and 4.4., respectively (Table 1).

Table 1: Depletion of maternal antibodies through the first 21 days of life in experimental chicks.

Age (days)	No. samples exam.	Positive samples per $\log_2$ dilution of serum									Mean $\log_2$	GMT*
		2^0	2^{-1}	2^{-2}	2^{-3}	2^{-4}	2^{-5}	2^{-6}	2^{-7}	2^{-8}		
1	60	0	0	5	18	21	8	5	3	0	3.98	16.0
7	60	0	2	10	20	16	7	3	2	0	3.05	8.6
14	60	0	5	22	18	10	4	1	0	0	2.82	7.0
21	30	2	7	12	5	2	2	0	0	0	2.13	4.4

* Geometric Mean Titre

Bursal weight indices:

The mean body weights, bursal weights and B/Bwt ratio are shown in Tables 2 and 3. The mean B/Bwt ratios of two-weeks-old chicks at day 3 post vaccination (p.v.) with IBDV were 2.85, 2.60, 3.00

and 2.94 for B, G, E and control groups, respectively; while they were 3.29, 1.90, 2.40 and 3.20 at day 9 p.v. (Table 2).

In elder birds (3 - week – old chicks) B/Bwt ratios were 2.82, 2.36, 3.18 and 2.89 at day 3 p.v. and 4.02, 3.30, 1.99 and 3.85 at day 9 p.v. for the vaccine strains B, G, E and the control groups, respectively (Table 3).

Table 2: B/Bwt ratios following administration of IBD vaccine to two- week-old chicks.

Vaccine strain	Days post vaccination	Mean body wt (g)	Bursa wt(g)	B/Bwt ratios (x1000)
B	3	70.52	0.202	2.85
	9	99.82	0.330	3.29
G	3	70.30	0.84	2.60
	9	79.60	0.153	1.90
E	3	72.73	0.218	3.00
	9	88.33	0.217	2.40
Control	3	64.52	0.188	2.94
	9	104	0.338	3.20

Table 3: B/Bwt ratios following administration of IBD vaccine to three- week-old chicks.

Vaccine strain	Days post vaccination	Mean body wt (g)	Bursa wt(g)	B/Bwt ratios (x1000)
B	3	101.39	0.289	2.82
	9	157.75	0.637	4.02
G	3	89.10	0.215	2.36
	9	141.30	0.464	3.30
E	3	103.00	0.328	3.18
	9	137.82	0.273	1.99
Control	3	104.86	0.303	2.89
	9	135.99	0.523	3.85

Humoral response to ND vaccination:

The lowest post-vaccinal HI antibodies titre to ND vaccine was obtained from two-week-old chicks of group E (Mean $\log_2$ 4.01 and GMT range 7.0-42.2). Markedly higher titre was reported in the group vaccinated by B strain of three-week-old chicks (mean $\log_2$ 6.02; GMT

range 26.0-128.0). Tables 4 and 5 show detailed results. No clinical signs were observed during the period of observation and all birds appeared healthy.

Table 4: Mean HI antibody titres (log_2) to ND vaccine in two-week-old chicks vaccinated with IBD vaccines.

Vaccine strain	HI antibody titres (log_2) at days post ND vaccination						Mean log_2
	7	15	22	30	43	57	
B	2.9	4.8	5.8	6.2	5.8	5.0	5.08
G	2.6	4.0	6.0	6.0	5.8	4.6	4.80
E	3.2	4.8	5.0	4.5	3.8	2.8	4.01
control	3.0	4.5	6.0	6.5	6.6	5.8	5.40

Table 5: Mean HI antibody titres (log2) to ND vaccine in chicks vaccinated with IBD vaccines at three weeks old.

Vaccine strain	HI antibody titres (log_2) at days post ND vaccination						Mean log_2
	7	15	22	30	43	57	
B	4.7	5.5	6.8	7.0	6.8	5.3	6.02
G	3.3	5.3	6.4	6.6	6.3	5.0	5.48
E	3.3	4.8	5.6	5.6	5.9	4.8	5.00
control	3.4	5.7	6.0	6.7	5.9	6.6	5.70

Discussion

The immuno-compromising effect of IBD vaccines was reflected by the response to ND vaccination (evaluated by HI antibody titres) and supported by concurrent bursal indices.

These parameters fulfilled the rule that denotes status of IS (Dohn and Saif, 1984). The findings are in agreement with that of Mazariegos *et al*. (1990) who have reported varying degrees of virulence and immunosuppressive properties for commercially available intermediate vaccine strains. IS due to IBD virus depends on age of birds (Faragher, 1972; Hirai *et al*., 1979) and its magnitude varies according to the nature of vaccine administered, since some strains are

less immunosuppressive when inoculated at 21st day than at first day of life (Higashihara *et al*., 1991). Moreover, their results revealed obvious variations in the magnitude of immunosuppressive effects of vaccine strains. Our results showed that strain B was safe and potent when used during relatively early life (less than two weeks). Strain E was markedly immunosuppressive and produced no appreciable immune response up to the 14th day of age, while strain G gave moderate protection and safety.

No initial enlargement of bursae was detected at day 3 post-vaccination. By day 9, the B/Bwt ratios indicated that strain B had no effect at both ages of vaccination. Evident bursal regression was caused by strain G in younger chicks and by strain E in chicks of both ages despite appreciable antibodies elicited by the two strains in elder birds. This is in agreement with Ivanyi and Morris (1976) who found that bursal but not peripheral B cells were targets for IBD virus, and immunodeficiency was mainly due to impaired peripheral seeding of B cells in infected juvenile birds. Sivanandan and Maheswaran (1980) have also suggested that IBD virus affected the immature or precursor B cells to a far extent than mature B lymphocytes. Bursal weight indices of control chicks were comparable and, corresponding to the normal curve of Glick (1956). Bursal atrophy and protection against challenge were more or less linked together and this is in agreement with Van den Berg and Meulemans (1991).

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