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Evaluation of Four Commercial Anti - Infectious Bursal Disease (IBD) Vaccines under Sudan Conditions

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Abstract: The potency of four anti-infectious bursal disease vaccines commercially available in Sudan was evaluated. For this purpose 125 layer chicks were divided into five groups of 25 birds each. Group A, B, C and D were vaccinated with 228E, D78, Bio-Gumboro and Gumboro3 strain vaccines respectively. 228E strain is a hot vaccine while the rest are intermediate strain vaccines. While group E remain without vaccination and acted as a negative control. Vaccination for each group was done twice at day 21 and 28 via drinking water route. ELISA test performed 15 days later revealed that vaccination using 228E vaccine induced the highest antibody titre and protected birds challenged at day 43 from clinical disease, postmortem gross lesions and mortality. In contrast birds vaccinated with Gumboro3 vaccine induced the lowest antibody titre. All vaccines used in the present study, except 228E vaccine, failed to protect birds fully from clinical signs, postmortem gross lesions and mortality caused by artificial challenge. The mean antibody titre of different groups was; 12521±2014.14, 4790±1234.95, 9903±966.91 and 1587±824.80 and the mortality rate was 00%, 40%, 12% and 40% for group A, B, C and D respectively compared to antibody titre of 363±196.22 and mortality rate of 64% for the control group.

Key words: IBD, vaccine, ELISA, protective efficacy, hot strain and intermediate strain

Introduction

Infectious bursal disease (IBD) is an acute highly contagious viral infection of young chickens associated with high morbidity and high mortality (Parkhurst, 1964; Lukert and Saif, 1991). It is a major poultry pathogen in the poultry industry (Hein *et al.*, 2002). One of the significant components of the control of the disease is its vaccination which if improved may help in lowering the incidence of the disease in poultry (Zaheer *et al.*, 2003). In practice different vaccination schedules have been recommended and used, but despite these vaccination schedules outbreaks of IBD are still reported throughout Pakistan (Zaheer and Saeed, 2003). Up to date more than 46 strains of imported IBD vaccines are used to control the disease in west Malaysia (Chin, 1993). In Pakistan a variety of imported IBD vaccine strains have been devised for their use. (Zaheer *et al.*, 2003). Two types of vaccines are used to control IBD. These are attenuated vaccine and inactivated oil emulsion vaccines (Thornton and Pattison, 1975). Recently recombinant vaccine expressing IBDV antigens has also been licensed (Wu *et al.*, 2004). Live attenuated vaccines are produced from fully or partially attenuated strains of the virus known as mild (intermediate) or hot (intermediate plus) respectively (Skeeles *et al.*, 1978). The mild (intermediate) strain vaccine was found to be unable to neutralize high level of maternally derived antibodies (MDA) in chickens and failed to induce IBD antibodies. In contrast hot (intermediate plus) strain vaccine cause severe bursal lesions similar to those observed in IBD field outbreaks (Hair-Bejo *et al.*, 2000).

The time of vaccination, type of the vaccine, maternally derived antibodies in the progeny chicks and pathogenicity of IBDV field challenge are the important factors determining the efficacy of the vaccination (Hair-Bejo *et al.*, 2004).

Suspicious about the efficacy of the vaccination in commercial poultry arise from the occurrence of low or high serological titre, clinical disease, reduced productivity and/or abnormal vaccine reaction. One of the important factors that can cause vaccination failure could be the vaccine itself. It is self evidence that the live virus vaccine must have an adequate titre and this titre must have sufficient stability (Mc Mullin, 1985). In Sudan up to date more than five IBD intermediate strain and two intermediate plus strain vaccines have been used to control the disease. But the disease despite vaccination occurred in poultry.

This study is an effort to evaluate the serological and protective efficacy of four of these vaccines under Sudan condition.

Materials and Methods

Experimental birds: A total of one hundred and twenty five day old commercial layer chicks of Hisex strain were reared in isolated pens for 21 days after which birds were divided into five groups based on infectious bursal disease (IBD) vaccine strain used for each group. Group A, group B, group C and group D birds were vaccinated using 228E, Nobilis D78, Bio-Gumboro and Gumboro3 strain vaccines respectively. 228E strain vaccine is a hot

vaccine, while the other strains are intermediate strain vaccines. Group E birds were not vaccinated and acted as negative control. 228E and Nobilis D78 vaccines were obtained from Detasi company and manufactured by Intervet company-Holland. Bio-Gumboro vaccine was obtained from Coral company and manufactured by Merial company - France. Gumboro3 vaccine was obtained from Dr. Aiad company and manufactured by Abkaras company-Italy. Vaccination of birds in each group was done via drinking water route twice at day 21 and day 28.

Sampling strategy and Sera collection: At day1, 5 birds were bled and blood was collected to measure MDA. At day 17, 5 birds were bled for sampling to insure that MDA has become low enough to vaccinate. At day 43 (15 days post booster dose of anti-IBD vaccination) 5 birds in each group were bled and blood samples were collected from wing vein and kept overnight and sera were then collected and stored at -20°C till used for serology.

Serology: Enzyme Linked Immuno-sorbent Assay (ELISA) technique was performed as described by the manufacturer of ELISA reader and infectious bursal disease ELISA kit that obtained from Bio - Check company - Holland Serum samples that preserved at -20°C, the antigen coated plate that consisted of 96 wells and the ELISA kit reagents that preserved at 2 - 4°C were adjusted to room temperature of 22 - 27°C prior to the test. Serum samples were diluted by adding 500 µL of the sample diluents to each 1µL of the sample prior to the assay using automatic pipette with disposable tips. Hundred µL of diluted sample was added into each well and 100µL of undiluted negative control into well A01 and well B01 and 100µL of undiluted positive control into well C01 and well D01. The plate was then covered with lid and incubated at room temperature for 30 minutes after which the contents of wells were aspirated and each well was washed with 300 µL of wash buffer for 4 times and wells were inverted and taped firmly on absorbent paper to dry. After which 100 µL of the conjugate reagent (sheep anti-chicken) was added into each well and the plate was covered with lid and incubated at room temperature for 30 minutes, the contents of the wells were then aspirated and washed 4 times with wash buffer (300 µL for each well) and the plate was inverted and taped firmly on absorbent paper to dry. Hundred µL of the substrate reagent was added into each well and the plate was covered with lid and incubated at room temperature for 15 minutes, after which 100 µL of the stop solution was added into each well to stop the reaction.

The absorbance values were measured and recorded at wavelength 405nm using ELISA reader. IBD antibodies titre and sample absorbance to positive control

absorbance (S / P) ratio of samples were calculated to interpret the results. Coefficient of variation of titres % (CV%) among birds in each group was calculated.

Inoculum preparation: IBDV inoculum for artificial challenge was prepared and performed as described by Khan *et al.* (1988) and Zaheer and Saeed (2003) bursae of Fabricious were collected from IBDV infected chicks. A 20% (W/V) bursae homogenate was prepared at the faculty of veterinary medicine - university of Khartoum and stored at -20°C till used. Each bird in each group was injected subcutaneously with 1 ml of the homogenate.

Mortality, clinical signs and gross lesions: Mortality was calculated as a percentage of the initial number of the birds. Clinical signs of Gumboro disease was considered as positive or negative. Postmortem gross lesions observed in every dead bird in each group were recorded and subjectively graded as normal (0), mild (1), mild to moderate (2) moderate (3) moderate to severe (4) and severe (5) based on the severity of the lesions on the bursa of Fabricious, body skeletal muscles surfaces, kidneys, erosions and hemorrhages of the mucosa of proventriculus (OIE manual, 2004).

Bursa to body weight ($\times 10^{-3}$) ratio: bursae of Fabricious were removed and their weights were determined using a sensitive balance and the weight was calculated. as a ratio of the body weight multiplied by 10^{-3} . and the bursae were preserved for histopathologic examination.

Histopathologic examination: bursae tissues were histopathologically examined and the pathologic changes were subjectively graded as normal (0) mild (1) mild to moderate (2) moderate (3) moderate to severe (4) and severe (5). This method was described by Hair-Bejo *et al.* (2000) as a modified old method.

Results and Discussion

Serology(ELISA): Serum samples tested at day 43 (15 days post booster dose of IBD vaccine) among different groups demonstrated that vaccination using 228E strain vaccine (group A) induced the highest anti-IBD antibody mean titre and S/P ratio mean value, followed by birds vaccinated using Bio-Gumboro strain vaccine (group C). CV% was low in these two groups. Antibody titre levels of group A and group C were significantly ($P<0.05$) higher than other groups. D78 vaccine (group B) induced an acceptable antibody titre. Group D showed low antibody titre with high CV% indicating poor uniformity. Unusual low titre in group D indicates that the vaccine did not raise the antibodies to acceptable level and hence suspicious about the efficacy of an immunization program (Mc Mullin, 1985).

The results also showed that all vaccine strains used in

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Table 1: Results of serologically (ELISA) tested serum samples for IBD antibody titre at day 1 and day 17

Age	Mean Titre	Min and Max Titre	CV%	S/P Ratio	N/S/P
1 day	25623±3005.61	18674-33888	26 (OK)	8.94±0.956	0/0/5
17 days	7323±2829.09	3343-18442	86 (High)	2.82±0.978	0/0/5

N = Negative, S = Suspicious, P = Positive, CV% = Coefficient of Variation, S/P Ratio = Sample to Positive Ratio.

Table 2: Results of serum samples serologically (ELISA) tested of different groups of birds gathered according to vaccine strain used

Group	Mean Titre	Min and Max Titre	CV%	S/P Ratio	N/S/P
A	12521±2014.14 (OK)	4665-15426	36 (OK)	4.85±0.795	0/0/5
B	4790±1234.95 (OK)	63-7027	58 (High)	1.92±0.847	1/0/4
C	9903±966.94 (OK)	7872-13349	22 (OK)	3.97±0.311	0/0/5
D	1587±824.80 (low)	63-3933	116 (High)	0.678±0.339	2/0/3
E	363±196.22 (low)	63-1031	121 (High)	0.170±8.92E-02	3/0/2

Table 3: Protective efficacy of different strains of anti-IBDV vaccines

Group	Gross lesion grade	Clinical signs	Mortality	Bursa to BW ($\times 10^{-3}$) Ratio	Histopathologic grade
A	00.00±00	Negative	0/25 (00%)	1.197±0.195	2.21±0.25
B	3.4±0.68	Positive	10/25 (40%)	3.772±0.611	2.69±0.32
C	1.8±0.37	Positive	3/25 (12%)	3.368±0.339	2.45±0.14
D	4.00±0.63	Positive	10/25 (40%)	4.302±0.319	4.24±0.21
E	4.4±0.40	Positive	16/25 (64%)	5.174±0.522	3.96±0.11

the present study failed to raise antibody titre to its level at day one (MDA). Results reported in this study was also recorded by Naji *et al.* (1980) who found marked differences in titre of antibody produced against IBD by different vaccines.

Protective efficacy: Artificial challenge 15 day post the booster dose of vaccination results in appearance of IBD clinical signs in all groups except group A. Severe clinical signs were observed in group B, D and the control causing mortality of 40%, 40% and 64% respectively. This finding disagreed with Hassan *et al.* (2004) who has reported that white leghorn birds vaccinated with a live intermediate vaccine did not lead to mortality when challenged 10 days later with vvIBDV. But agreed with Zaheer *et al.*, 2003 who observed outbreaks of IBD in flocks vaccinated with a variety of vaccines.

In contrast only moderate signs were seen in group C causing 12% mortality. No clinical signs and no mortality were reported in group A. Group A also showed no postmortem gross lesions. Challenge of birds in group C lead to mild to moderate lesions. which is significantly ($P < 0.05$) lower than in group B, D and the control. The greatest bursa to body weight ($\times 10^{-3}$) ratio was reported in group D. All groups showed histopathologic changes in the bursae tissues (Table 3). Type of the vaccine is one of the important factors that determined the efficacy of IBD vaccination (Hair-Bejo *et al.*, 2004).

Vaccination against IBD with 228E strain gave a solid protective immunity which is obvious when considering that 228E vaccine is a hot (intermediate plus) vaccine compared to other mild (intermediate) vaccines and it is recommended for use when MDA is high or moderate.

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