Assessment of serological and defensive effectiveness of the merchants live IBD vaccines in Ghana to shield chicks

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Abstract

In spite of the intensive and varied vaccination procedures to control Infectious Bursa Disease (IBD), the emergence of a very virulent (vv) IBD pathotype in Ghana has led to high economic losses in the poultry industry. The potencies of two live intermediate IBD vaccine strains (TAD and Nobilis D78) and an intermediate-plus (hot) vaccine strain (228E) used in Ghana were evaluated by challenging Specific Antibody Negative (SAN) chicks with a vvIBD virus (LV/G19) which was recently isolated in Ghana. Serum samples were obtained from all chicks before vaccination and virus challenge. Antibody titres were measured by Enzyme-linked Immunosorbent assay (ELISA). Body weight, bursal weight and bursa to body weight measurements were also taken. All vaccinated chicks were fully protected from the vvIBD virus, as neither morbidity nor mortality was observed in the vaccinated chicks after challenge. The intermediate–plus (hot) IBD vaccine elicited the highest antibody titers but caused reduction in the size of the bursa of Fabricius. Taken together, the results of this study showed that vaccines currently in use in Ghana can protect chicken against the locally-isolated vvIBD virus.

Keywords: Infectious bursal disease, vaccine efficacy, intermediate, intermediate-plus (hot).

INTRODUCTION

Infectious bursal disease (IBD) or Gumboro Disease is a highly contagious viral disease of young chickens cha-racterized by a haemorrhagic syndrome, immuno-suppression and high mortality generally at 3 to 6 weeks of age (Cosgrove, 1962; Hitchner, 1970).

The causative agent is a double stranded RNA virus that has a bi-segmented genome and belongs to the genus *Avibirnavirus* of the family *Birnaviridae* (Lukert et al., 1991). It is a nonenveloped icosahedral virus with a diameter of about 55 - 60 nm (Ismail and Saif, 1990). This virus can be differentiated into two serotypes by virus the neutralization test (McFerran et al., 1980). Serotype 1 contains the pathogenic strains while serotype 2 strains are not pathogenic to chickens (Ismail and Saif, 1990). Pathogenic serotype 1 IBDV field strains can be grouped into classical, antigenic variant and very virulent (vv) strains (Brown et al., 1994).

The bursa of Fabricius is the primary target organ of IBDV. The virus replicates in immature bursa-derived lymphocytes (B-lymphocytes) of chickens. One form of the disease associated with high mortality has been endemic in Ghana since 1990 (Amakye-Anim et al., 2007). VvIBD virus induces severe lesions in the bursa of Fabricius, caecal tonsils, kidneys and spleen (Nunoya et al., 1992).

Several IBDV vaccine types are commercially available (Winterfield et al., 1978). In terms of virulence these vaccines range from "mild" to intermediate and interme-diate-plus (hot). Since the response to intermediate vaccines is less affected by maternal antibodies they are superior to the "mild" vaccine in providing immunity to commercial chickens with maternal antibodies (Guittet et al., 1992).

Since the 1990s it has been difficult to control field IBD with vaccines that are available in Ghana. Three interme-

Table 1. Experimental Design.

Groups	No of chicks	Vaccine type*	Challenge virus[§]
Group 1	20	TAD (I)	GHLV/G19
Group 2	20	D78 (I)	GHLV/G19
Group 3	20	288E (I-P)	GHLV/G19
Group 4	20	Unvaccinated control	GHLV/G19

*I = Intermediate vaccine; I-P = inteRmediate-plus vaccine
[§]GHLV/G19 = Ghanaian stock field vvIBD virus strain.

diate and two intermediate-plus vaccine types have been used in various vaccination schedules in Ghana. However, vaccinated birds were not always effectively protected in the field, and it has been speculated that the vaccine types used were, therefore, inappropriate given that an important cause of vaccination failure is the vaccine type used. In order to be effective a vaccine must elicit an adequate antibody titre and this titre should be of sufficient duration (Mc Mullin, 1985).

The present work was conducted to assess the serological and protective efficacy of the commercial live IBD vaccines available in Ghana to protect chicks against a vvIBDV strain.

MATERIALS AND METHODS

Experimental chickens

One-week-old Specific Antibody Negative (SAN) White Leghorn chickens (Lohmann, Germany) were used in this study. The parent flock was raised at the CSIR-Animal Research Institute, Katamanso Station, under strict hygienic and biosecurity conditions. The parent stock (from day-old) was not vaccinated against IBD. Progenies hatched from the SAN parent stock were used for all the experiments. Feed and water were provided *ad libitum* for the duration of the experiment. All experiments were performed in animal facilities following international ethical guidelines on animal welfare.

Vaccines

Three vaccine types commonly used against IBD in Ghana were tested in this study. They are the Intermediate [Nobilis Gumboro vaccine strains D78 ($40\log^{10}TCID_{50}$)] and TAD (10^2 EID_{50} per dose, Lohmann Animal Health, Germany), and an Intermediate–Plus (hot) strain [Nobilis Gumboro 228E ($\log^{10}$ EID_{50}) Intervet International B.V. Boxmeer. The Netherlands]. The vaccines were administered to the chicks at 7 days of age via the oral route according to the manufacturers' instructions.

Experimental design

Eighty SAN chickens were randomly selected, wing tagged and divided into 4 groups and immunized with the various vaccines used in the study on the 7th day after hatching. Group 1 (G1, n = 20) were vaccinated with TAD intermediate vaccine, Group 2 (G2, n = 20) with D78 intermediate vaccine, and group 3(G3, n = 20) with 228E intermediate-plus vaccine. Twenty unvaccinated chicks served as the control group (G4) (Table 1). Two weeks post

vaccination (pv), 15 birds in each group (G1 to G4) were challenged with LV/G19 ($10^{6.3}$ ELD_{50} /100 µl) via eye drops. The remaining five birds in each group were treated with the same volume (100 µl) of physiological saline (PBS) via the intraocular route. All birds were monitored for overt signs of disease (e.g. depression, inappetence, diarrhoea) and mortality over 15 days Post Challenge (pc).

Serum samples were taken by wing vein bleeding before vaccination and before challenge and stored at - 20^oC until tested. The experimental birds were also weighed weekly until 35 days old. Necropsy was conducted on birds that died after challenge with the challenge virus to study gross pathologic lesions and also the integrity of the bursa of Fabricius. Surviving birds were euthanized and the bursa of Fabricius removed, weighed and used to determine the bursa to body weight ratio.

Serology

Antibody titres to IBDV were determined by Enzyme-Linked Immunosorbent Assay (ELISA) . The ELISA technique was carried out according to the methods described by IDEXX Laboratories Incorporation, USA. Briefly, the antigen-coated plates and the ELISA kit reagents were adjusted to room temperature prior to the test. The test sample was diluted 1:500 with sample diluent prior to the assay. Diluted sample (100 l) was then put into each well of the plate. This was followed by 100 l of undiluted negative control into well A1 and A2, 100 l of undiluted positive control into well A3 and A4. The plate was incubated for 30 min at room temperature. Each well was then washed with approximately 350 l of distilled water 3 times. Goat anti- chicken conjugate (100 l) was dispensed into each well. The plate was incubated at room temperature for 30 min, followed by washing each well with 350 l of distilled water 3 times. Tetramethylbenzidine (TMB) solution (100 l) was dispensed into each well. The plate was then incubated at room temperature for 15 min. Finally, 100 l of stop solution was dispensed into each well to stop the reaction. The absorbance values were measured and recorded at 650 nm. The presence or absence of antibody to IBDV was determined by relating the A (650 nm) value of the unknown to the positive control mean. The positive control had previously been standardized and represented significant antibody levels to IBD in chicken serum. The relative level of antibody in the unknown was determined by calculating the sample to positive (S/P) ratio. The equation for calculation provided in the ELISA kit was used in calculating the antibody titre as follows:

a)   Positive Control Mean (PCX)

$$\frac{Well\ A1(650nm) + Well\ A2(650nm)}{2} = PCX$$

b)   Negative Control Mean

Table 2. Serologic response of SAN chicken before and after vaccination with Commercial live attenuated vaccines and challenge with Ghanaian vvIBD virus strain.

Groups	Vaccine Strain	Antibody titres	
		Prior to vaccination on Day 7	Prior to challenge on day 21
G1	TAD	7.75 ± 1.71	1571.45 ± 103.39[a]
G2	D78	1.58 ± 1.02	1784.63 ± 213.90[a]
G3	288E	24.65 ± 3.22	1870.25 ± 163.56[a]
G4	Unvaccinated	24.09 ± 3.06	1.17 ± 1.02[b]

The data are ELISA titers ± Standard Deviation (SD).
[a,b] Means with different superscripts within column differ significantly ($p < 0.05$).

$$\frac{\text{Well A3(650nm) + Well A4(650nm)}}{2} = \text{NCX}$$

c) $\text{S/P Ratio} = \dfrac{\text{Sample mean} - \text{NCX}}{\text{PCX} - \text{NCX}}$

d)   Titre relates S/P at a 1:500 dilution to an end point titre:
$\mathrm{Log_{10}}$ Titre 1.09 ($\log_{10}$ S/P) + 3.36

Serum samples with S/P ratios of less than or equal to 0.2 were considered negative. S/P ratios greater than 0.2 (titres greater than 396) were considered positive and indicated either vaccination or exposure to IBD virus.

Challenge virus

A vvIBD virus pathotype was used for challenge in the study. The virus was isolated from a six-week-old pullet from Akim Oda in the Eastern region of Ghana during an IBD outbreak in 2004 (Amakye-Anim et al., 2007). In this previous study, the virus designated LV/G19 caused 100% mortality in SAN chicken and has $\mathrm{ELD_{50}} = 10^{6.3}$. The virus was propagated using 10-day- old SAN embryo-nating eggs and titrated as described by Hitchner (1970).

Calculation of bursa-to-body weight ratio

Individual bursa to body weight ratio was calculated by dividing bursal weight by body weight and multiplying by 10^{3} as previously described by Giambrone and Closser (1990).

Statistical analysis

Data were subjected to a one-way analysis of variance (ANOVA). Statements of statistical significance were based on *p < 0.05.* Variations among groups were determined using Tukey's comparative test (Myra, 1980).

RESULTS

Serologic response of SAN chicken to IBD vaccination

All birds in the experiment showed very low antibody titres (varying from 1.58 ± 1.02 in G2 to 24.65 ± 3.22 in G3) before vaccination (Table 2). Prior to challenge, TAD, D78 and 228E -vaccinated birds showed large increases in titres ($p < 0.05$) that did not differ between the vaccinated groups. The titres in unvaccinated birds remained low.

Protection of vaccinated SAN chicken against challenge

No overt clinical signs of IBD were observed in any of the chicks before challenge. Protection from vvIBDV was determined by the absence of clinical signs or death during the 15 days post challenge period. None of the chicks that had been vaccinated with TAD, D78 or 228E vaccine showed clinical signs after challenge with locally-isolated vvIBDV (LV/G19) (Table 3). In contrast 80% of the unvaccinated birds that had been challenged with LV/G19 died within 4 days post challenge. All dead birds exhibited severe depression, yellowish white diarrhoea, lethargy and droopy wings between days 2 and 4 post challenge. All unchallenged birds in each of the four groups remained normal during the course of the experiment.

Bursa to body weight ratios

The mean bodyweight of the unvaccinated control group was significantly lower than that of the vaccinated groups (Table 4). In addition, the mean bursal weight of the unvaccinated control group birds was significantly less ($p < 0.05$) than those of the vaccinated birds. Birds vaccinated with the 228E vaccine showed significantly lower mean bursa weight and bursa weight/body weight ratio compared to birds vaccinated with TAD and D78 vaccines (Table 4).

DISCUSSION

Characterization of the IBDV strain that was recently isolated in Ghana confirmed that the local vvIBDV pathotype may be responsible for the continuous outbreaks of IBD in commer-

Table 3. Protection of vaccinated SAN chicken with live attenuated vaccines against challenge with Ghanaian vvIB virus strain.

Group	Vaccine	Challenge*			Protection	
		Strain	Route	No. Birds	Clinical signs	Mortality
G1	TAD	GHLV/19	I/O	15	0/15	0%(0/15)
		PBS	I/O	5	0/5	0% (0/5)
G2	D78	GHLV/19	I/O	15	0/15	0% (0/15)
		PBS	I/O	5	0/5	0% (0/5)
G3	228E	GHLV/19	I/O	15	0/15	0% (0/15)
		PBS	I/O	5	0/5	0% (0/5)
G4	Unvaccinated	GHLV/19	I/O	15	12/15	80% (12/15)
		PBS	I/O	5	0/5	0% (0/5)

*1 week-old SAN birds were vaccinated with a commercial live attenuated vaccine and challenged two weeks later with $10^{6.3}$ EID_{50} (100µl) of vvIBD virus via the intraocular route.
   Protection of chickens against challenge was observed for 15 day post-challenge. I/O = intraocular route.

Table 4. Mean body and bursa weights of SAN chicken with live attenuated vaccines and challenge with Ghanaian vvIBD virus strain.

Group	Vaccine Strain	No of Birds	Weights at 35 Days of Age		
			Mean Body weight	Mean Bursa weight	Bursa weight/Body weight ($\times 10^3$) ratio
G1	TAD	5	268.70 ± 5.50^a	1.19 ± 0.12^a	4.43 ± 0.06^a
G2	D78	5	264.40 ± 9.01^a	1.07 ± 0.08^a	4.05 ± 0.03^a
G3	228E	5	251.60 ± 5.99^a	0.63 ± 0.77^b	2.50 ± 0.03^b
G4	Unvaccinated	3	189.06 ± 22.66^b	0.26 ± 0.03^b	1.37 ± 0.04^{bc}

*The data are mean weights ± S.E. All chickens were challenged at 21 days of age with $10^{6.3}$ EID_{50} (100 µl) of vvIBD virus via the intraocular route and observed for 15 day post-challenge. The birds were euthanized on day 35 to study integrity of the bursa of Fabricius.
[a,b,c] Means with different superscripts within column differ significantly ($P < 0.05$).

cial poultry in Ghana (Amakye-Anim et al., 2007). In this study we investigated the ability of available commercial vaccines to protect birds against the vvIBDV pathotype. Both the intermediate and intermediate-plus vaccines provided full protection to chickens from vvIBDV-induced disease. No mortality or disease symptoms were observed in any of the vaccinated chickens.

This agrees with findings of Hassan et al. (2004) who reported that white Leghorn chickens vaccinated with a live intermediate vaccine were protected when challenged 10 days later with vvIBDV and also those of Van den Berg et al. (2000) who demonstrated that chickens vaccinated with intermediate or intermediate-plus vaccines were fully protected from challenge with vvIBDV strains. Despite this, Zaheer et al. (2003) observed outbreaks of IBD in flocks vaccinated with a variety of vaccines. Therefore, it appears that other factors such as immune status of chicks at the time of vaccination, immunosuppression by other agents, poor handling and administration of vaccines or inappropriate vaccination schedules may affect the success of immuni-zation (Hair Bejo et al., 2004).

In IBD outbreaks acute disease and death are due to the necrotizing effect of the infecting viruses on the tissue of the bursa of Fabricius (Van den Berg, 1998). If birds survive and recover from this phase of the disease, they remain immuno compromised and may be more susceptible to other infectious diseases and immunization against other viruses may not be effective. Hair-Bejo et al. (2000) have reported that intermediate-plus strain vaccines may cause severe bursal lesions similar to those observed in IBD field outbreaks. They suggested that vaccine type is one of the important factors that determine the efficacy of IBD vaccination. The intermediate-plus vaccine has also been associated with subclinical infections which lead to a reduced performance of birds (Boot, 2001). Our results appear to confirm the deleterious effect of the intermediate-plus vaccine on the bursa of vaccinated birds. The use of the intermediate-plus vaccine caused reduction in the weight of the bursae which would indicate changes in bursal integrity (Hair-Bejo et al., 2000). It is known that intermediate vaccines also cause some bursal degeneration although recovery is faster than in birds vaccinated with intermediate-

plus strains.

In conclusion, the vaccination and virus challenge results obtained in this study show that the three vaccines used in the study provided solid protective immunity to the SAN chicken used in the experiments. It is concluded that vaccines currently in use in Ghana might effectively protect chicken against highly virulent infectious bursal disease circulating locally. However, caution should be exercised when using intermediate-plus vaccines as they could affect the integrity of the bursa of Fabricius and hence affect the efficacy of vaccination against other infectious diseases.

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