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ARTICLE ORIGINAL

Improving the control of Gumboro disease in commercial poultry in Ghana: viral isolation and vaccine trial studies

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Abstract

Isolation and characterization of Ghanaian field IBD virus were undertaken to establish an efficacious vaccination program against the disease in the country. Bursal homogenates were prepared from chickens that died of IBD in five different locations of the country. Batches of 11- day old Specific Antibody Negative (SAN) embryonating eggs were inoculated with 0.2ml of homogenate each on the chorio-allantoic membrane. The eggs were incubated and candled daily and all embryonic deaths were examined for gross IBD lesions. In addition, batches of 3-week and 6-week old SAN chickens were inoculated intra-ocularly with 10Ìl of the bursal homogenate and observed over 10 days for clinical signs and gross lesions of IBD. Confirmation of isolate was by RT-PCR/RFLP. Embryos inoculated with homogenates from all five locations died 3-5 days PI, showing characteristic IBD lesions of extensive haemorrhages, congestion of limbs and stunted growth. Inoculated SAN chickens showed 100% cumulative mortality similar lesions. One isolate LV /G19 standardized for viral challenge studies had an ELD50 value of 106.3. This study confirms the presence of vvIBDV in Ghana. In a second study, different vaccination programs using intermediate and intermediate-plus vaccines were investigated for the control of vvIBD in commercial poultry. SAN chicken and commercial poultry birds were used. Various groups of chicken were vaccinated either once, twice, thrice on days 7, 14, 23, 28 or 35. All chickens were bled before each vaccination and their antibody titer levels determined by ELISA. The birds were inoculated intra-ocularly on day 49 with the isolated vvIBD virus pathotype and observed for 10 day PI. Dead chickens were examined for gross pathologies, while surviving birds were sacrificed on day 59 to study bursal integrity. The most significant finding is that, intermediate vaccines are most effective for the control of Gumboro disease in Ghanaian poultry, when administered twice on day 14 and 28. This is recommended for use by Ghanaian poultry farmers. (*RASPA,* 7 (S) : 129-133).

Key – Words: Gumboro disease - Vaccine trials - Commercial poultry - Ghana.

Résumé

Amélioration de la lutte contre la maladie de Gumboro en aviculture semi-industrielle au Ghana: isolement du virus et essai vaccinal

L'isolement et la caractérisation du virus IBD ghanéen ont été entrepris pour établir un programme de vaccination efficace contre la maladie dans le pays. Des homogénats de la bourse de Fabricius ont été préparés à partir de poulets morts de la maladie de Gumboro dans cinq localités du pays. Des lots d'oeufs embryonnés de 11 jours dépourvus d'anticorps spécifiques ont été inoculés avec 0,2 ml d'homogénat sur la membrane chorio-allantoïde. Les ?ufs ont été incubés et mirés quotidiennement et tous les embryons morts ont été examinés pour mettre en évidence les lésions macroscopiques de IBD. En outre, les lots de poulets de 3 et 6 semaines d'âge dépourvus d'antigène spécifique ont reçu une inoculation intra-oculaire de 10 Ìl de l'homogénat de la bourse de Fabricius. Ils ont été observés pour les symptômes et les lésions de la maladie de Gumboro. La confirmation de l'isolat a été réalisée par RT-PCR/RFLP. Les embryons inoculés avec des homogénats sont morts 3-5 jours après l'inoculation, montrant des lésions caractéristiques de la Gumboro : hémorragies, congestion des membres et retard de croissance. Un isolat LV/ G19 normalisé des essais vaccinaux ELD50 a eu une valeur de 106,3. Cette étude a confirmé la présence du virus de la maladie de Gumboro au Ghana. Dans une deuxième étude, les programmes de vaccination basés sur différents vaccins intermédiaires et intermédiaires plus ont été utilisés pour prévenir la maladie de Gumboro dans les élevages semi-industriels. Divers groupes de poulets ont été vaccinés, soit une fois, deux fois, trois fois à 7, 14, 23, 28 ou 35 jours. Avant la vaccination des prélèvements de sang ont été réalisés pour le dosage du niveau d'anticorps par ELISA. L'inoculation à été faite par voie intra intra-oculaire à 49 jours d'âge avec le pathotype isolé du virus IBD ; les oiseaux étant observés pendant 10 jours post-inoculation. Les poulets morts ont été examinés pour des pathologies graves, alors que les oiseaux survivant ont été abattus à J59 pour l'étude de l'intégrité de la bourse de Fabricius. Le résultat le plus significatif est que, les vaccins intermédiaires sont les plus efficaces pour la prévention de la maladie de Gumboro chez les volailles au Ghana, quand ils sont administrés deux fois à J14 et 28.

Mots – Clés : Maladie de Gumboro - Essai vaccinal - Aviculture industrielle - Ghana.

Introduction

Infectious Bursal Disease (IBD) also known as Gumboro disease of poultry is an acute highly contagious viral disease of chickens between the ages of 3 and 6 weeks. The disease was first definitively diagnosed in Ghana in 1977 [4] but had been suspected to be endemic in the country since 1973. Then, the disease was recognized only as a mild one accounting for about 2-5% mortality and was effectively controlled with the available vaccine using a vaccination programme recommended by the Veterinary Services Department (VSD). Over the past decade, IBD has become the most important health problem in commercial poultry in the country.

It is considered a highly devastating disease accounting for as much as 60% in layer chick mortality and 25% in broilers [13].

In spite of a nationally adopted vaccination program, outbreaks still occur. It has been speculated that new strains and pathotypes of the virus may have been introduced into the country resulting in vaccination failures. As a result of recurrent vaccination failures many poultry farmers have adopted their own vaccination strategies, with different vaccine types from various manufacturers. The result of the farmers' practices has been mixed.

For vaccination programs to be effective, the prevailing pathotypes of IBDV need to be identified, since different IBD strains and pathotypes may require specific vaccines and vaccination programs among other measures to achieve effective control [12]. Such comprehensive studies of IBD have not been carried out in Ghana. The purpose of this study therefore was to isolate and characterize the field IBD virus pathotypes and develop a vaccination strategy for the control of the disease in chicken in Ghana.

Materials and Methods

1. Viral isolation studies

1.1. Experimental chicken

Specific Antibody Negative (SAN) White Leghorn chickens were raised at the CSIR-Animal Research Institute, Katamanso Station is used for the experiments.

1.2. Preparation of bursal homogenate (inoculum)

Twenty grams of bursal tissue were homogenised in 100 ml of sterile PBS. The suspension was centrifuged at 1500 rpm for 20 minutes. The supernatant obtained was mixed with 10 000 IU / ml penicillin and 2.0ml streptomycin to prepare bursal homogenate used in the viral isolation experiments (Hitchner, 1970).

1.3. Virus isolation and characterization in SAN chickens

Twenty, 3- week and 6- week old SAN chickens were inoculated intra-ocularly with 10Ìl of bursal homogenates prepared from field cases of IBD as described above. Another 10 chicken served as uninoculated control. Clinical signs, morbidity and mortality rates were observed over 10 days post inoculation (PI). Bursae of Fabricius of all dead chickens were examined for IBD lesions, harvested and stored at – 70oC for use. Birds surviving beyond 10 days were sacrificed to assess their bursal integrity.

1.4. Preparation of standardized virus challenge material

The isolate LV/G19 that was one of the most virulent (Table 1) was selected for use as standard challenge virus. The bursal homogenate of this isolate was titrated by inoculating 10-day old SAN embryonating eggs via the CAM route with 0.1ml of inoculum and the ELD50 was calculated following the method of REED and MUNCH (1938)

1.5. Molecular diagnosis

Twenty samples of bursa of Fabricius from the five sites of studies were sent to Hipra Laboratories in Spain under strict protocol for transporting biological samples. Samples were pooled into five, Pool 1: ARI; Pool 2: LV/G11; Pool 3: LV/G13; Pool 4: LVG19 and Pool 5: LV/G23 and analyzed by RT-PCR/ RFLP.

Table 1. Morbidity and mortality rates induced in 6-week old Specific Antibody Negative (SAN) chickens by field isolates of IBDV from different locations in Ghana.

Isolate	Source	ID Number	No of chicken inoculated	% Morbidity	% Mortality
Pokoase	ARI	ARI	20	100	80
Nungua	La Vet	LV/G11	20	100	80
Kasoa	La Vet	LV/G13	20	100	90
Akim Oda	La Vet	LV/G19	20	100	100
Lashibi	La Vet	LV/G23	20	100	100

NB: Ten un-inoculated chickens used as control did not show signs of IBD.

2. Vaccine trial studies

2.1. Experimental chicken

Specific Antibody Negative (SAN) White Leghorn commercial replacement pullets were used in the study.

2.2. Vaccines

The two IBD vaccine types used in the country, the "Intermediate" (TAD, Laprovet, France and Intervet D78) and "Intermediate-Plus" (Nobilis 228E, Intervet company, Netherlands) were used in this experiment.

2.3. Testing of Vaccination Schedules

A total of 960 SAN and commercial replacement pullets were used for the trial in which vaccine types were evaluated. The intermediate vaccines (TAD, D78) and intermediate plus vaccine 228E were administered to the various groups of chicks. Twenty chicks from each group were bled weekly for the whole period of the experiment and sera harvested for serology. The chicks were inoculated with the standardized the stock field challenge virus intra-ocularly on day 49 and observed for 10 days PI, to study clinical patterns and pathologic lesions. The bursae of Fabricius of chicks that died after challenge were harvested to study their integrity. Surviving chicks were sacrificed on

day 59 to also study bursal integrity. Enzyme-linked immunosorbent assay (ELISA) was used to evaluate responses to vaccinations and determination of the best ages of vaccination.

2.4. Statistical analysis

Data was analyzed in ANOVA following GLM procedures of SPSS for windows (version 10.0).

Results

1. Virus isolation in SAN embryos

The infected embryos showed mortalities after 3 to 5 days PI. Lesions included extensive body haemorrhages, congestion of limbs and stunted growth. None of the surviving embryos showed signs of splenomegaly.

2. Virus isolation in SAN chickens

After inoculation with the field isolate homogenate, 100% cumulative mortality was observed over 10 days PI. Post mortem findings included enlarged haemorrhagic and oedematous bursae with haemorrhages in the breast and thigh muscles. No mortality occurred and gross lesions were observed in the bursae of control birds.

3. Biological Characterization of the isolated IBDV strains

Biological characterization of isolated strains was based on clinical signs and morbidity and mortality rates in challenged birds. Clinical signs appeared after 3 days post inoculation. These included lethargy, anorexia, ruffled feathers and sudden death. Morbidity and mortality rates were 100% within 10 days PI (Table 1). Post mortem examination showed enlarged, haemorrhagic and edematous bursae with haemorrhages in the thigh and pectoral muscles.

4. Standardization of Challenge virus

The embryo lethal dose ELD50 recorded for LV/G19 selected for viral challenge studies was $10^{6.3}$.

5. Molecular Diagnosis

All samples (Pools 1-5) were identified to be very virulent infectious Bursal Disease (vv IBDV) at Hipra Laboratories, Spain (M. Bentue, 2005, Personal Communication).

6. SAN chickens

All SAN chicks vaccinated with either of the vaccines and challenged a with the Ghanaian vvIBD, showed no sign of disease while the unvaccinated controls exhibited clinical signs and gross pathologies typical of vvIBD with 100% morbidity and 75% mortality rates. There were significant differences ($p>0.05$) in the mean titers of vaccinated SAN chicks (Table 2). While titers for the control group were different from those of the other groups ($p<0.05$).

Table 2. Mean ELISA antibody titers of chicken without maternal antibodies (SAN) vaccinated in different vaccination regimen.

Time of vaccination (Days)	Antibody titers			
	Group 1	Group 2	Group 3	Group 4
7	-	4.49±1.71	4.39±3.22	-
14	-	-	-	-
23	2.06 ±1.02	1260.80±103.39	2076.90±329.39	-
28	-	-	-	-
35	-	-	2252.89±213.90	-
49	3983.87± 312.75	2173.34±294.74	4227.16±163.56	4.60±3.06

Gp 1 - vaccinated only on day 23; Gp 2 - vaccinated on days 7 and 23; Gp 3 - vaccinated on days 7 , 23 and 35; Gp 4 - control - no vaccination

6. Commercial replacement pullets

Vaccinated and challenged, commercial chicken showed results similar to those of the SAN chickens (Table 3). There were significant differences in the mean titers between the control group and the vaccinated groups ($p>0.05$), mortality rates in the control group was however lower (60%) than in SAN chicken.

Discussion

Gumboro Disease outbreaks even in vaccinated flocks have caused serious frustration in the poultry industry in Ghana with some farmers actually abandoning their farming enterprises in recent years. Several workers have blamed vaccination failure on many factors including the overwhelming of vaccinal immunity induced by highly pathogenic field strain of the virus [10].

Table 3. Mean ELISA antibody titers of locally produced commercial replacement pullets vaccinated in different regimen

Time of Vaccination (Days)	Antibody titers							
	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8
7	-	-	371.97 ±138.22	-	975.5± 66.13	-	-	-
14	72.76± 14.64	-	-	208.59 ±43.21	-	91.85±39.78	236.98±54.36	-
23	-	105.90 ±51.59	43.76 ±19.32	-	43.22 ±19.54	-	-	-
28	-	-	-	3215.28 ±54.32	-	-	4119.16±67.84	-
35	-	-	-	-	2303.73± 114.36	-	-	-
49	4886.96± 782.72	3436.00±454.50	4227.10±527.13	5384.30±1470.31	4735.30± 668.30	4921.70±43.19	5690.34± 205.47	140.17± 36.45

Gp 1 –vaccinated only on day 14; Gp 2 - vaccinated only on day 23; Gp 3 - vaccinated on days 7 and 23; Gp 4 - vaccinated on days 14 and 28; Gp 5 - vaccinated on days 7, 23 and 35; Gp 6 - Inter-plus day 14; Gp 7 – Inter-plus day 14 and 28; Gp8 - no vaccination

McFERRAN *et al.* [7] showed differences in pathotypes among field isolates and vaccine strains of serotype 1 IBDV and postulated the presence of antigenically distinct viruses within this serotype to explain poor vaccination results. It has been speculated that, the Ghanaian stock field virus may be more virulent than the vaccinal viruses and hence able to break through vaccinal immunity to cause outbreaks. The economical importance of both the clinical disease and sub-clinical disease resulting from frequent outbreaks of Gumboro disease, especially in vaccinated chickens in Ghana has led to the search for an efficient vaccine and vaccination regime.

Our studies were based on the assertion by VAN DEN BERG *et al.* [12] that the only criterion for the classification of IBD strains as pathotypes should be their virulence in SPF or SAN chickens or embryonating eggs. Accordingly SAN chickens and embryonating eggs were used in these studies. One of the isolates, LV/G19 induced death, dwarfing, haemorrhages, or edema in the embryos when inoculated on to the CAM of 11-day old embryonated SAN chicken eggs. These lesions are considered pathognomonic for IBD [9].

In reproducing the disease in 3-week and 6-week old SAN chickens, with 100% mortality rates using a field isolate in the present studies, there are strong indications that the isolates are of the very virulent biotype. Similar findings were reported by NUNOYA *et al.*[8] using 3-week old SAN chickens.

Molecular tests at the Hipra Laboratories, S.A. Spain using RT-PCR-RFLP technique confirmed that the isolates form Ghana indeed belong to the vvIBD biotype (M. Bentue, 2005, Personal communication).

The ELD50 of 106.3 used in the studies for LV/G19 was ideal for Gumboro disease challenge work. The STC strain of serotype 1 IBDV standardized for challenge work by AMAKYE-ANIM *et al.* [2] had a similar ELD50 value of 106.8.

The study to test various vaccination programs showed that SAN chicken responded well to vaccination to produce antibodies. This is probably due to the fact that they did not have MDAs that could interfere or react with the vaccine virus and so they sero-converted after primary vaccination on day 7. It is observed from this study also (Table 3) that before primary vaccination of commercial chicken on day 7, antibody levels were higher than protective levels. But after vaccination the titers decreased for intermediate and 'hot' vaccines used. This is an indication that the vaccines failed to stimulate immune response due perhaps to interference by MDAs. However, after primary vaccination on day 14 immune response takes place and antibody titers

increased. KNEZEVIC *et al.* (1999) reported similar observations in broiler chicken. Chicken vaccinated on day 14 with either of the vaccines used produced immune response but with lower antibody titers, however after booster dose at day 28, secondary immune response takes place and the titer becomes increased. In summary, intermediate and 'hot' vaccines both produced similar responses in eliciting antibody titers. Though the 'hot' vaccine administered primarily on day 14 and boosted on day 28 produced higher titers on day 49 (5690.34) than the intermediate vaccine (5384.31), the difference was not significant. More so the hot vaccine is more expensive and recommended only for farms where IBD has become a notorious problem.

It is evident from the foregoing that commercial pullets, when primed with an intermediate vaccine on the 14 day and boosted on day 28 with the same type of vaccine, will develop an immunity that protects them against the disease and reduce losses.

It is concluded from the above findings that the prevalent IBD virus strain currently in Ghana is the vvIBD pathotype and the best vaccination regime against this pathotype is priming chicks first at 14 days of age and administering the same vaccine on day 28 as a booster dose. This will produce antibodies to achieve the protection required for both pullets and broilers against the disease.

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