Isolation and characterization of infectious bursal disease virus (IBDV) field strains and pathotypes in Ghana

J. AMAKYE-ANIM, H. R. OTSYINA, A. OSEI-SOMUAH & K. G. ANING
CSIR-Animal Research Institute, P. O. Box AH 20, Achimota, Accra, Ghana

ABSTRACT

Isolation and characterisation of Ghanaian field IBD virus were undertaken as part of a comprehensive study to establish an efficacious vaccination programme 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 0.2 ml 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-old SAN chickens were inoculated intra-occularly with 10 µl of the bursal homogenate and observed over 10 days for clinical signs and gross lesions of IBD. Pathotyping of the virus isolates was done by the biological method, using groups of 10, 6-week-old SAN chickens and confirmed by the reverse transcriptase-polymerase chain reaction-restriction fragment length polymorphism (RT-PCR-RFLP) technique. Embryos inoculated with homogenates from all five locations died 3 to 5 days pest inoculation (PI), showing characteristic IBD lesions of extensive haemorrhages, congestion of limbs and stunted growth. Inoculated 3-week-old SAN chickens showed 100 per cent cumulative mortality, enlarged haemorrhagic and oedematous bursae, and haemorrhages in the breast and thigh muscles. Six-week-old SAN chickens inoculated with bursal homogenates showed similar lesions. One isolate LV/G19 selected and standardized for viral challenge studies had an ELD_{50} value of $10^{6.3}$. The SAN chickens challenged with this isolate were more susceptible between the ages of 3 and 6 weeks, but morbidity and mortality were seen up to 10 weeks of age. This study confirms the presence of the very virulent Infectious Bursal Disease Virus (vvIBDV) in Ghana and the urgent need for its control.

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RÉSUMÉ

AMAKYE-ANIM, J., OTSYINA, H. R., OSEI-SOMUAH, A. & ANING, K. G.: *Isolement et caractérisation de virus de la maladie bursale infectieuse (VMBI) de la souche de champ et les pathotypes au Ghana.* Isolement et caractérisation de virus MBI du champ ghanéen étaient entreprises comme une part d'une étude compréhensive pour établir un programme de vaccination efficace contre la maladie dans le pays. Les homogenates bursales étaient préparées de poulets qui étaient morts de MBI à cinq emplacements différents dans le pays. Les groupes des œufs à l'état embryonnaire de l'âge de 11 ans d'anticorps spécifique négatif (ASN) étaient vaccinés avec 0.2 ml de homogenate par chacun sur la membrane de chorio-allantoïde. Les œufs étaient incubés et éclairés aux bougies chaque jour et tous les morts embryonnaires étaient étudiées pour les lésions totales. En plus, les groupes des œufs de poulets de ASN de l'âge de 3-semaines étaient vaccinés intra-occulaires avec 10 µl de homogenate bursale et observés sur une période de 10 jours pour des signes cliniques et les lésions totales de MBI. Les pathotypes d'isolat de virus ont été fait par la méthode biologique, employant des groupes de 10 poulets de ASN de l'âge de 6 semaines et confirmé par l'inverse de transcriptase-réaction en chaîne par polymérase-technique de polymorphisme de la longueur du fragment de restriction (IT-RCP-PLFR). Les embryons vaccinés avec les homogenates de tous les cinq emplacements étaient morts de 3-5 jours post inoculation (PI), montrant les caractéristiques de lésions de MBI de hémorragies extensives, de congestion de membres et la croissance retardée. Les poulets de ASN de l'âge de 3-semaines vaccinés montraient 100% de mortalité cumulative, les bursae œdematous et hémorragique hypertrophié et les hémorragies dans les muscles de blanc et de cuissardes. Les poulets de ASN de l'âge de 6-semaines vaccinés avec les homogenates bursales montraient les lésions semblables. Un isolat LV/G19 selectionné et standardisé pour les études de sérieux défi viral avait une valeur de ELD_{50} de $10^{6.3}$. Les poulets ASN stimulés avec cet isolat étaient plus susceptibles entre les âges de 3-6 semaines mais la morbidité et la mortalité étaient observées jusqu' à l'âge de 10 semaines. Cette étude confirme la présence de vvVMBI au Ghana et la nécessité urgente pour son contrôle.

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 (Gyening & Corkish, 1977), 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 per cent mortality, and was effectively controlled with the available vaccine, using a vaccination programme recommended by the Veterinary Services Department of the Ministry of Food and Agriculture.

Over the past decade, IBD has become the most important health problem in commercial poultry in Ghana. It is considered a highly devastating disease, accounting for as much as 60 per cent in layer chick mortality and 25 per cent in broilers (VSD, 2001). In spite of a nationally adopted vaccination programme, 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. Elsewhere, the emergence of a new strain, very virulent Infectious Bursal Disease Virus (vvIBDV), has resulted in identifying a new form of the disease (Chettle, Stuart & Wyeth, 1989).

Comprehensive studies of IBD outbreaks in Ghana have not been carried out; and as a result of recurrent vaccination failures many poultry farmers have, out of desperation, adopted their own vaccination strategies, including the use of different vaccine types from various manufacturers. The result of the farmers' practices has been mixed.

For vaccination programmes to be effective, the prevailing pathotypes of IBDV need to be identified, because different IBD strains and pathotypes may require specific vaccines and vaccination programmes, among other measures, for effective control (Van den Berg, 1998).

The purpose of this study was to isolate and characterise field IBD virus pathotypes in Ghana.

Materials and methods

Experimental chicken

Specific Antibody Negative (SAN) White Leghorn chickens were used in the study. The parent flock was raised at the CSIR-Animal Research Institute, Katamanso Station, under strict hygienic and biosecurity conditions. The birds from day-old were not vaccinated against IBD. Embryonating eggs and progenies hatched from the SAN flock were used for the experiments.

Sourcing of viral material

Bursae of Fabricius of chicken diagnosed to have died of IBD were collected from the Veterinary Diagnostic Laboratories at CSIR-ARI and La Veterinary Laboratories. The samples came from five different locations in Ghana and were identified as Pokoase (ARI), Nungua (LV/G11), Kasoa (LV/G13), Lashibi (LV/G23), and Akim-Oda (LV/G19).

Preparation of bursal homogenate (inoculum)

Twenty grams of bursal tissue were homogenised in 100 ml of sterile PBS. The suspensions were centrifuged at 1500 rpm for 20 min. The supernatants collected were mixed with 10 000 IU ml^{-1} penicillin and 2.0 ml streptomycin to prepare bursal homogenates used in the viral isolation experiments (Hitchner, 1970). The homogenates were cultured on blood and nutrient agar overnight to ensure there was no bacterial contamination.

Virus isolation in SAN embryonating eggs

Ten groups of 11-day embryonating eggs (n =12) collected from the SAN flock were used for virus isolation according to the method described by Hitchner (1970). Briefly, the eggs were marked on the side about midway along the long axis that has the vein structure well developed. The egg cell end and the marked side were disinfected.

Small holes were drilled at the centre of the egg cell and at the marked point on the side of the egg. The eggs were candled and a vacuum applied gently to the hole at the egg cell end to drop the

Chorio-Allantoic Membrane (CAM). A 25-gauge needle was used to introduce 0.2 ml of bursal homogenate (inoculum) onto the CAM. The holes were sealed with wax and the eggs rocked gently to distribute the inoculum evenly on the CAM surface. Eggs were further incubated horizontally for 7 days at 37 °C with daily candling. All embryonic deaths beyond 24 h were examined for gross IBD lesions. The CAM of eggs with embryos dying after 24 h were harvested and stored at - 70 °C.

Virus isolation in SAN chickens

Twenty 3-week-old SAN chickens were inoculated intra-occularly with 10 µl of bursal homogenates prepared from field cases of IBD as described above. Ten other chickens served as uninoculated control. Clinical signs and morbidity and mortality rates were observed over 10 days PI. Bursae of Fabricius of all dead chickens were examined for IBD lesions, harvested and stored at - 70 °C for further use. Birds surviving beyond 10 days were killed to assess their bursal integrity.

Biological characterisation of IBDV field isolates in Ghana

Bursal homogenates prepared from the isolation studies above were subsequently inoculated into 6-week-old SAN chickens in groups of 10. Another group of 10 chickens served as uninoculated control. All birds were observed for 10 days PI for clinical signs, morbidity and mortality rates, and for gross lesions. Surviving chickens were killed after 10 days to study the integrity of the bursa of Fabricius.

Preparation of standardized virus challenge material

The isolate LV/G19, 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 l0-day-old SAN embryonating eggs *via* the CAM route with 0.1ml of inoculum according to the method described by Reed & Munch (1938). Briefly, the homogenate was serially diluted 10-fold with sterile nutrient broth in the range of 10^{-1} to10^{-9}. Embryonating eggs in 10 groups of five each received 0.1ml of the homogenate and were incubated at 37 °C for 6 days. The 10th group served as uninoculated control. The eggs were candled daily for dead and dying embryos. The ELD_{50} was calculated following the method of Reed & Munch (1938).

Age susceptibility of chicken standardized IBDV isolate

The SAN chickens in groups of 10, ranging in age from 1 to 10 weeks (Table 2), were challenged intra-occularly with inoculum prepared from the standardized IBDV isolate to check their susceptibility to the virus at the various ages. Each age group had a group of 10 chickens serving

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	10	100	80
Nungua	La Vet	LV/G11	10	100	80
Kasoa	La Vet	LV/GI3	10	100	90
Akim Oda	La Vet	LV/G19	10	100	100
Lashibi	La Vet	LV/G23	10	100	100

NB: Ten uninoculated chickens used because control did not show signs of IBD

TABLE 2

Age Susceptibility of Specific Antibody Negative (SAN) Chickens to Standardized IBD Challenged Virus (Strain LV/G19)

Age (weeks)	** Challenged chicken Morbidity (%)*	*Mortality (%)*
1	60	10
2	100	50
3	100	70
4	100	90
5	80	80
6	80	80
7	20	60
8	20	10
9	20	10
10	20	10

* 10 chickens were challenged in each age group. For each group, 10 chickens served as unchallenged control birds; no morbidity and mortality were observed in these birds.

as uninoculated controls. The challenged birds and the controls were observed for 10 days PI for clinical signs, morbidity, mortality and gross lesions.

Molecular diagnosis

Twenty samples of bursa of Fabricius from the five sites studied were sent to Hipra Laboratories in Spain under strict protocol for transporting biological samples. Samples were pooled into five. Pool 1: ARI (4 samples); Pool 2: LV/G11 (4 samples); Pool 3: LV/G13 (2 samples); Pool 4:LV/GI9 (4 samples); and Pool 5: LV/G23 (4 samples). The samples were analyzed by reverse transcriptase-polymerase chain reaction-restriction fragment length polymorphism (RT-PCR-RFLP) technique at Hipra Laboratories, Spain (M. Bentue, 2005, Personal Communication).

Results

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.

Virus isolation in SAN chickens

After inoculation with the field isolate homogenates, 100 per cent 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 was recorded in the control. There were no gross lesions in the bursae of control birds.

Biological characterization of the isolated IBDV strains

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

Standardization of challenge virus

The embryo lethal dose ELD_{50} recorded for LV/G19 selected for viral challenge studies was $10^{6.3}$

Age susceptibility to standardized isolated IBDV strain

The SAN chickens showed limited susceptibility during the 1st week of life. Table 2 shows morbidity and mortality rates recorded. Susceptibility was highest at the 4th, 5th and 6th week of age. Morbidity and death occurred in SAN chickens up to the age of 10 weeks.

Molecular diagnosis

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

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 such factors as interference with maternal antibodies (Abdu, 1986), improper brooding temperatures and duration (Anon., 1994), poor feed quality and high levels of aflatoxins that prevent vaccine uptake (Anon., 1994), and overwhelming of vaccinal immunity induced by a highly pathogenic field strain of the virus (Okoye & Uzoukwu, 1981). McFerran *et al.* (1980) 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.

Ismail & Saif (1991) also showed that vaccination with one subtype of serotype 1 did not always protect chickens from challenge with another subtype of serotype 1. No such investigations had been conducted in Ghana previously. Therefore, it was important in this work, aimed at identifying an effective immunization programme for chickens against IBD, to isolate and characterize the prevailing field pathotypes in Ghana.

The studies were based on the assertion by Van den Berg, Gonze & Muelemans (1991) that the only criterion for classifying 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 (OIE, 1997).

In reproducing the disease in 3 and 6-week-old SAN chickens, with 100 per cent mortality rates using a field isolate in this study, there are strong indications that the isolates are of the very virulent biotype. Nunoya *et al.* (1992) reported that SAN chickens inoculated with vvIBDV field isolates produced clinical signs typical of IBD and 100 per cent mortality rates. Similar findings were reported by Chettle *et al.* (1989) and Van den Berg (1998) using 3-week-old SAN chickens.

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

In the age susceptibility tests, chickens showed lower susceptibility during the 1st week of age. However, higher morbidity and mortality rates were recorded between the ages of 3 and 6 weeks, although deaths continued up to 10 weeks of age. Cosgrove (1962) attributed resistance of very young chicks to the non-development of the bursa of Fabricius, the main target organ for the IBD virus.

The ELD_{50} of $10^{6.3}$ used in the studies for LV/G 19 was ideal for Gumboro disease challenge work. The STC strain of serotype 1 IBDV standardized for challenge work by Amakye-Anim *et al.* (2000) had a similar ELD_{50} value of $10^{6.3}$.

It is concluded from the above findings that the prevalent IBD virus strain in Ghana now is the vvIBD pathotype. This provides an important basis for selecting vaccines to control the disease in commercial poultry in Ghana. A single vaccination of chicks at 23 days old with an intermediate vaccine as practised by many farmers in Ghana may not be protective against vvIBD. The above findings also provide a basis for formulating an effective vaccination schedule against the disease and producing IBD vaccines in Ghana, using the locally prevalent pathotype.

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