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SERO-PREVALENCE OF INFECTIOUS BURSAL DISEASE IN DAY OLD CHICKS AND IMMUNE RESPONSE OF CHICKS TO VACCINATION AGAINST THE DISEASE

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ABSTRACT

A survey for maternal antibodies to infectious bursal disease virus in day old chicks hatched in Nigeria and the immune response of such chicks to IBD vaccination were studied. Also, agar gel precipitation antibody titre that protects chicks against challenge with Nigerian isolates of the infectious bursal disease virus was determined. Ten chicks were bled in each of 10 hatcheries and their sera separated and used to detect antibodies to infectious bursal disease virus using the agar gel precipitation test. Also, four groups of chicks vaccinated with infectious bursal disease vaccine were bled and their whole blood used for total and differential white blood cells count while their sera were used for quantitative agar gel precipitation test to assess infectious bursal disease virus antibodies. To determine level of humoral immunity that would protect chicks against Nigerian virulent infectious bursal disease virus isolate, groups of chicks with different quantitative agar gel precipitation test titres were challenged with the virus. All 100 sera of day old chicks tested were negative for infectious bursal disease antibodies. Mean humoral immune response of Nigerian chicks to infectious bursal disease vaccination was 9 ± 2.06 while their mean cellular immune response was 216,500 ± 20,900 lymphocytes/µl of blood. There was a direct relationship (r =0.002) between lymphocyte counts in blood of chicks vaccinated against infectious bursal disease virus and infectious bursal disease virus antibody titres (quantitative agar gel precipitation test) in their sera. The results showed that chicks with antibody titres ≥ 8 or ≥ 212,000 lymphocytes/µl of blood were protected against the virulent IBDV isolate.

Keywords: Infectious Bursal Disease, Seroprevalence, Immune Response, Vaccination

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INTRODUCTION

Infectious Bursal Disease (IBD) is an acute, highly contagious viral disease of young chickens [1]. It is characterized by enlargement of the bursa of fabricius and renal damage [2]. These clinical signs were first described by Cosgrove [3] in Gumboro area of southern Delaware, United States of America, hence

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the common name Gumboro disease. The virus was isolated from bursae of infected chicks in embryonated chicken eggs [4].

The first outbreak of IBD in Nigeria was reported from Plateau state [5,6]. Since then, IBD has been reported from all parts of the country. A survey of free range and commercial chickens in Borno state of Nigeria showed a very high prevalence of 67 % [7].The disease has also been reported from all the poultry producing countries of the world except New Zealand [8]. Infectious bursal disease has attracted more research attention than any other poultry disease in recent years because of virulence of the virus and the difficulty encountered in its control [9].

Natural IBD has been reported mainly in fowls and rarely in turkeys, guinea fowls and ostriches [10]. An experimental infection of turkeys and ducks failed to produce clinical IBD but precipitin and virus neutralizing antibodies were detected. Meroz [11] had reported that there is no difference in mortality due to IBD among breeds of chickens. However, differences in mortality have been recorded between the Nigerian local breeds and the exotic breeds of chicken [12].

The economic effects of IBD manifest in two ways; the first is the high mortality rates associated with the clinical disease while the second and more important is the severe and prolonged immunosupression in chickens infected early in life [8,13]. Chickens that survived IBD usually have retarded growth and reduced response to vaccination against other diseases [12]. The consequences of such IBD induced immunosupression have been reported to include flareup of diseases such as gangrenous dermatitis, inclusion body hepatitis – anaemia syndrome, colibacillosis and coccidiosis. Affected broilers take longer days to get to market weight [14].

Infectious bursal disease has defied most control measures. Vaccination which is the principal method of controlling the disease has become of limited success in recent times. Consequently, many countries now adopt new measures in an attempt to control the disease. In China, yolk IgG and hyperimmune serum have been employed for prevention and for treatment of the disease [15]. Researchers in the USA have produced special IBD vaccine by mixing IBD virus strain 2512 with bursal disease antibodies to produce the IBDV – BDA complex vaccine which has been reported to produce immunity even in day – old chicks that have maternal antibodies [16].

There is therefore, need to study factors that affect outbreaks of IBD and the pattern of immune response of chicks to vaccination against the disease in Nigeria, as a step towards developing more effective control measures for the disease.

MATERIALS AND METHODS

Ten hatcheries in Oyo, Ogun, Lagos and Edo States were randomly selected for the study. Ten (10) chicks aged between one and seven days were also randomly selected from each of the hatcheries and bled through the jugular vein or by cardiac punture. The blood samples were deposited in test tubes and kept at 4℃ over night to clot. They were then centrifuged at 3,000 revolutions per minute (for 10 minutes). The sera were used for qauntitative agar gel precipitation test (QAGPT) as described by Herbert [17].

Agar gel was prepared by boiling 1 g agarose, 10 g sodium chloride and 0.1 g sodium azide in 100 ml of distilled water. The molten agar was then poured into Petri dishes and allowed to gel. A well was bored at the centre of the gelled agar in each Petri dish. Six wells were bored round the central well on the agar plate. Each of the serum samples was serially double diluted and each dilution was deposited in a peripheral well round the central well which contained a known virulent IBD virus sourced from the National Veterinary Research Institute, Vom, Nigeria. The setup was then incubated in a humidified incubator, at 37 ℃. It was observed every 24 hours for 5 days, for white precipitation lines between the

central well (antigen) and the peripheral wells (dilutions of sera). The reciprocal of highest dilution of a serum which produced precipitation line with the antigen was recorded as titre for that serum.

In another experiment to determine QAGPT antibody titre that would protect chicks against Nigerian isolates of the IBDV, 6 groups of day old chicks, each of 10 chicks, were used. To get different antibody levels in the various groups of chicks different vitamin medication strategies and repeat vaccinations were adopted. Group 1 was given vitamins for 3 days after vaccination. Group 2 was given the vitamins for 3 days before vaccination. Group 3 was vitamins 3 days before and 3 days after vaccination. Groups 4 and 5 did not receive any vitamin medication. Groups 1 – 4 were vaccinated once while group 5 received repeat vaccination 3 weeks after the first. Group 6 received the vitamin medication and the vaccination was also repeated. Group 7 was not vaccinated and served as control.

Three weeks post vaccination; the chicks in the seven groups were bled before they were each challenged by intra-ocular inoculation with a drop of virulent IBDV (NVRI, Vom, Nigeria).

Pooled serum from each group was used for QAGPT to determine antibody titre for the group while the chicks were observed for clinical signs of IBD and for mortality. Mortality rates and AGPT titres of the different groups of chicks were compared. The lowest AGPT titre that gave zero mortality was taken as the protective QAGPT antibody titre against IBDV in Nigeria.

To study the pattern of immune response to IBD vaccination, four groups of 10 chicks each were vaccinated at 10 days of life and bled 3 weeks post vaccination. Part of each blood sample was used for differential lymphocyte count while the remaining portion was allowed to clot and the serum harvested was used for QAGPT [17]. Mean lyphocyte counts and AGPT titres were compared [18].

RESULTS

Quantitative agar gel antibody titre of each of the 100 chicks screened was zero. Mean immune response of Nigerian chicks following IBDV vaccination was 9 ± 2.06 (QAGPT) and 216,500 ± 20,900 lymphocytes/µl of blood (Tables 1 and 2). Quantitative agar gel precipitation antibody titres of chicks vaccinated with the IBDV vaccine and their lymphocytes counts were directly related ($P < 0.05$).

Table 1. Mortality pattern and antibody titres of day old chicks challenged with a virulent Nigerian isolate of IBDV.

Groups	No. of chicks	Mortality (%)	Antibody titre
1	10	0	32
2	10	80	1
3	10	70	2
4	10	0	8
5	10	0	16
6	10	0	32
7	10	100	0

Discussion

Failure to detect antibodies against IBDV in all ten hatcheries in Nigeria suggests that the chicks lack maternal immunity and are not protected against infection with IBDV. The high percentage mortality recorded among the chicks with AGPT titres of 0, 1 and 2 suggest the lack of maternal immunity as well as vaccine protection and that the birds could come down with clinical disease if exposed. This may be a major contributor to the high incidences of IBD in the country.

Maternally derived antibodies to IBD are very important as they provide considerable protection against the disease in young chicks [19,20,21] . infection with the virus at an early age causes serious immunosupression [22, 23] which may lead to reduced growth rate and increased incidences of other diseases [14].Vaccination of breeder flocks with live IBD vaccine, followed by inactivated vaccines is recomended, to boost antibody titre in the hens so that they can pass high titre of maternal antibodies to their progenies [24]. Giving breeding hens only live IBD vaccine without booster vaccination results in antibody levels that are not high enough to protect the chicks [14].

Baxendale and Lutticken [25],reported that when the level of neutralizing antibodies against IBDV was high in parent stock hens, it persisted throughout the laying period and even chicks from eggs taken at end of lay had sufficient maternal antibodies to protect them against IBDV challenge, up to 4 weeks of age. Vaccinating breeding hens with live IBD vaccine, followed with inactivated IBD vaccine close to start of lay and annual repeat vaccinations, by Nigerian breeder farms, is suggested, so that the hens can transfer enough maternally derived antibodies to protect the chicks during their early weeks of life.

Table 2. AGPT titres and lymphocyte counts of chicks vaccinated with IBD vaccine

AGPT titres	Lymphocyte counts/µl of blood
8	212,000
4	168,000
16	270,000
8	216,000

The results of this study suggest that antiboby levels of AGPT ≥ 8 may be the protective antiboby titre against the virulent Nigerian IBDV isolate. Giving chicks vitamin medication 3 days before and 3 days after the IBDV vaccination and repeating the vaccination are necessary to achieve this levels of immunity/protection [26]. The association between humoral immune response (AGPT antibody titre) and lymphocyte count seen in this experiment is in agreement with Roilf [27] and Stites *et al* [28] who noted that when an antigen enters a host, the body produces both cellular and humoral immune responses specific to that antigen. Humoral and cell mediated immunity develop concurrently [29].

Most laboratories in Nigeria and other countries in the tropics where IBD causes high mortality [30] can run both differential WBC counts and the QAGPT test. Consequently, poultry farmers should be educated on the need to check immune responses of their flocks after IBD vaccination by either the QAGPT or differential WBC counts for lymphocytes/µl of blood. In Nigeria, AGPT titre of ≥ 8 or ≥ 212,000 lymphocytes/ml of blood would protect chicks against IBDV challenge.

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