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SEROLOGICAL STATUS OF UNVACCINATED INDIGENOUS CHICKENS FOR INFECTIOUS BURSAL DISEASE VIRUS ANTIBODY IN ABEOKUTA

[1]O.O. ONI, [2]O.L. AJAYI AND [1]I.T. OGUNYEYE

[1]Department of Veterinary Medicine and Surgery,
[2]Department of Veterinary Pathology,
University of Agriculture, Abeokuta, Nigeria.

ABSTRACT

This study was carried out to evaluate the seroprevalence of infectious bursal disease virus (IBDV) antibody in unvaccinated local chickens in Abeokuta. A total of 58 unvaccinated chickens were randomly sampled, all in Abeokuta area of Ogun State, Nigeria. The indirect Enzyme Linked Immunosorbent Assay (ELISA) was the technique used for antibody detection. The result showed the presence of IBDV antibody in 89.7% of chickens evaluated while 10.3% were negative. It is recommended to isolate and characterize the DNA of IBDV infecting chickens in this region. This finding also implies a need for the vaccination of local or free-range birds to induce vaccine rather than field virus immunity which would prevent or control the spread of infectious bursal disease.

Keywords: Serology, Indigenous chicken, IBDV antibody

INTRODUCTION

Infectious bursal disease (IBD) is an acute, highly contagious viral infection of young chickens that has lymphoid tissue as its primary target with a special predilection for the bursa of Fabricius (Lukert and Saif, 2003). In Nigeria, it is estimated that poultry supplies about 10% of the total meat needs and out of about 150 million poultry birds, 102 million are indigenous (Majiyagbe and Lamorde, 1997). The indigenous village chicken is identified as a way of providing rural women with diverse income earnings and employment (Alexander, 2001). Infections with serotype 1 of infectious bursal disease virus (IBDV) are of worldwide distribution, occurring in all major poultry producing areas. The incidence of infection in these areas is high; essentially, all flocks are exposed to the virus during the early stages of life, either by natural exposure or vaccination (Lukert and Saif, 2003).

Chickens are the only animals known to develop clinical disease and distinct lesions when exposed to IBDV (Lukert and Saif, 2003). Serotype 1 of the virus is known to be the major cause of the disease in chicken, however works by Sivanandan *et al.* (1986) on the infectivity of serotype 2 isolates showed that some of the isolates could be incriminated in eliciting a clinical from of IBD, but serotype 1 is the more important causal agents in chicken (Jackwood and Saif, 1983). Fifty-eight outbreaks of infectious bursal disease virus

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(IBDV) were observed in vaccinated chicken flocks in four southwestern states of Nigeria between 1995 and 2000. Bursa samples from 40 flocks were found virus-positive in VP2 specific nested RT-PCR. Sequence analysis revealed that all 40 Nigerian isolates belonged to the very virulent (vv) variant (Owoade *et al.,* 2004).

The economic importance of this disease is manifested in two ways. First some virus strains may cause up to 20% mortality in chickens, 3 weeks of age and older. The second and more important manifestation is a severe prolonged immunosuppression of chickens infected at an early age (Lukert and Saif, 2003). This study determines infectious bursal disease antibody status of the unvaccinated indigenous village chickens in Abeokuta, Ogun State, Nigeria.

MATERIALS AND METHODS

The Flocks

Backyard poultry birds of mixed age groups from 11 households with bird population ranging between 20 and 150 were used for the study. At least 5% of birds in each household were bled and 5-6 sera samples were randomly selected from these for analysis. None of the birds showed clinical signs of IBD.

Sampling

A total of 58 infectious bursal disease unvaccinated indigenous chicken sera were sampled for the purpose of this study. Blood was collected from birds through the Jugular vein with the use of syringe and 21G needles into sterile Bijou bottles. These were slanted for the blood to clot and placed in ice packs during transportation to the laboratory. Bijou bottles containing clotted blood were then left on the bench for 1 hour at room temperature for sera to separate from the clotted blood. Separated sera were transferred into 1.5ml eppendorf tubes and stored in deep freezers until used.

Enzyme Linked Immunosorbent Assay (ELISA)

Elisa Procedure

The Enzyme Linked Immunosorbent Assay method used was as described by the manufacturer (FlockChek IBD, IDEXX®) of the kits used for the purpose of this study. The kit consisted, IBD coated plates; IBD positive control sera, negative control sera, goat anti-chicken (IgG) horseradish peroxidase conjugate, sample diluent buffer, substrate and stop solution.

Briefly, Dilutions of test sera were made in the sample diluent buffer. Fifty microlitres (50µl) of each including the positive and negative control sera were added into the IBD (antigen) coated plates and incubated for 1 hour at room temperature. The plates were then emptied and washed thrice. Horseradish peroxidase (HRP) conjugate (Goat anti-chicken HRP conjugate) was then added to the wells and allowed to incubate for 20 minutes at room temperature. The wash cycle was then repeated three times at 15 minutes interval. Finally, 50µl TMB substrate was added to the wells at room temperature for color development and stopped after 15 minutes. The optical densities were then measured with an ELISA plate reader at 405nm.

Interpretation of optical density results

Upper limit of negativity (ULN) was determined by adding 0.0155nm to mean O.D value (0.0845nm) of negative control sera.

Any serum with O.D value greater than ULN (0.10nm) is regarded as containing antibody.

RESULTS

From the ELISA results, 52 (89.7%) samples were positive, while 6 (10.3%) samples were negative (Table 1).

Optical density readings ranged from 0.100 -1.060nm and O.D of the negative and positive control sera were 0.0845nm and 0.3285nm respectively (Table 2).

Table 1: Sera samples positive or negative for IBD antibody

Age (weeks)	Number positive	Number negative	Total
24	2	0	2
25	15	1	16
36	35	5	40
Total	52	6	58

Table 2: Distribution of birds on basis of antibody titre level

O.D reading(nm)	Antibody titre level	Number in group
0.100-0.340	118-1966	22
0.341-0.580	3047-4845	12
0.581-0.820	5007-7430	12
0.821-1.060	7678-10404	6
Total		52

DISCUSSION

The result of this study demonstrates serological evidence of infectious bursal disease virus in unvaccinated indigenous chicken. From 58 chicken sera samples, 52 (89.7%) samples were positive, while 6 (10.3%) samples were negative for IBD antibody. The presence of IBD antibody in these birds will either be due to maternally derived antibody or as a result of survival from natural infection. However, maternal antibodies to IBD in unvaccinated chickens persist in chicks up to 21 days as determined by ELISA with complete decay by 28 and 35 days (Zaheer and Saeed, 2003). Antibody detected in these birds cannot be maternally derived because the age range of birds used for this study was between 25-36weeks. Also, since the birds were not vaccinated, it is expected that they would not have antibodies against IBD. With maternally derived antibody and vaccination ruled out, the antibody detected in the birds would have been caused by a field virus, since the birds were on free range. The presence of IBD antibody in unvacci-

nated chicken has also been reported by Vui *et al*. (2002) in Vietnam and also in cattle egrets and pigeon in Nigeria by Fagbohun *et al*. (2000). It should however be noted that there are different strains of the IBD virus. Serotype 1 of the virus is known to be the major cause of the disease in chicken, Works by Sivanandan *et al.* (1986) on the infectivity of serotype 2 isolates showed that some of the isolates could be incriminated in eliciting a clinical form of IBD, but serotype 1 is the more important causal agents in chicken (Jackwood and Saif, 1983). The serotype 2 strains have been predominantly isolated from turkeys. It therefore will be necessary to sequence the DNA of the viruses eliciting antibodies to determine the strain infecting chicken in this region.

The optical density of the positive birds ranged between 0.100-1.060nm. Twenty two birds had optical density readings between 0.100-0.340nm. The optical density of the positive control sera also falls within this group. A higher optical density value with corresponding high antibody titre levels was detected in 30 other positive birds. This implies the field virus is capable of inducing a higher antibody titre level than those of the vaccinated positive control bird. This could have occurred because of constant re-infection of the free range birds with the field virus which is endemic in the environment.

The rate of growth in the poultry industry could have been much more impressive but for several limiting factors such as disease which constitute a unique problem in poultry production (Durojaiye and Adene, 2004). One of such leading disease problem of commercial poultry is IBD (Adene and Akpavie, 2004). This is as seen in this study were unvaccinated birds have high antibody titre against IBD. In such birds mortalities occur, which are not clinically diagnosed. Such mortalities would have been caused by diseases including IBD. Therefore, the potential economic resources of the poultry industry may not be fully utilized until the etiological agent of IBD is controlled among the local village chickens which account for about 90% of the total poultry population in Nigeria (Sonaiya *et al.,* 1999). The local husbandry practices where different species of birds are raised together in the same open range environment encourages cross infection between birds. This cross infection and maintenance of the virus in the environment would also serve as a source of the virus to the commercial poultry farms as reported by Owoade *et al*. (2004) where 58 of infectious bursal disease virus (IBDV) were observed in vaccinated chicken flocks in four southwestern states of Nigeria between 1995 and 2000. From these findings, it may be concluded that there is need to routinely vaccinate local chickens against IBD and restrict their movement especially around the commercial flock. It is also important to fully characterize and identify the strains of IBDV infecting the local birds in our environment.

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