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Serological Status of an Unvaccinated Turkey Flock for Infectious Bursal Disease Virus Antibody in Abeokuta, Nigeria

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ABSTRACT

The serological status of unvaccinated turkey birds to infectious bursal disease virus (IBDV) antibody on a livestock farm in Abeokuta was determined using the Enzyme Linked Immunosorbent Assay (ELISA) technique. A total of 51 turkeys were sampled and the result indicated the presence of IBDV antibody in 37 (72.5%) birds. Since turkeys may serve as carriers of chicken serotype 1 virus, it is recommended that the virus eliciting antibody in the birds be isolated and characterized.

Key words: IBDV antibody, unvaccinated turkeys, ELISA

INTRODUCTION

Infectious bursal disease (IBD) is an acute viral disease of young chickens which was first reported from the U.S.A by Cosgrove (1962) and subsequently from other countries including Nigeria (Ojo *et al.,* 1973; Onunkwo, 1975). Clinical IBD in turkeys has also been reported in Nigeria by Owoade and Durojaiye (1995). Fifty-eight outbreaks of infectious bursal disease (IBD) were observed in vaccinated chicken flocks in four southwestern states of Nigeria between 1995 and 2000. Bursa samples from 40 of these 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). Serotype 1 IBDV was also detected in four symptomatic turkey flocks. The turkey isolates were found within 2 of the 3 VV-clusters of chicken isolates. Full length sequence of a turkey isolate (NIE009t) confirmed its close relation to vvIBDV strain D6948NET for both segment A (1.4% sequence diversity) and segment B (2.1%). Thus, turkeys should be considered to be susceptible to vvIBDV infection (Owoade *et al.,* 2004).

The IBD virus does not affect humans and it is of no public health significance (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 the 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. The serotype 1 isolate has also been recovered from symptomatic turkey flocks; serotype 2 strains have been predominantly isolated from turkeys (Jackwood and Saif, 1983).This study was carried out to determine the IBDV antibody status of unvaccinated local turkeys.

MATERIALS AND METHODS

A total of 51 (9 weeks old) infectious bursal disease unvaccinated turkeys were bled for the purpose of this study. Blood was collected from birds through the Jugular vein with the use of syringe and 21 G 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 1.5 ml Eppendorf tubes and stored in deep freezer until used.

Enzyme linked immunosorbent assay (ELISA)

Indirect ELISA procedure

The indirect Enzyme Linked Immunosorbent Assay method was carried out using FlockChek IBD (IDEXX®)

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kit.

Briefly, Dilutions of test sera were made in the sample diluent buffer. Fifty microlitres (50μl) of each including the positive and negative controls was added into the IBD (antigen) coated plates and incubated for 1 hour at room temperature. The plates were then emptied and washed thrice. Goat anti-turkey horseradish peroxidase (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 tetramethylbenzidine (TMB) substrate was added to the wells at room temperature for color development and 50μl of stop solution added after 15 minutes. The optical densities were then measured using a Sunrise® (Touch screen model) ELISA plate reader at 650 nm.

Interpretation of optical density results

Upper limit of negativity (ULN) was determined by adding 0.0155 nm to mean O.D value (0.0845 nm) of negative control sera. Any serum with OD value greater than ULN (0.1 nm) is regarded as containing IBDV antibody.

RESULTS

From the ELISA results, 37 (72.5%) samples were positive. Optical density readings ranged from low (0.100 - 0.136 nm), medium (0.137 - 0.200 nm), high (0.201 - 0.264 nm) to very high (0.265 - 0.328 nm) when compared with the positive control (0.328 nm).

Twenty five (49%) birds had low OD reading, 10 (19.6%) birds had medium OD reading, while 2 (3.9%) birds had high OD reading. There was no bird with a very high OD reading as shown in Table 1.

Table 1. Number in antibody titre groups.

Antibody titre level	OD reading (nm)	Number in group
Low	0.100-0.136	25
Medium	0.137-0.200	10
High	0.201-0.264	2
Very high	0.265-0.328	0
Total		37

Positive control (OD) = 0.3285 nm; negative control (OD) = 0.0845 nm

DISCUSSION

Natural IBD infection with clinical manifestation in turkeys is very rare worldwide. The only report of IBD outbreak in turkey in Nigeria was by Owoade and Durojaiye (1995). In this study, out of 51 turkey samples, 37 samples were positive, while 14 samples were negative. This implies that the viral antigen eliciting the detected antibodies is a field virus, since the birds were not vaccinated against IBD. Such virus could have also caused mortalities in infected turkeys which would have been misdiagnosed, because of previous reports that turkeys are less susceptible to IBD. The serologic evidence of the antibody however, confirms the presence of IBD virus infecting turkeys in the environment. 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, 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 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.

In a sequential serological study of IBDV serotypes in turkeys in Germany, Neumann *et al.* (1994), observed the turkey dominant serotype 2 in only 2 of 4 flocks examined, and a high proportion of the birds in all the flocks possessed antibodies to the chicken dominant serotype 1. Thus turkeys may serve as carriers for the serotype 1 virus infecting chicken.

Antibodies have been shown to occur in turkey as a result of natural infection (Barnes *et al.,* 1982) and experimental infection with strains of IBD isolate from chickens or turkeys (Giambrone *et al.,* 1978). The antibody titre detected was low in most (25) of the positive birds, while only 2 birds had a high IBD antibody titre. This could be attributed to the less susceptible nature of turkeys to IBDV.

It is recommended that the virus eliciting antibody in turkeys in this region be characterized. This would aid in the control of the disease, since turkeys may serve as carriers of the virus and consequently be a source of infection of the virus to chickens. Also, cross infection and disease between chicken and turkeys could be reduced by proper vaccination and rearing each species separately.

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