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Serum Antibody Levels Against Infectious Bursal Disease (IBD) Virus in Village Chickens Using Indirect Haemagglutination (IHA) Test

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Abstract: The serum antibody levels in village chickens reared in and around Nsukka, Southeast Nigeria was studied using Indirect Haemagglutination (IHA) test. A total of 484 serum samples were collected from these predominantly unvaccinated village chickens and examined. Result showed a high seroprevalence of 88.4%. This indicates the endemic nature of this infection among the village poultry population in the study area. These village poultry are by this result being incriminated as an important factor in the epidemiology of the disease in commercial and exotic chickens where frequent outbreaks occur. Education of the rural farmers and mass immunization of the villagers chickens were suggested as a way of reducing the maintenance and dissemination of the virus in the environment.

Key words: Serum antibody, IBD, village chickens, IHT, Nsukka, Nigeria

INTRODUCTION

Infectious Bursal Disease (IBD) is an acute, highly contagious viral disease of young chickens and it affects the poultry industries worldwide (Hassan *et al*., 1996; Lukert and Saif, 1991). The disease causes heavy economic losses in poultry industries due to immunosuppression in subclinical cases (Domanska *et al*., 2002) and in acute cases; it is associated with mortalities, hemorrhages and also bursal damage (Nakamura *et al*., 1994; Shane *et al*., 1994).

The causative agent is Infections Bursal Disease Virus (IBDV) which is a bisegmented nonenveloped double stranded RNA virus that belongs to the family Birnaviridae (Brown, 1986). The disease is seen mostly in birds between 3 and 6 weeks of age causing severe outbreaks characterized by sudden outset of depression in susceptible flocks (Barlic-Maganja *et al*., 2003). The bursa of fabricius is the target and diagnostic organ which becomes turgid, edematous, swollen haemorrhagic and turns atrophic within 7-10 days in acute cases (Van den Berg, 2000).

The researcher also described dehydration, nephrosis with swollen kidney, haemorrhages in the muscles and mucosa of the proventriculus as common signs in acute cases.

Rural poultry keeping is the dominant form of poultry production in the developing world. In some of these countries, scavenging and backyard chicken production systems are more important than the modern intensive poultry production (Vui *et al*., 2002). These backyard birds are kept in larger numbers in fenced areas or kept in cages while scavenging village birds are allowed to roam around the village in search of feed and go home for only for egg laying and for the night. Nigeria is one of such developing countries and many families especially those in rural communities keep backyard and village chickens. The poultry population of Nigeria have once been estimated to be 150 million (RIM, 1993) and out of this a far greater percentage are believed to be indigenous. Village chickens provide protein in the form of meat and eggs for special festivals, offerings for some traditional ceremonies and serve as a source of income for the payment of school fees and purchase of some food and drugs.

Among many other factors infectious diseases have been an important factor, constraining village poultry production in Africa. Over the years, all attentions have been focused on broiler and other intensively managed exotic birds with regards to the control and preventions of infectious diseases in a bid to improve production and reduce economic losses in this industry. The stakeholders have ignored the fact that local chickens that are not managed intensively are also susceptible to these diseases and contribute significantly in the maintenance and spread of the diseases. Infectious bursal disease which has been diagnosed and identified in Nigeria (Onunkwo, 1975) is one of such diseases. Therefore, there is a constant need to frequently monitor such infection. This present study investigated the prevalence of antibodies to IBD in village chickens in and around Nsukka, Nigeria.

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MATERIALS AND METHODS

Antigen: The antigen used was procured from the National Veterinary Research Institute (NVRI), Vom, Nigeria. The bursae of Fabricius of Infectious Bursal Disease Virus (IBDV) infected chickens were collected, processed and the isolate from the tissues identified as IBDV using IBDV hyperimmune serum.

Serum sample collection: A total of 484 serum samples were collected from village chickens kept at homes in slaughter shops and market places in Nsukka and neighbouring communities. Samples were collected from grower and matured birds. About 2-3 mL of blood was collected per bird through the ulnar vein into a sterile sample bottle and allowed to clot. The samples were thereafter transferred to a refrigerator at 2-8°C and allowed to stand overnight for the clot to retract. The serum samples were harvested and used in an Indirect Haemagglutination Test (IHT) as described by Aliev *et al.* (1990) and Hussain *et al.* (2003) for the detection of antibodies against IBD.

Indirect haemagglutination test

Sensitization of red blood cells: With sterile disposable hypodermic syringe, 5 mL of human blood group O was collected in a bottle containing 4% sodium citrate solution as anticoagulant. The blood was centrifuged at 1500×g for 5 min and the straw coloured supernatant and White Blood Cell (WBC) layers were discarded. The packed RBCS were washed 3 times with Phosphate Buttered Saline (PBS). About 1 mL of washed RBCs was mixed with 1 mL of the antigen and 2 mL of PBS.

The test tube containing the mixture was shaken gently and incubated at 37°C for 45 min. At short intervals during incubation, the test tube was shaken gently to help the erythrocytes come in contact the antigens (ag). After incubation, the sensitized RBC suspension was centrifuged at 1500×g for 5 min to remove the excessive free or loosely attached ag on RBCS's surfaces. The supernatant was discarded. Then PBS was added to the RBCS once more and washed again. The sensitization of the RBC was checked using Slide agglutination test.

Indirect Haemagglutination (IHA) testing: The serum samples examined were inactivated using heat in a water bath at 56°C for 30 min. Then 1% suspension of the sensitized RBCs was prepared in PBS. Using U-bottom microtitre plate and a multi-channel micropipette, 50 μL of PBS was added in a row from well No. 1-12 of the microtitre plate. Then 50 μL of the serum sample was added in well No. 1 and a 2-fold serial dilution made up till well No. 12. Then 50 μL of 1% sensitized RBCS was added in all the wells. Negative and positive controls were set. The plates were gently tapped to ensure even dispersion of the RBCS and then incubated at 37°C for 30 min. The rows were examined for agglutination and the IHA titre of each sample was recorded as the reciprocal of the last dilution showing completed agglutination.

RESULTS AND DISCUSSION

A number of serodiagnostic tests are available for the detection of serum antibodies against IBD (Hussain *et al.*, 2003). Enzyme Linked Immunosorbent Assay (ELISA) and IHT have been reported to be very sensitive in the diagnosis of IBD in chickens (Amin *et al.*, 1999). IHT is cheap and easy to perform. A total of 484 sera were collected from village chickens in and around Nsukka and subjected to IHT for the detection of antibodies against IBD. Out of this number, 428 samples tested positive while 56 samples tested negative. This gave a positive percentage of 88.4%. The 56 samples negative for antibodies against IBD accounted for 11.6% of the total sample. The present study was conducted for the provision of reliable information regarding the actual seroprevalence of IBD in village chickens in Nsukka area. It will also give information on the role played by village chickens in the maintenance and spread of the disease.

The existence of IBD in Nigeria was confirmed by Onunkwo (1978). Since, then IBD have been reported in other parts of the country (Okoye and Uzoukwu, 1982; Durojaiye *et al.*, 1984). Subsequent studies by other scientist in Nigeria show that IBD has acquired an endemic status among the Nigerian poultry population (Oluwayelu *et al.*, 2007). Oni *et al.* (2008) in their study on the serological status of unvaccinated indigenous using ELISA in Abeokuta, Nigeria found a seroprevalence rate of 89.7%. In Ibadan, Fagbolum *et al.* (2000) reported a seroprevalence rate of 36.7% in pigeons and 20% in cattle egrets. Okoye and Uche (1985) demonstrated the presence of IBDV antibodies in the sera of wild rats.

One of the major problems in the development of poultry industry in developing countries is the outbreak of various disease that cause significant mortalities in chickens (Shrestha *et al.*, 2003). Among such diseases in chicken, IBD takes a major position as it causes high morbidity and mortality (Ceribasi *et al.*, 2007). Improvement in the poultry industry should incorporate emphasis on the prevention and control of diseases that cause economic losses. The prevalence of diseases in a particular area will depend on various factors like geoclimatic conditions, biological barriers, age, breed, sex of chickens, immune status of the chickens and social

awareness (Shrestha *et al*., 2003). In Nigeria, village chickens are not vaccinated against most infectious diseases including IBD. Several factors contribute to the lack of vaccination of village chickens against infectious disease in Nigeria and other development countries. One major factor is the lack of social awareness among the farmers on the control and prevention of diseases. Other factors will include lack of veterinary services in the rural areas, unavailability of electricity and power for the maintenance of cold chain for vaccines and other biological in these areas, most of the vaccines come in large doses and are both expensive and too large for the number of birds kept by the farmers. The high poverty level among these rural dwellers makes it difficult for them to take care of their up keep and still buy drugs and vaccines for the low productive village chickens. The absence of preventive measures against infectious diseases in village chickens has lead to the widespread dissemination of these infections among this poultry population. Many of these birds succumb to the infectious and those that survive carry circulating antibodies in their blood. Some become carriers and disseminate the virus in the environment. Their free range roaming system where they scavenge for food encourages thin dissemination.

Village chickens are highly susceptible to IBD (Okoye *et al*., 1999). The seroprevalence of 88.4% as found in this study is high. This indicates that this disease is highly prevalent among the village poultry population in this area. This high prevalence of IBD in village chickens may contribute significantly in the epidemiology of this disease in exotic chickens reared in the same area. Exotic or commercial chickens suffer from sporadic and frequent outbreaks of IBD. This is seen mostly in unvaccinated commercial flocks or in those flocks where poorly stored vaccines have been used. This dissemination of the virus by the village chicken contributes to the maintenance of endemicity and spread of this disease to the commercial birds. Most of the field strains in Nigeria are believed to be the virulent strain of IBDV (Okoye, 1984; Okoye *et al*., 1999). Considering this, the prevention of this disease in village chickens will go a long way to controlling this disease in the environment, reducing the endemicity and therefore, achieving a more productive commercial farming. This will increase the quantity of protein available to every Nigerian table. Government should be involved and should develop policies that will encourage mass vaccination of village poultry populations.

CONCLUSION

The result of the serological survey showed a high prevalence of IBD (88.4%) in village chickens reared in Nsukka area. These birds are predominantly unvaccinated against this disease. Consequently, there is a need for government involvement in education of the farmers and also in mass immunization of this poultry population. This will control this disease in village chickens and reduce their role in the epidemiology of this disease.

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