Comparative evaluation of agar gel precipitation test (AGPT) and indirect haemagglutination test (IHA) for the detection of antibodies against infectious bursal disease (IBD) virus in village chickens

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This study compared the antibody titers to infectious bursal disease (IBD) virus in village chickens using agar gel precipitation test (AGPT) and indirect haemagglutination test (IHA). 484 serum samples were collected from these chickens, which were rarely vaccinated against IBD. In the AGPT, 282 (52.1%) of the serum samples tested positive and in the IHA, 428 (88.4%) tested positive for presence of IBD antibody. IHA was therefore found to be more sensitive than AGPT. IHA was also observed to be comparatively inexpensive and rapid in the diagnosis of IBD especially in developing countries where diagnostic facilities are limited.

Key words: Serum samples, agar gel precipitation test, indirect haemagglutination, IBD, village chickens.

INTRODUCTION

Infectious bursal disease (IBD) was first described in 1962 (Cosgroove, 1962). IBD is one of the most economically important diseases that affect chickens and it possesses a great threat to the poultry industry worldwide (Hussain et al., 2004). The economic importance of the disease is manifested in two ways. Firstly, some virus strains may cause mortalities up to 30% or more in three to six weeks old birds (Hussain et al., 2004). Secondly, and very importantly, the manifestation of severe, prolonged immunosuppression in chickens infected at early age and which have recovered (Van-der-Berg, 2000). The immunosuppression increases the risk of secondary infection and reduces antibody response to vaccinations (Muller et al., 2003). The etiological agent of IBD is a double stranded RNA virus which is non-enveloped and icosahedral in symmetry (Kibenge et al., 1988). The virus is resistant to ether and chloroform, to extremes of PH, high temperatures and some disinfectants, which is why the viral particles persist in poultry farms (Lukert and Saif, 1997). The disease is seen mostly in chickens between 3 to 6 weeks old, and common signs will include rapid drop in feed and water consumption, whitish to greenish-white diarrhea with soiled vent, ruffled feathers and listlessness with unsteady gait or sitting in hunched position, vent picking and sleeping with beak touching the floor. IBD virus primarily affects the bursa of Fabricius, resulting in inflammation and swelling and leading to damage of the organ with consequent immunosuppression (Roy et al., 2008). Biosecurity measures and proper vaccinations are used in the control of IBD (Vieltiz, 1993).

Accurate and rapid diagnosis of IBD is important in order to implement effective control measures. It is also important in the study of the epidemiology and prevalence of the disease in a given area. Serological tests such as agar gel precipitation test (Castello et al., 1987), counter-immunoelectrophoresis (Durojaiye et al., 1985), immunofluorescence (Allan et al., 1984), immunoperoxidase (Johnson and Engstrom, 1986), reverse passive haemagglutination (Nachimuthu et al., 1995), indirect haemagglutination test (Aliiev et al., 1990) and
enzyme linked immunosorbent assay (Cao et al., 1995) have been used in the diagnosis or confirmation of IBD with variable results (Amin et al., 1999). There are however, different degrees in their sensitivity and specificity (Okoye, 1984). In Nigeria, about 90% of the poultry population was estimated to be indigenous or village poultry (Ogundipe, 1998). IBD is endemic in Nigeria and these indigenous or village chickens are highly susceptible to the infection (Okoye et al., 1999). Moreover, in Nigeria, the village chickens are not vaccinated against most infectious diseases including IBD. Therefore, it is important and of immense advantage that any serological technique employed in studying IBD in these birds should be highly sensitive to ensure the detection of disease in most of the infected birds. This study therefore compared agar gel precipitation test (AGPT) and indirect haemagglutination test (IHT) in the diagnosis of IBD in village chickens.

MATERIALS AND METHODS

Collection of serum samples

Growing and mature village chickens in and around Nsukka, Southeast Nigeria were used in this study. A total of 484 birds were sampled from homes, poultry slaughter slabs and live poultry markets. Approximately 3 to 4 ml of blood samples were collected from each bird through the ulnar vein using sterile disposable hypodermic syringes and needles. The blood samples were dispensed into sterile containers and allowed to clot, after which the containers were stored in a refrigerator overnight. Thereafter, the formed sera were harvested into other sterile containers and serum samples were stored at 4°C until used.

Virus (antigen)

The bursa of IBD infected chickens were collected and processed for virus isolation. The isolate from the processed tissue was identified as infectious bursal disease virus (IBDV) using IBDV specific hyperimmune serum. The serum samples collected and the virus antigen prepared were used in AGPT and IHT for the detection of antibodies against IBDV.

Agar gel precipitation test (AGPT)

AGPT was carried out on the serum samples using the methods described by Wood et al. (1979) and Castello (1987). It was performed using immunodiffusion plates with 10 ml of 1% agar noble gel containing 8% sodium azide at pH 7.2 ± 0.1. Using a template and cutter wells of 4 mm diameter and 4 mm interspace (apart) were cut, the plates were set up with groups of 6 wells in a circle surrounding a centre well. The peripheral wells were filled with the serum samples to be tested, while the centre well was filled with the antigen. Known IBD- positive antigen and serum, as well as known IBD - negative serum samples were also added as controls. The plates were incubated at 37°C and read at 24, 48, and 72 h under diffused light. The observations were compared with positive and negative controls. Positive serum samples showed a line of precipitation between the serum and antigen wells, while negative serum samples showed no line of precipitation.

Indirect haemagglutination test (IHT)

Sensitization of erythrocytes

Human blood group O was collected in a sterile bottle containing 4% sodium citrate as anticoagulant using a sterile disposable hypodermic syringe. The blood was separated by centrifuging at 1500 × g for 5 min after which the straw coloured supernatant and white blood cell layers were discarded. The packed erythrocytes were washed three times with phosphate buffered saline (PBS). One part of the washed erythrocytes was mixed with 1 part of antigen and 2 parts PBS. The mixture contained in a test tube was shaken gently and incubated at 37°C for 45 min. At short intervals during incubation, the test tube was shaken gently; this was to help the erythrocytes come in contact with the antigen. After the period of incubation, the sensitized erythrocyte suspension was centrifuged at 1500 × g for 5 min to remove the free or loosely attached antigen on erythrocyte surface and the supernatant was discarded. PBS was added to the RBC once more and washed again. The sensitization of the erythrocytes was checked using slide agglutination test.

Test procedure

The test was performed according to the method of Aliev et al. (1990) and Hussain et al. (2003). The test serum samples were heat-inactivated in a water bath at 56°C for 30 min, and then 1% suspension of sensitized erythrocyte was prepared. Using a U-bottom microtitre plate and a multichannel micropipette, 50 µL of PBS was added in a row from well number 1 to well number 12 of the microtitre plate. Then 50 µL of the serum sample was added in well number 1 and a twofold serial dilution was made up till well number 12. Also, 50 µL of the 1% suspension of sensitized erythrocyte was added in all wells. Negative and positive controls were included in the test. The plates were gently tapped to ensure even dispersion of the erythrocytes and then kept at 37°C for 30 min. Positive serum samples caused a distinct erythrocytic agglutination resulting in a characteristic reticulum settling of the erythrocytes throughout the bottom of the well. The samples that showed peculiar central button-shaped settling of erythrocytes were recorded as negative. In addition, the IHT titre of each serum sample was taken as the reciprocal of its end point dilution.

RESULTS AND DISCUSSION

Out of the 484 serum samples examined, 252 (52.1%) tested positive for the presence of IBD antibody by showing a line of precipitation when examined between 24 and 72 h in the AGPT, while 428 (88.4%) serum samples tested positive in the IHT (Table 1) by showing characteristic reticulum settling of the erythrocytes in the bottom of the wells. The principal aim of this study was to compare the sensitivities of AGPT and IHT in the diagnosis of IBD in village chickens. These chickens are reared on free range system and they fend for themselves with no administration of medication and vaccination. In Nigeria, these chickens are rarely vaccinated against IBD. Though IBD is easily recognized by clinical signs and gross pathological lesions of the bursa of Fabricius with swelling, edema and hemorrhages of the bursa being the characteristic features (Roy et al., 2008). Such lesions in the bursa may not be common in...
Table 1. Comparison of the results of (AGPT) and (IHT) used for the diagnosis of infectious bursal disease in village chickens.

<table>
<thead>
<tr>
<th>Test procedure</th>
<th>Number of serum sample</th>
<th>Number positive</th>
<th>Number of negative</th>
<th>% positive</th>
<th>% negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGPT</td>
<td>484</td>
<td>252</td>
<td>232</td>
<td>52.1</td>
<td>47.9</td>
</tr>
<tr>
<td>IHT</td>
<td>484</td>
<td>428</td>
<td>56</td>
<td>88.4</td>
<td>11.6</td>
</tr>
</tbody>
</table>

Table 2. Indirect haemagglutination antibody titre against IBDV in village chickens.

<table>
<thead>
<tr>
<th>Titre</th>
<th>$2^2$</th>
<th>$2^3$</th>
<th>$2^4$</th>
<th>$2^5$</th>
<th>$2^6$</th>
<th>$2^7$</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number positive</td>
<td>5</td>
<td>24</td>
<td>36</td>
<td>25</td>
<td>22</td>
<td>7</td>
<td>428</td>
</tr>
</tbody>
</table>

some acute and sub-acute cases (Durojaiye et al., 1985). Moreover, other infections such as *Escherichia coli* infections can also produce similar kinds of lesion in the bursa of Fabricius (Venugopalan et al., 1995). Confirmation will therefore require serodiagnosis or demonstration of the virus in infected tissues. Since village chickens are rarely vaccinated against IBD and there are other diseases with similar symptoms in Nigeria, it is important to employ a very sensitive technique for its confirmatory diagnosis. Again, Nigeria like other African countries is still developing and the technology applied in disease diagnosis must be cheap and affordable by most laboratories.

The result of this study showed that IHAT is more sensitive than AGPT in the serodiagnosis of IBD in village chickens. This observation is in agreement with what was recorded previously by some authors. Amin et al. (1999) in their evaluation of some tests for the diagnosis of IBD in broilers, found a sensitivity of 91.53% for IHT and 66.92% for AGPT. AGPT was found to be less sensitive than passive haemagglutination test (PHAT), which is a modification of IHAT for the diagnosis of IBD (Mahmood and Saddique, 2006). AGPT is routinely used in the diagnosis of IBD in Nigeria. The test is relatively cheap and easy to perform and can be carried out by most laboratories and also other investigators. The limitation as can be seen in this investigation is the low sensitivity of AGPT when compared to other tests (Mahmood and Saddique, 2006; DeWit et al., 1992). Moreover, the test will require more than 24 h to run and sometimes up to 72 h especially when the levels of antigens and antibodies are low in the samples.

IBD virus is a non-haemagglutinating virus. However, agglutination can be induced after coating the erythrocytes with specific coupling reagents and sensitizing these coupled erythrocytes with specific hyperimmune serum against IBD virus (Mahmood and Saddique, 2006). This procedure has also been used in the diagnosis of other infections like rinderpest, infectious bronchitis and hydro-pericardium syndrome. This study showed that IHAT is more sensitive, cheaper and more rapid than AGPT. It can be afforded by most laboratories in developing countries as all the reagents required can be sourced or prepared locally. Another advantage is that the test can be performed in one and half to two hours. This is good in IBD as accurate and rapid diagnosis of the disease is important for the implementation of effective control measures that will reduce mortalities and stop spread of infections. Another finding was that it is easier to obtain quantitative results using IHAT than AGPT. The IHAT titre is obtained immediately as the endpoint and this will reveal the level of antibody in the serum (Table 2). To obtain this type of result with AGPT however, further titrations are required and this may further reduce the sensitivity of the test procedure. We also observed that most of the serum samples that were positive by AGPT were the ones that had higher IHAT titres. Therefore, IHAT was able to detect lower antibodies in the serum samples.

Generally, the antibody levels as observed in the village chickens were low. The reason for the low level could be as a result of primary infection of the birds by IBD virus and/or low level of activity of the virus in village chicken population. Moreover, the IHAT assessed both homologous and heterologous antibodies in the local chickens. Amin et al. (1999) were able to get homologous antibody titers of $2^9$ to $2^{12}$ when specific IBD strains were used to sensitizie human group O erythrocytes, and combined IBD antibody titre of $2^5$ to $2^{10}$ in the heterologous system. The result also showed high prevalence of IBD in village chickens in Nigeria.

Conclusion

The IHAT was found to be more sensitive than AGPT in the diagnosis of IBD in village chickens in Nigeria. AGPT is routinely used in Nigeria in the diagnosis of IBD under field and laboratory conditions, but this test has low sensitivity. We therefore recommend the use of IHAT under field condition in Nigeria as this test is cheap, fast and more sensitive than AGPT.

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