STUDIES ON POTENCY OF NEWCASTLE DISEASE AND INFECTION BURSAL DISEASE VACCINES IN EXOTIC BIRDS IN JOS, PLATEAU STATE, NIGERIA

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Abstract: The potency of some of the Newcastle Disease Vaccines - Lasota (NDV-L) and Infectious Bursal Diseases Vaccines(IBDV) were determined in sixty-six (66) exotic white leghorn birds. The vaccines were tested by culturing in bacteriological media and using Hemagglutination Test (HA), Hemagglutination Inhibition Test (HI), Egg Infective Dose 50% (EID50), Tissue Culture Infective Dose 50% (TCID50), Neutralization Tests (NT) and Agar Gel Precipitation Test (AGPT). The Vom vaccines gave the best results compared to the foreign vaccines. Vom NDV-L had 512 HA units and 10^-7.25 EID50 pre-vaccination titer compared to Ornibur vaccines with 128 HA units and 10^-6.25 ELD50 liters. Also in the post vaccination antibody responses, Vom NDV-L had 1024 HI, 512 in AGPT and 2048 NT liters as compared to Biovacc and Ornibur ND-L with 256 HI, 64 and 128 in AGPT, and 1024 and 512 NT antibody liters respectively. After challenges with field strains, 81.82% birds and 72.73% birds survived with Vom and Biovac vaccines respectively while Ornibur vaccines had 64.64% and 54.55% birds survived with NDV and IBDV. We wish to advocate the use of indigenous vaccines in protecting poultry birds. However, the foreign vaccines may be used where the indigenous vaccines are not available.

Key words: Vaccines, Hemagglutination, Antibody, NDV, IBDV.

INTRODUCTION:

Newcastle disease (ND) is a contagious viral fatal disease which affects all ages and species of birds (Alexander, 1995; Olabode and Chukwuego, 2005). Over the years the diseases have occurred in both vaccinated and unvaccinated birds which may suggest the failure of the live vaccine used in poultry against the disease (Olabode and Chukwuego 2005; Ibu et al., 2009). The ND and IBD are dreaded diseases to both exotic and local village birds with devastating consequences on the poultry farmer (Onunkwo and Momoh 1981, Chukwuego et al., 2006). ND is caused by an enveloped single stranded ribonucleic acid virus of the paramyxoviridac family (Spradbrow, 1993-94). The symptoms include dullness, cough, sneezes, gasping, depression, prostration and profuse greenish charrheos (Alexander, 1991; Olabode et al, 1992).

Infectious bursal disease (IBD) is caused by a non-enveloped, double stranded RNA virus, belonging to the genus avibirnavirus and Birnaviridae. In infected birds, it is highly contagious, appearing suddenly and spreading rapidly with symptoms of depression with ruffled feathers, diarrhoea, dehydration, trembling prostration followed by death (cosgrove 1962, Okey and Uzoukwu, 1982a; Chukwuego et al, 2006). The disease is worldwild and was first reported in Nigeria in 1973 and confirmed in 1975( Ojo et al., 1973; Onunkwo, 1975). Morbidity and mortality could be as high as 30-50% in naïve birds (Saif et al., 2000).

In Nigeria poultry production has witnessed a very rapid growth in the recent times due to high demand of poultry

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products for the increase human population (Chukwuedo et al 2006; 1bu et al, 2009). Newcastle disease and infection bursal disease are the most important economic diseases posing major problems in the poultry industry. There have been many reported cases of post vaccination disease outbreaks due to ND and IBD. Meanwhile prevention is by vaccinations of birds using live attenuated vaccines developed through passages in chicken embryos. Due to reports of post vaccination outbreaks, this study was designed to check the potency of the commonly used commercial poultry vaccines in Plateau State.

MATERIALS AND METHODS:

Vaccines: The Vaccines were purchase from registered Veterinary clinics (Evangelical church of West Africa (ECWA) clinic, Bukuru in Plateau state and National Vet. Res. Institute (NVRI- Vom) in Jos South Local Government, Plateau state. The vaccine brands include: Vom-IBDV, Vom-NDV-Lasota vaccine, Bio-NDV-Lasota and Bio-IBDV vaccines ( from Israel), Ornibur – NDV and IBDV vaccines (from Czech. Republic).

Anti-Serum and Antigen: The Standard specific Antiserum and Antigens of NDV and IBDV were obtained from the Virology Research Department of National Vet. Res. Institution Vom, Plateau State.

Sterility, Safety and Potency Tests: Sterility check of the vaccines was carried out in artificial bacteriological media (Blood agar, Nutrient agar, MacConkey agar, Sabouraud broth).The vaccines were reconstituted as per manufacturer guideline and 50ul of each of the vaccines were cultured in the media, incubated at 37°C for 72hrs. and checked for evidence of contaminations. In safety test, each vaccine suspension was inoculated into eleven (11) exotic white legions (4 to 8 weeks old). The vaccines were given orally through the chlorine free drinking water. Morbidity and Mortality were checked each day until they were bled for antibody assay before the birds were disposed.

Hemagglutination Test (HA): The U-shaped polystyrene disposable plates with 96 wells were used for the hemagglutination test. The phosphate buffered saline (PBS pH 7.2) was used as diluent. The ND vaccines and the antigens were diluted in 2 - fold serial dilution in PBS. 50ul of the vaccine and antigen suspensions were diluted serially from wells 2 to 12 while well 1 contained only the neat samples. 50ul of 1% chick red blood cells was added to all well including the controls. The plates were incubated at + 4°C for 45 minutes and later read.

Egg Infection Dose (EID<sub>50</sub>): The EID<sub>50</sub> test was carried out in 9 days old chick embryonated eggs. Two holes were drilled on the egg shelf (one on the air space and the other 3mm below the air region). The 10 - fold serial dilutions of the vaccines and space the antigen were prepared in PBS, PH 7.2 (10<sup>-1</sup> to 10<sup>-10</sup>). 0.1ml of each dilution was inoculated into each egg and five eggs per dilution. The eggs were sealed and incubated for 48 to 72hours after which they were chilled at 4°C over night, spot tested with 10% chick rbc and scored as positive or negative. The EID<sub>30</sub> was determined by Reed and Muench (1938) simple virus estimation method.

Hemagglutination Inhibition Test (HI): The HI test was carried out in U-shaped disposable polystyrene plates. 50ul of PBS, pH 7.2 was added to well 2 through to well 12. The serum was added to well 2 and diluted serially in 2-fold serial dilution to well 12. The well 1 contained only the neat serum samples. The four hemagglutinating unit (4 HAUnits) of the virus was added to all well and mixed thoroughly by gentle tapping at the four edges including the controls. The tests were incubated at 4°C for 60-90 min. after which the 1% chick red blood cell was added to all wells. The plates
were again incubated at 4°C for 45-60 min. after which the tests were read and scored.

**Agar Gel Precipitation Test (AGPT):** Seven (7) wells of 3mm in diameter were made on semi solid agar gel in petri dishes. 6 wells were arranged in a circular form with one well at the centre. The adjacent wells contain the various dilutions of the anti-sera while the antigen was placed in the central well. The distance between two antiserum wells were arranged in a circular form with one well at the centre. The adjacent wells contain the various dilutions of the anti-sera while the antigen was placed in the central well. The distance between two antiserum wells were 4mm. the wells were filled with the corresponding reagents (avoiding spillage). The plates with the controls were incubated at room temperature for 48-72 hours in a humidified chambers and as well as at 37°C incubator.

**Neutralization test (NT):** The antiserum – IBD virus neutralization assay was carried out by mixing equal volume of IBD test serum (dilutions) with virus suspension (0.5ml each) in separate tubes, incubated at 37°C for 60-90 minutes in B and T (searle Co.) incubator. The mixtures were inoculated into chicken embryo fibroblast cells in monolayer and in suspensions and incubated for 24-96 hours at 37°C. The culture were observed for the presence or absence of cytopathic effect (CPE). The controls (infected and uninfected monolayers) were also set up along with the test.

**Tissue Culture Infective Dose 50% (TCID50):** The tissue culture infective dose 50% was carried out in chicken embryo fibroblast (CEF) monolayer in cell culture tubes. 0.1ul of the various IBDV suspensions were serially diluted in PBS, pH 7.2 using 10 Fold serial diluted (10^1 to 10^-10). Five tubes were inoculated per dilution and the tubes were incubated at 37°C in humidified (merrmnet) incubator for 96 hours. The tubes were observed daily for evidence of cytopathic effect (CPE) and the virus titre was determined by Reed and Munch (1938) virus estimation method.

**Challenge of Birds:** The experimental and the control birds were all challenged with Hertz 33 strain of Newcastle disease virus (virulent strain) for the NDV vaccines birds while the birds given the IBD vaccines were challenged with the wild field strain of the infectious bursal disease virus. The challenged virus was inoculated intramuscularly through the thigh muscle. All the birds were monitored for a period of two weeks for morbidity and mortality before they were disposed.

**RESULTS :**

The results of the sterility and safety tests on the vaccines were presented in Table 1. All the vaccines were sterile with no microbial growth. The safety test shows that in Vom-ND-L vaccine, Bio-ND-L vaccine and Ornibur ND-L vaccines all the birds survived, 11/11 (100%) respectively. In the IBDV vaccines, 9/11 (81.82%) and 8/11 (72.73%) survived in Bio-IBDV, Vom-IBDV and Ornibur-IBDV vaccines respectively (Table 1).

The pre- vaccination vaccine antigen titres showed that VOM-ND-L vaccine HA-titre was highest with 512/0.05ml unit while the Ornibur vaccine has the least (128/0.05ml unit). The EID50 was highest in Bio-ND-L vaccine with 10^8.5/0.01ml followed by Vom-ND-L vaccine with 10^7.25/0.01ml while ornibur had the least with 10^6.25/0.01ml. The tissue culture infective close 50% (TCID50) was highest in Vom-IBDV vaccine with 10^-6.0/0.1ml while Bio-IBDV vaccine with 10^-6.3/0.01ml had the least. The pre- vaccination HI, AGPT and the neutralization tests antibody titres in the birds were less than 2 (Table 2).

The post- vaccination antibodies titres in the vaccinated birds showed that Omibur ND Lasota and Bio-ND Lasota vaccines had 256HIunits antibody titres respectively while Vom-ND Lasota had the
highest titre of 1024 HI units. In AGPT assay, vaccines had 512, 256 and 128 antibody titres respectively (Table 3).

**Table 1: Shows Sterility and Safety Test of Vaccines**

<table>
<thead>
<tr>
<th>Media</th>
<th>BA</th>
<th>MCA</th>
<th>NA</th>
<th>TGB</th>
<th>SDA</th>
<th>Safety Test in chicken (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vom-ND-L vacc.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>11/11 (100%)</td>
</tr>
<tr>
<td>Bio-ND-L vacc.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>11/11 (100%)</td>
</tr>
<tr>
<td>Ornibur-ND-L vacc</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>11/11 (100%)</td>
</tr>
<tr>
<td>Vom-IBDV vacc.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8/11 (72.73%)</td>
</tr>
<tr>
<td>Bio-IBDV vacc.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9/11 (81.82%)</td>
</tr>
<tr>
<td>Ornibur-IBDV vacc</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8/11 (72.73%)</td>
</tr>
</tbody>
</table>

**Key:** L = Lasota, IBDV = Infectious bursal disease vaccine, Vac= Vaccine, BA = Blood agar, NA = Nutrient agar, MCA = MacConkey agar, SDA = Sabouraud dextrose agar, TGB = Thioglycolate broth.

**Table 2: Shows Vaccines Antigens Titres and Pre-Vaccination Antibodies Titres in Birds**

<table>
<thead>
<tr>
<th>Test</th>
<th>NVRI ND-L</th>
<th>BioVac ND-L</th>
<th>Ornibur ND-L</th>
<th>NVRI IBDV</th>
<th>BioVac IBDV</th>
<th>Ornibur IBDV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccine Antigen titles</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HA</td>
<td>512</td>
<td>512</td>
<td>128</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>EID50</td>
<td>10^{-7.25}</td>
<td>10^{-8.5}</td>
<td>10^{-6.75}</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TCID50</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10^{-8.0}</td>
<td>10^{-6.5}</td>
<td>10^{-7.0}</td>
</tr>
<tr>
<td>Pre-Vaccination Antibody titres</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HI</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AGPT</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
<tr>
<td>NT</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
</tbody>
</table>

**Table 3: Shows Post-Vaccination Antibody Titres and Birds survival After Challenge**

<table>
<thead>
<tr>
<th>Test</th>
<th>Vom ND-L</th>
<th>BioVac ND-L</th>
<th>Ornibur ND-L</th>
<th>Vom IBDV</th>
<th>BioVac IBDV</th>
<th>Ornibur IBDV</th>
</tr>
</thead>
<tbody>
<tr>
<td>HI</td>
<td>1024</td>
<td>256</td>
<td>256</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AGPT</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>512</td>
<td>256</td>
<td>128</td>
</tr>
<tr>
<td>NT</td>
<td>256</td>
<td>128</td>
<td>64</td>
<td>1024</td>
<td>1024</td>
<td>512</td>
</tr>
<tr>
<td>Post-Vacc. Challenge Survivals</td>
<td>(81.82)</td>
<td>(72.73)</td>
<td>(64.64)</td>
<td>(81.82)</td>
<td>(72.73)</td>
<td>(72.73)</td>
</tr>
</tbody>
</table>

**Key:** ( ) = % percentage
DISCUSSIONS:

Newcastle disease (ND) and infectious bursal disease (IBD) are enzootic in Nigeria (Fatunbi and Adene, 1979; Gomwalk et al., 1985). In this present study the vaccines culture in bacteriological media showed that the various vaccines were sterile hence there are no contaminants that will interfere with the birds immune responses to the vaccine inoculations and the birds showed over 70% safety rates (Table 1).

The neutralization antibody assay was 1024 in both Vom-IBD and Bio-IBD while Ornibur-IBD vaccine gave 512 titre, less by one log. The results of the post-vaccination challenges of the birds given the different ND and IBD vaccines, showed that protection was best with Vom vaccines, 9/11(81.82%) followed by Bio vaccine with 8/11(72.73%) while the Ornibur vaccine had the least with 7/11(64.64%) in the ND challenge (Table 3).

The neutralizing antibody titres were quite above the base line titre of 64 required to protect the birds (NVRI Vaccine Manual, 1984). The vaccines maintained their quality and integrity due to proper storage and handling by the various clinics. In the post-vaccination antibody assay, the birds showed good responses to the various vaccines as well as survival after post vaccination challenge. However, Vom-vaccine both ND and IBDV vaccines gave the best results compared to the Israel and Czech Republic vaccines. The lower protection rates obtained from the foreign vaccines may be attributed to differences the antigenic properties of the vaccine antigens compared to the challenge with the indigenous strains in the field responsible for the outbreaks. This agreed with Alexander (1995) who reported that indigenous strain in vaccines protect better than the foreign vaccines. Also Nawathe and Lamorde (1987) strongly advocated the use of indigenous vaccine in protecting poultry bird against viral diseases. It also inline with the results of previous similar studies carried out by Chukwuedo and Olabode (2005) in Nigeria on IBD and ND vaccines.

Over 70% of the birds given Vom and Bio vaccines survival post vaccination challenges with ND and IBD field strain viruses. In Ornibur vaccines, 64.64% with NDV and 54.55% with IBDV survived post vaccination challenges. The results strongly suggests that foreign vaccines do not adequately protect our birds against ND and IBD viruses (Table 2). The foreign vaccines may continue to receive patronage because they are cheaper, available with high doses. Sometimes, ineffectiveness or failure of the vaccines may be attributed to poor storage and handling by vaccine vendors who are not authorized by law to handle and administer vaccines. Other factors responsible for vaccine failure may include, time of vaccination, nutritional and immune status of the birds, route of vaccination and endemicity of the disease.

Fatunbi and Adene (1979), has demonstrated that type of feed (low protein feed) and Chukwuedo and Mbakwe (2007) has demonstrated in their study that nutritional status of the birds played a vital role in the birds responses to vaccination and disease resistance. However, efforts should be made to regulate and control the importation and handling poultry vaccines in the country to ensure that they are properly maintained and potent at the time and point of use. From the results of this study, evidence has shown that the local and the imported foreign vaccines are efficacious. We therefore recommend that, there should be more legislative laws and proper control measures in the importation, handling, storage and administration of poultry vaccines in Nigeria.

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REFERENCES: