EVALUATING THE PROTECTIVE EFFICACY OF A LIVE AND TWO KILLED VACCINES AGAINST INFECTIOUS BURSAL DISEASE IN COMMERCIAL CHICKS IN ZARIA, NIGERIA

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ABSTRACT

This study evaluated and compared the efficacy of a live (intermediate plus strain) and two killed (produced from intermediate plus and intermediate strains of viruses) infectious bursal disease vaccines in commercial Isa brown cockerel chicks. Two hundred and eighty, one day old chicks were divided into four groups of seventy chicks each. Group 1, 2 and 3 were vaccinated with live vaccine, killed vaccine A (produced from intermediate plus strain) and killed vaccine B (produced from intermediate strain) respectively while group 4 was not vaccinated. Vaccination was conducted at 9 and 16 days of age with group 1 vaccinated orally and groups 2 and 3 vaccinated intramuscularly via the leg muscles. Blood was collected from 15 chicks in each group weekly from 2 to 44 days of age. Twenty chicks from each group were challenged with a very virulent infectious bursal disease virus (vvIBDV) at 30 days of age. Agar gel precipitation test (AGPT) performed on the sera revealed that the group vaccinated with live vaccine had the highest percentage of chicks with antibody at all ages followed by group 2 (killed vaccine A). Precipitin antibodies were not detected in chicks in groups 3 (killed vaccine B) and 4 (unvaccinated) at all ages. Enzyme-linked immunosorbent assay (ELISA) performed on the same set of sera revealed that
vaccination with live vaccine induced the highest antibody titre (2,757±897) followed by group 2 (893±458) then group 3 (404±179) and group 4 (331±168) had the least. ELISA titre in group 1 was significantly different from groups 2, 3 and 4 at p ≤ 0.05 while group 2 was significantly different from groups 3 and 4 at p ≤ 0.05. There was no significant difference between the ELISA titre of groups 3 and 4. Chicks in group 1 and group 2 did not exhibit clinical disease and no mortality was recorded following challenge. Chicks in group 3 and group 4 manifested clinical disease and mortalities and gross lesions were seen upon postmortem examination. The mortality rates in groups 1 to 4 were 0, 0, 55, 65 per cent respectively. In conclusion, the live vaccine and killed vaccine A were efficacious. The killed vaccine B did not fully protect the chicks against IBD. Live vaccine and killed IBD vaccine produced from intermediate plus IBDV strain are recommended for vaccination in Nigeria.

1 Introduction

One of the major constraints to the development of the poultry industry is the outbreak of diseases (Rashid et al., 2003; Mbuko et al., 2010). Among the various diseases that cause significant losses in chicken in developing countries, infectious bursal disease (IBD) takes a major position. Infectious bursal disease has been of great concern to the poultry industry for a long time, particularly in the past three decades (Lukert & Saif, 2003; Mbuko et al., 2010).

The disease is caused by a bi segmented, double stranded RNA virus which belongs to the family Bibrnaviridae (Lukert & Saif, 2003). After infection, the virus destroys lymphocytes and macrophages as a result suppresses the immune system leading to vaccination failures, breaks and concurrent infections (Lukert & Saif, 2003). Infectious bursal disease causes economic losses through morbidity, mortality and increased susceptibility to other diseases as a result of its immunosuppressive effect (Owoade et al., 2004).

Improvement in the poultry industry should incorporate emphasis on the prevention and control of diseases that cause economic losses (Okwor et al., 2009). The use of conventional live vaccines for the control of IBD has not yielded the desired results as outbreaks of IBD in flocks that have been vaccinated with live vaccines have been reported and are still being reported in Nigeria (Abdu, 1986; Owoade et al., 2004; Mbuko, 2010). Vaccination and good biosecurity measures still remain the most effective method of prevention and control of IBD (Chansiripornchai & Sasipreeyajan, 2009; de Wit & Baxendale, 2013).

There have been reports of immunosuppression due to some live IBD vaccines (Rautenschlein et al., 2007). Killed vaccines which are not commonly used, minimally affected by maternal antibodies and not known to spread, mutate or revert to virulence and may prevent the vaccination failures in Nigeria. Information regarding the comparison between live and killed IBD vaccination in Nigeria is scarce. It was therefore necessary to investigate the role of killed vaccines in the prevention and control of IBD and to recommend an effective vaccination program for chickens to suit the poultry industry in Nigeria.

2 Materials and Methods

2.1 Experimental chickens

Two hundred and eighty one day-old Isa brown breed of cockerel chicks were purchased from a commercial hatchery in Ibadan, Nigeria. The chicks were fed on chick mash purchased from a commercial feed distributor. The feed contained 22% crude protein, 4.2% fibre and 2,800 kilo calories of energy.

2.2 Vaccines

A live (intermediate plus strain) and two killed (produced from intermediate plus strain and intermediate strain) commercially available infectious bursal disease vaccines were purchased from an agro-veterinary company in Ibadan, Nigeria.

2.3 Challenge virus

The infectious bursal disease virus used for challenge was a very virulent strain obtained from previously vaccinated commercial layers that died of a natural outbreak of IBD. Sixty five per cent of commercial cockerels inoculated at 30 days of age with 50 µL of bursal suspension (v/w) in PBS (pH 7.4) from the cockerels died. One millilitre of bursal suspension (v/w) in PBS (pH 7.4) contained 10^{6.5} CID_{50} of IBDV.

2.4 Housing and Feeding

The chicks were housed in an experimental animal house and divided randomly into four groups (groups 1, 2, 3 and 4) of 70 chicks per group, housed under deep litter system. Water and feeds were provided to the chicks ad libitum.

2.5 Experimental Design

Group 1 was vaccinated with a live vaccine; group 2 was vaccinated with killed vaccine A, group 3 was vaccinated with killed vaccine B while group 4 remained unvaccinated and served as the control. Vaccination was conducted at 9 and 16 days of age with the live vaccine administered orally (diluted to 0.5mls per chick) and killed vaccine injected intramuscularly (0.5mls) via the leg muscles. The chicks were
challenged at 30 days of age with vvIBDV and observed for clinical signs and mortality.

2.6 Agar Gel Precipitation Test

2.6.1 Agar gel

Agar gel was prepared by weighing out 1.3 g of agarose (Life Technologies, USA), 80 g of sodium chloride, 0.2 g of sodium azide and these were reconstituted in 100 ml of distilled water. The sodium chloride was boiled for 2 minutes and later autoclaved at 115°C for 15 min. According to modified method of Abdu (1997), the agar plate was made by dispensing 25.7 ml of agar into 9 × 9.5 cm petri dishes to give a depth of 3 mm. Wells of 6 mm width with 3 mm separation were cut into already set agar. A parallel six vertical rows of 6 wells with a middle row having three wells which are at the centre of 4 peripheral wells were created on the agar gel plate using a template.

2.6.2 Precipitation Test

Agar gel precipitation test (AGPT) as described by OIE (2008) was performed to detect IBD antibody in the sera. The central wells were filled with 0.02 ml of positive antigen and 0.02 ml of the test serum was dispensed into the wells in the parallel vertical columns. The petri dishes were incubated in a humid chamber at room temperature and observed after 24 and 48 hours for precipitin lines.

2.6.3 Enzyme-linked Immunosorbent Assay

The enzyme linked immunosorbent assay (ELISA) technique was carried out according to the methods described by IDEXX Laboratories Incorporation, USA. Briefly the antigen coated plates and the ELISA kit reagents were adjusted at room temperature prior to the test. The test sample was diluted to five hundred folds (1:500) with sample diluents prior to the assay. A 100 µl of diluted sample was then placed into each well of the plate. This was followed by 100 µl of undiluted negative control into the well A1 and A2, 100 µl of undiluted positive control into well A3 and A4.

The plate was incubated for 30 minutes at room temperature. Each well was then washed with approximately 350 µl of distilled water 3 times. Goat anti-chicken conjugate (100 µl) was dispensed into each well. The plate was incubated at room temperature for 30 minutes, followed by washing each well with 350 µl of distilled water 3 times. Tetramethylbenzidine (TBM) solution (100 µl) was dispensed into each well. The plate was then incubated at room temperature for 15 minutes. Finally 100 µl of stop solution was dispensed into each well to stop the reaction. The absorbance values were measured and recorded at 650 nm. Infectious bursal disease antibody titre was calculated automatically, using software by Blankfard & Silk (1989).

2.7 Challenge of Chicks with Infectious Bursal Disease Virus

Twenty from each group were challenged and each chick was inoculated with 0.05 ml of a virulent IBDV suspension containing 10⁴.9% CID₅₀ per ml via conjunctival instillation at 30 days of age.

2.8 Determination of Mortality Rate

Mortality rate recorded in different groups after challenge with IBD virus at the end of the experiment was calculated as number of chicks dead divided by number of chicks challenged multiplied by 100 as described by Babiker et al. (2008).

\[ MR = \frac{NCD \times 100}{NCC} \]

Where NCD = Number of chicks dead, NCC = Number of chicks challenged.

2.9 Statistical Analysis

Descriptive statistics was used to express agar gel precipitin antibodies in chicks, morbidity rate, mortality rate and mortality pattern. Using Blankfard and Silk software, ELISA antibody titre levels were reduced to means and standard deviations. Coefficients of variation (CV) were calculated in percentages. With the aid of Graph Pad Prism, body weight, were subjected to a one-way analysis of variance (ANOVA). Statements of statistical significance were based on p ≤ 0.05. Variations among groups were determined using Tukey’s comparative test.

3 Results

A few (6.7%) of the chicks in group 2 had precipitin antibodies at 2 days of age while they were not detected in chicks in other groups. Precipitin antibodies were not detected at 9 and 16 days of life of the chicks in all the groups. At day 23, 33.3% and 46.7% of chicks in group 1 and group 2 respectively had precipitin antibodies. At 30 days of age, 92.9% and 53.3% of chicks in groups 1 and 2 had precipitin antibodies. At 37 days of age, 80.0% and 46.7% of chicks in groups 1 and 2 had precipitin antibodies respectively. Precipitin antibodies were not detected at 44 days of age in the chicks in all the groups (Figure 1).

Group 1 had the highest mean ELISA antibody titre, (2,757±897) followed by group 2 (893±458), then group 3 (404±179) at 30 days of age. Group 4 had the lowest antibody titre (331± 168) at 30 days of age (Table 1). There was a significant difference (p < 0.05) in the ELISA titre of group 1 and the other groups. There was also a significant difference (p < 0.05) in the ELISA titre between group 2 and group 3 and 4. The coefficient of variation (CV) in the chicks in group 1 was 32.5%, 51.3% in chicks in group 2, 44.3% in the chicks in group 3 and 50.7% in the chicks in group 4 at 30 days of age.

Chicks challenged with vvIBDV at 30 days of age showed whitish-yellowish diarrhoea, anorexia, depression, ruffled feathers and somnolence. These signs were observed in chicks
in groups 3 and 4 commencing from the 2nd day post challenge with eighteen chicks in group 3 and all the 20 chicks in group were 4 sick (Figure 2). On the 3rd day post challenge, no chick was sick in groups 1 and 2, nine chicks in group 3 and 10 chicks in group 4 were sick. On the 4th day post challenge, one chick was sick in group 3 and four chicks were sick in group 4. On the 5th and 6th days post challenge, no chick was sick in group 3 but two chicks were sick in group 4. By the 7th day post challenge, the chicks in all groups had fully recovered. Mortality commenced on the 2nd day post challenge with one chick in group 3. Mortality peaked on the 3rd day with eight chicks in group 3 and 10 chicks in group 4 and declined on the 4th day with two chicks in group 3 and three chicks in group 4. By the 5th day post challenge chicks in all groups were fully recovered. Mortality rate of 55% and 65% were recorded in groups 3 and 4 respectively (Table 2). Of the chicks that died, the gross lesions seen in chicks in group 3 and 4 included; enlarged bursa, spleen, kidneys (with prominent tubules), and liver; distended gall bladder; empty crop; oedematous bursa; congested bursa, thymus, spleen, muscles, duodenum, pancreas, lungs and trachea; haemorrhages in the bursa, duodenum, jejunum, ileum; and skeletal muscles (Plate 1).

Figure 1 Agar gel precipitin antibody in chicks vaccinated at 9 and 16 days with different infectious bursal disease vaccines (Key: Group 1: Live vaccine, Group 2: Killed vaccine A).

Table 1 ELISA antibody response of chicks before and after vaccination (9 and 16 days) with a live and two killed infectious bursal disease vaccines.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean antibody titre before vaccination (day 9)</th>
<th>% CV day 9</th>
<th>Mean antibody titre before challenge (day 30)</th>
<th>% CV day 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Live)</td>
<td>1162±654</td>
<td>56.3</td>
<td>2757±897a</td>
<td>32.5</td>
</tr>
<tr>
<td>2 (Killed A)</td>
<td>1209±1461</td>
<td>30.8</td>
<td>893±458b</td>
<td>51.3</td>
</tr>
<tr>
<td>3 (Killed B)</td>
<td>1230±537</td>
<td>43.6</td>
<td>404±179c</td>
<td>44.3</td>
</tr>
<tr>
<td>4 (Unvaccinated)</td>
<td>927±497</td>
<td>27.3</td>
<td>331±168c</td>
<td>50.7</td>
</tr>
</tbody>
</table>

The data are ELISA titres ± Standard Deviations (SD). a, b and c Means with different superscripts within column differ significantly (p ≤ 0.05).
Figure 2 Number of chicks showing clinical signs after vaccination at 9 and 16 days of age and challenge with very virulent infectious bursal disease virus at 30 days of age (Key: Group 3: Killed vaccine B, Group 4: Unvaccinated).

Plate I Diffused haemorrhages in the leg muscles of chicks in group 4 that died after challenge with very virulent infectious bursal disease virus.
lesions observed in this study have been reported earlier in vvIBD outbreaks (Lukert & Saif, 1997; Abdu, 1997; Abdu, 2007; Cereno, 2013; de Wit & Baxendale, 2013). The morbidity rate of up to 100 per cent and mortality rate of up to 65 per cent recorded in group 4 was highly suggestive of vvIBD. An indication that the chicks were highly susceptible to IBD at the time of challenge. The chicks in the killed vaccine B vaccinated group had a lower morbidity and mortality rate compared to the unvaccinated group. It therefore appeared that vaccination reduced the impact of clinical signs, lesions and mortality following exposure to vvIBD. This study’s findings agrees with those of Lone et al. (2012) who reported a 65% mortality rate in unvaccinated control chicks challenged with vvIBD virus and Otsyina et al. (2009) who reported an 80% mortality rate in unvaccinated control chicks challenged with vvIBDV. In the findings of this study, the course of mortality after challenge of the chicks with vvIBDV followed the normal course of disease in vvIBD; a peak between 2-3 days post infection and receded in a period of 5-7 days (Lukert & Saif, 1997; Abdu, 2007; de Wit & Baxendale, 2013). This further confirms the fact that the chicks died as a result of IBD infection and possibly, there was no complicating pathogen.

In conclusion, the live vaccine and killed vaccine A were efficacious. The killed vaccine B did not fully prevent the chicks against IBD. The live vaccine and killed vaccine produced from intermediate plus IBDV strain are recommended for vaccination in Nigeria.

References
Babiker MAA, Tawfeeg E (2008) Role of administration routes of anti-infectious bursal disease virus (Gumboro)

Table 2 Mortality rates post challenge of chicks with very virulent infectious bursal disease virus at 30 days of age.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number challenged</th>
<th>Number dead</th>
<th>Mortality rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>11</td>
<td>55</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>13</td>
<td>65</td>
</tr>
</tbody>
</table>

KEY: Group 1: Live vaccine vaccinated group; Group 2: Killed vaccine A vaccinated group; Group 3: Killed vaccine B vaccinated group; Group 4: Unvaccinated group.

4 Discussions

Chicks screened for antibodies to IBDV at 2 days old showed that they had a protective ELISA antibody titre level to IBD which dropped at 16 days of age. This finding agrees with the that of Babiker & Tawfeeg (2008) and Abdu (1986) who reported a drop in MDA after 17 days and Hair-Bejo et al. (2004) who reported a drop in MDA levels at 14 days of age. The presence of MDA could be attributed to passive transfer of antibodies from parent to chicks. The implication of this finding is that if the chicks are exposed to IBDV at 2 days of age, they will not succumb to the disease.

The live vaccine evaluated in this study was more immunogenic than the two killed vaccines because it elicited the highest ELISA antibody titre level and the highest percentage of chicks with precipitin antibodies were in the group vaccinated with the live vaccine. There was also a faster seroconversion rate after vaccination with the live vaccine compared to the killed vaccines. This finding is in contrast with the findings of Begum et al. (2004) who reported a higher antibody titre level and highest percentage of chicks having precipitin antibodies in chicks vaccinated with inactivated IBD vaccine compared to chicks vaccinated with live IBD vaccines. The implication of the present finding is that if the chicks were exposed to field challenge, chicks vaccinated with the live vaccine will be better equipped to face the challenge.

The fact that less than 10 per cent of chicks in group 2 had detectable MDA by AGPT at 2 days of age and antibodies were not detected in chicks in other groups but were detected at 2 days of age in the ELISA can be attributed to the fact that AGPT is not a very sensitive test (Harris, 2010). Our inability to detect precipitin antibodies in groups 3 and 4 throughout the course of the experiment can also be attributed to the less sensitive nature of the test. This is in conformity with the findings of Lone et al. (2012) who evaluated the efficacies of live attenuated IBD vaccine and inactivated oil emulsion IBD virus vaccines in broiler chicks and did not detect precipitin antibodies in chicks at 2 days of age but antibodies were detected using ELISA.

The relatively higher % CV seen in chicks in group 2 suggests lack of uniformity in the antibody titre and this can be attributed to the fact that chicks in that group had the highest MDA titre and possibly, variable levels of antibodies and vaccinations conserved this variation. Clinical signs and gross
Evaluating the protective efficacy of a live and two killed vaccines against infectious bursal disease in commercial chicks in Zaria, Nigeria.


Blankfard M, Silk BC (1989) Enzyme-linked immunosorbent assay software R. Gaithersburg, Md. USA.


