COMPARISON OF THE SAFETY, IMMUNOGENI CITY AND POTENCY OF LA SOTA AND V4 NEWCASTLE DISEASE VACCINES

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ABSTRACI
Some Poultry vaccines found in Nigeria are imported. Therefore, there is a need to compare the safety, immunogenicity and potency of some of the vaccines used to control Newcastle disease (ND) particularly La Sota ND vaccines and the V4 ND vaccine. A total of 120 day — o Id pullets were obtained from a commercial hatchery at Kaduna. The pullets were brooded for 3 weeks (wks), after which they were divided into 12 groups each, consisting 10 pullets. Groups 1, 2, 3 and 4 were vaccinated using one field dose with ND La Sota vaccine from four different sources, namely National Veterinary Research Institute (NVRI), Vom, Intervet (Netherland), Shafit (Israel), and Fort Dodge (United States of America). Group 5 were accinated with one field dose (0.2 mis) of ND V4 vaccine. Groups 6, 7, 8 and 9 were vaccinated with 10 times the field doses (2 mis) of La Sota vaccines from the four different sources, while group 10 were vaccinated with 10 times the field dose(2 mis) of ND V4 vaccine, Group 11 served as the challenged control, while group 12 served as the unchallenged control. At 3 weeks post vaccination (PV), the pullets in groups 1—11 were challenged with virulent strain (Hert's "33") of Newcastle disease virus (NDV). The mean antibody (Ab') titres at 3 wk PV for groups 1, 4 and 5 were: 5.4 ± 0.7 log2, 7.0 ± 0.8 log2 and 5.0 ± 0.8 log2 respectively. The morbidity rates post challenge (PC) with Hert's "33" strain of NDV were 70%, 20%, and 100% for groups 1, 2 and 11, respectively. The postmortem lesions such as congestion of the trachea, Necrotic foci in the intestinal tract and the ceacal tonsils seen in group 11 were those typical of the velogenic viscerotropic ND (WND). There is the need to improve on the quality of the locally produced La Sota ND vaccine by NVRI.
Key words: Pullets, La Sota, V4, Vaccine, Newcastle disease

INTRODUCTION

The first confirmed outbreak of Newcasle disease in Nigeria was in 1953 by Hill *et al.* (1953). Newcastle disease is said to be the major disease affecting the development of the poultry industry in Nigeria (Ambali *et al.,* 1995). The disease is also a major problem to the production of village chicken in developing countries like Nigeria (Spradbrow, 1988; Alexander, 1997). It is also known to cause severe economic losses in many poultry producing areas of the world (Philips, 1973; Lancaster, 1981). In countries like Nigeria, ND is enzootic (Fatumbi and Adene, 1979; Adu *et al.,* 1986; Echeonwu *et al.,* 1994; Sa'idu *et al.,* 1994; Alders and Spradbrow, 2001). It is therefore necessary to vaccinate commercially reared birds in order to reduce the risk of outbreak ofND and also to minimize production losses. Vaccination against ND has been practiced worldwide since the development of ND vaccines in 1940 (Her and Dobson, 1940; Philips, 1973; Marlikayev *et. al.,* 1988; Mestecky and McGhee, 1989; Rajiswar and Masillamamy, 1991; Jackson, 1992). The most popular live ND vaccines are developed from the "lentogenic viruses. La Sota ND vaccine belongs to this group (Alexander, 1995). The immune

response following live ND vaccines is related to the virulence of the virus strain (Reeve, 1974). For instance, La Sota ND vaccine gives better protective antibody litres than Hitchner Bl ND vaccine (Shamaki *et al.,* 1989). A more recent vaccine, the V4 ND vaccine (thermostable) was introduced to other parts of the world from Australia. Thereafter a number of studies have been conducted on the vaccine and it has been reported to be highly immunogenic by seversi workers from different parts of the world (Kirn, 1977; Ibrahim, *et al.,* 1981; Spradbrow *et al.,* 1988; Ambali *et al.,* 1995; Usman, 2002; Nwanta, 2003). Some poultry vaccines in use in Nigeria are imported from different parts of the world such as Europe, United States of America, Asia and Middle East. Despite the availability of ND vaccines at affordable prices in Nigeria, ND has continued to be a problem in vaccinated and unvaccinated commercial poultry (Halle *et al.,* 1999). This study was therefore designed to compare the safety, immunogenicity and potency of ND La Sota vaccines from four different sources and ND V4 vaccine.

MATERIALS AND METHODS

Experimental birds

One hundred and twenty (120) day — old Bovans brown pullets were obtained from a commercial hatchery in Kaduna. The pullets were brooded for 3 wks in a clean house. At 3 wks of age, the pullets were divided into 12 groups each, consisting of 10 pullets and were housed in 56.5 cm x 56.5 cm wire floored cages in 4 different rooms. Groups 1, 2, 3, 4 and 5 were housed in room 1, Groups 6, 7, 8, 9 and 10 were housed in room 2, Groups 11 was housed in room 3 and Group 12 was housed in room 4. Separate caretakers were assigned to each room. The birds were fed on a commercial chick mash up to the end of the experiment.

Vaccines

The La Sota ND vaccines were bought from Agro Veterinary shops in Kaduna and Kano. The V4 ND vaccine was obtained from the National Veterinary Research Institute (NVRI), Vom, Nigeria. The titre per dose for the ND La Sota vaccines and the V4 ND vaccines used were as follows: The titre per dose of La Sota ND vaccine from NVRI was 10^{8} ° Embryo Infective Dose 50, (EID5o),while the titre per dose of La Sota ND vaccine from Intervet was 10^{6} ° EID 50, the titre per dose for La Sota ND vaccine from Shafit was $10^{6\ 5}$ EID 50, La Sota ND vaccine from Fort Dodge had titre per dose of $10^{6\ 5}$ EIDso and the titre per dose for ND V4 vaccine from NVRI was $10^{6\ 8}$ EID 50

Vaccination

Two hundred doses of ND La Sota vaccines from each of the 4 sources and ND V4 vaccines were diluted with 40 ml of sterile physiological saline (PBS), 0.2 ml of the diluted vaccine represented one field dose of the vaccine. Each of the chicks in all the first four groups were vaccinated with 0.2 ml of ND La Sota vaccine from sources 1, 2, 3, 4 and ND V4 for group 5 per os. Chicks in groups 6—9 were vaccinated with 2 ml of ND La Sota vaccine from sources 1, 2, 3 and 4 per, os respectively, while chicks in group 10 were vaccinated with 2 ml of ND V4 per os. Chicks in groups 11 and 12 were not vaccinated, but 0.2 ml ofPBS was administered to each chick in these groups per os.

Challenge virus The Hert's "33" strain of the ND virus with titre of lO^EU^o per dose was obtained from **NVRI.Vom,** Nigeria.

Challenge of Experimental Birds

At 3wks PV all the pullets in groups 1—11 were challenged with 0 .2ml (10^{96} EID50) of the Hert's "33" strain ND virus intramuscularly at the breast muscles. The chicks were observed daily for clinical signs and mortality on daily basis for 4 wks PC. Postmortem examination was conducted on any pullet that died and the lesions observed were recorded. The morbidity and mortality rates were calculated. The protection rates were also calculated for each of the vaccines. Serum samples The birds were bled through the wing vein using 5ml syringe and 21G needle on weekly basis, for 3 wks PV and for 4 wks PC. Serum was extracted from the sample and stored at

-20°C until used. Preparation of Chicken red blood cells (RBC) Five milliliter of chicken blood was collected into 5 ml of Alsever's anticoagulant solution and gently mixed. The RBC was washed 3 times with phosphate buffered saline (PBS) pH 7.4 by centrifugation at 1400 g for 5 minutes. A 0.25% suspension ofRBC was prepared by mixing 0.05 ml fRBC in 20 ml of PBS for use in haemagglutination (HA) and haemagglutination inhibition (HI) tests (Allan and Gough, 1974).

Antigen

La Sota ND vaccine obtained from the N V R I, Vom, Nigeria was used as the antigen for the HI test. The HA titre of the antigen was determined as described by Allan and Gough (1974).

Serological test procedure

Haemagglutination Inhibition (HI test)

The HI test was used for the detection and quantification of antibodies against NDV in the sera as described by Allan and Gough (1974). The HI titre of each bird was determined and expressed in log 2 and the mean for each group was calculated.

Statistical Analysis

Duncans multiple range test (DMRT) was employed to compare the mean ND HI titre of the various groups before vaccination, PV and PC (Harnett and Murphy, 1974; SAS, 1987)

RESULTS

The prevaccination mean NDV, Antibody titre in all the groups ranged from 0.6 ± 0.7 log2 -1.9 ± 2.1 log2 (Table l). The mean NDV Antibody titre for the pullets a week PV was: 1.7 ± 1.9 log2, 1.7 ± 2.5 log2 and 1.4 ± 1.3 for groups 1, 4 and 5 respectively. The litres at 3 wk PV were: 5.4 ± 0.7 log2, 7.0 ± 0.8 log2 and 5.0 ± 0.8 log2 for groups 1, 4 and 5 respectively (Table l). The mean HI titre for groups 8, 9, 10 and 11 were:, 2.5 ± 1.4 log2, 2.6 ±0.1 log2, 2.4 ± 0.8 log2 and 0.9 ±0.1 log2, respectively a wk PV. The titres at 3 wks PV were, 8.7 ± 0.5 log2 and 0.4 ± 5.2 log2 for groups 8 and 11 respectively (Table 1). The clinical signs observed in group 11 were somnolence, diarrhoea, droppy wings and tail feathers, ruffled feathers, coughing, sneezing and facial swelling. The pullets in group 4 had diarrhoea, somnolence, ruffled feathers. The chicks in groups 2 and 5 had diarrhoea, droppy wings and tail feathers, ruffled feathers and congested eyes. The pullets in group 1 had diarrhoea, somonolence, droppy wings and tail feathers, ruffled feathers, cloudy eyes, sitting on the hock, torticollis, clonic spasm and moving backward. There was drop in feed and water consumption in all the challenged groups (Table 2). The post mortem lesions seen in all the groups are shown in Table 3 The lesions seen in groups 1 and 11 were enlargement and congestion of liver and kidneys, congested trachea, thymus, lungs and skeletal muscles, presence of urates in the ureters, haemorrhages in the proventriculus, and rectum, haemorrhages with necrotic centres in the duodenum, jejunum, ileum and cecal tonsils. The lesions seen in groups 2, 4 and 5 were enlargement and congestion of liver, spleen and kidneys, congested skeletal muscles, haemorrhages in the duodenum, jejunum, and rectum, enlarged and haemorrhagic caecal tonsils The morbidity rate post challenge with virulent NDV Hert's "33" was 20%, and 100% in groups 2 and 11, respectively. The mortality rate was 20% and 80% in groups 2 and 11, respectively. The protection rate was 75%, and 25% in groups 2 and 5, respectively while protection rate was 100% in groups 3, 6, 7, 8, 9 and 10, respectively. (Table 4). The mean ND litre a week PC was 10.0 log2, , 8.1 ± 0.8 log2 and 0.4 ± 0.7 log2 for groups 6, 5 and 12, respectively. At 2 wk PC the mean titre was 10.0 log2 for all the challenged groups. While at 4 wk PC the mean titre was 9.6 ± 0.6 log2, 9.7 ± 0.5 log2, and 8.0 ± 1.8 log2, for groups, 6, 4 and 5, respectively (Table 5).

DISCUSSION

In this study, the immune response produced by the V4 ND vaccine was significantly low ($P < 0.05$) at 3 weeks PV. This result contradict the reports of other workers were high titres were produced by birds vaccinated with the V4 ND vaccine (Westbury, 1984; Ambali *et al.,* 1995).

This may explain why high morbidity and mortality rates were recorded in birds vaccinated with V4 ND vaccine in this sturdy. Clinical signs such as diarrheoa, somnolence, and drop in feed and water consumption and ruffled feathers were first noticed in chicks vaccinated with La Sota ND vaccines from NVRI and Fort Dodge before those vaccinated with Intervet vaccine.

This may be a function of the protection rate of the La Sota vaccine from NVRI. Because, the protection rate is higher for the Intervet vaccine than the rates for both NVRI and Fort Dodge vaccines. Therefore the birds vaccinated with the the Intervet La Sota were able to resist challenge with the Hert's "33" virus better than the groups vaccinated with NVRI and Fort Dodge La Sota vaccines. At three wk PV, the NDV HI titre was higher than the minimum level (3.0 log2) for protection against mortality (Philips, 1973) in all the pullets vaccinated with 10 times the normal dose of the vaccines from all the 4 sources and V4 vaccine. Despite the high HI Antibody titre, morbidity rate of 6% and 10% were noticed in chicks vaccinated with vaccine from Fort Dodge and V4 vaccine. Which means that even at higher doses ND vaccine from Fort Dodge and V4 vaccine were not able to protect against morbidity. This observation is different from what was reported by Heath *et al.* (1992) that V4 and V4 HR vaccines confer 100% protection to birds against challenge with Hert's "33/56" strain of ND virus. The observed higher protection rate of all the imported La Sota ND vaccines from Intervet, Shafit and Fort Dodge, despite the fact that the Vom ND La Sota vaccine had the highest EID50 may be due to differences in production procedures or the immunogenicity of the isolates used, while all the imported vaccines were produced using the eggs of specific pathogen free chicks. The Vom vaccines are not produced using the eggs of specific pathogen free chicks. The presence of Antibodies to ND in the egg yolk is known to adversely affect the titre of the vaccine (Nawathe, 1985; FAO, 1992). At higher doses (10 times the one field dose) the protection was very high (100%) in all the ND La Sota vaccines (both local and imported) including the V4 vaccine meaning that chicks vaccinated with La Sota ND vaccines or V4 ND vaccine at higher doses may be able to resist challenge with field strain of NDV. The NDV Ab' litres after challenge were very high in all challenged chicks, particularly in the unvaccinated challenged chicks. This finding is similar to what was reported by Philips (1973) that following challenge with ND virus the NDV Ab' titre was usually high even as high as 11.0 log2. The clinical signs seen in unvaccinated challenged pullets such as coughing a nd sneezing, facial swelling and cloudy eyes were signs seen in velogenic ND outbreak (Alders and Spradbrow, 2001; Abdu *et al.,* 2004). In the present sturdy, the nervous signs were only observed in vaccinated groups and not in unvaccinated group. This may indicate that the disease was probably acute in the unvaccinated challenged group while it was chronic in the vaccinated groups. This is similar to what was reported by earlier workers (Fatumbi and Adene, 1984; Abdu *et al.,* 1999; Alders and Spradbrow, 2001), that the nervous signs tend to denote chronic infection ofND and recovery from the disease. The postmortem lesions such as congestion of trachea, necrotic foci in the intestinal tract (duodenum, jejunum and ileum) and necrotic foci in the ceacal tonsils seen in the unvaccinated challenged pullets and those vaccinated with ND La Sota vaccine from NVRI were those of the velogenic viscerotropic form of ND, (Alexander, 1995; Alders and Spradbrow, 2001; Abdu *et al.,* 2004). Those seen in birds vaccinated with vaccines from Intervet, Fort Dodge and ND V4 vaccine were generalized congestion of the viscera and some haemorrhages in the gastro — intestinal tract. This finding showed that the La Sota vaccines from Intervet and Fort Dodge and the ND V4 vaccine were able to reduce the severity of the postmortem lesions. It was concluded that the vaccine from Shafit was the best in terms of stimulating Ab' production, protection against morbidity and mortality, and that at the recommended dose ND V4 vaccine was less immunogenic than ND La Sota in pullets raised intensively.

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