Evaluation of Efficacy of LaSota® Vaccine against Circulating Newcastle virus strains from Morogoro, Tanzania

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SUMMARY

Newcastle disease (ND) outbreaks in flocks vaccinated with LaSota® vaccine have been reported around Morogoro municipality. This study was conducted to evaluate the efficacy of commercially available LaSota® vaccine against virulent strains of newcastle disease virus (NDV). One hundred day-old chicks were randomly allocated to five groups of 20 chicks each. Group I and II were vaccinated at the age of 5 days through oral and ocular routes, respectively, and boosted at the age of 20 days. Groups III and IV were vaccinated once through oral and ocular routes, respectively, at the age of 10 days. Group V served as a negative control. Immune response against NDV was measured by the level of antibodies using Haemagglutination Inhibition (HI) test and resistance to challenge with virulent strain of NDV. All birds were challenged with virulent NDV at 32 days of age and monitored for 21 days. Regardless of the route, there was no statistical significant difference ($p > 0.05$) between the mean HI titres in the four vaccinated groups. Significant differences, however, existed between regimes ($p < 0.05$). The morbidity and mortality in vaccinated birds were 20% and 10% respectively, while in un-vaccinated birds the corresponding values were 95% and 65%. In conclusion, the used LaSota® strain ND vaccine available in Morogoro, Tanzania produced enough protection against ND. Both oral and ocular routes provided the same level of protection, however, regardless of route of vaccination, booster dose produced higher level of protection.

Key words: Newcastle disease, oral and ocular routes, layer chicks, vaccination regime, antibodies

INTRODUCTION

Poultry farming forms an important economic activity in Tanzania especially in rural settings with estimated total of 42.0 million birds (MoALF, 2016). It contributes about 19% of the total meat consumed in the country (Anonymous, 2008) thereby improving nutrition and reducing household food insecurity especially in rural communities (Knueppel *et al*., 2010). However, poultry productivity is hampered by diseases and other factors. Newcastle disease (ND) being considered the most important cause of death in chickens of all ages in Tanzania and other developing countries (Foster *et al*., 1997).

Limited biosecurity measures expose the poultry to infectious diseases like ND (Yongolo *et al*., 2002). The disease remains a major threat to the development, sustainability and profitability of both village and commercial poultry industry (Munir *et al*., 2012; Waheed *et al*., 2013; Ashra and Shah, 2014). It is caused by virulent strains of avian paramyxovirus – 1, being transmitted by direct or indirect contact with infected birds (Ashra and Shah, 2014). The disease has extremely high morbidity and mortality rates of nearly 100% in unvaccinated flock (Shabbir *et al*., 2013). Newcastle disease has no treatment; however, the use of prophylactic vaccines and biosecurity control measures reduces the likelihood of the disease outbreak (Komba *et al*., 2012; Waheed *et al*., 2013; Shabbir *et al*., 2013; Ashra and Shah, 2014). Different types of vaccines have been developed, these include Lentogenic vaccines: Hitchner-$B_{1,}$ La Sota, V_4 NDW, I-2 and F, Mesogenic vaccines: Roakin, Mukteswar and Komarov (Senne *et al.,* 2004). Types of vaccines and vaccination schedules used vary depending on the potential threat, virulence of the field challenge virus, type of production, and production schedules (Senne *et al.,* 2004). In Tanzania, the vaccines used for control of Newcastle disease virus (NDV) are LaSota® and I-2. LaSota® vaccine is commonly used in commercial poultry farms and I-2 in the village chickens. Chickens are routinely vaccinated against NDV through drinking water (LaSota®) and intraocular eye drops (I-2).

For a long time lentogenic LaSota® vaccine has been used by farmers in the country for prevention of ND (Temba, 2013 - person communication). The oral route of vaccination through drinking water is the commonly used method. The vaccine can also be applied via other routes such as the ocular (eye drop) and spray. The recommended regime for ND vaccination in the country is to vaccinate birds at

day three and boost them at 21 days of age and then booted every after three months. Other regimes employed elsewhere include vaccinating the birds once at day 10 followed by three monthly boosters. For safe recommendation on continue use of LaSota® vaccine for ND control it was important to evaluate the effects of different routes and regimes of administration of the vaccine on its effectiveness against NDV infection in the country and this was what prompted to conduct this study.

Materials and methods

Study area

This study was conducted at the College of Veterinary and Medical Sciences, Sokoine University of Agriculture (SUA), Morogoro, Tanzania from November, 2013 to January 2014. Chicks were housed at the poultry unit in the Department of Animal Science and Production.

Source of birds and group formation

Day old Red star chicks (n = 100) were purchased from a commercial hatchery located in Dar es Salaam. At day two they were individually tagged with numbered wing tags and then randomly assigned to five groups of 20 birds each. Groups were labeled I, II, III, IV and V. Groups I, II, III, and IV the treatment groups and V the control group. They were raised on deep litter and fed on commercial feeds according to manufacturer's instructions; and water was made available *ad libitum.* Sample size was estimated according to OIE protocol for testing the potency of ND live vaccine and inactivated oil vaccines (OIE, 2004). All the vaccination and sampling procedures were performed at the chicken units.

Vaccination schedule

Vials of freeze dried live ND vaccine-LaSota strain (Biovac Ltd, City, Israel) were purchased from veterinary products supplier in Morogoro. At 5-days of age, birds in the treatment group I and II were vaccinated through oral and ocular routes, respectively. At 10 days of age, birds in the treatment groups III and IV were vaccinated through oral and ocular routes, respectively. At 20 days of age, birds in the treatment group I and II were vaccinated with a booster dose of LaSota®. Birds in the control group were not vaccinated. All groups were vaccinated against infectious bursa disease at 14 days of age.

Challenge virus and challenge procedure

The NDV strain used as challenge virus in this study was isolated from chickens in Morogoro in 2012 disease outbreak. At 32-days of age, each bird was inoculated with 0.1ml of inoculum intramuscularly in the thigh muscle. The parameters used to evaluate protection from NDV were the levels of HI geometric mean titer, development of clinical signs and mortality. Protection was mornitored for a period of 21 days after challenge. In addition, postmortem examination was carried out for dying birds.

Sample collection and handling

Blood samples were collected from all chicks on day 3 to test for the presence of NDV maternal antibodies prior to vaccination. On day 20 blood samples were collected and used to estimate the levels of NDV antibodies from birds in groups I and II post vaccination. Birds in group III and IV were sampled on day 25 for the same purpose. Birds in all study groups were blood sampled on 30th day of age to determine the pre-challenge NDV antibodies levels. In all sampling occasions the samples were collected from the wing vein. Blood samples were allowed to clot at room temperature to separate serum which was stored at -20 and later subjected to HI test.

Serological analysis of sera samples

Haemagglutination Inhibition (HI) test based on the inhibition of viral agglutination by the specific serum antibody was performed. The protocol developed by Allan and Gough (1974) was adopted.

Data analysis

Data were cleaned in Microsoft Excel® and analyzed in StatView® computer software. The geometric mean antibody titers between groups and within groups were analyzed using Student t-test. A p-value equal to or less than 0.05 was considered significant.

RESULTS

Haemagglutination inhibition (HI) titration results

Pre-vaccination results

Serological analysis of sera samples collected on day three of age indicated that all birds were negative for NDV antibodies. This implies that none of the birds was exposed to NDV.

Post-vaccination results

Post-vaccination analysis of sera samples was conducted for all birds regardless of the route and regime of vaccination. For the control group the HI test geometric mean titers (GMTs) were still negative for NDV antibodies (Table 1). For the vaccinated groups different proportions of birds attained protective levels of antibodies against NDV as shown on Fig. 1. The differences in proportions among the groups were not statistically significant (P>0.05). The HI GMT for individual groups are presented in Table 1. The lowest protective haemagglutination inhibition titre among the vaccinated birds was $\log 2^2$ titre units. Comparisons of the GMTs between vaccinated groups revealed significant differences as follows; titres were higher for oral boosted as opposed to oral unboosted group (p=0.0376), oral unboosted and ocular unboosted (P=0.2037), oral boosted vs ocular boosted (P=0.2083), higher for ocular boosted as opposed to ocular unboosted group (p=0.0028).

Table 1. Geometric mean NDV antibody titers in chicks vaccinated with live NDV LaSota® strain vaccine using different routes and regimes

Treatment groups	Geometric titer 14 days after first vaccination	Geometric mean titer before challenge
Group I ORB	58	66.4
Group II OCB	60.4	106.8
Group III ORUB	49.6	29
Group IV OCUB	72	18.6
Control	-	4.3

Legend: NDV – Newcastle disease virus, ORB – Oral boosted, ORUB - Oral unboosted, OCB – Ocular boosted and OCUB – Ocular unboosted

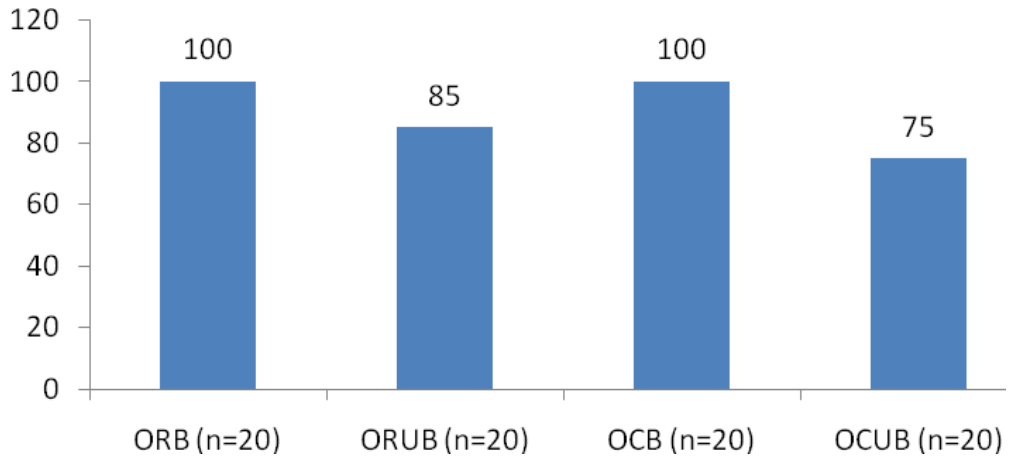

Figure 1. Proportions of protected chicks against NDV following vaccination with LaSota® vaccine (Pre-challenge results). ORB – oral route boosted, ORUB – oral route unboosted, OCB – ocular route and OCUB – ocular route unboosted.

Challenge results

Morbidities and mortalities

Two days post challenge two birds in the control group developed clinical signs of ND and died on day seven. Other birds started showing clinical signs on day four post challenge. On the fourth day

3 - 4 birds in all vaccinated groups were depressed, had greenish diarrhoea and later developed nervous signs. The frequently observed clinical sign in most of the affected birds was greenish watery diarrhea. Other clinical signs included depression, paralysis of legs and wings and elevated head, ruffled feathers, dropped wings, reluctance to move, anorexia, difficulties in breathing, coughing, sneezing, gasping and torticollis (Fig. 2A & B). During post - challenge observation period the recorded morbidities were: 95% in control group, 20% in oral boosted, 20% in ocular boosted, 15% in oral unboosted and 20% in ocular unboosted, while mortalities were 65%, 10%, 10%, 5% and 5% in control group, oral boosted, ocular boosted, oral unboosted and ocular unboosted, respectively. All the morbidities in the control group occurred within 7 days post challenge.

Figure 2. Newcastele disease clinical signs post-challenge; A - torticollis and B – depression.

Post-mortem lesions

Newcastle disease lesions were petecheal or ecchymotic haemorrhages on intestinal and cloacal mucosa, haemorrhages on proventricullus and cecal tonsils (Fig. 3C & D), pneumonic lungs, severe tracheitis and enteritis. Diagnosis of ND was confirmed by HI test from blood samples and internal organs from sick and dying birds.

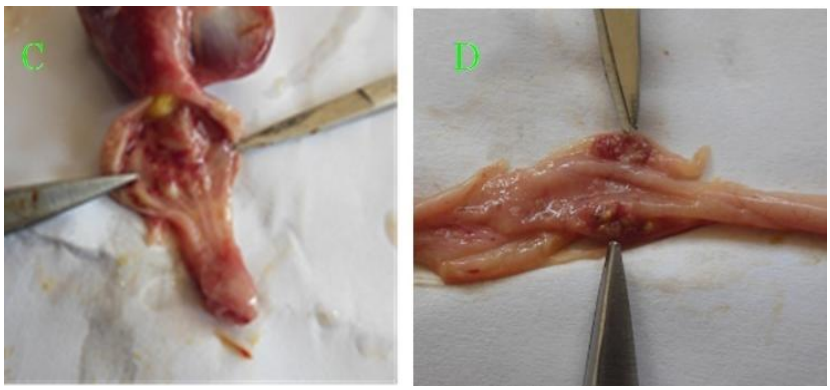

Figure 3: Post-mortem lesions in birds that died of Newcastle disease displaying haemorrhagic lesions; C - proventricullus, D - caecal tonsils.

DISCUSSION

This study demonstrated lack of significant differences on level of antibodies devepoled against NDV following LaSota® vaccination through oral and ocular routes in chicks and protection following challenge using locally circulating virus.

These results are consistent with findings from previous studies which reported lack of statistical significant differences on level of antibodies in

chickens vaccinated by the two routes (Allan *et al.*, 1978; Orthel *et al.*, 1981). Similarly studies conducted earlier found no differences in seroconversion in chickens vaccinated using different regimes (Folitse *et al.*, 1998). Furthermore, other researchers also found that intraocular route of vaccination gave better immune responses compared to oral route of vaccination similar to our obsevations (Salam, *et al.*, 2003; Degefa *et al.*, 2004; Anebo *et al.*, 2013; Okwor *et al.*, 2013). The differences reported in these studies might be attributed by the differences in frequency of sampling and combination of live and inactivated vaccines used in other studies.

Significant differences in GMTs were observed between regime of vaccination.

The serologic responses were particularly greater in birds receiving a booster vaccination compared to unboosted ones (Table. 1). A similar observation was made in previous studies in which the authors reported greatest serologic response and best resistance to clinical ND in birds receiving a booster vaccination (Mazengia *et al.*, 2009; Bwala *et al.*, 2011; Palya *et al.*, 2013). The higher protective antibody titre in the two boosted groups was probably attributed to a booster dose and hence stronger protection.

The effectiveness of ND vaccines is determined mainly by the assessment of antibody response in chickens and the ability of vaccinated chickens to resist exposure to virulent NDV when compared with unvaccinated control (Spradbrow, 1994). In the present study the two assessments were adopted to evaluate the effectiveness of LaSota strain in protecting chickens against ND. It was clear that all unvaccinated chickens were negative to NDV antibodies throughout the study (Table. 1). Serologic conversion and challenge data indicated that majority of vaccinated chickens produced measurable HI titers and were refractory to challenge with virulent NDV field isolate (Table 1). Higher protection levels (75-100%) in vaccinated birds revealed by both antibody response and the ability to resist exposure to virulent NDV field isolate is an indication that the vaccine is effective in preventing ND outbreaks (Fig. 1). The epidemic theory suggests that; if 70% of the population is immune, the disease outbreak is unlikely to occur because there will be no enough susceptible hosts to propagate an epidemic (Thrusfield, 1995). Nearly all unvaccinated control chickens challenged on the same day succumbed to the infection indicating high sucseptibility to the disease. In a protection challenge studies the degree of protection conferred by the vaccines have been found to be related to serum HI antibody profile of chickens (Abbas *et al*., 2006).

Findings of this study show that LaSota® strain ND vaccine commercially available in the study area can produce an adequate serological response following a single vaccination by either oral or ocular route at 10 days of age. This confirms an observation made by Bwala *et al.* (2011) that a single application of ND vaccine (LaSota®) conferred protection against clinical ND in naive specific pathogen free (SPF) birds. For economic purposes this regime could be adopted in the study area where multiple vaccinations are employed for the initial immunization before the three monthly boosters. However, boosting produced stronger protection in both routes.

The observed clinical signs in this study were very suggestive of ND as also reported elsewhere (Aldous *et al*., 2001; Alexander *et al*., 2004). These clinical signs were evident in challenged birds more so in those from the control group (unvaccinated) (Fig. 2A&B). Similarly, PM examination of the challenged birds showed lesions commonly reported in natural infections of ND (Fig. 3C&D) (Alexander *et al.*, 2000).

In conclusion, the LaSota strain ND vaccine commercially available in Morogoro, Tanzania protected vaccinated birds against clinical disease following exposure to virulent NDV strains. Both the oral and ocular routes of vaccination provided the same level of protection. Regardless of route of vacination, booster dose produced higher level of protection. Therefore the vaccine is still useful and the oral route remains the route of choice, because it is easy to perform especially when large numbers of birds are involved.

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