Immunogenicity and protection ability of candidate Newcastle disease virus isolates for vaccine production

M G S Yongolo, A P Muhairwa*, M M A Mtambo*, U M Minga, M Minja and R H Mdegela***

Animal Disease Research Institute, Ministry of Water and Livestock Development, Dar es Salaam, Tanzania
myongolo@yahoo.co.uk
**Department of Veterinary Medicine, Faculty of Veterinary Medicine, Sokoine University of Agriculture,*
P. O. Box 3021, Chuo Kikuu, Morogoro, Tanzania
***Open University of Tanzania (OUT), P.O. Box 23409, Dar-es Salaam, Tanzania*

Abstract

Virulence of 33 Tanzanian Newcastle disease virus (NDV) isolates was determined by Mean Death Time, Intracerebral Pathogenicity Index and Intravenous Pathogenicity Index with the aim of identifying candidate viral isolates for production of ND vaccine. Five out of 12 lentogenic isolates were of low virulence. Immunogenicity and protection ability of the 5 isolates and a commercially available LaSota vaccinal strain were determined in 10-weeks old chickens against a known field virulent NDV isolate. Eight groups of birds comprising 10-15 chickens each were used. Each of the five groups was inoculated one of the 5 candidate vaccine isolates. Group 6 was inoculated with LaSota, while group seven was a negative control and group 8 was a positive control. HI test was used to determine the seroconversion before and after challenge Two chickens infected with virulent NDV strain were used for contact challenge in each group 21 days after inoculation. After challenge, clinical signs and mortality were monitored for two weeks.

At day 21 LaSota strain had significantly higher ($p<0.01$) HI titre ($6.7\log_2$) than the remaining. By day 35, chickens inoculated with LaSota and one of the 5 isolates showed 100% protection against mortality. The field isolate that showed 100% protection ability did not revert to virulence following serial passage in naïve chickens indicating its suitability as a local vaccine strain.

These findings provide for the first time the existence of a potential local vaccine candidate in Tanzania. Further work on dosage to optimise duration of protective immunity is indicated. It is also recommended that more field isolates should be screened to increase the spectrum and diversity of vaccine candidates.

Key words: Immunogenicity, Newcastle disease virus, vaccine, virulence

Introduction

Free-ranging chickens (FRC) forma a significant socio-economic set up in rural communities of Tanzania (Minga et al 1996) and in other developing countries (Spradbrow 2001). In Tanzania about 97% of all households in villages keep chickens (MOA 1994), which provide a cheap source of animal protein and serves as an income generating activity especially for women. Commercial market for chickens has markedly increased in the recent years, which indicates a need for increased productivity to generate surplus for sale (Mlozi et al 2001). However, health and productivity of these chickens are highly constrained by diseases and husbandry problems (Minga et al 1996). Results by Yongolo (1996) showed that Newcastle disease is still the leading killer of chickens in the villages in Tanzania. Similar results have been indicated elsewhere in developing countries where free-ranging chickens rearing is the main form of poultry raising (Saidu et al 1994; Thitisak et al 1988; Alders 2002).

Different from commercial poultry industry where Newcastle disease has been controlled by vaccination, there is little success in free-ranging chickens. Available vaccines are designed for large commercial flocks (1000 doses) against small number of chickens kept by farmers in the villages. These significantly raise cost of vaccination for poor farmers in the villages. Successful vaccination is further hindered by lack of cold chain facilities, poor transport to the remote villages and physical control over the chickens and the vaccine. Records show that little effort has been done to control Newcastle disease using locally obtained strains.

Lentogenic and mild virulent isolates have been used for production of live and inactivated vaccines in Europe and Asia (Palya 1991; Mowat and Rweyemamu 1997).

A study by Yongolo (1996) found lentogenic and mild virulent Newcastle disease viruses from birds with clinical Newcastle disease as well as from healthy carrier birds. However, immunogenicity potential of these local isolates in preparation vaccines against birds in Tanzania is not known. Production of vaccines from local strains and resources may provide a cheap and relevant way of controlling Newcastle disease in the country, as production and dosages may be adapted to the chicken management employed by farmers in Tanzania.

The present study aimed at identifying potential NDV vaccine candidates from chicken and duck isolates obtained in Tanzania. Protective ability and immunogenicity of the selected strains were compared to examine their use to a live vaccine.

Materials and Methods

NDV isolates

A total of 33 NDV isolates from ducks and chickens were used in this study. After initial isolation from the hosts the strains were passaged five times in embryonated chicken eggs (ECE) and stored at - 20^{o}C and were grown in chicken egg embryo before use in the present experiment. Mean Death time (MDT), Intracerebral Pathogenicity Index (ICPI) and Intravenous Pathogenicity Index (IVPI) were carried out according to the procedures described by OIE (1996) and 12 isolates were found to be lentogenic. Five of these isolates (MG/07/10/C, MG/06/11/C, MG/10/03/C, TB/02/27/C and TB/05/29/D) were selected for determination of immunogenicity. The selected isolates all had MDT > 96 hour, ICPI 0.0 to 0.9 and IVPI 0.0-0.5. The challenge NDV isolate(MG/06/28/C) had a Mean Death Time < 60 hours in ECE, Intracerebral Pathogenicity Index of 1.7 and EID_{50} of 8.6 $\log_{10}$ per ml.

Control vaccine virus

La Sota ® vaccine (SHAFIT biological laboratories: Kibutz Shefayim Ltd, 60990 Israel) was used as a control strain. The vaccine was used as per manufacturers instructions.

Embryonated Chicken Eggs (ECE)

Locally obtained ECE were used for ND virus multiplication, virulence determination for reversion to virulence study and for virulent NDV seeding chickens. The flocks were selected on the basis of having an average ND antibody flock titre of less than 4 $\log_2$. ECE were incubated at 37^{o}C and 60% relative humidity at the Sokoine University of Agriculture (SUA) virology laboratory and incubated for 9 - 11 days before inoculation.

Experimental chickens

All chicks were hatched at SUA virology laboratory and were raised at SUA poultry experimental house. The chicks were used for ICPI, IVPI and immunogenicity, protective ability determination of the strains. Determination of protective ability was made using ND naïve 10-week-old chickens. Additional 20, five-weeks old chickens were used for serial passage of the selected local strain to test reversion.

Determination of the presence of NDV and serology

Haemagglutination (HA) and Haemagglutination Inhibition test (HI) were done using the protocol described by Allan and Gough (1974). ND antiserum and antigen used were produced at SUA virology laboratory.

Determination of protective ability

One-hundred and ten experimental chickens were divided into 8 groups, six groups (group 1-6) having 15 birds each and two groups (7 and 8) with 10 chickens each. Ten chickens in groups 1 to 5 were directly inoculated with a test virus and group 6 with a known LaSota vaccine strain. Group 6 was inoculated with LaSota, and group Group 7 was a negative control. Group 8 was challenged with a virulent strain. Isolates used in different groups of chickens are shown in Table 1.

Table 1. Comparison of overall pre challenge Newcastle disease HI Lsmean titres elicited in chickens after inoculation with field NDV isolates

Isolate (Group)	Post inoculation HI titre Lsmean								Survival rate(%) after challenge
	Day 1		Day 7		Day 14		Day 21		
	Direct	In contact	Direct	In contact	Direct	In contact	Direct	In contact	
MG/10/03/C (1)	0.00 ± 0.36 [a]	0.00 ± 0.39 [a]	0.30 ± 0.36 [a]	0.00 ± 0.39 [a]	4.90± 0.36 [b]	3.00 ± 0.39 [b]	4.90± 0.36 [b]	3.00 ± 0.39 [b]	100
MG/07/10/C (2)	0.00 ± 0.36 [a]	0.00 ± 0.39 [a]	0.00 ± 0.36 [a]	0.00 ± 0.39 [a]	3.50± 0.36 [b]	2.60 ± 0.39 [b]	3.50± 0.36 [b]	2.60 ± 0.39 [b]	66.7
MG/06/11/C (3)	0.00 ± 0.36 [a]	0.00 ± 0.39 [a]	0.00 ± 0.36 [a]	0.00 ± 0.39 [a]	2.40± 0.36 [b]	2.00 ± 0.39 [b]	2.40± 0.36 [b]	2.00 ± 0.39 [b]	66.7
TB/05/20/D (4)	0.00 ± 0.34 [a]	0.00 ± 0.43 [a]	0.00 ± 0.34 [a]	0.00 ± 0.43 [a]	1.60± 0.36 [b]	2.50 ± 0.43 [b]	1.60± 0.36 [b]	2.50 ± 0.43 [b]	20
TB/02/27/C (5)	0.00 ± 0.36 [a]	0.00 ± 0.39 [a]	0.10 ± 0.36 [a]	0.00 ± 0.39 [a]	2.40± 0.36 [b]	3.20 ± 0.39 [b]	2.40± 0.36 [b]	3.20 ± 0.39 [b]	6.7
La Sota (6)	0.00 ± 0.33 [a]	0.00 ± 0.51 [a]	2.75 ± 0.33	0.00 ± 0.51 [a]	6.70± 0.36 [b]	5.00 ± 0.51 [b]	6.70± 0.36 [b]	5.00 ± 0.51 [b]	100
Uninoculated (7)	0.00 ± 0.27 [a]	0.00 ± 0.21 [a]	0.00 ± 0.27 [a]	0.00 ± 0.21 [a]	0.00 ± 0.27[a]	0.00 ± 0.21 [a]	0.00 ± 0.27 [a]	0.00 ± 0.21 [a]	26.7

Fresh allantoic harvests of the isolates were diluted to 10^{-5} before inoculation into birds in each group. Before each experiment the allantoic harvests were tested for bacteria sterility by plating into 5% blood agar at 37^{o}C overnight. Only harvests with no bacterial growth on blood agar were used. One ml of the inoculum was given by applying one drop on each eye and the remaining volume was given orally. Bleeding for serum was made at day 1, 7, 14, 21, 28, and 35. In contact birds were introduced on the 4th day in groups 1, 2, 3, 4, and 5.

On day 18 the 21 seed chicken were each directly challenged using one ml containing neat virulent isolate MG/06/28/C orally. They were kept separately until day 21 when 3 birds were introduced onto each of the seven groups.

Data collection

Individual bird daily recording of the health status was made in a data sheet where apparently normal birds were recorded as 2, sick as 1 and dead as 0. Survival was coded as 1 if present and 0 if dead. Serology titre results after HI test were in Log_2.

Statistical analysis of data

A general linear model (GLM) of the Statistical Analysis System (SAS Institute **1996**) 6.1 programme was used to perform multiway analysis of variance and interaction instead of ANOVA. The statistical model for the experimental design used was $Y_{ix} = \mu + R_i + D_x + E_{ix}$. Where Y_{ix} = Haemagglutination Inhibition test titre results of health outcome or Survivability of X^{th} day; μ = Constant term, $R_I = i^{th}$ effect due to NDV isolate, D_x = xth effect due to day post inoculation or post challenge; E_{ix} = A random error effect.

Results

Serology, health status and survivability after inoculation with candidate NDV isolates

All experimental chickens were ND negative at day 1 before inoculation with test Newcastle Disease (NDV) isolates. The overall HI titre Least square means (Lsmeans) for 21 days is shown in Table 1.

The trend on HI test ND antibody response from day 1, 7, 14, and 21 is shown in Table 1 All groups were ND negative at day 1. However, at day 7 groups inoculates with MG/10/03/C, TB/02/27/C and LaSota strains ND seroconverted. On day 14 and 21 all groups had seroconverted except the uninoculated group. All chickens in all groups showed no clinical signs and no deaths were observed. In the same period the trend in incontact chicken is shown in Table 1

From day 1 to day 21 there was no change of the health status of all birds in all the experimental groups. No clinical signs were observed in birds after inoculation with vaccine test stains

Post challenge results

The trend in ND HI titres after challenge is shown in figure 1. With exception of LaSota and isolate MG/10/03/c where no mortality was observed, variable mortality was observed in groups infected with other isolates

Figure 1. Pre and post challenge seropositivity in six week old chickens elicited by different field ND virus isolates

Test for virulence reversion

One isolate MG/10/03/C was tested in ECE and the MDT was > 96 hours post inoculation in all five in five ECE for each passage. Even after passage in 10-week-old chickens the chickens did not show any clinical signs. After the last passage in chickens the isolate was passaged again in ECE and the MDT was > 96 hours.

Discussion

The present results demonstrated antibody response to NDV from all isolates tested as indicated by HI titres. LaSota vaccine strain had the highest HI titres in chicken which persisted from day seven to day 35, followed in decreasing order by isolates

MG/10/03/C, TB/02/27/C, MG/07/10/C and TB/05/20/D (Table 1 and Figure 1). Although these isolates had the potential to elicit immune response their protection ability against virulent NDV differed substantially. Only one isolate MG/10/03/C had the same protection rate as the conventional vaccine strain LaSota with 100% survival rate. This isolate could not revert to virulence after five passages in ECE and in live birds. These results are therefore suggestive that isolate MG/10/03/C is a suitable ND vaccine candidate. Further investigations in a bigger population under laboratory conditions remains to be done before final trials in the field.

Two isolates MG/07/03/C and MG/06/11/C had 66.7% protection against ND which was lower than that of LaSota vaccine and MG/10/03/C. Variation on protectivity when developing isolates for vaccine production is in agreement with Goldhaft (1980) findings when developing LaSota strain. These results might mean that the two isolates with slightly lower protection rate could be candidate vaccine strains if the right inoculation dose is determined against the present blanket dose. These results highlights that the dose universally recommended by manufactures might not be enough under certain conditions. Whether the antigenicity of these isolates and the challenge isolate were different could not be determined in the present experiments. However, the present results may partly be explained by Alexander (2001) and Westbury (2001) findings, which suggested that NDV are not always antigenically homogeneous. In view of these results it can be speculated that the reported failures in some vaccination attempts in FRC could be associated with antigenic differences with the virulent strain causing disease at that particular time.

Conclusions

- In summary this study has shown the protection ability to ND by local isolates of NDV.
- One strain MG/10/03/C had equivalent protection ability to LaSota vaccine.
- Variation in protection ability of immunogenic strains underlines the presence of antigenic heterogeneity of NDV suggesting further testing of local strains for their suitability as vaccines against Newcastle disease.

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