

Laboratory and Field Trials with Thermostable Live Newcastle Disease Vaccines in Mozambique

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Abstract

The Australian strains of avirulent, thermostable Newcastle disease virus designated NDV4-HR and I-2 were tested in the laboratory and under field conditions in Mozambique. An overview of this work is presented. Under experimental conditions, both vaccine strains provided protection to all vaccinated birds and those in contact with them against a local virulent strain of ND virus, V868. Field trials confirmed these results. In addition, it was shown that vaccine administered by eye-drop gave better results than vaccine administered in the drinking water or by oral drench. There were no adverse reactions to the vaccines and it was concluded that NDV4-HR and I-2 strains are avirulent, innocuous, efficacious, immunogenic and suitable for use in the control of ND in Mozambique. Factors contributing to the successful implementation of the field trials are also discussed.

NEWCASTLE DISEASE (ND) is endemic in Mozambique, occurring every year in the rural poultry sector (National Directorate of Livestock 1992). Although few surveys of the prevalence of Newcastle disease (ND) in Mozambique have been undertaken, available information indicates that this disease is the most important constraint to the rearing of rural chickens (Fringe and Dias 1991; Mavale 1995; Wethli 1995).

Vaccination is the most effective means of controlling ND and has been used successfully throughout the world since the 1940s (Beard and Hanson 1984). In 1955 the National Veterinary Research Institute (INIVE) in Maputo commenced production of attenuated, live ND vaccines and has produced vaccines based on strains F, B1, La Sota and Komarov. These vaccines are used mainly in the commercial poultry sector. They are of limited application in rural areas due to problems of heat lability of the vaccine strain, large dose presentation, affordability, and shortage of government staff, transport and cold chain for effective administration of the vaccine.

The avirulent, thermostable ND vaccine strains NDV4-HR (Ideris et al. 1987) and I-2 (Bensink and

Spradbrow 1999) were developed by researchers at The University of Queensland in Australia to provide rural poultry farmers with an effective, affordable means of controlling ND in their flocks. These vaccines have been used successfully in village chicken populations in many countries in Asia and Africa. In 1996, The Australian Centre for International Agricultural Research (ACIAR) project AS1/96/96 commenced with the major objective of producing I-2 vaccine and testing its use in the sustainable control of ND in rural areas of Mozambique. Initial laboratory and field trials were conducted using NDV4-HR since this was available commercially and could easily be replaced by I-2 once local production commenced. This paper briefly describes the results of the laboratory and field trials with NDV4-HR and I-2 vaccines and describes the activities undertaken to ensure the successful implementation of the field trials.

Materials and Methods

NDV4-HR vaccine laboratory and field trials

NDV4-HR vaccine was supplied by Fort Dodge Pty. Ltd, Australia, as infected allantoic fluid in lyophilised form and was reconstituted and diluted in potable water prior to use.

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Laboratory trials

Commercial broiler day old chickens were purchased locally. At three weeks of age, the birds were tagged individually with numbered wing tags and randomly allocated into three experimental groups (Figure 1). Ten birds (vaccinated, group 1a) were vaccinated with 10^6 EID₅₀ via eye-drop on two occasions two weeks apart. They were housed with a group of six unvaccinated (in contact) birds (in-contact, group 1b). Two weeks after the second dose of vaccine, all birds were challenged by contact with five non-vaccinated birds which had been inoculated intranasally with a local virulent isolate of ND virus (directly challenged, group 1c).

Non-vaccinated control birds were housed separately and attended by a different technician. This group was challenged by randomly selecting five birds (group 2b) that received a suspension of challenge virus intranasally and kept in contact with group 2a consisting of the 13 other non-vaccinated birds.

All birds were observed for clinical signs during the vaccination and challenge periods, and number of deaths in each group was recorded.

Serum was collected at key stages of the laboratory trial and from all surviving birds but the results of the haemagglutination inhibition (HI) tests will not be presented in this paper.

At the end of the laboratory trial, the extensionists and district livestock officer from the field trial site

were invited to INIVE to participate in the clinical and post mortem examination of trial birds. The clinical signs and lesions of ND were demonstrated, and the efficacy of the vaccine against a local virulent strain of ND virus demonstrated.

Field trial

Five hundred chickens in each of three villages were vaccinated with the NDV4-HR vaccine administered by community assistants. A different route of administration was used in each village: eye-drop, oral drench and drinking water. In a fourth village, approximately 500 chickens were mock-vaccinated with water and served as controls. The dose rate was one drop of vaccine (10^6 EID₅₀) per bird every four months. Approximately 10% of birds in each group were identified using individually numbered wing tags and serum samples were collected from each bird. Monthly serum samples were collected from approximately 20 identified birds from each group for titration of HI antibodies. The status of individually tagged birds and the number of birds per household were recorded every two weeks over a 12-month period. Postmortem samples from dead birds were subjected to ND virus isolation.

I-2 Vaccine laboratory and field trials

The vaccine strain I-2 was supplied by the Virus Laboratory, the University of Queensland, Australia. It was propagated in 9-day-old embryonated chicken

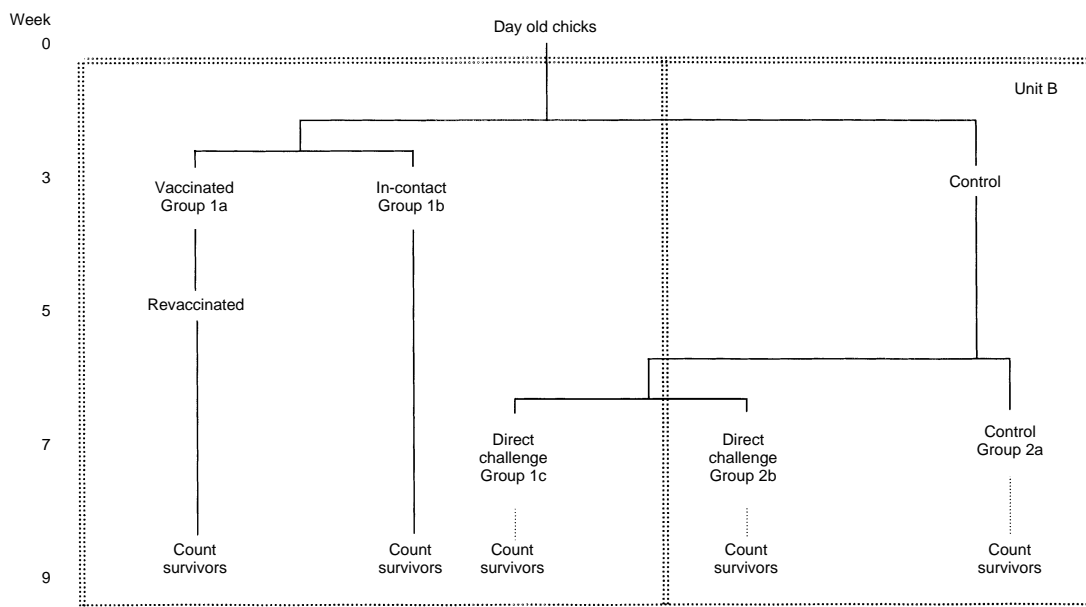


Figure 1. Design of NDV4-HR and I-2 vaccine laboratory trials. The double line indicates the separate housing of the groups.

eggs (from a minimum disease free flock) at INIVE, Maputo, Mozambique. The vaccine was prepared as a wet vaccine (Spradbrow et al. 1995).

Laboratory trial

Day old chickens were purchased from a local commercial hatchery and raised in the isolation unit at INIVE. After three weeks they were identified with individual numbered wing tags (Figure 1), randomly divided into groups and serum was collected to determine the level of antibodies against ND.

Ten birds (vaccinated, group 1a) were vaccinated with 10^6 EID₅₀ via eye-drop, on two occasions two weeks apart. The first vaccination was given at three weeks of age. The in-contact group (1b) consisting of six unvaccinated birds was kept in contact with the vaccinated group (1a). Serum was collected from the birds two weeks after the second dose of vaccine to determine the level of antibodies to ND. The vaccinated (1a) and in-contact (1b) groups were challenged immediately following serum collection by contact with an unvaccinated group of 5 birds (group 1c) which had been inoculated via eye-drop with virulent ND virus.

Unvaccinated control birds were housed separately and attended by a different technician. This group was challenged by randomly selecting five birds (Group 2b) that received a suspension of challenge virus via eye-drop and kept in contact with group 2a consisting of the 20 other non-vaccinated control birds. The number of birds that survived challenge was recorded.

Serum was collected at key stages of the laboratory trial and from all surviving birds but the results of the haemagglutination inhibition (HI) tests will not be presented in this paper.

Field trial

From 70 to 230 chickens in each of three villages were vaccinated against ND using the wet I-2 vaccine administered by community assistants. In each village, a different route of administration was used: eye-drop (once only); eye-drop (twice, three weeks between doses) and drinking water. In a fourth village, approximately 200 chickens were mock vaccinated as controls. In each group, about 10% of chickens were identified using numbered wing tags and serum samples collected. Approximately 30 serum samples were collected from each group of identified birds four to seven weeks after vaccination depending on the treatment group. Fewer serum samples were collected from the control group as a ND outbreak commenced shortly after the start of the trial and greatly reduced chicken numbers in the control area. The status of individually tagged birds and the number of birds per household were recorded every two weeks over a five-month period.

Serology

HI titres were measured on the day of vaccination and at monthly intervals during the field trials. The HI test was performed using four HI units of NDV4-HR virus strain and a 1% suspension of chicken red blood cells (Allan and Gough 1974). Tests were conducted on sera collected before and after vaccination. Sera were subjected to doubling dilutions eight times and for statistical purposes all sera with titres equal to or greater than 8 (log to base 2) were given a value of 8.

Challenge virus

The virus used as the challenge virus was isolated by the Virology section at INIVE from birds in the commercial sector that had died of ND. The reference material, designated V868, was passaged twice in 9-day-old embryonated chicken eggs from a minimum disease free flock. The average time for embryo death (mean death time) was 51 hours. Birds inoculated directly with the virus received a dose of $10^{7.7}$ ELD₅₀ in the NDV4-HR vaccine laboratory trial and 10^7 ELD₅₀ in the I-2 vaccine laboratory trial.

Pre-trial activities

Experimental sites were selected in collaboration with National Directorate of Rural Extension at meetings of project staff with the chiefs of Provincial Veterinary Services and with the Provincial Rural Extension Service.

All fieldwork was conducted in collaboration with the District Extension and Livestock Services. Meetings were held with the community leaders and village poultry farmers to discuss the objectives of the trial, to explain that some groups of birds would probably not be protected by the vaccine and that the vaccine would only protect against ND. The need for collecting blood samples and using wing tags to identify trial birds was also discussed. Members of the community approved the proposal and volunteered to cooperate with the investigation.

A questionnaire was designed to collect information on poultry husbandry practices and problems in the trial area. The questionnaire was prepared in both Portuguese and Shangana (the local language) with the help of the Poultry Working group that consisted of representatives of INIVE, Rural Extension, Institute of Animal Production, Veterinary Faculty, Institute of Rural Development and Provincial Service of Agriculture in Maputo.

An assistant was selected from each community with the assistance of community members and trained to carry out the village poultry survey, collect data on chicken numbers, health status and production in each participating household, and the seasonal

occurrence of ND outbreaks. Trial groups were allocated randomly by a lottery draw at a community meeting.

Comprehensive records of meetings, decisions, vaccine used and participating farmers were kept. A form of compensation or incentive for participating farmers was also discussed. To avoid disrupting normal village activities, all meetings, vaccinations and blood collection were arranged at times suitable to the farmers.

Trial extension activities

During the trial, monthly meetings were held with the leaders and members of the community, and local assistants to present the results of serological monitoring, to discuss the health status of poultry in the trial sites and to encourage continued cooperation.

Results

NDV4-HR

Laboratory trials

No signs of clinical illness were observed in any of the vaccinated birds or birds in contact with them after vaccination or challenge, and all survived challenge with the virulent ND virus V868. All chickens in group 1c (100%) and four chickens in group 2b (80%) died after challenge with V868 ND virus showing typical clinical signs and lesions of ND. All 13 birds challenged by contact (group 2a) also died (100%). Results are shown in Table 1.

Table 1. Results of the vaccine laboratory trial conducted using NDV4-HR vaccine in broilers in Mozambique.

Group	No. of chickens per group	Vaccination procedure *	Challenge procedure **	No. of survivors/No. challenged	Mortality (%)
1a	10	ED	IC-1c***	10/10	0
1b	6	IC	IC-1c	6/6	0
1c	5	Nil	IN	0/5	100
2a	13	Nil	IC-2b#	0/13	100
2b	5	Nil	IN	1/5##	80

ED – Eye-drop.

IC – In contact.

IN – Intranasal.

* – 10^6 EID₅₀.

** – $10^{7.7}$ ELD₅₀, Local isolate No. V868.

*** – In contact with group 1c.

– In contact with group 2b.

– The chicken that survived the challenge demonstrated clinical signs of Newcastle disease, developed an elevated HI titre and remained uncoordinated at the end of the trial.

Field trials

Administration of NDV4-HR by eye-drop provoked a greater antibody response than administration via drinking water or oral drench (Table 2). More birds vaccinated by eye-drop survived an outbreak of ND than birds vaccinated by the other routes and this group also showed the greatest population increase after 1 year. Results are shown in Table 3.

Table 2. Geometric mean HI titre (log to base 2) of tagged birds on day 0 and day 30 of the NDV4-HR vaccine field trial.

Route of vaccination	GMT day 0	GMT day 30
Eye-drop*	3.6	6.0
Oral drench*	3.0	4.3
Drinking water*	1.9	3.9
Control	3.3	3.4

GMT – Geometric mean titre (log to base 2).

* – NDV4-HR vaccine administered once.

Table 3. Survival of tagged birds to six months and changes in the chicken population to 12 months of the field trial with NDV4-HR vaccine.

Route of vaccination	Survival of tagged birds to 6 months	Changes in general chicken population after 1 year
Eye-drop*	77%	+144%
Oral Drench*	56%	+25%
Drinking Water*	50%	-22%
Control	32%	-46%

* – NDV4-HR vaccine administered once every four months.

Of the birds vaccinated with NDV4-HR vaccine by eye-drop, an additional 17% were slaughtered for consumption and 6% were stolen, sold, lost or transferred to another area. Of birds vaccinated via drinking water and oral drench, an additional 5% and 2% respectively were stolen, sold, lost or transferred to another area. No birds in these groups were slaughtered. In the control group, 10% of birds were slaughtered, stolen, sold, lost or transferred to another area.

Virulent ND virus was isolated during the natural outbreak that occurred during the field trial.

I-2

Laboratory trials

No adverse reactions to the vaccine were observed in vaccinated and in contact birds. I-2 induced HI

antibodies in these birds and the level of HI antibody and survival after challenge were positively correlated. All vaccinated and in contact birds survived challenge. In contrast, all birds in groups 1c, 2a and 2b died between 4 and 6 days after infection showing typical clinical signs and lesions of ND. Results are shown in Table 4.

Table 4: Results of the vaccine trial using the heat-tolerant I-2 vaccine against Newcastle disease in broilers in Mozambique.

Group	No. of chickens per group	Vaccination procedure *	Challenge procedure **	No. of survivors/No. challenged	Mortality (%)
1a	10	ED	IC-1c***	10/10	0
1b	6	IC	IC-1c	6/6	0
1c	5	Nil	ED	0/5	100
2a	16	Nil	IC-2b#	0/16	100
2b	5	Nil	ED	0/5	100

ED – Eye-drop.

IC – In contact.

* – 10^6 EID₅₀.

** – 10^7 ELD₅₀, Local isolate No. V868.

*** – In contact with group 1c.

– In contact with group 2b.

Field trials

The questionnaire showed that ND was considered by farmers to be the most important constraint to village poultry production.

Administration of the vaccine by eye-drop provoked a higher antibody response than administration by drinking water, with little difference between birds vaccinated once or twice (Table 5). After five months, there were marked differences in chicken numbers between treatment groups. Results are shown in Table 6.

Table 5. Geometric mean HI titre (log to base 2) of tagged birds on day 0 and day 30 of the I-2 vaccine field trial.

Route of administration	GMT Day 0	GMT 30 days post vaccination
Eye-drop (twice)*	3.8	7.1
Eye-drop (once)**	3.3	6.6
Drinking water*	4.8	6.3
Control	2.6	2.0

GMT – Geometric mean titre (log to base 2).

* – I-2 vaccine given on two occasions, 3 weeks apart.

** – I-2 vaccine given once.

Table 6. Survival of tagged birds and changes in the chicken population at the end of the five month field trial with I-2 vaccine.

Route of vaccination	Survival of tagged birds	Change in general chicken population (%)
Eye-drop (twice)*	83%	+54%
Eye-drop (once)**	82%	+50%
Drinking water*	65%	+17%
Control	0%	-71%

* – I-2 vaccine administered twice, three weeks apart at the start of the trial and then given once every 4 months.

** – I-2 vaccine administered once only with revaccination occurring after 4 months.

Of the birds vaccinated twice with I-2 vaccine by eye-drop, an additional 6% were slaughtered for consumption and 9% were stolen, sold, lost or transferred to another area. 4% of birds vaccinated once by eye-drop were slaughtered for consumption and 10% were stolen, sold, lost or transferred to another area. Of the birds vaccinated via drinking water, an additional 12% were stolen, sold, lost or transferred to another area. In the control group, 10% of birds were slaughtered, stolen, sold, lost or transferred to another area.

Discussion and Conclusions

Laboratory trials showed that chickens vaccinated with NDV4-HR and I-2 vaccines were protected against a local virulent isolate of NDV. The virus strains were transmitted by contact between chickens and provoked no clinical response in vaccinated or in contact birds. This is in agreement with other laboratory trials with NDV4-HR vaccine in Zambia (Alders et al. 1994) and with I-2 vaccine in Vietnam.

The results obtained in the field trials show that the vaccines can be successfully administered via different routes. In each trial, the highest titres were achieved in the groups where the vaccine was administered via eye-drop. The vaccination regime employed in the NDV4-HR vaccine field trial was unusual in that the vaccine was given once only every four months for each route of administration. This was done to determine the most economically viable strategy for vaccination. After a natural outbreak of ND, it was clear that eye-drop was the only administration route that gave adequate protection when employing such a vaccination regime. Bell et al. (1995) found that a single eye-drop administration of NDV4-HR vaccine provided acceptable protection of village chickens in Cameroon.

In both trials, approximately 80% of chickens vaccinated via eye-drop (irrespective of whether the vaccine was administered once or twice initially) survived natural challenge with virulent ND virus. When the vaccine was administered twice via drinking water, a survival rate of 65% was achieved. Although protection of 60% of birds in a village is considered sufficient to limit the propagation of ND outbreaks, the protection of 60% of birds within a household was rarely acceptable to the farmers concerned.

Although eye-drop administration requires that each bird be caught, this was the method preferred by farmers because of the better protection experienced when this route of vaccination was used. As farmers gain confidence in the ND control activities, they should be encouraged to improve the housing provided for their chickens. Improved housing will facilitate the catching of birds for eye-drop administration of the ND vaccine and should reduce losses due to predation. Bell et al. (1995) also recommended improved housing for village chickens, especially for young chicks up to four weeks of age, as this was the group that suffered the highest losses from predation.

There was little difference in antibody response and survival after challenge between birds vaccinated once or twice with I-2 by eye-drop. The results also clearly indicate that eye-drop administration is more effective than oral vaccination when vaccine is given once every four months. Application by drinking water is easier especially where around 50% of birds are housed but regrettably, this route provokes a lower level of immunity.

Successful implementation of field trials depended on good laboratory-field communications. Pre-trial planning and extension were crucial to ensure farmer cooperation in these trials, and in any future vaccination programs.

A strategy for the control of ND in village chickens must be economically feasible. The costs associated with vaccination campaigns are purchase of the vaccine, transport and administration of the vaccine. Therefore, less frequent but effective vaccination is preferred. If the seasonal prevalence of ND is known, vaccination campaigns can be timed appropriately. Currently in Mozambique vaccination with I-2 vaccine via eye-drop is recommended every four months in village chickens.

Acknowledgments

Support provided by ACIAR and INIVE to enable the authors to undertake investigations into the control of Newcastle disease in village chickens in Mozambique is gratefully acknowledged. The time and information provided by village chicken farmers in Bilene and Manhiça districts of Mozambique was invaluable. Thanks also go to Dr Mary Young for assistance with the editing of this manuscript.

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