African Journal of Biotechnology Vol. 7 (10), pp. 1594-1599, 16 May, 2008
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2008 Academic Journals

Full Length Research Paper

Response of chickens to oral vaccination with Newcastle disease virus vaccine strain I_2 coated on maize offal

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Accepted 14 April, 2008

Thermostable Newcastle disease (ND) vaccine virus strain I_2 was investigated for its efficacy as food-borne vaccine, using maize offal as the vehicle. Immune response to vaccination and resistance to challenge were assessed by standard methods. Results showed that following primary vaccination, 40 (64.5%) out of the 62 birds produced detectable haemagglutination inhibiting (HI) antibody, but only 4 (6.5%) produced HI (log_2) antibody titre ≥ 3.0 regarded as protective with a geometric mean titre (GMT) of 3.1. After a booster dose, 49 (79.0%) seroconverted and 20 (32.3%) had HI (log_2) titres ≥ 3.0 with GMT of 4.9. When challenged all vaccinated birds survived while all control (unvaccinated) birds died. Pre-challenge HI antibody titre of 50 vaccinated birds selected for challenge showed that 13 (26.0%) had titres ≥ 3.0 and GMT = 4.5, while post-challenge, 31 (62.0%) had HI (log_2) ≥ 3.0 with GMT of 7.2. Using Student t test analysis of significance, the birds were observed to show 70% HI antibody production at a P ≥ 0.3 and 3 degree of freedom (df), and 70% secondary immune response on challenge at 4df. It is therefore concluded that the vaccine could be effective for protection of village chickens as food-borne vaccine provided the carrier foods are adequately treated.

Key words: Newcastle disease, maize offal, I_2 vaccine, chickens.

INTRODUCTION

Newcastle disease (ND) is the most important limiting factor in village chicken farming in most developing countries of the world and also a serious threat to intensely reared chickens. The disease is characterized by signs and symptoms that vary with the pathotypes involved in particular outbreaks. Infection with very virulent strains such as viscerotropic or neurotropic velogenic ND viruses may lead to high mortality occurring sometimes in the absence of discernible clinical signs. In some flocks, depending on the pathotype, mortality may range from 50 to 90%, and in fully susceptible flocks up to 100% (Alexander, 1997). In severe cases observable signs may include listlessness, increased respiration, cough, weakness, prostration, greenish watery diarrhoea, torticollis, paralysis of legs and wings, and death in that order (Gordon and Jordan, 1983).

Effective control of the disease is by vaccination with thermostable avirulent ND vaccines (Spradbrow, 1990). Although the conventional ND vaccines may be even more efficacious than their thermostable counterparts in terms of measurable immune response, the near-feral nature of the birds has made it impossible for conventional vaccines to be reliably administered to village chickens (Echeonwu, 2006). In addition, their multiage, difficulty of catching and inoculating individual chickens

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or of vaccinating them in an enclosure by drinking water or aerosol sprays are other problems that need to be solved (Spradbrow 1993, 1994; Thekisoe et al., 2004).

To obviate these problems, the use of food-based thermostable vaccine for the vaccination of village chickens has been found to be very useful (Aini et al., 1990a). The only problem with this method is that most foods possess antiviral agents that often inactivate such coated vaccine viruses (Cumming, 1992). Various methods for removal of antiviral properties from foods have been suggested by some workers. These include washing, boiling or heating of the chosen or available food type before coating with vaccine virus (Cumming, 1992; Jackson, 1992). In some cases, the food is coated with vaccine and fed to the target birds immediately to prevent inactivation of coated virus before consumption (Samuel et al., 1993). This method does not make room for storage of the food vaccine for transportation to rural areas and for use at a later date at the convenience of the rural farmers.

A thermostable ND vaccine strain I_2 has been recommended for use in developing countries for the protection of village chickens against ND (Bensink and Spradbrow, 1999). However, the efficacy of this vaccine in Nigerian environment has not been tested with food stuffs available locally. Reports from elsewhere show varying degrees of success with the vaccine in both laboratory and field trials (Nasser et al., 1998; Wambura et al., 2000; Amakye-Anim et al., 2000).

We report our method of carrier food treatment that sustained infectivity and immunogenicity of coated vaccine virus and delivery of same in dry form after days of storage to target chicken population as assessed by levels of haemagglutination inhibition antibody production and resistance to velogenic NDV challenge.

MATERIALS AND METHOD

Experimental design

Day-old chicks were purchased, brooded and raised to 6 weeks and screened for maternal HI antibody. Maize was obtained, processed, coated with NDV strain I_2 vaccine and dried at room temperature (RT). Chickens were separated into two groups and one was fed with the food-borne vaccine and the other with food containing no vaccine. Immunogenicity of food-borne vaccine was evaluated by testing chicken blood for HI antibodies. Finally the ability of food-delivered I_2 vaccine to protect against ND was evaluated by challenge of vaccinated and unvaccinated control chickens with velogenic NDV

Study area

The study was conducted in Vom, Jos South Local Government Area, Plateau State, Nigeria. Vom lies at 80° 45' east and 90° 43' north of the state. It has a cold climate as a result of its high altitude measuring well over 1,450 m above sea level. The average rainfall is between 1300 and 1500 mm with average daily maximum temperature of 28.6°C and minimum of 17°C while relative humidity varies between 14 and 74%.

Source of viruses

The virus strain (NDV- I_2) was obtained from Australia through the assistance of Dr. T. M. Joannis of Viral Research Department, National Veterinary Research Institute NVRI), Vom, Plateau State, Nigeria while the challenge velogenic NDV (strain VGF-1) was isolated and characterized in a previous study (Echeonwu et al., 1993). The ampoule of I_2 virus was propagated in large number of chick embryos, harvested, freeze dried and stored as the stock virus for the investigation.

Source of chicken

Day-old cockerels (Bovan Brown strain) were purchased from ECWA Farm Hatcheries, Bukuru, Jos South Local Government Council of Plateau State while white maize (*Zea mays*) used as vaccine carrier was purchased from Vom local market.

The chicks were brooded in experimental laboratory animal house for six weeks by providing adequate heat, water, feed, vitamins, drugs to control worm infestation and coccidiosis. The birds were also vaccinated against infectious bursal disease with vaccine obtained from Viral Vaccines Production Department, NVRI Vom.

Treatment and coating carrier food

The maize was soaked in tap water in a bowl for 48 h, washed, rinsed and milled to a smooth paste. The paste was then sieved through fine muslin cloths in water to remove the starch content (often used for food called pap or akamu) while the maize waste or offal was sun-dried and stored at room temperature (RT). Ten ampoules of the freeze dried vaccine were reconstituted in 100 ml of phosphate buffered saline (PBS) pH 7.2 and used to mix with 1.0 kg (at a ratio of 1.0 ml to 10 g) (Alders and Spradbrow, 2001) of the dried maize offal thoroughly in a plastic bowl and spread on trays to dry at RT overnight. The virus content of the dried coated maize offal was estimated to be $\geq 10^{6.0}$ EID_{50}/g.

Administration of food vaccine

The birds numbering eighty two (82) were divided into two groups after screening for residual haemagglutination inhibiting (HI) antibody and kept in separate rooms before vaccination. Group A contained 62 birds and Group B (for control experiment) contained 20 birds. Group A was fed with about 1.0 kg of vaccine-coated maize offal, while Group B was fed with uncoated offal after starving both groups of food for 5 h. All the birds were bled three weeks post primary vaccination and the serum samples assessed for antibody by the HI test technique (OIE, 2000). Group A was administered with a booster dose of the vaccine while Group B still received uncoated maize offal; all birds in the two groups were once again bled for serum for HI assay three weeks post booster vaccination. Geometric mean titre (GMT) was computed by the method of Reid (1968), while statistical analysis was done using Student t Test of Significance as described by Howell (1995).

Challenge experiment

Challenge experiment was carried out with 50 vaccinated birds taken from Group A and the 20 unvaccinated (controls) birds in Group B after screening them for pre-challenge HI antibody titres. They were then administering with the NDV (VGF-1) in drinking water containing $10^{5.5}$ ELD_{50}/ml of challenge virus after starving them of water for 4 h making provision for 10 ml per bird (Allan et al., 1978). They were observed for ten days following challenge for

Table 1. Immune response of chickens given primary dose of food-based NDV I_2 vaccine and assessed after 3 weeks by HI test.

HI (log_2)	No. of birds	% (total)	% HI (log_2) ≥ 3.0
<1	22	35.5	0
1	19	30.7	0
2	17	27.4	0
3	4	6.5	6.5
Total	62	100	6.5
Unvaccinated controls	20	100	0

Geometric mean titre (GMT) = 3.1.

Table 2. Immune response of chickens given booster dose of food-based NDV I_2 vaccine and assessed after 3 weeks by HI test.

HI (log_2)	No. of birds	% (total)	% HI (log_2) ≥ 3.0
<1	13	20.9	0
1	14	22.6	0
2	15	24.2	0
3	12	19.4	19.3
4	8	12.9	12.9
Total	62	100	32.2
Unvaccinated controls	20	100	0

GMT = 4.9

signs of Newcastle disease. Dead birds were subjected to postmortem examination while the survivors were bled for serum for HI assay. Pooled organs (proventriculus, spleen, lungs and intestine) were collected at postmortem and processed for challenge virus re-isolation by chick embryo inoculation (NAS, 1971).

RESULTS

Response of group A birds to primary vaccination

All birds screened prior to administration of vaccine were negative for HI antibody. Out of 62 birds vaccinated, 40 (65.52%) produced detectable HI antibody with GMT of 3.08, while 22 (35.48%) produced no HI antibody. Distribution of the HI log_2 titres among the 40 chickens that produced antibody showed that 19 (30.65%) had titre of 1, 17 (27.2%) had titre of 2, while only 4 (6.45%) had titre of 3 regarded to be protective. All control (unvaccinated) birds had titre of zero (Table 1).

Response of group A chickens to booster vaccination

When administered with a booster dose of the food vaccine, the chickens further seroconverted to the extent that 49 (79.03%) out of the 62 birds produced detectable antibody with GMT of 4.88, while 13 (20.97%) did not produce any HI antibody. Details of the antibody distribution are presented on Table 2. The control birds remained negative for HI antibody. Using the Student t Test of Significance to analyze the results, the birds were observed to show 70% of antibody production at a P ≥ 0.3 and 3 degree of freedom (df).

Post challenge observations

The pattern and rate of morbidity and mortality showed that between the first and the third day of challenge, there were no mortality recorded among all the vaccinated and control (unvaccinated) birds. However, during that period, six out of the twenty control birds challenged showed signs of ND (morbidity). By the 9th day of challenge all the control birds had succumbed to challenge and died (100% mortality) while all vaccinated chickens survived (100% survival).

Post challenge assessment of secondary immune response by chickens resisting the challenge velogenic virus

The fifty (50) chickens screened for pre-challenge HI antibody status showed that only 13 (26.0%) had log_2 titres ≥ 3.0 regarded as protective, 12 (24.0%) had no detectable antibody while 25 (50.0%) had HI titres ≥ 3.0

Table 3. Post challenge HI (log_2) titres of 50 chickens surviving challenge.

HI (log_2)	No. of birds	% (total)	% HI (log_2) ≥ 3.0
<1	2	4.0	0
1	9	18.0	0
2	8	16.0	0
3	15	30.0	30.0
4	13	26.0	26.0
5	3	6.0	6.0
Total	50	100	62

GMT = 7.2

Table 4. Challenge experiment results of chickens given booster dose of food-based NDV I_2 vaccine.

No. of chickens challenged	Number dead	Percentage dead	Number of survivors	Percentage of survivors
Vaccinated				
50	0	0	50	100
Unvaccinated				
20	20	100	0	0

and the GMT was 4.5. A post challenge screening of all the 50 birds that survived challenge showed that there was a further increase in antibody production. Out of the 50 surviving birds, 31 (62.0%) had HI log_2 ≥ 3.0, only 2 (4.0%) had no detectable antibody and the rest, 19 (34.0%) had titres ≤ 3.0. The GMT was 7.18 (Table 3). Result of challenge experiment is shown on Table 4.

Using the Student t Test of Significance analysis, the birds were observed to show 70% response to challenge by increased secondary HI antibody production against the challenge virus at P ≥ 0.3 and 4 df.

DISCUSSION

Apart from slaughter to stamp out, the most welcome and reliable control method for ND is by vaccination. The vaccines used for ND control must be able to protect the target birds to a reasonable level to be regarded as useful. In this study, an attempt was made to establish the efficacy of a vaccine primarily designed for use in village chickens (Bensink and Spradbrow, 1999), and to evaluate the mode of delivery of same to target chickens in the laboratory.

The virus was delivered to the laboratory birds on maize meal waste (maize offal). The result obtained showed that 79% of the target birds fed with maize offal containing the vaccine virus produced HI antibodies against the virus. Some of the characteristics of the virus strain have been reported (Alders et al., 2000). The performance of the vaccine under laboratory condition was expected to give an insight to what would be expected in the field. The efficacy of any vaccine is determined mainly by assessment of the level of antibody produced in the target animal and ability of the vaccinated animal to resist exposure to the virulent agent when compared with unvaccinated control (Spradbrow, 1993, 1994). These parameters were determined in this work with the NDV I_2 vaccine virus strain and were found to agree with the findings of other workers (Ideris, 1990a; Jayawardane et al., 1990), albeit with some variations.

The suggested and reported protective antibody titres for ND vaccines are HI (log_2) of 2^3 and above (Allan and Gough, 1974b). This was based on the level of HI antibody detected in the chicken serum. By implication antibody titre less than 2^3 would not be protective. In this study about two thirds of the birds produced detectable antibody following primary administration of the vaccine. This showed that the vaccine virus was immunogenic although only 4(6.45%) of the target birds attained HI log_2 titre of 2^3 on initial administration. The level of HI antibody appreciated when a booster dose of the vaccine was administered bringing the number producing the putative protective antibody level of 2^3 to 20%. This result also agrees with findings of previous workers with other thermostable ND viruses (Samuel and Spradbrow, 1989; Iroegbu and Nchinda, 1999). This means that technically, only 20% of the birds so vaccinated would be protected on exposure to virulent NDV. However, the results of the challenge experiment proved otherwise. When both vaccinated and unvaccinated (control) birds were exposed to velogenic challenge ND virus, through drinking water, all vaccinated birds resisted the challenge, including those with HI (log_2) titre of <1, while all control birds succumbed

and died. This result was explained by reports of some previous workers (Jayawardane and Spradbrow, 1995ab; Spradbrow, 1992b) that following the administration of challenge or vaccine virus by the natural route of infection: oral and/or respiratory, the aim of antibody intervention would be mucosal (secretory) rather than serum. Previous works with orally administered ND vaccines showed that there were increased number of plasma cells secreting immunoglobulin A (IgA) which is referred to as secretory antibody on the mucosal surfaces of avian intestine, bronchi and oviduct (Jayawardane and Spradbrow, 1995ab). The result is consistent with many reports that most orally administered ND vaccines do not produce reasonable levels of HI antibody following a dose of the vaccine (Ideris et al., 1990a; Jayawardane et al., 1990; Iroegbu and Nchinda, 1999).

Apart from serum and secretory antibodies, cell mediated immunity (CMI) has been reported to contribute to resistance of vaccinated birds to challenge with velogenic viruses (Reynolds and Maraqa, 2000). Demonstration of secretory and cell mediated immunity involve sophisticated techniques, and were not attempted in this work. However, it is believed that basically, mucosal immunity and some level of CMI may have been responsible for the protection of the vaccinated chickens recorded in this work. It was interesting to note that during post challenge observation, not even one chicken among the vaccinated flock showed any sign of disease including those with <1 HI (log_2) level of serum antibody. Although this result was obtained in the laboratory with laboratory bred chickens, there was nothing indicating that there would be significant difference in the field with village chickens that the vaccine is designed for.

The use of food-scrap (maize offal) was meant to obviate the problem of individual vaccination which is difficult in the natural habitat of the village chicken and to demonstrate that food waste may be good vaccine carrier. The immune response of the chickens to feeding with the dried vaccine-coated maize offal showed that the carrier food sustained virus infectivity and immune-genicity. Those that did not produce detectable antibody in the serum could be assumed to have secreted sufficient antibodies on the respiratory and intestinal surfaces to neutralize the challenge virus. This observation also agrees with reports of trials with the same virus in Tanzania (Wambura et al., 2000; Amakye-Anim et al., 2000) with commercial chickens, and in Vietnam (Tu et al., 1998) with village chickens. Post challenge signs observed among the control birds and the lesions observed at post mortem of deceased chickens and some vaccinated birds were identical with those described for ND (Gordon and Jordan, 1983).

It is therefore concluded that the vaccine could be useful for protection of rural chickens against ND. However it is also recommended that pilot field trials be carried out to establish its efficacy outside the laboratory environment.

ACKNOWLEDGEMENTS

We thank the Executive Director for permission to publish this work. We are also grateful to Mr. Elisha Tiyagnet for his technical assistance.

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