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Full Length Research Paper

Immune response and protection of free range chickens vaccinated orally with feeding of newcastle disease vaccine-coated cassava granules

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Cassava granules coated with Newcastle disease virus (NDV) strain V4-UPM was used to vaccinate free-range chickens in their natural habitat. Immune response, vaccine virus excretion and the efficacy of the food vaccine were assessed by standard methods. Results show that out of 218 chickens given initial food vaccine in the four locations, 138 (63.3%) produced detectable HI antibody while 202 (92.7%) had titres < 3.0. However, only 16 (7.3%) attained log2 3.0 with GMT of 3.2. This was made up of Nchara- Akanu 7(12.7%), Vandekya 0(0.0%), Fadan Karshi 1(1.7%), and Turu 8(15.1%) with GMTs of 3.3, 2.9, 3.0, and 3.6 in that order. Following the administration of a booster dose of vaccine on 194 birds in the same flocks, 170(87.6%) sero-converted with 118(60.8%) attaining log2 3.0 and GMT of 9.7. Chickens attaining HI titres up to log2 3.0 from the locations were as follows, Nchara-Akanu 26(51.0%), Vandekya 22 (51.2%), Fadan Karshi 28(53.8%), and Turu 33(68.8%) with GMTs of 12.8, 7.5, 7.0, and 12.6 respectively. Vaccinated birds excreted infective vaccine virus. Out of 55 buyback chickens challenged, 15(27.3%) died while 40(72.7%) survived. Twenty two (22) out of 24 (91.7%) unvaccinated birds challenged died and only 2(8.3%) survived. It is therefore concluded that cassava granules could be good carrier for food-borne ND vaccine delivery to village chickens in Nigeria.

Key words: Cassava, V_4-UPM virus, village chickens, Newcastle disease.

INTRODUCTION

The epizootiology and economic importance of Newcastle disease often times have been described mainly with reference to commercial, intensely reared poultry in organized farms (Alexander, 1997). Attention, however, turned to the free range village chicken flocks and other free-roaming avian species when various strains and pathotypes of NDV were isolated from apparently healthy individuals among these avian species including village chickens (Majiyabge and Nawathe, 1981; Bell and Mouloudi, 1988; Spradbrow 1993, 1994).

Thereafter, the results of several epizootiological studies pointed to these avian species and village chickens as important factors in transmission and enzootic maintenance of NDV in various localities (Echeonwu et al., 1993, Iroegbu and Amadi, 2004). Indeed, this has given rise to the speculation that apparently healthy free-roaming birds, including the village chickens, may be important in transmission of velogenic virus to organized poultry farms in their neighborhood thus giving rise to epizootics in the farms (Iroegbu and Amadi 2004, Spradbrow 1991).

The importance of the village chickens may not be limited to their undesirable role in transmission of veloge-

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nic NDV to organized poultry farms or in enzootic maintenance of NDV in the environment. They are important and valuable in their own right, and therefore need as much protection as the intensely reared flocks.

Studies have shown that protection of intensely reared chickens and other poultry against ND or control of the disease can only be achieved practically by vaccination (Allan et al., 1978; Aini, 1990). It is widely speculated that vaccination of the free roaming village chicken flock, particularly, and other avian species against ND would not only protect them for their own value but would also form a useful intervention against transmission of NDV to intensely reared poultry in organized farms. Indeed, a successful village chicken vaccination programme would improve the confidence of the rural farmer regarding the profitability of village chicken farming; and herald a realizable poverty alleviation strategy.

However, the free-range nature of the village chickens renders them not amenable to the conventional vaccine delivery methods. These modes of vaccine administration were designed for intensely reared commercial poultry but are not feasible for village chicken flock. The major limiting factors of the conventional live vaccines in use over the years are (1), they need cold-chain storage to maintain the viability and hence the immunogenic potency of the vaccine virus and (2), the price of such vaccines is not easily affordable to the poor village farmers.

The introduction of heat stable vaccines led to an innovative mode of ND vaccination, namely, oral delivery through chicken feeds (Spradbrow et al., 1978; Aini, 1990), thereby providing a feasible method for vaccination of large scattered population of free-roaming village chickens flocks. It was suggested that an ideal vaccine carrier food should be cheap, readily available in the target locality and should not contain substances that would inactivate the vaccine virus (Spradbrow, 1993, 1994). Although the carrier food need not be nutritious for it to be effective in conveying the vaccine virus, it ought to be palatable and desirable to the chickens (Iroegbu and Nchinda, 1999). Elsewhere other potential ND vaccine carrier food stuffs have been investigated for survivability of virus coated on them (Wambura et al., 2007). Thus, there is need to study the suitability of readily available potential carrier foods in each locality in Nigeria where mass ND vaccination of free-range village chicken flock is intended. Cassava is a foodstuff that is very popular especially in many parts of Nigeria. Its potential as a carrier food for delivery of ND vaccine to free range chickens in their natural habitats is the objective of the work herein reported.

MATERIALS AND METHOD

Experimental design

1. Coating of cassava granules with NDV strain V4-UPM, drying at RT and determination of titre per gram of food vaccine (EID_{50}/gm).
2. Vaccination of selected free range chickens flocks by oral feeding with virus-coated cassava granules.
3. Evaluation of immunogenicity of food vaccine by testing for HI antibody in sera of chickens fed with the food vaccine.
4. Assessment of infective virus excretion by chickens fed with the food vaccine
5. Evaluation of the efficacy of the food vaccine by challenge experiment with vaccinated and unvaccinated (control) birds

The virus strains used for the investigation

The seed virus was a lentogenic NDV strain V_4–UPM obtained from Professor Aini Ideris of the Faculty of Veterinary Medicine and Animal Science, University Pertanian, Malaysia (UPM). The virus was propagated in embryonated hen eggs and the stock virus titrated and stored as previously reported (Echeonwu et al., 2007). The velogenic virus strain used for challenge experiments was the NDV-VGF-1 isolated locally in Nigeria from sick guinea fowls and characterized (Echeonwu et al., 1993), freeze-dried and stored among the reference virus stock of the Virology Division, NVRI, Vom.

Preparation and coating of carrier food with vaccine virus

Some tubers of cassava were purchased from the market in Vom. They were peeled, cut in pieces, washed and sun-dried. The dried material was ground into granules in commercial milling machine and stored in polythene bags at room temperature (RT) (18 - 27°C) until used for coating with the vaccine virus by the method described by Alders and Spradbrow (2001). The vaccine-coated food was allowed to dry at RT under a gentle air current overnight and the virus content per gram of carrier food (EID_{50}/gm) was estimated to be approximately $10^{8.0}$ by the method of Samuel et al. (1993). Thereafter the dried food vaccine was packaged in 500 g quantities ready for field vaccination trials.

Pilot field vaccination trials

Description of field project locations

Field work was done in 4 locations (villages) selected for convenience, consent, willingness to participate in the pilot project, availability of reasonable chicken population and easy accessibility to birds. They comprised of 24 compounds or households each owning between 5 and 12 village chickens with an average of about 8 chickens per household or compound. The farmers practiced the age old husbandry in which birds were allowed to freely roam the village environment scavenging for food and roosting at night on tree branches around the houses and any available makeshift shelter. These households were located in four states of Nigeria, (namely Abia, Benue, Plateau and Kaduna States). The locations or villages were Nchara-Akanu (Abia), Vandekya (Benue), Fadan Karshi (Kaduna), and Turu (Plateau). Abia State is located on latitude 5°30 N and longitude 7°30 E, Benue State on latitude 7°47 N and longitude 6°46 E, Kaduna State on latitude 10°31 N and longitude 7°26 E and Plateau State is on 9°56 N and 8°53 E.

Method of entry into the villages and obtaining consent from participants

The method described by Alders and Spradbrow (2001) was adopted for all field vaccinations, evaluation of immune responses and efficacy of the food-based vaccination method. Chicken farmers were instructed on how to handle, store and apply the food vaccines. They were specifically told to spread the vaccine on their

Table 1. Antibody response of village chickens fed with vaccine coated on cassava granules – Primary dose.

Location	No. of vaccinations	Pre-vaccination Mean HI (log_2) titre	Vaccine dosage (EID_{50}/gm)	No. of chickens vaccinated	No of chickens showing HI (log_2) titre spread					No (%) HI (log2) Titre	Geometric mean titre (GMT)
					<1	1	2	3	4	3.0	
Nchara-Akanu	1	0.82	8.0	55	20	16	12	7	0	7(12.7)	3.3
Vandekya	1	0.11	8.0	51	19	15	17	0	0	0(0.0)	2.9
Fadan Karshi	1	0.17	8.0	59	22	17	19	1	0	1(1.7)	3.0
Turu	1	0.95	8.0	53	19	16	10	5	3	8(15.1)	3.6
Total	-	-	-	218	80	64	58	13	3	16(7.3)	3.2

local trays or ground first thing in the morning under a shade near where the birds roosted so that they would consume it before wandering away. On the day of bleeding they were told to keep the birds where they roosted in their makeshift housing until bled and released.

Method of vaccination in the field

Food without vaccine was supplied along with food vaccine to the participating chicken owners. Birds were screened by HI test technique for NDV antibody prior to initial vaccine feeding with standard antigen titre of 4 haemagglutinating unit (4HAU) = 1/64. The uncoated foods were used as bait to draw the birds to a particular spot under a shaded area near their roosting place. This was done for 2 – 3 days before the actual food vaccine was placed at the same spot for the chickens to consume. This ensured that the birds consumed the food vaccine. This method was adopted for all the field vaccination exercises. The quantity of vaccine-coated food supplied was such that each bird had the chance of consuming between 10 and 20 g in one feeding event with some leftover. Faecal samples were collected daily after food vaccine feeding for five days and screened by standard method (NAS 1971) for infective vaccine virus excretion. Booster vaccinations were done 2 weeks after the primary dose was administered. Blood samples were collected for HI assay two weeks post primary and booster vaccinations while the method of Reid (1968) was used to compute the geometric mean titres (GMTs).

Challenge experiments

Chickens vaccinated with food vaccines in the field were challenged by exposure to (velogenic) NDV ($10^{7.5}$ ELD_{50} /ml.) by the oral (drinking water) route (Spradbrow, 1993, 1994). Two weeks after the booster vaccination, samples of vaccinated village chickens were bought back (buybacks) from their owners and some unvaccinated (controls) samples from outside vaccination areas were also bought and taken to the laboratory for challenge experiments. Chickens were observed for 10 days post challenge. A total of 55 buy-backs (30% of vaccinated chickens from each location) and 24 control birds were purchased and comprised mostly of those at the grower age and size to avoid mutual aggression. The buy-backs were quarantined for 7 days for observation for any sign of disease – especially, ND before challenge com-menced.

RESULTS

The mean pre-vaccination HI (log_2) titres were 0.82, 0.11, 0.17 and 0.95 respectively for Nchara-Akanu, Vandekya, Fadan Karshi and Turu.

Out of a total of 218 village chickens fed vaccine-coated cassava granules as primary vaccination in all the four (4) locations, 138 (63.3%) produced detectable HI antibody, but only 16 (7.3%) produced HI (log_2) antibody titres 3.0 with overall GMT = 3.2 and 202 (92.7%) produced titre < 3.0. A breakdown of results from each of the locations showed that Nchara-Akanu (55 birds), Vandekya (51 birds), Fadan-Karshi (59 birds) and Turu (53 birds) had only 7(12.7%), 0(0.0%), 1(1.7%) and 8(15.1%) producing HI (log_2) titre 3.0, and GMTs of 3.3, 2.9, 3.0, and 3.6 respectively following primary administration of the food vaccine, Table 1. When booster doses were administered to a total of 194 chickens in the same flocks, 118 (60.8%) seroconverted to HI (log_2) titre 3.0 and overall GMT of 9.7. The number of chickens that produced log_2 HI antibody titres below 3.0 was 76 (39.2%). Breakdown showed that Nchara-Akanu (51 birds), Vandekya (43 birds), Fadan-Karshi (52 birds), and Turu (48 birds) had 26(51.0%), 22(51.2%), 28(53.8%), and 33(68.8%) producing HI (log_2) titre 3.0, and GMTs of 12.8, 7.5, 7.0, and 12.6 respectively, Table 2.

Out of a total of 55 buyback chickens from the four locations challenged by exposure to velogenic NDV, 40(72.7%) survived while 15(27.3%) succumbed and died. From each of the locations, 15, 10, 16, and 14 birds (Nchara- Akanu, Vandekya, Fadan Karshi, and Turu) respectively were challenged and 10(66.7%), 7(70.0%), 12(75.0%) and 11(78.6%) survived while 5(33.3%), 3(30.0%), 4(25.0%), and 3(21.4%) died in that order. For control challenge experiment, 22 out of 24 (91.7%) unvaccinated birds challenged died and only 2(8.3%) survived, Table 3.

There was evidence of infective vaccine virus excretion because isolates from the faecal samples inoculated into 10-day old chick embryo failed to kill chick embryos even after 96 h of incubation.

DISCUSSION

Cassava was found to adequately support coated vaccine virus in the study with equally good level of recovery of infective virus. It would appear that cassava contains no virus inhibitory factors like the grains. This assump

Table 2. Antibody response of village chickens fed with vaccine coated on cassava granules – Booster dose

Location	Number of Vaccinations	Vaccine Dosage (EID_{50}/gm)	Number of Chickens Vaccinated	No. of chickens showing HI (log_2) titre spread									No. (%)HI (log_2) titre 3.0	Geometric mean titre (GMT)
				<1	1	2	3	4	5	6	7	8		
Nchara-Akanu	2	8.0	51	5	3	8	9	13	8	4	1	0	26(51.0)	12.8
Vandekya	2	8.0	43	9	5	7	8	14	0	0	0	0	22(51.2)	7.5
Fadan Karshi	2	8.0	52	5	11	8	11	13	4	0	0	0	28(53.8)	7.0
Turu	2	8.0	48	5	3	7	9	12	7	5	0	0	33(68.8)	12.6
Total	-	-	194	24	22	30	37	52	19	9	1	0	118(60.8)	9.7

Table 3. Results of challenge experiments with chickens from the four locations

Location	No. of chickens challenged	HI (log_2) titre range	Mean (log_2) titre	No. dead	% dead	No. surviving	% surviving
Nchara-Akanu	15	<1-3	2.8	5	33.3	10	66.7
Vandekya	10	<1-3	2.3	3	30.0	7	70
Fadan Karshi	16	<1-4	4.0	4	25.0	12	75
Turu	14	<1-5	4.2	3	21.4	11	78.6
Total	55	-	-	15	27.3	40	72.7
Controls	24	<1	0	22	91.7	2	8.3

tion is supported by reports of its successful use in vaccination trials in Indonesia (Spradbrow, 1992b), while Salum et al., (1997) also reported its successful use with V_4 for vaccination in Tanzania. When supplemented with a protein source (5% crayfish) (Iroegbu and Nchinda, 1999), it was found that the palatability of cassava as carrier food to chickens was improved during a trial vaccination. Cassava is the commonest staple food in the eastern part of Nigeria and its abundance in this area could be exploited for large-scale food based vaccination program.

If the carrier food delivered viable virus to the chickens fed with it, the birds would be expected to excrete viable vaccine virus as well as be stimulated to produce antibody against the virus. Vaccinated chickens did excrete infective virus for up to 8 days post primary and booster application of the food vaccine. This proved that the coated food was able to deliver viable vaccine virus to the birds fed with it and this result agrees with the findings of other investigators (Iroegbu and Nchinda, 1999; Spradbrow et al., 1988; Samuel and Spradbrow, 1989; Ideris et al., 1990a) who recorded virus excretion from food-based vaccinated birds for up to 10 - 30 days with peak between the 5^{th} and 6^{th} day after administration of vaccine.

Excretion of the vaccine virus is an important condition for adequate spread of vaccine virus among the flock of chickens and ensures that all birds in the flock housed or roosting together become infected and therefore immunized by the vaccine virus. Longer duration of virus excretion observed with the booster vaccinations even ensured better herd immunity. However, this becomes a different matter with the observation that vaccinated challenge birds could excrete velogenic NDV and hence could be source of infection to non-immune birds. Mahmood (1998) reported that low levels of antibody have been shown to encourage prolonged excretion and hence persistence of virulent virus post-challenge. It is possible to have this situation among group-vaccinated chickens by drinking water or food vaccine since it has been demonstrated that all birds in a flock do not consume equal amounts of vaccine (Spradbrow 1993/94).

Immunogenicity of the vaccine virus was assessed based on the production of detectable HI antibody to the level accepted as protective. It was also observed that a single administration of the vaccine did not induce the production of protective level of HI antibody in most birds, while still in some no detectable antibody was recorded. However, administration of booster dose of vaccine led to a good number of chickens producing HI antibody up to and above the putative protective log_2 HI titre of 3.0 (Allan and Gough 1974a). The result also agrees with the report of some other workers that a second administration of vaccine 10 – 14 days after the first one was necessary for effective production of HI antibody by vaccinated chickens (Iroegbu and Nchinda, 1999; Samuel and Spradbrow, 1989; Ideris et al., 1990a; Samuel and Spradbrow, 1991; Jayawardane et al., 1990; Echeonwu et al., 2007). We consider HI log_2 titre of 2^3 (3.0) as ade-

quate and protective enough for village chickens although 2^4 (4.0) was recommended by OIE (2000) for vaccines designed for commercial intensely reared chickens.

Orally administered vaccines have been reported to primarily provoke mucosal immunity (Parry and Aitken, 1977; Jayawardane and Sppradbrow, 1995a, b). It is thought that this is the first line of defense against NDV infection, which occurs either by inhalation or ingestion or both in nature (Alexander, 1997). This may explain why the percentage of chickens resisting challenge in this work was higher than the percentage producing HI ($\log_2$) antibody titre 2^3. The variations in the level of immune response and protection observed from location to location may be due to variable environmental conditions such as ambient temperatures, humidity, rainfall, and health status of the chicken as well as human factors in the vaccine administration which may also vary from location to location.

Following challenge experiments to assess the efficacy of the vaccination method and the immunogenicity of the vaccine, clinical signs observed were similar to those described by Gordon and Jordan (1983) and lesions observed at post mortem examination were identical with lesions described by McFerran and McCraken (1988) for Newcastle disease. The challenge experiments followed a natural route of infection in the field, namely, by drinking water in line with the suggestion of Spradbrow (1993, 1994) that the conventional intramuscular route would by-pass the natural route of infection in the field. Iroegbu and Nchinda (1999) employed the drinking water route for challenge experiment with satisfactory results.

It is therefore concluded that cassava, if properly treated and processed, could be a reliable vehicle for delivery of thermostable ND vaccines to free range chickens in Nigerian rural localities for reasonable protection of the birds against the disease. This would go a long way to reducing ND related mortalities, improve confidence in village chicken farming and contribute to poverty alleviation especially in the rural areas.

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