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Full Length Research Paper

Delivery of thermostable Newcastle disease (ND) vaccine to chickens with broken millet grains as the vehicle

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The efficacy of treated broken millet grains as a carrier for delivery of thermostable Newcastle disease (ND) vaccine HRV_4 to free-range chickens in three locations was assessed by haemagglutination inhibition (HI) test and challenge experiment. Out of 256 birds fed with first dose of the vaccine, 130 (50.8%) produced detectable HI antibody but only 16 (6.3%) attained serum antibody level of $log_2 \geq 3.0$ adjudged protective. From the locations, Igumale (74 birds), Kuru (88 birds) Riyom (94 birds), only 1 (1.4%), 8 (9.1%) and 7 (7.4%) attained log_2 titre ≥ 3.0, respectively. A booster vaccine dose 2 weeks later on 236 of the birds led to 126 (53.4%) attaining HI (log_2) titre ≥ 3.0 [Igumale (63 birds), Kuru (86 birds) Riyom (87 birds) had 33 (53.5%), 46 (53.5%) and 42 (48.3%) respectively attaining log_2 titres of ≥ 3.0]. Out of a total of 70 buy-back chickens challenged, 49 (70.0%) survived. The break down showed that from Igumale (20 birds), Kuru (25 birds) Riyom (25 birds), the survivors were 13 (65.0%), 19 (76.0%) and 17 (68.0%), respectively. Out of 28 unvaccinated control birds challenged, only 4 survived. The overall results showed that millet, if properly treated, could be a good vaccine carrier and that the method of vaccination was relatively efficacious.

Key words: Broken millet, village chicken, Newcastle disease, vaccination.

INTRODUCTION

The epizootiology and economic importance of Newcastle disease have been described mainly with reference to commercial, intensely reared poultry in organized farms. 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 (Alexander, 1986; Bell and Mouloudi, 1988). 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 (Kaleta and Baldauf, 1988; Echeonwu et al., 1993; Iroegbu and Amadi, 2004). This has, indeed, 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 (Spradbrow, 1992a; Iroegbu and Amadi, 2004).

The village chickens are important and valuable in their own right as sources of animal protein and cash, and therefore need as much protection as the intensely reared flocks. Despite the recognized importance of the village chicken, the rural farmers are most unwilling to commit their scarce resources and time to village chicken farming because of the high annual mortality among the

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village chicken flocks during ND epizootics (Olabode et.,1992; Iroegbu and Amadi, 2004).

Studies among intensely reared poultry have shown that protection of chickens against ND or control of the disease can only be achieved practically by vaccination (Allan et al., 1978). 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 farmers regarding the profitability of village chicken farming; and herald a realizable poverty alleviation strategy.

However, village chicken vaccination efforts would meet with a number of obstacles, one of which is their free-range life style which renders them not amenable to the conventional vaccine delivery methods, namely, aerosol/sprays or drinking water methods only practiced in enclosures for mass vaccination, and eye-drop and injection methods applied individually. These modes of vaccine administration were designed for intensely reared commercial poultry but are not feasible for village chicken flock in their feral nature.

Hope for village chicken vaccination came with advent of heat stable vaccines which in turn heralded the introduction of an innovative mode of ND vaccination, namely, oral delivery through chicken feeds (Spradbrow et al., 1978; Aini, 1990). This presented a feasible method for vaccination of the village chicken flock. Spradbrow (1993/94) suggested oral delivery of food-borne vaccine to large scattered population of free-roaming village chickens as convenient means of protecting then and other poultry in the locality against ND. At the same time, 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, 1993/94). 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).

Preliminary work with food-coated V_4 virus vaccine administered to chickens showed that it was possible to deliver the vaccine virus to chickens on food (Ibrahim et al*.,* 1981). Subsequent trials with different types of food carriers have produced variable results due to variations in the characteristics of food types, including their constituents (Spradbrow, 1989). Several workers have suggested various treatments to be given the carrier foods so as to enhance the viability of the vaccine. These include prior washing before coating or soaking overnight followed by washing (Cumming, 1992; Johnston et al., 1992). These treatments are expected to render foods, erstwhile observed to be “unfriendly” to vaccine virus, satisfactory as vaccine carriers (Darminto and Daniels, 1992b; Ibrahim, et al., 1992; Spradbrow, 1992b). 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. The primary objective of this work was to assess the efficacy of millet as a potential carrier for the V4-UPM vaccine virus and our findings are herein reported.

MATERIALS AND METHOD

The vaccine and challenge virus strains

The seed virus was NDV strain V_4–UPM obtained from Professor Aini Ideris of the Faculty of Veterinary Medicine and Animal Science, University Pertanian, Malaysia (UPM), propagated in chick embryos and titrated by the techniques recommended by (NAS, 1971), freeze-dried in 2.0 ml vials and stored at –70°C ultra-low temperature freezer (Revco Scien Temp Corp Adrian Michigan) prior to use for food vaccine preparation. The virus strain used for challenge experiment was the VGF-1 isolated locally from apprently healthy guinea fowl, characterized by Echeonwu et al. (1993), freeze dried in 0.5 ml ampoules and stored at –70°C in another ultra-low temperature freezer of the same make as above. in Virology Department of National Veterinary Research Institute (NVRI), Vom, Nigeria.

Preparation and coating of carrier food with vaccine virus

Millet, which is readily available, was purchased from a local market in Vom, Plateau State, Nigeria and ground to produce coarse particles of the foodstuff and was soaked in tap water for 72 h and washed with clean tap water and sieved three times. After thorough drying, the material was packed in polythene bags and stored at room temperature until used for coating with vaccine virus. The method described by Alders and Spradbrow (2001), was used for coating with vaccine virus. Ten vials of the freeze-dried vaccine were reconstituted in 100 ml well water and was sprayed onto the dried carrier food in a bowl at a ratio of 1.0 ml of reconstituted vaccine, 10.0 g of carrier food and thoroughly mixed manually. After mixing, the coated food vaccine was spread on metal trays and kept at room temperature (RT) to dry under gentle air current overnight. The dried food vaccine was placed in polythene bags and stored at RT until used for pilot food-based vaccination trials. Residual infectivity titre of coated virus per gram of carrier food was determined by the method of Samuel et al. (1993) and the 50% egg infective dose (EID_{50}) was computed by the method of Reed and Muench (1938).

Description of field pilot vaccination trial locations

Field work was done in 3 locations (village/wards) selected for convenience and consent of willing chicken owners, comprising between 6 and 8 compounds or households each owning between 7 and 15 village chickens with an average of about 11 chickens per household or compound. These households were located in two (2) states of Nigeria. The higher number of locations was in Plateau State, representing area with low ambient temperature (18 – 27°C) with 2 villages comprising 13 households with 182 birds while the least number of households, seven (7), was in Benue State representing area of high ambient temperature (31-38°C) in one village with 74 birds. Chickens were the indigenous breeds that have long existed in the areas and the sample size (population)

Table 1. Antibody response of village chickens in three locations fed with first dose of vaccine coated on broken millet.

Location	Vaccine dosage (EID_{50}/gm)	Pre-vaccination HI (log_2) titre	No. of chickens vaccinated	HI (Log_2) titre spread amongst number of vaccinated chicken						No. (%) HI log_2 ≥ 3.0	Geometric mean titre
				0	1	2	3	4	5		
Igumale	7.6	1.7	74	42	19	12	1	0	0	1 (1.4)	2.7
Kuru	7.6	1.3	88	41	22	17	7	1	0	8 (9.1)	3.3
Riyom	7.6	1.1	94	43	25	19	5	2	0	7 (7.4)	3.2
Total	-	1.4*	256	126	66	48	13	3	0	16 (6.3)	3.1*

* = Mean values

was considered adequate for pilot food-based vaccine field trial. The method of husbandry was the age old one in which birds were left to roam the village environment scavenging for their food and roost in any available shade, including tree branches at night. Twenty eight (28) unvaccinated control chickens used in the challenge experiment were obtained from another village not participating in the pilot vaccination trial. Plateau State is located on latitude 9 °56′N and longitude 8 °53′E while Benue State is on latitude 7°47′N and longitude 6 °46′E. The villagers in both states are mainly farmers and keep sizable number of indigenous chicken and other livestock.

Method of vaccination in the field

The methods described by Alders and Spradbrow (2001) were adopted for obtaining consent from participants, field vaccinations, evaluation of immune responses and efficacy of the food-based vaccination method. Birds were screened by haemagglutination inhibition (HI) test technique for NDV antibody prior to initial vaccine feeding with food-borne vaccine. Plain carrier food without vaccine was supplied along with vaccine-coated food to the participating chicken owners. The uncoated food was used as a bait to draw the chickens to a particular spot under a shade near their roosting places. 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. Blood samples were collected according to the method described by Alders and Spradbrow (2001) two weeks post primary and two weeks post booster vaccinations for HI assays which were done following the method described in OIE (2000) Manual. The method of Reid (1968) was used to compute the geometric mean titres (GMTs). For post vaccination challenge experiments, a total of 70 vaccinated (buy-back) birds were purchased from pilot vaccination locations (20 from Igumale, 25 from Kuru and 25 from Riyom). Another 28 unvaccinated (control) birds for challenge control were purchased from locations that did not participate in the pilot vaccine trial and all were brought to the laboratory for the experiment. Post mortem examination was done on chickens that died due to exposure to challenge virus.

RESULTS

The mean pre-vaccination haemagglutination inhibition (HI) (log_2) titre recorded for all the birds was 1.4, while for each of the three locations, Igumale was 1.7, Kuru was 1.3, and Riyom was 1.1. Out of the 256 birds receiving the initial food-borne vaccine, only 16 (6.3%) produced detectable serum HI (log2) ≥ 3.0 and GMT of 3.1; 126 (49.2%) produced no detectable HI antibody while the rest of the birds produced antibody that ranged from HI (log_2) 1 – 2. A breakdown from the locations showed that Igumale (74 birds), only 1 (1.4%); Kuru (88 birds), 8 (9.1%); and Riyom (94 birds), 7 (7.4%) had HI (log_2) titre ≥ 3.0 with GMTs of 2.7, 3.3 and 3.2 respectively. Out of the number of chickens in each location, 42, 41 and 43 produced no detectable HI antibody respectively following primary vaccination. Details are presented in Table 1.

However, following administration of booster dose of the food vaccine on a total of 336 birds in the same flocks, 121 (51.3%) seroconverted and attained HI (log_2) titre ≥ 3.0 with GMT of 8.9; only 40 (16.9%) had no detectable HI antibody and the remaining birds HI (log_2) titres of 1 – 2. From the locations, Igumale (63 birds), 33 (52.4%); Kuru (86 birds), 46 (53.5%), and Riyom (87 birds), 42 (48.3%) produced HI (log_2) titres ≥ 3.0 with GMTs of 8.2, 10.0 and 8.2 respectively. Out of the above number of birds per location, only 11, 14 and 15 still had no HI antibody while the rest had HI (log_2) titre of 1 – 2 (Table 2).

Challenge experiment results showed that out of a total of 70 chickens from the three locations exposed to velogenic challenge virus, 49 (70.0%) survived. Details from locations showed that Igumle (20 birds), Kuru (25 birds) and Riyom (25 birds), 13 (65.0%), 19 (76.0%) and 17 (68.0%) survived, respectively. The control challenge experiment showed that only 4 out of 28 birds (14.3%) survived Table 3.

DISCUSSION

The ability of the carrier food to deliver viable vaccine virus to the chickens' intestinal tract and to stimulate the production of protective HI antibody were the main parameters investigated. If the carrier food delivered via-

Table 2. Antibody response of village chickens in three locations fed with a booster dose of vaccine coated on broken millet

Location	Vaccine dosage (EID_{50}/gm)	Number of chickens vaccinated	HI (Log_2) titre spread amongst number of vaccinated chickens									No. (%)HI (log_2) titre ≥ 3.0	Geometric mean titre
			0	1	2	3	4	5	6	7	8		
Igumale	7.6	63	11	6	13	14	11	8	0	0	0	33 (52.4)	8.2
Kuru	7.6	86	14	9	17	16	15	6	5	4	0	46 (53.5)	10.0
Riyom	7.6	87	15	8	22	15	14	9	3	1	0	42 (48.3)	8.6
Total		236	40	23	52	45	40	23	8	5	0	121 (51.3)	8.9*

* = Mean value

Table 3. Results of challenge experiment with buy-back chickens from vaccination locations and unvaccinated controls.

Location	No. of birds Challenged	No. of surviving birds	% of surviving birds
Igumale	20	13	65.0
Kuru	25	19	76.0
Riyom	25	17	68.0
Total	70	49	70.0
Controls	28	4	14.3

ble virus to the chickens fed with it, the birds would be expected to produce antibody against the virus. This was assessed based on the production of detectable HI antibody in the chicken serum to the level accepted as protective. HI antibody was produced following primary vaccination by about 47% but only very few of the chickens (6%) attained HI (log_2) titre of 3.0 and above conventionally quoted as protective (Allan and Gough, 1974a). However, administration of booster dose of vaccine led to a good number of chickens (83%) producing HI antibody with about 51% up to and above HI (log_2) titre of 3.0. These results also agree with the findings of some other investigators who observed that a second administration of vaccine 10 – 14 days after the first one was necessary for effective production of HI antibody by vaccinated chickens (Samuel and Spradbrow, 1991; Jayawardane, et al., 1990; Ideris et al., 1990a; Iroegbu and Nchinda, 1999).

Newcastle disease vaccines administered orally have been reported to primarily provoke mucosal immunity (Parry and Aitken, 1977; Jayawardane and Spradbrow, 1995a, b), which attempt was not made to detect in this study. 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, 1988). This arm of humoral immunity is reported to be responsible for protection of the chickens even before detectable HI antibody is found in the serum (Spradbrow, 1992b). Although OIE (2000) recommended HI (log_2) titre of 4.0 as protective with reference to conventional ND vaccines designed for intensely reared commercial chickens, we consider log_2 titre of 3.0 adequate for food-borne vaccines administered orally to scavenger chickens. This is more so since it has been found that even chickens with HI log_2 titre of zero resist challenge with velogenic ND virus indicating that serum antibody alone may not be responsible for the resistance to challenge. Judging from the level of protective serum antibody attained following booster dose of the vaccine, only 51% of the vaccinated birds would be expected to be protected in the event of velogenic virus infection of the flock. However, orally routed challenge experiment results showed that higher percentage of vaccinated birds (70%) survived.

Perhaps, many of the birds which resisted challenge with velogenic NDV were protected through IgA-dependent mucosal immunity. According to Jayawardane and Spradbrow, (1995a, b), factors other than serum and secretory antibodies contribute to resistance of vaccinated birds to challenge with velogenic virus. These include cell-mediated immunity, which has always been

assumed to be part of the complex immune responses to a variety of ND vaccines. However, Reynolds and Maraqa (2000) reported that specific CMI to ND vaccine by itself was not protective against virulent virus, rather virus neutralizing (VN) and HI antibodies were necessary in providing protection against ND. Cell mediated immunity was not specifically studied in this work.

Earlier reports on similar investigations showed varying outcomes (Ideris et al., 1978a, Aini et al., 1990b). Failure of some of these trials was blamed on antiviral factors constituent in the seeds or introduced as preservatives. This necessitated the cracking, soaking and washing treatments given the grain investigated in this work. Cumming, (1992) and Jackson, (1992) suggested that heat, washing and cracking might be useful in developing a successful carrier food. The level of successful coating of the virus recorded could be attributed to the type of treatment given to the grain. In addition to other physical and/or chemical factors (eg. lectins) that may aid virus binding (Rehmani and Spradbrow, 1995), the dried food grain would be expected to have high affinity for moisture and hence, readily absorb the virus with the suspending fluid.

In an initial trial (results not shown) involving coating the grain with virus after cracking (but without soaking or washing), there was significant loss of virus infectivity within few hours of storage at room temperature (RT). This observation could be explained, as suggested by Spradbrow (1993/94), by the presence of antiviral factors in grains and certain seeds. The antimicrobial compounds are thought to dissolve in any available moisture and diffuse to the surface of the grains or seeds where they act on the adsorbed virus (Spradbrow 1992b). With the soaking treatment adopted for the selected grain, the antiviral agents were likely to have leached out and eventually eliminated during washing and rinsing. Soaking is presumed a critical protocol because cracking and washing alone may not allow enough time and condition for antiviral elements inside the grain material to leach out. On the contrary, the treatment, which involves only cracking and washing might just enhance diffusion of the dissolved antiviral compounds to the surface and hence inactivation of the vaccine virus. As opined by Spradbrow (1993, 1994), the binding may be reversible or irreversible. In any of the cases, the virus, if viable, would still be available to initiate infection in the digestive tract of the chicken, thereby provoking immune response

Following challenge experiments to assess the efficacy of the vaccination method with millet-borne vaccine, clinical signs of the chickens observed were similar to those described by Alexander (1997). These were listlessness, increased respiration, weakness, prostration, greenish watery diarrhoea, torticollis, paralysis of legs and wings, and death in that order within 3-5 days; the lesions at post mortem examination were identical with lesions described by McFerran and McCraken (1988) for Newcastle disease. These included haemorrhagic lesions in the small intestines, proventriculus and caecal tonsils. Other features observed were tracheal congestion, air saculitis and thickening of the air sac with catarrhal or caseous exudates.

Subsequent isolation of virus from pooled organs of deceased birds following challenge and inhibition of haemagglutinating activity of the isolate with standard NDV antiserum further proved that the challenge birds died of the challenge virus infection. The challenge experiments followed one of the natural routes of infection in the field, namely oral by drinking water in line with the suggestion of Spradbrow (1993/94) 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 hereby concluded that millet, if adequately treated, could be useful carrier for thermostable ND vaccine designed for protection of village chicken against the disease.

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