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## Research Article



### Appraisal of NDV<sub>4</sub> thermo-stable Per-os vaccination amongst free-ranged local poultry keepers in Adamawa state, North-eastern Nigeria

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#### Abstract

Field trial of the V<sub>4</sub> thermostable Newcastle disease vaccine (NDV) was carried out in rural poultry in Adamawa state Northeastern Nigeria. This was to establish the effect of V<sub>4</sub> thermostable NDV on the productivity of rural poultry, breed, feeding, medication and general husbandry. Structured questionnaire on general ecology of rural poultry including hatchability, marketing, culling and socio-economic characteristics of rural poultry farmers were administered thrice to respondents in the study villages along side oral vaccination with V<sub>4</sub> thermostable NDV. Sera obtained from blood samples collected from birds during pre-vaccination, post-vaccination and post-booster vaccination with NDV<sub>4</sub> thermostable vaccine was subjected to Hemagglutination Inhibition (HI) test. Vaccination showed insignificant effect on productivity, hatchability, mortality and morbidity. There was no significant Geometric Mean Titre (GMT) change in trial and control groups as well as variable investigated during pre-vaccination, post-vaccination and post-booster vaccination. Both groups showed no evidence of seroconversion, although high GMT of 1:14 was observed during the post-booster vaccination. Therefore, per-os vaccine administration via drinking water is not an efficient means of delivery for V<sub>4</sub> thermostable NDV. Thus, use of suitable feed as vehicle for V<sub>4</sub> thermostable ND vaccination is recommended to facilitate vaccine uptake and immune protection amongst scavenging village poultry, in addition to improvement in other health and management practices that would enhance productivity.

**Keywords:** Rural appraisal, NDV<sub>4</sub> thermo-stable, Per-os vaccination, rural poultry, Nigeria.

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#### Introduction

Newcastle Disease (ND) is one of the most important endemic diseases of chickens (Okor, et al., 2010) characterized by high mortality in susceptible flock with consequent economic losses in Nigerian poultry industry (Saidu et al., 2006) especially amongst local chickens (Echeonwu, et al., 2008). The disease have been reported amongst most poultry producing countries globally with most devastating velogenic forms especially in tropical Asia, Middle East and Africa (Spradbrow, 1987). The disease in commercial poultry is adequately controlled by vaccination using live vaccines (Abdu et al., 2005). However, limitations

associated with use of live vaccines in local free-ranged, small sized chicken flocks have been documented (Ibrahim and Idris, 1988). This includes cumbersome nature of gathering and handling the scattered birds during vaccination, multiple needs for vaccination and instability of live-vaccines under tropical field conditions. The development of V<sub>4</sub> thermo-stable ND vaccine using selected resistant avirulent V<sub>4</sub>HR form (French *et al*, 1967; Westbury *et al*, 1984) which is highly immunogenic (Webster *et al*., 1976) with good transmissibility (Westbury, 1979) has demonstrated possible and efficient vaccination of

local poultry in different tropical countries including Nigeria (Nwanta, 2003). However, there exists insufficient information on the effects of V<sub>4</sub> thermo-stable NDV on free-ranged local poultry in these study areas of North-Eastern Nigeria characterized by high ambient temperatures especially in the dry season when the ND is most likely to occur (Olabode et al., 2012). Participatory rural appraisal (PRA) evolved out of Rapid Rural appraisal (RRA) and placed more emphasis on community empowerment to process and utilizes locally generated information (McCracken et al., 1988). The use of rapid rural appraisal or participatory rural appraisal techniques as project design and monitoring tool have been shown to be timely, accurate and cost-effective means of collecting essential information for project formulation (Moris and Copestake, 1993) at community level (Theis and Grady, 1991). These livestock owners are active participants in design, implementation, monitoring and review that intellectually contribute towards successful development of trustworthy projects conducted for better acceptance of disease control interventions by the animal-owning public (FAO, 2011). This study

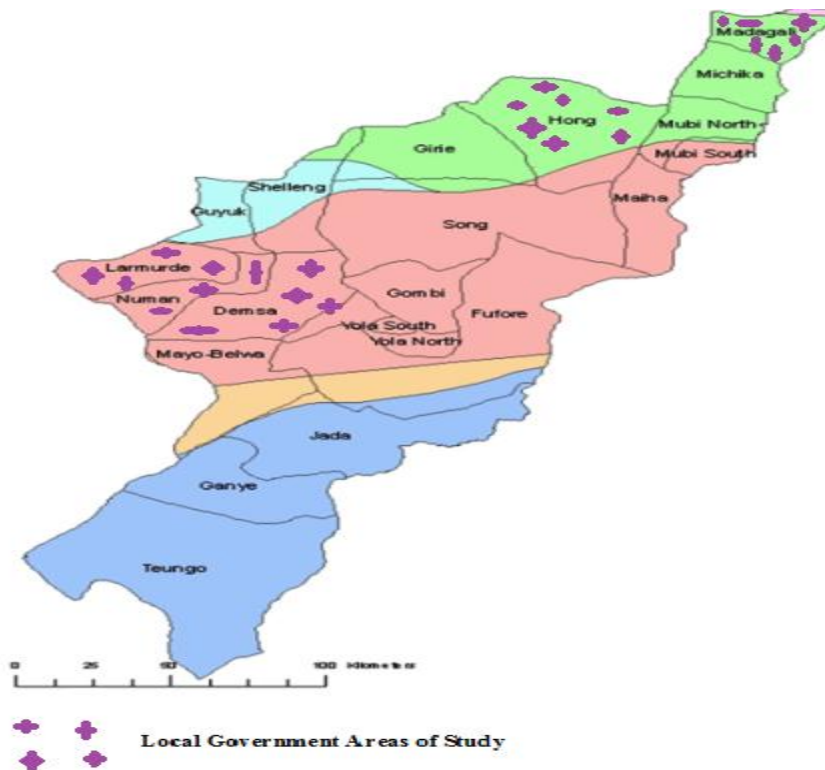
therefore was designed to establish the effect of NDV<sub>4</sub> thermo-stable vaccine amongst local poultry under field condition in these study area in optimism that it would enhance ND control amongst village flocks thereby improving livelihood and the income generating capacity of rural poultry keepers.

## Materials and Methods

### Study area

The study was carried out in ten villages (Sabonpegi, Goningora, Fari, Uding, Sabongari, Ngalang, Pella, Shuwa, Nassarawo and Gyawana) of five local government areas of Adamawa State in the Northeastern Nigeria. It is one of the thirty-six (36) States which constitute the Federal Republic of Nigeria. Map of the study area is shown in Figure 1: Adamawa state of Nigeria showing the local governments (Demsa, Numan, Lamurde, Hong and Madagali Local government areas of Adamawa State of Nigeria) where the study was conducted indicated by purple colour cross signs.

**Figure 1:** Map of Adamawa state of Nigeria showing Demsa, Numan, Lamurde, Hong and Madagali Local government areas where the study was conducted indicated by purple colour cross signs.



Ten villages located in five local government areas of Adamawa state in Northern Nigeria were selected and designated for sampling. The choice of sampling location was based on high concentration of local free ranged birds in the selected villages and compliance of their owners. Stratified random sampling was employed in this study carried out between November 2013 and May 2014 from the seven villages designated as the vaccine trial group namely Sabonpegi, Goningora, Fari, Uding, Sabongari, Ngalang and Pella, as well as the three villages considered as control group namely Shuwa, Nassarawo and Gyawana. The ten villages were tagged A - J. In each village, four households were selected for both trial and control groups. Twenty eight households were used as trial groups while twelve households served as the controls. In each of these households, there was an average of twenty birds. The trial group comprised of five hundred and forty birds while the control group had two hundred and forty birds. The control household groups were numbered 1- 4. The vaccine was administered orally in drinking water as recommended by the manufacturer.

### **Thermo-stable Vaccine**

NDV<sub>4</sub> strain vaccine (Arthur Webster Pty Limited, Australia) was used in this study. This Webster ND V<sub>4</sub> thermo-stable strain vaccine is a freeze-dried live virus preparation from heat resistant V<sub>4</sub> viral strain designed for use in temperate climate to improve viral antigen stability in feed and or oral administration and also to reduce dependence on cold chain during transportation. The ND V<sub>4</sub> thermo-stable vaccine is available in 100, 500 and 1,000 doses in a 3 ml glass vial.

### **Questionnaire survey**

Appraisal of ND V<sub>4</sub> thermo-stable vaccination effect on socio-economic characteristics of rural poultry keeping was conducted using well structured questionnaires administered to farmers in both test and control groups in three phases: before vaccination, post vaccination and post booster vaccination. These questionnaires were administered to determine the productivity, health, population dynamics and general ecology of village chickens. Open ended discussions were also conducted with the farmers and the responses noted accordingly.

### **Experimental design**

Birds in selected trial villages were vaccinated with V<sub>4</sub> thermo-stable ND vaccine while those in the control villages were given placebo vaccination using normal saline in drinking water. The vaccination was conducted in the early morning hours post water starvation. This vaccination was repeated twice on a monthly interval.

### **Blood sample collection using filter paper strip**

Blood samples from one third of the vaccinated birds were collected on filter paper strips, as described by Brugh and Beard (1980). The filter paper strip was placed on the blood pool formed at the wing vein punctured point and allowed to saturate up to distance of 1-2cm of the length of the strip. Samples were dried and stored in plastic cellophane bags and stored at 4°C until use.

### **Serological test**

The diluted sera collected from the birds were assayed for the presence of antibodies against ND virus using the haemagglutination inhibition (HI) test. The HI was conducted as described by Allan and Gough (1974). NDV antibody titers (HI) were expressed as Geometric Mean Titre (GMT) values.

### **Red Blood cells**

Blood was collected in an anticoagulant tube (Alsevers) for ND virus antibody from chicken under sterile condition. The blood was washed with Alser solution and twice with normal saline solution at 1,500 rpm for 10 minutes per each wash. The obtained packed cells were diluted in normal saline to 0.9% and were used as indicator in the haemagglutination and haemagglutination inhibition test.

### **Haemagglutination (HA) test**

Newcastle LaSota vaccine from the National Veterinary Research Institute, Vom was used as antigen source for HA test. Each vial was reconstituted in 1ml normal saline and divided into aliquots. A two-fold antigen serial dilution was carried out in a polystyrene (V-shaped) microtitre plate using 0.025ml normal saline. The mixture was looped out (wells 2 through 12) and 0.05ml of 0.09% chicken RBC was finally added to all wells. The plate was incubated at

room temperature for 30 minutes before the results were read. The dilution showing 50% haemagglutination was considered as end-point (1 HA unit). The dilution representing 8 HA units was determined and corresponding antigen was diluted for HI test.

### Haemagglutination inhibition (HI) test

The test sera were screened for NDV antibody presence using modified methods (Allan and Gough, 1974). This involved dispensing 0.25ml treated sera into duplicate wells of polystyrene microtitre plates. Equal antigen volumes were added to one of the duplicate wells containing the test serum and normal saline in the second well. Finally, 0.05ml of 0.09% chicken RBC were added to the plate duplicate wells and incubated at room temperature for 45 minutes before result interpretation. Inhibition of haemagglutination was indicated as button formation at the bottom of the well and was interpreted as positive presence for ND virus antibodies.

### Positive sera end-point determination

Positive sera (0.5ml) were added to the first well on each row of a polystyrene microtitre plate and 0.025ml added to the last well. 0.025ml of normal saline was added to all the other wells (2-11). A two-fold serial dilution of the test sera was carried out by looping out samples in the first wells through the other wells (2-11). Well 12 served as serum control. Appropriately diluted antigen (8 HA units) was added at 0.025ml volume to all the wells except the last (well 12) where

### Field trial of V<sub>4</sub> thermostable ND vaccine

Table II: showed antibody profile V<sub>4</sub> thermostable ND vaccine in free ranged local birds as determined by Geometric Mean Titre (GMT). Prior to vaccination, NDV (HI) antibody GMT value was 1:2 in both trial and control villages. The GMT values increased slightly to 1:13 after first vaccination in trial group and 1:8 in control group. The second vaccination booster did not show much difference in the trial village with GMT values of 1:14 while the control village had 1:1 GMT values for NDV antibodies. There was no significant difference ( $P>0.05$ ) in NDV antibody GMT values in all stages of vaccination. Antibody profile V<sub>4</sub> thermostable ND vaccine in free ranged local birds as determined by Geometric Mean Titre (GMT)

### Discussion

Equivalent amount of normal saline was added. 0.05ml of 0.9% chicken RBC was added to all the wells and plates were incubated at room temperature for 45 minutes prior to result interpretation and documentation.

### Statistical analysis

The antibody titer with minimum of 4 HA units were expressed as positives. Other socio-economic variables appraised in both groups were also expressed as percentages

### Results

#### Socio-economic characteristics

Table 1 showed socio-economic characteristics and variables of respondents at different periods of the study (pre-vaccination, post-vaccination and post booster vaccination), which was not significant ( $P>0.05$ ). However, level of education, age and sex of respondents who care for the poultry were important factors affecting production in the study areas. Significant numbers of respondents had elementary education whom were mostly women aged 31-40 years. Most households had been keeping poultry for 5-10 years with local chicken being the most domesticated for household income generation and little family consumption. Purchase of foundation and replacement stock is often practice among flock holders.

Field trials of V<sub>4</sub> thermostable ND vaccination indicates insufficient immune response in vaccinated local experimental birds with a low (1:2) geometric mean titre (GMT) of ND antibody. This observation is inconsistent with previous report of Bancroft and Spradbrow (1978). Drinking water method employed for vaccine delivery in this study has advantage of administration ease as reported by Ibrahim *et al.*, (1980, 1981) for chickens kept under intensive or semi-intensive system of poultry production. However, this technique indicates in-effective means of vaccination in birds under extensive scavenging system of poultry keeping.

**Table I:** Socio-economic characteristics associated with rural poultry production

Indicators	Response	Pre-vaccination		Post-vaccination		Post-booster vaccination	
		Trial group	Control	Trial group	Control	Trial group	Control
General husbandry	Extensive Others	27 (96%) 1 (4%)	11 (92%) 1 (8%)	27 (96%) 1 (4%)	11 (91%) 1 (4%)	27 (96%) 1 (4%)	11 (91%) 1 (4%)
Species	Turkey Guinea Fowl Duck Chicken Pigeon	10 (36%) 19 (4%) 31 (%) 460 (86%) 28 (5%)	3 (25%) 10 (4%) 20 (8%) 190 (76%) 31 (12%)	12 (42%) 25 (5%) 31 (6%) 456 (86%) 70 (13%)	3 (25%) 30 (12%) 10 (4%) 157 (75%) 30 (12%)	11 (39%) 31 (6%) 12 (2%) 480 (89%) 70 (13%)	5 (42%) 35 (14%) 8 (3%) 180 (72%) 30 (12%)
Feeding	No supplement Yes (a times)	14 (50%) 14 (50%)	5 (42%) 7 (58%)	14 (50%) 14 (50%)	5 (42%) 7 (58%)	14 (50%) 14 (50%)	5 (42%) 7 (58%)
Medication	Self Vet.Doc Slaughter None	5 (18%) 6 (21%) 10 (30%) 4 (14%)	2 (17%) 1 (8%) 10 (33%) 3 (25%)	5 (18%) 6 (21%) 9 (32%) 4 (14%)	1 (8%) 3 (25%) 3 (25%) 3 (25%)	6 (21%) 5 (18%) 10 (36%) 4 (14%)	1 (8%) 2 (7%) 4 (33%) 2 (17%)
Hatchability	Once/year Twice Three times More 3X	1 (4%) 6 (21%) 15 (64%) 3 (11%)	11 (8%) 2 (17%) 7 (58%) 2 (17%)	0 7 (25%) 20 (71%) 1 (4%)	1 (8%) 3 (25%) 5 (42%) 3 (25%)	1 (4%) 5 (18%) 19 (67%) 3 (11%)	0 3 (25%) 4 (33%) 1 (8%)
Marketing	Xmas festival Muslim festival Traditional	16 (57%) 4 (14%) 3 (11%)	7 (58%) 2 (17%) 2 (17%)	16 (57%) 3 (11%) 4 (14%)	8 (67%) 1 (8%) 2 (17%)	17 (61%) 4 (14%) 2 (7%)	5 (42%) 3 (25%) 3 (25%)
Culling	Yes No	20 (71%) 8 (21%)	8 (67%) 4 (33%)	21 (75%) 7 (25%)	9 (75%) 3 (25%)	22 (79%) 6 (21%)	8 (67%) 4 (33%)

**Table II:** Antibody profile V<sub>4</sub> thermostable ND vaccine in free ranged local birds as determined by Geometric Mean Titre (GMT)

GMT	Pre-Vaccination	3 weeks Post Vaccination	6 weeks Post Vaccination
Test	1:2	1:13	1:14
Control	1:2	1:8	1:1

Geometric mean titre, 21 days post vaccination was 12.9 in trial village and 8.3 in control village. This increase GMT in trial village indicates sero-conversion in some birds post vaccination. This observation is similar to the report of Darminto and Daniels (1991). However, degree of immune protection was not assessed in this study. The poor sero-conversion observed in this study could be associated with management factors in local free ranged, unconfined scavenger poultry where

insufficient dose of the vaccine is taken by the roaming birds. The milky (vaccine stabilizer) colour of the diluted vaccine could also be a factor preventing the birds from drinking the water. In addition, the uncontrolled use and disposal of water by household members especially women during washing of cooking utensils and cloths provides ready source of water for scavenging birds especially in the morning when the birds were expected to consume vaccine water. Other factors that could be associated with

poor sero-conversion include poor field conditions where these village chickens are reared as well as poor nutrition, and immune status as compared with confined commercial chickens.

The vaccinated birds with apparently zero or less antibody titre might not necessarily be unprotected as cell mediated and mucosal immunity may have played role in immune protection amongst birds against ND. Previous studies of Cumming *et al.*, (1991) indicate antibody titre of GMT values 1:14 provided good field protection. The dramatic rise of HI from 1:2 to 1:8 in the control village could be due to natural infection during the course of the study as previously reported by Baba *et al.* (1998) as observed by slight decrease in the number of chickens from [190 (76%) to 180 (74%)] amongst control group after collecting post vaccination questionnaire, while the chickens in the trial group slightly increased from [460 (86%) to 480 (87%)]. Thus, V<sub>4</sub> thermostable ND vaccination effects on other independent rural poultry production variables and response investigated were not statistically significant.

### Conclusion and Recommendations

In conclusion, this study confirms poor immune response and protection in free ranged birds vaccinated with NDV<sub>4</sub> vaccine administered in drinking water. Therefore, further study is required to source for alternative vaccine delivery vehicle under field conditions. It is also envisaged that feed-based vaccine would be more efficient than water in vaccine delivery so as to reduce losses associated with the ND which would enhance the livelihood of rural poultry farmers who are mostly women and younger people. Increased awareness amongst rural household farmers on vaccine use and methodology of administration is thereby suggested.

### Conflict of Interest

There is no any conflict of interest by the authors.

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