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RESEARCH

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Evaluation of NDV4 thermo stable feed-based vaccination effects on rural chicken productivity in Adamawa State, Nigeria

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ABSTRACT

Background: Newcastle disease (ND) has been documented as one of the major viral diseases that hampers poultry productivity. Oral Newcastle Disease (ND) vaccination has been documented as a method for control of the disease with varied effects in scavenging rural poultry especially in Nigeria.
Aim: This cross sectional study was conducted to establish the impact of V4 thermostable ND vaccination on rural poultry productivity using corn bran (dusa) as alternative vaccine carrier in five Local Government Areas (LGA) in Adamawa state, Nigeria.
Method: Structured questionnaire on general knowledge, practice and productivity indicators was administered thrice to participating household poultry farmers alongside administration of feed based V4 thermostable ND vaccination in chickens. Sera obtained from forty (40) chickens per LGA during pre-vaccination, post-vaccination and post-booster vaccination with NDV4 thermostable vaccine were subjected to Hemagglutination Inhibition (HI) test.
Result: Antibody detection showed seroconversion in chickens 28day post vaccination and post booster vaccination with significant ($P<0.05$) antibody titer increase from 1:16 - 1:1024 and mean GMT 2.87 - 5.09 amongst 61.5 - 87.0% tested chickens. Questionnaire further showed V4 thermostable ND vaccination had significant effect on morbidity, mortality, hatchability, culling and ultimately the flock productivity.
Conclusion: Corn-bran feed based vaccination demonstrated effective delivery and suitability of V4 thermostable ND vaccine use in scavenging village chickens in Adamawa State, Nigeria.

Key Words :NDV4 thermo-stable, vaccine, feed-based vaccination, HI test, rural chicken, Adamawa State, Nigeria.

BACKGROUND

Rural poultry keeping is a major source of income and livelihood especially in low income, food deficient countries [LIFDC] (Branckaert and Guèye, 1999) where free-ranged and backyard extensive system of poultry keeping is practiced such as Latin America, South East Asia (Saleque, 1999), Africa (Sonaiya, 1990) and Nigeria (Adene and Oguntade, 2006). However, Newcastle disease (ND) has been documented as one of the major viral diseases that hampers productivity in poultry keeping (Olabode *et al.,* 2008) with disease occurrence in both commercial (Olabode *et al.*, 2012) and local (Ameh *et al.,* 2016) poultry characterized by serious economic losses especially in local birds (Nwanta *et al.,* 2006). Vaccination has been reported as the principal means of ND control (Abdu et al., 2005). Despite advent of V4 thermostable ND vaccine using selected resistant avirulent V4H form (Westbury *et al.,* 1984) and vaccination in local chickens (Nwanta *et al.,* 2005), report of in-efficient vaccine delivery in orally vaccinated backyard scavenging poultry was documented (Nafarnda *et al.,* 2015). However, Heat Resistant (HR) V4 feed-based vaccine trials using commercial feeds (Copland, 1987), processed rice grains (Jayawardane *et al.,* 1990), cooked rice (Samuel *et al.,* 1993), wheat (Oh, 1987), barley and crushed maize (Jagne *et al.,* 1991) and maize grit (Lawal *et al.*, 2016) have been documented

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with varied promising effects in different countries. The advocated low cost smallholder poultry vaccination scheme (Sonaiya and Swan, 2004) necessitated this study which sought to establish the effect of V_4 thermo-stable NDV administration on local poultry productivity using corn bran "dusa" produced by participatory households as a cheap, alternative vehicle for vaccine delivery in ND control amongst scavenging poultry so as to improve rural poultry productivity and income generating capacity of the rural poultry keepers

MATERIALS AND METHODS

Study Area

Adamawa is a state in northeastern Nigeria, with its capital at Yola. It lies between 8O0 N and 11N and longitude 11.50 and 13.50 E (Anonymous, 2019). Adamawa is bordered on the north and northwest by Borno and Gombes, on the west and southwards by Taraba state, and on the southeast and east by Cameroon (Encyclopædia Britannica, 2013). The state consist of 21 local government areas namely: Fufore, Ganye, Gombi, Guyuk, Hong, Jada, Shelleng, Demsa, Madagali, Maiha, Mayo-Belwa, Michika, Mubi, Numan, Song, Yola, Mubi-South, Jimeta, Girei, Toungo and Lamurde. Majority of the people in Adamawa State are farmers. Cattle rearing are also a major occupation, while village communities living on the banks of Rivers Gongola and Benue and their tributaries in the State engage in fishing and farming (Anonymous, 2019). This study was conducted in Demsa, Numan, Lamurde, Hong and Madagali as shown in the map below.

Figure 1: Map of Adamawa state in Nigeria showing Demsa, Numan, Lamurde, Hong and Madagali Local Government Areas. The study locations indicated in purple colour cross signs.

Study design and Sampling technique

Stratified random sampling was employed in this study carried out between May 2014 and November 2014 in Five Local Government Areas of Adamawa state, Nigeria namely Demsa, Numan, Lamurde, Hong and Madagali. In each LGA, four (4) rural poultry keeping households in different villages were identified and included in this study following owner compliance. The study comprised of two phases conducted simultaneously: questionnaire survey and vaccine trial phase. A total of twenty (20) poultry keepers were served with questionnaires and ten (10) chickens in each household were tagged for the vaccine trials and the sum total of forty (40) birds per LGA from all the flock of chickens exposed to NDV4 thermostable vaccine and screened for ND antibodies during Pre-vaccination, post-vaccination and post-booster vaccination stages.

Thermo-stable Vaccine

NDV4 strain vaccine (Arthur Webster Pty Limited, Australia) was used in this study. This Webster ND V4 thermo-stable strain vaccine is a freeze-dried live virus preparation from heat resistant V4 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 V4 thermo-stable vaccine available in 100 doses was used for this study.

Vaccine Carrier (Dusa)

During pre-sampling visits, the poultry keepers that agreed to participate in this study were directed to gather and dry properly corn bran (dusa) from their household kitchen waste. During the vaccine trials, "dusa" from each household was mixed with reconstituted NDV4 thermostable vaccine in dechlorinated water (100 dose in 1 liters dechlorinated water). The

vaccination was conducted in the early morning hours post overnight water starvation. This vaccination was repeated twice on a monthly interval.

Questionnaire survey

Structured questionnaire on general knowledge, practice and productivity indicators was administered thrice to participating household farmers alongside administration of feed based V4 thermostable ND vaccination in birds. The effect of NDV4 thermo-stable vaccination on rural poultry productivity was observed and documented before vaccination, post vaccination and post booster vaccination based on morbidity, mortality, hatchability, culling rates and the frequency of medication. Open ended discussions were also conducted with the farmers and their responses noted accordingly.

Blood sample collection and serum processing using filter paper strip

The rural chickens were bled on day 1 before the vaccination, day 28 after the initial vaccination and day 56 after the first vaccination with NDV4 thermo-stable vaccine. Blood samples from vaccinated chickens were collected on Whatman 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 placed in petri dish then wrapped in plastic cellophane bags for transportation under cool chain to the Department of Veterinary Microbiology Laboratory, University of Maiduguri and stored at 4^0 C until use. In the laboratory, serum were eluted from the strips using punched paper disks from blood stained filter paper placed in microtiter plate wells containing 100 µl of normal saline incubated at 4^0 C overnight.

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). Positive sera end-point determination was also established in accordance with standard methods and the NDV antibody titers (HI) were expressed as Geometric Mean Titre (GMT) values as described by Garner et al., (1988).

Statistical analysis

The antibody titer with minimum of 4HA units were expressed as positives and the GMT values calculated using the formula Xgeo= antilog10 {1/n (Σfilog10Xi)} where n=number tested, Xi=the reciprocal of dilution and fi=frequency. The data were presented in a tabular manner for further analysis with SPSS package at a significance p-value <0.05. Other productivity variables appraised in the vaccinated flocks were also expressed as percentages.

RESULTS

V4 thermostable ND vaccine field trials

The distribution of ND titers in rural chickens screened in this study prior to vaccination (Table 1) and post vaccination trials with NDV4 thermo-stable vaccine are indicated (Table 2 and 3). The ND pre- vaccination HI antibody titers in different LGA showed overall 16.0% seroconversion rate with no significant difference (P>0.05) in both occurrence and GMT values (Table 1). NDV4 thermo-stable vaccine seroconversion of 61.5% was observed on day 28 which increased to 87.0% on day 56 with antibody titer range of 1:2 - 1:128 and 1:2 – 1:1024 as well as GMT values of 14.36 25.45 with corresponding mean GMT 2.87 and 5.09 respectively (Table 2 and 3)

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Table 1: Distribution of Serum Newcastle disease HI antibody titers in village chickens prior to NDV4 thermo-stable feed-based vaccination in some Local Government Areas of Adamawa state.

LGA	No. tested	No. (%) Positive	Distribution of the reciprocal of HI antibody titers			
			2	4	8	GMT
Demsa	40	8(20.0)	4	3	1	0.265
Numan	40	7(17.5)	5	2	0	0.245
Lamurde	40	5(12.5)	3	1	1	0.163
Hong	40	6(15.0)	2	3	1	0.225
Madagali	40	6(15.0)	5	1	0	0.143
Total	**200**	**32(16.0)**	**19**	**10**	**3**	**1.041**

Mean GMT=0.21

Table 2: Distribution of Serum Newcastle disease HI antibody titers in village chickens 28days post vaccination with feed based NDV4 thermo-stable vaccine in some Local Government Areas of Adamawa state.

LGA	No. tested	No. (%) Positive	Distribution of the reciprocal of HI antibody titers							
			2	4	8	16	32	64	128	GMT
Demsa	40	21(52.5)	0	0	1	3	3	5	9	2.516
Numan	40	28(70.0)	0	0	3	4	5	9	7	2.925
Lamurde	40	30(75.0)	0	0	2	3	5	7	13	3.600
Hong	40	25(62.5)	0	0	1	3	4	7	10	3.007
Madagali	40	19(47.5)	0	0	1	2	2	6	8	2.311
Total	**200**	**123(61.5)**	**0**	**0**	**8**	**15**	**19**	**34**	**47**	**14.359**

Mean GMT=2.87

Table 3: Distribution of Serum Newcastle disease HI antibody titers in village chickens 28days post booster vaccination with feed based NDV4 thermo-stable vaccine in some Local Government Areas of Adamawa state.

LGA	No. tested	No. (%) Positive	Distribution of the reciprocal of HI antibody titers										
			2	4	8	16	32	64	128	256	512	1024	GMT
Demsa	40	37(92.5)	0	0	1	1	2	4	12	10	6	1	5.502
Numan	40	34(85.0)	0	0	1	2	1	3	14	7	5	1	4.970
Lamurde	40	35(87.5)	0	0	0	1	3	4	13	9	4	1	5.155
Hong	40	36(90.0)	0	0	1	1	3	2	13	7	6	3	5.285
Madagali	40	32(80.0)	0	0	0	1	2	2	10	9	6	2	4.541
Total	**200**	**174(87.0)**	**0**	**0**	**3**	**6**	**11**	**15**	**62**	**42**	**27**	**8**	**25.453**

Mean GMT=5.09

Questionnaire Response

Questionnaire responses indicated extensive husbandry system of poultry keeping was commonly practiced in the study area with rural chickens been mostly domesticated on household feeds with no supplements. The effect of ND vaccination on flock morbidity and mortality showed a remarkable reduction by 70% and 75% respectively on day 56 post booster vaccination with a corresponding reduction on the use of medication (55%). The culling rate was

also reduced by 80%, consequently, the hatchability rate improved by 95% amongst study birds (Table 4).

Table 4: **Questionnaire response to NDV4 thermo-stable feed-based vaccination trial in some Local Government Areas of Adamawa state.**

Indicators	Response	Pre-Vaccination	Post-Vaccination	Post Booster Vaccination
General husbandry	Extensive	19 (95%)	19 (95%)	19 (95%)
	Others	1 (5%)	1 (5%)	1 (5%)
Species	Turkey	3 (15%)	3 (15%)	3 (15%)
	Guinea Fowl	2 (10%)	2 (10%)	2 (10%)
	Duck	2 (10%)	2 (10%)	2 (10%)
	Chicken	12 (60%)	12 (60%)	12 (60%)
	Pigeon	1 (5%)	1 (5%)	1 (5%)
Feeding	No	12 (60%)	12 (60%)	12 (60%)
	supplement	8 (40%)	8 (40%)	8 (40%)
	Yes (a times)			
Morbidity	Yes	17 (85%)	10 (50%)	6 (30%)
	No	3 (15%)	10 (50%)	14 (70%)
Mortality	Yes	19 (95%)	8 (40%)	5 (25%)
	No	1 (5%)	12 (60%)	15 (75%)
Medication	Frequent	10 (50%)	6 (30%)	1 (5%)
	Less frequent	8 (40%)	9 (45%)	8 (40%)
	None	2 (10%)	5 (25%)	11 (55%)
Hatchability	Once/year	6 (30%)	4 (20%)	1 (5%)
	Twice -			
	Three times	12 (60%)	15(75%)	19 (95%)
	More 3X	2 (10%)	1 (5%)	-
Culling	Yes	19 (95%)	9 (45%)	4 (20%)
	No	1 (5%)	11 (55%)	16 (80%)

DISCUSSION

In this study 30 farms from different governorates (Alexandria, Gharbia, Giza and Qalubia) of age 40 to 100-days-old were examined for presence of CAV with real-time PCR, all examined farms were clinically characterised by anorexia, weakness, unthriftiness, weight loss, severe anemia, (paleness of comb, wattle, eyelids and legs) and sudden death, these signs were mentioned before in Japan, China, Australia, New Zealand, India, Slovenia, Brazil, Nigeria and South Africa Kuscu and Gurel, 2008. The clinical signs of CAV infection, especially in chicks above 3 weeks were not seen, but the lesions which suggestive to be CAV such as both lymphoid depletion and atrophy of thymus were recorded. These were compatible with that previously mentioned by Ledesma *et al.*, (2001) who recorded that, the chicks infected with virulence strain or high doses of virus after the maternal antibodies were decline usually suffering from lymphoid lesions without anemia. Moreover, the previous studies find the absence of clinical signs after about 3 weeks of age and the immunocompetent chickens were resistant to disease, but they can gain asymptomatic infections (Schat, 2003).

The histopathological examination of the positive farm showing generalized lymphoid aplasia in thymus, spleen and bone marrow; these findings were as similar as described by (Dhama *et al.*, 2002; Smyth *et al.*, 1993). The severe depletion of hematopoietic cells in bone marrow replaced by adipocytes

and this is due to the destruction of hemocytoblasts in bone marrow leading to severe depletion of myeloid and erethroid cells producing anemia, theses were agreement with Adair, 2000. Spleen exhibited depletion of lymphocytes with focal coagulative necrosis, in addition to congested blood vessels due to the infection of mature lymphocytes with CAV. The recorded lymphoid aplasia could be bravely described by the strong immunosuppressive effect of CAV which induces marked destruction both to bone marrow stem cell and precursor T-lymphocytes in thymus (Goryo *et al.,* 1989; Smyth *et al.,* 1993). Focal starry sky depletion in cortex of thymus with cellular necrotic foci and severe hemorrhage in the medulla. Moreover, widening of few hussal's corpuscles forming cysts containing necrotic debris and granulocytes. Thymic lesions confirmed with the results of (Adair, 2000) as he proved that the destruction of T lymphocytes progenitor cells in thymus results in depletion of helper and cytotoxic cells inducing severe atrophy and depletion of lymphocytes.

PCR is the most advantage of adequate, faster and sensitive detection of more fastidious viral pathogens that might require several days and sequential passages in culturing for virus isolation and provide the faster diagnosis of viruses that's usually to be isolated in vitro cell culture (Cavanagh, 2002 and Dhama *et al.,* 2002). The 30 farms which were examined by real-time PCR, one farm is positive with C.T. reading =26. This sample is of age 100-days-old of ISA type from Qalubia governorate, our results was confirmed with Adair (2000) who's said that, the complete protection against CAV induced disease was grant by maternal antibodies which confirmed by many studies that are showed CAV infection and development of specific antibodies in chickens of eight and 12 weeks age as maternal antibodies completely disappear at 2-3 weeks of age so such infections are mostly due to horizontal transmission.

Sequence of the VP1 gene is commonly used to determine the relationship of different CAV isolates due to the fact that most of the amino acid substitutions between isolates lie in VP1 gene and more specifically in the N-terminal half of VP1 gene (Craig *et al.,* 2009 and Hailemariam *et al.,* 2008). PCR assay performed on the extracted DNA from infected tissues of SPF, giving positive reactions with DNA fragment at 418 bp (Hussein et al., 2002). This was sequenced and submitted to the GenBank under accession number MH260568 for the partial sequenced to VP1 gene fragments.

The strategies to control immunosuppression are mostly depending on vaccination programs for breeders and their progeny. The most suitable way to minimize the prevalence of this disease is to use the best progress vaccines available, correctly apply them and ensure the good level of nutrition to get the highest possible response to these vaccines (Cloud *et al.,* 1992). Currently, characterize circulating CAVs in Egypt is so important to improve methods of virus control and to knew the relationship of circulating CAV with vaccine strains and other CAV strains. Now a large number of isolates have been fully or partially sequenced as we done on this work, partially sequenced VP1gene revealed that ours strain according to phylogenic tree was markedly varied from a commercial CAV vaccine (Nobilis® CAIV P4 and Cux-1N/Germany) which used as vaccine in our filed. Also according to phylogenic tree, they were present in two clusters our strain with Egypt I/ Giza2009, Egypt II/Fayoum I and Egypt III/ Fayoum II, India and Japan in one and the other cluster include old CAV isolates in 1990s Egy-1, 2, 3 and 4 by Abo Elkhair *et al.,* 2014 and 5 vaccinal strains (Cux-1M/Germany, Cux-1N/Germany, CAV/Nobilis®P4, CAV/26P4/USA and CAV/Del-Rose/USA,).

The Immunoperoxidase staining techniques are most sensitive in detecting infected cells and the stained tissue sections can be re-examined several times and stored for a long time. Also the identification of cells is possible due to the counterstaining with haematoxylin. This is because the methods consist of two-step antibody reaction results of thymus revealed high level of expressed CAV antigen-specific staining (high positive signal) in the nuclei of lymphocytes, thymocytes and connective tissue this is agreement with (Hoop and Reece, 1991).

The indirect immunofluorescence staining techniques detected the infected cells in bone marrow. The Fluorescent green apple color was seen on organs of SPF infected chicks with positive real-time PCR sample and stained with fluorescence. These observations matched with the histological lesions in the same tissues and correspond with other reports of light microscopic changes due to infection with CAV (Yuasa *et al.,* 1979 and Goryo *et al.,* 1989). Our observations for immunohistochemically examination are the largest amounts of CAV antigen were detected in the thymus, spleen, bone marrow and these are matched with Smyth *et al.,* 1993.

CONCLUSION

In conclusion, this study showed "corn bran" as an alternative feed based vaccine carrier and suitability of V_4 Thermo stable ND vaccine for rural setting vaccination in Adamawa, Nigeria, characterized by high immune response, reduced morbidity and mortality as well increased flock productivity.

RECOMMENDATIONS

Public awareness amongst rural household farmers on the adoption of feed-based vaccination method using cheap "corn bran" waste in free ranged rural chicken to reduce ND associated losses is necessary. This would foster enhanced livelihood and income generation of rural poultry farmers who are mostly women.

Conflict of Interest: Authors declares no conflict of interest

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