Nigerian Veterinary Journal 2010 Vol 31(1):46-55

VACCINATION WITH NEWCASTLE DISEASE VACCINES STRAIN I_2 AND LASOTA IN COMMERCIAL AND LOCAL CHICKENS IN PLATEAU STATE NIGERIA

MUSA[*1], U., ABDU[2], P.A, MERA[3], U.M., EMMENNA[1], P.E. and AHMED[1], M.S.

[1] *National Veterinary Research Institute, Vom, Nigeria*
[2]*Veterinary Teaching Hospital, Ahmadu Bello University, Zaria, Nigeria*
[3]*Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria*

***Corresponding Author Email: usmanmusagulma@yahoo.com ; Tel: 2348034537443**

SUMMARY

Vaccination trials and comparative immunogenicity study using Newcastle disease vaccine strain I_2 ($NDVI_2$) and NDV La Sota administered to commercial and local chickens through intraocular (i/o), intramuscular (i/m), drinking water (dw), untreated sorghum, parboiled sorghum, sorghum coated with gum Arabic or commercial chick mash feed as vaccine carriers was conducted. Newcastle disease vaccine strain I_2 and NDV La Sota vaccines provided protection to commercial and local chickens vaccinated through i/o, i/m or dw. No significant difference ($P \leq 0.05$) was observed in the antibody titre of commercial or local chickens vaccinated with either $NDVI_2$ or NDV La Sota vaccines administered via commercial feed, parboiled sorghum, parboiled sorghum coated with gum Arabic and untreated sorghum. $NDVI_2$ or NDV La Sota vaccines administered through commercial feed, parboiled sorghum, parboiled sorghum coated with gum Arabic and untreated sorghum gave no or limited protection (0-22%) to the birds when challenged with a local strain of velogenic viscerotropic Newcastle disease virus Kudu 113 strain. It was concluded that the vaccine carriers used in this study were not suitable for delivery of $NDVI_2$ or NDVS La Sota vaccines to local or commercial chickens.

Keywords: ***Chickens, Newcastle Disease vaccines, Vaccine carriers.***

INTRODUCTION

Newcastle disease is the most important limiting factor in rural chicken farming in most developing countries of the world and a serious threat to intensively reared chickens (Echeonwu *et al.,* 2008a). A thermostable $NDVI_2$ has been recommended for use in developing countries for the protection of rural chickens against ND (Bensink and Spradbow, 1999). However, the efficacy of this vaccine has not been tested with foodstuffs available in rural Nigeria (Echeonwu *et al.,* 2008a). Reports from elsewhere show varying degrees of success with the vaccine in laboratory and field trials (Amakye-Anim *et al.,* 2000; Wambura *et al.,* 2000).

The control of ND in rural poultry can make a vital contribution to the

improvement of household food security and poverty reduction in many developing countries. Vaccination against ND virus (NDV) is routinely practiced throughout the world. Intensive poultry farmers in Nigeria vaccinate poultry routinely, but poultry in extensive production systems are not (Sa'idu, 2006).

In an attempt to make delivery of ND vaccine easier to rural chickens different types of feed stuffs have already been tested as carriers for the vaccine. Not all feed stuffs were found to be suitable and some staple foods such as sorghum, millet and other grains produced in many areas of Nigeria have not yet been studied in detail (Spradbrow, 1992b; Musa, 2002). The objectives of this study were to compare the efficacy of NDV I_2 and ND La Sota vaccine strain administered to local and commercial chickens via different routes and common feedstuff as possible vaccine carriers.

MATERIALS AND METHODS

Commercial chickens

One hundred and twenty day old unsexed Shika Brown chicks were obtained from the Poultry Research Farm, National Veterinary Research Institute (NVRI), Vom. The chicks were housed in a room previously cleaned, washed, disinfected and fumigated. They were provided with chick mash and water *ad libitum.* At three weeks of age, the birds were identified individually with numbered wing tags and randomly allocated into twelve experimental groups of ten chicks each and vaccinated with NDV La Sota or $NDVI_2$ vaccines via i/o, i/m, dw, sorghum coated with gum Arabic, parboiled sorghum, untreated sorghum and a commercial chick mash feed.

Local chickens

One thousand local chicken eggs were purchased from live bird markets in Jos South and Kanam Local Government Areas (LGA) of Plateau State. After selection, 876 eggs were found to be suitable for incubation. The eggs were then candled on the 18^{th} day of incubation. One hundred and seventy eight chicks (20.3% hatchability) were hatched and harvested between the 21^{st} and 22^{nd} day of incubation. The chicks were selected on the basis of fitness, uniformity and body weight. The chicks were identified, grouped and vaccinated as outlined for commercial chickens.

Vaccine food carriers

Commercial chick mash feed, sorghum and gum Arabic were purchased from a market in Bukuru, Jos South LGA of Plateau State.

Vaccines and vaccination

Newcastle disease vaccine strain I_2 with a titre of $10^{10.2}$ EID_{50} per vial and ND La Sota vaccine with a titre of $10^{9.8}$ EID_{50} per vial in lyophilized form were obtained from the Virology Division of NVRI, Vom. A 100 and 200 dose vial of $NDVI_2$ and NDV LaSota vaccines were reconstituted in 50mls and 100mls of normal saline respectively and each bird was given 0.5mls of the reconstituted vaccine i/o. For im vaccination, 100 and 200 dose vials of $NDVI_2$ and NDV La Sota vaccines were reconstituted in 20mls and 40mls of normal saline respectively and each bird was injected with 0.2mls of the reconstituted vaccines.

Phytochemical and proximate analysis of the food carrier

Proximate and phytochemical analysis of sorghum and gum Arabic was carried out as described by AOAC, (1995) in the Biochemistry and Molecular Biology Division of the NVRI, Vom.

Preparation and coating of food carrier with vaccine virus

The method described by Alders and Spradbrow (2001), with slight modification was used for coating the sorghum with vaccine virus. About 100 g of gum Arabic was dissolved in 1,000 ml of distilled water and boiled for 30 minutes and allowed to cool. One vial of the freeze-dried $NDVI_2$ vaccine containing 100 doses was reconstituted in 10 ml of PBS (pH 7.4). Then 40 ml of diluted gum Arabic was thoroughly mixed with the reconstituted vaccine (100 doses). One kilogram of sorghum was added to the mixture of the vaccine and gum Arabic and thoroughly mixed manually. After mixing, the coated food vaccine (Vaccine carrier) was spread on metal trays and kept at room temperature to dry under gentle air current for 30 minutes and 10 g of the coated food vaccine was presented to 10 birds.

Challenge of experimental chickens

At 3 weeks post vaccination each bird was inoculated with 0.20 ml containing $10^{6.5}$ EID_{50}/ml of the NDV kudu 113 virus strain (Echeonwu *et al.*, 1993) i/m at the leg muscle. All birds inoculated were observed for two weeks for clinical signs and the number of sick and dead in each group were recorded.

Serology

Sera collected were tested for NDV antibodies by the haemagglutination inhibition tests as described by Allan and Gough (1974).

Data Analysis

Geometric mean of HI antibody titre (GMT) and percentage of birds with detectable ND antibody were calculated. The Statistical Package for Social Sciences (SPSS) Programme (version 13) using excel was used to determine if there was any significance difference between the mean HI titre of different groups. Morbidity, mortality, case fatality and protection rates for each group was calculated.

RESULTS

Phytochemical analysis showed that tannins and saponins were detected in gum Arabic, while anthraquinone and alkaloids were in sorghum. Steroids and flavonoids were absent in sorghum and gum Arabic, while cardiac glycosides were present in sorghum and gum Arabic. Sorghum contained a higher percentage of crude protein (12.0.4%) and crude fibre (8.97%) than gum Arabic (4.29% C.P and 5.09% crude fibre).

There were no significant differences ($P \geq 0.05$) in the prevaccination HI antibody titres. Furthermore, there was no significant difference ($P \geq 0.05$) in HI antibody titres in chicks vaccinated with $NDVI_2$ or ND La Sota vaccines administered through intramuscular route or via drinking water at two and three week post vaccination. The highest HI antibody titre (Log_2 6.3$\pm$1.3 and Log_2 9.8 $\pm$ 1.2) were observed in chicks vaccinated with ND La Sota via intraocular route 2 and 3 weeks post vaccination (PV). The lowest HI antibody titre (Log_2 0.0 $\pm$ 0.0) was observed in unvaccinated control group. All the groups responded to challenge with velogenic NDV and no significant difference in titre was observed two weeks post challenge (Table I). The highest HI antibody titre (Log_2 7.8$\pm$ 0.9 and Log_2 9.3 $\pm$ 1.4) in local chicks vaccinated with $NDVI_2$ or ND La Sota vaccine via different routes and vaccine carriers was observed at two and three weeks PV, respectively in group LCdw vaccinated with $NDVI_2$ via drinking water. The lowest HI antibody titre (Log_2 0.0 $\pm$ 0.0) was observed in the unvaccinated control group. There was no significant difference in HI antibody titre of chicks vaccinated via i/o, im, dw and commercial

feed, parboiled sorghum, parboiled sorghum coated with gum Arabic with $NDVI_2$ and ND La Sota vaccines. The HI antibody titres were also not significantly different in birds vaccinated two weeks post challenge (Table III).

Table I: Geometric mean antibody titres (GMT) in serum of commercial chickens vaccinated with $NDVI_2$ or NDV La Sota vaccine administered through different routes and vaccine carriers.

			GMT ± SD (Log_2)			
Group	Vaccine	Route/vehicle of administration	Pre-vaccination (n)	2 weeks post vaccination (n)	3 weeks post vaccination (n)	2 weeks post challenge (n)
CCi/o	$NDVI_2$	Intraocular	0.0 ± 0.0^{a} (10)	5.2 ± 1.4^{b} (9)	7.9 ± 2.1^{c}(9)	10.8 ± 1.6^{d} (9)
CCi/m	$NDVI_2$	Intramuscular	0.0 ± 0.0^{a} (10)	6.7 ± 1.6^{b} (9)	8.6 ± 2.2^{c} (8)	11.3 ± 0.9^{d} (8)
CCdw	$NDVI_2$	Drinking water	0.0 ± 0.0^{a} (10)	4.8 ± 2.2^{b} (9)	8.8 ± 3.2^{c} (8)	11.4 ± 0.8^{d} (7)
CCF1a	$NDVI_2$	Commercial feed	0.0 ± 0.0^{a} (10)	0.3 ± 0.5^{ab} (9)	1.0 ± 0.7^{ac}(9)	11.7 ± 0.6^{d} (2)
CCF2a	$NDVI_2$	Parboiled sorghum	0.3 ± 0.2^{a} (10)	0.4 ± 0.7^{ab} (9)	1.7 ± 2.0^{ac} (9)	12.0 ± 0.0^{d} (2)
CCF3a	$NDVI_2$	Parboiled sorghum coated with gum Arabic	0.2 ± 0.4^{a} (10)	0.4 ± 0.7^{ab} (9)	0.4 ± 0.7^{ac} (9)	12^{d} (1)
CCF5a	$NDVI_2$	Untreated sorghum	0.2 ± 0.4^{a} (10)	$0.5 \pm .7^{ab}$ (10)	0.5 ± 1.0^{ac} (10)	12^{d} (1)
CCLi/o	NDV La Sota	Intraocular	0.0 ± 0.0^{a} (10)	6.3 ± 1.3^{b} (10)	9.8 ± 1.2^{c} (10)	11.4 ± 0.7^{d} (10)
CCLi/m	NDV La Sota	Intramuscular	0.0 ± 0.0^{a} (10)	4.9 ± 1.1^{b}(8)	6.3 ± 1.6^{c} (8)	10.6 ± 1.0^{d} (8)
CCLdw	NDV La Sota	Drinking water	0.0 ± 0.0^{a} (10)	5.3 ± 2.3^{b} (10)	8.1 ± 3.1^{c} (10)	10.6 ± 1.9^{d} (10)
CCLF1a	NDV La Sota	Commercial feed	0.0 ± 0.0^{a} (10)	0.1 ± 0.3^{ab} (9)	1.2 ± 1.1^{ac} (9)	8.0^{d} (1)
Control	None	None	0.0 ± 0.0^{a} (10)	$0.0 \pm .0^{ab}$ (10)	0.0 ± 0.0^{ac} (10)	12^{d} (1)

(n)* = number of chicks in the group, CC = commercial chicken, i/o = intraocular, i/m = intramuscular, dw = drinking water, F= feed, La Sota $^{a,b,\ ab,\ ac,c\ \text{and}\ d}$ = Means with the same letters in the same column are not significantly different at 0.05 confidence level.

Table II: Morbidity, mortality case fatality and protection rates in commercial chickens vaccinated with $NDVI_2$ or NDV La Sota vaccine administered through different routes and vaccine carriers and challenged with a Local strain of velogenic NDV intramuscularly.

Group	Vaccine	Route/vehicle of administration	Morbidity rate	Mortality rate	Case fatality rate	Protection rate
CCIi/o	$NDVI_2$	Intraocular	0	0	0	100
CCIi/m	$NDVI_2$	Intramuscular	0	0	0	100
CCIdw	$NDVI_2$	Drinking water	30.0	20.0	67.0	78.0
CCIF1a	$NDVI_2$	Commercial feed	100	80.0	80.0	11.0
CCIF2a	$NDVI_2$	Parboiled sorghum				
CCIF3a	$NDVI_2$	Parboiled sorghum coated with gum Arabic	100	90.0	90.0	0.0
CCIF5a	$NDVI_2$	Untreated sorghum				
CCLi/o	NDV La Sota	Intraocular	0	0	0	100
CCLi/m	NDV La Sota	Intramuscular	20.0	20.0	67.0	78.0
CCLdw	NDV La Sota	Drinking water	0	0	0	100
CCLF1a	NDV La Sota	Commercial feed	100	90.0	80.0	11.0
Unvaccinated Control	None	None	100	90.0	90.0	10.0

CC = commercial chicken, i/o = intraocular, i/m = intramuscular, dw = drinking water, F= feed, L= La Sota, I = $NDVI_2$

Clinical signs and gross lesions in local chickens after challenge were similar to those observed in commercial chickens and included proventricular and skeletal haemorrhages on the breast muscles, haemorrhagic enteritis in groups vaccinated via commercial feed, sorghum coated with gum Arabic, parboiled sorghum and unvaccinated controls .

Commercial and local chickens vaccinated with $NDVI_2$ or ND La Sota vaccine through i/o, im routes and dw respectively had complete (100%) protection against challenge with a velogenic NDV. The least protected chicks were those vaccinated with $NDVI_2$ via commercial feed, parboiled sorghum, parboiled sorghum coated with gum Arabic and ND La Sota vaccine via commercial feed. High morbidity (100%), mortality and case fatality rates were observed in groups that were either vaccinated with $NVDI_2$ or La Sota via parboiled sorghum, sorghum coated with gum Arabic, untreated sorghum and commercial feed (Tables II and IV).

Table III: Geometric mean antibody titre (GMT) Log_2 of Local chickens vaccinated with $NDVI_2$ or NDV La Sota vaccine administered through different routes and vaccine carriers.

			GMT ± SD (Log_2)			
Group	Vaccine	Route/vehicle of administration	Pre-vaccination (n)	2 weeks post vaccination(n)	3 weeks post vaccination(n)	2 weeks post challenge (n)
LCIi/o	$NDVI_2$	Intraocular	0.0 ± 0.0^{a} (10)	6.4 ± 1.5^{b} (9)	8.7 ± 2.8^{c}(9)	11.0 ± 0.8^{d} (9)
LCIi/m	$NDVI_2$	Intramuscular	0.0 ± 0.0^{a} (10)	5.9 ± 1.1^{b} (8)	8.0 ± 1.4^{c}(8)	10.9 ± 0.8^{d} (8)
LCIdw	$NDVI_2$	Drinking water	0.0 ± 0.0^{a} (10)	7.8 ± 0.9^{b} (8)	9.3 ± 1.4^{c}(8)	10.3 ± 1.0^{d} (8)
LCIF1b	$NDVI_2$	Commercial feed	0.0 ± 0.0^{a} (10)	0.3 ± 0.5^{ab} (8)	2.6 ± 0.7^{ac}(8)	11^{d} (1)
LCIF2b	$NDVI_2$	Parboiled sorghum	0.0 ± 0.0^{a} (10)	0.4 ± 0.5^{ab} (7)	1.3 ± 0.8^{ac}(7)	12^{d} (1)
LCIF3b	$NDVI_2$	Parboiled sorghum coated with gum Arabic	0.0 ± 0.0^{a} (10)	0.8 ± 0.4^{ab} (8)	1.7 ± 0.4^{ac}(8)	12^{d} (1)
LCIF5b	$NDVI_2$	Untreated sorghum	0.0 ± 0.0^{a} (10)	0.3 ± 0.4^{ab} (8)	1.2 ± 0.4^{ac}(8)	12^{d} (2)
LCLi/o	NDV La Sota	Intraocular	0.0 ± 0.0^{a} (10)	6.6 ± 1.8^{b} (9)	7.3 ± 1.7^{c}(9)	9.8 ± 2.1^{d} (9)
LCLi/m	NDV La Sota	Intramuscular	0.0 ± 0.0^{a} (10)	5.1 ± 1.2^{b} (9)	5.7 ± 3.9^{c}(9)	10.3 ± 1.3^{d} (9)
LCLdw	NDV La Sota	Drinking water	0.0 ± 0.0^{a} (10)	5.3 ± 1.0^{b} (9)	6.4 ± 2.4^{c}(9)	10.3 ± 1.7^{d} (9)
LCLF1b	NDV La Sota	Commercial feed	0.0 ± 0.0^{a} (10)	0.8 ± 0.8^{ab} (9)	0.7 ± 0.7^{ac}(9)	12^{d} (1)
Control	None	None	0.0 ± 0.0^{a} (10)	0.0 ± 0.0^{ab} (9)	0.0 ± 0.0^{ac} (9)	12.0 ± 0.0^{d} (2)

LC = Local chicken, i/o = intraocular, i/m = intramuscular, dw = drinking water, F= feed, L= La Sota, I = $NDVI_2$ $^{a,b,\ ab,\ ac,c\ \text{and}\ d}$ = Means with the same letters in the same column are not significantly different at 0.05 level of significance.

Table IV: Morbidity, mortality case fatality and protection rate of Local chickens vaccinated with $NDVI_2$ or ND La Sota vaccine administered through different route and vaccine carrier and challenged with Local strain of velogenic NDV intramuscularly.

Group	Vaccine	Route/vehicle of administration	Morbidity rate	Mortality rate	Case fatality rate	Protection rate
LCIi/o	$NDVI_2$	Intraocular	0	0	0	100
LCIi/m	$NDVI_2$	Intramuscular	0	0	0	100
LCIdw	$NDVI_2$	Drinking water	0	0	0	100
LCIF1a	$NDVI_2$	Commercial feed	100	70.0	87.5	22.0
LCIF2a	$NDVI_2$	Parboiled sorghum	100	70.0	87.5	22.0
LCIF3a	$NDVI_2$	Parboiled sorghum coated with gum Arabic	100	70.0	87.5	22.0
LCIF5a	$NDVI_2$	Untreated sorghum	100	80.0	75.0	11.0
LCLi/o	NDV La Sota	Intraocular	0	0	0	100
LCLi/m	NDV La Sota	Intramuscular	0	0	0	100
LCLdw	NDV La Sota	Drinking water	0	0	0	100
LCLF1a	NDV La Sota	Commercial feed	100	90.0	89.0	10.0
Control	None	None	100	90.0	100	10.0

LC = Local chicken, i/o = intraocular, i/m = intramuscular, dw = drinking water, F= feed, L= La Sota, I = $NDVI_2$

DISCUSSION

The efficacy of any vaccine is determined mainly by assessment of the level of antibody produced in the target bird and the ability of the vaccinated bird to resist exposure to the virulent agent when compared with unvaccinated control (Spradbrow, 1993/1994; Allan *et al.*, 1978). The suggested and reported protective antibody titres for ND vaccines are HI $\geq$ Log_2 4 (OIE, 2000). By implication, antibody titre less than Log_2 4 may not be protective. The vaccine viruses ($NDVI_2$ and NDV La Sota) as observed in this study were immunogenic and protective only in commercial or local chickens that received the vaccines via i/o, im or dw at 3 weeks PV and not protective in birds vaccinated via commercial feed, parboiled sorghum, parboiled sorghum coated with gum Arabic and untreated sorghum. Earlier reports on similar investigations showed varying outcomes (Aini *et al.*, 1990; Echeonwu *et al.*, 2008b). Failure of some of these trials using grains was blamed on antiviral factors constituent in the seed or introduced as preservatives. The presence of tannins in sorghum and gum Arabic as observed in this study might have been responsible for inactivating the vaccine virus. As tannins are known to chellates ND vaccine virus making it unavailable to the birds, this effect of tannins on NDV may probably be associated with substances that inactivate the NDV and

with binding to food lectins (Spradbrow, 1992b).Bioactive chemical compounds like saponins present in gum Arabic are known to be immune pontentiators in birds and other mammals (Hughes *et al.,* 1958).
Newcastle disease vaccines administered orally have been reported to primarily provoke mucosal immunity (Jayawardane and Spradbrow, 1995). It is thought that this is the first line of defense against NDV infection, which occurs either by inhalation or ingestion or both (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 Log_2 4.0 as protective with reference to conventional ND vaccines designed for intensively reared commercial chickens, HI antibody titre of Log_2 3.0 was considered to be adequate for food-based vaccines administered orally to scavenging chickens (Echeonwu *et al*., 2007). This is more so since it has been found that even chickens with HI antibody titre of $\leq Log_2$ 3.0 resist challenge with velogenic ND virus as observed in both the commercial and local chickens, indicating that serum antibody alone may not be responsible for the resistance to challenge.
Following challenge experiments to assess the efficacy of the vaccination method, clinical signs of ND observed in the chickens were similar to those described by Alexander (1997). The gross lesions observed 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 lesions observed were tracheal congestion and air sacculitis.
The challenge experiments did not follow 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. Though, Iroegbu and Nchinda (1999) employed the drinking water route for challenge experiments with satisfactory results, it was however, considered in this experiment that for birds to receive equal doses of challenge virus and to assess the efficacy of vaccination on protection of the birds against velogenic ND virus in the laboratory, im route was considered the method of choice.

CONCLUSIONS

It was concluded that i/o, i.m, dw routes of $NDVI_2$ vaccine administration gave higher HI antibody titre and protection rate in commercial or local chickens. Untreated sorghum, parboiled sorghum, sorghum coated with gum Arabic and a commercial feed mash when used as feed carriers for $NDVI_2$ vaccine gave low antibody titre and less protection following challenge with velogenic NDV.
Different processing methods of traditional feeds or their offals should be utilized to reduce or eliminate possible virucidal substances that may be present in feed grains which affect the survival of the $NDVI_2$ vaccine virus.

ACKNOWLEDGEMENTS

The authors are grateful to the Dr (Mrs.) L. H. Lombin Executive Director, NVRI, Vom for providing the facilities for the research and the Staff of Viral Research, Virology and Poultry Divisions of the NVRI, Vom for sample analysis and management of the birds.

REFERENCES

AINI, A., IBRAHIM, A. L. AND MUSTAFFA, B. (1990): Feed based Newcastle disease vaccine for rural chickens. *Poult. Inter.,* December edition, Pp. 24-28.

ALDERS, R. AND SPRADBROW, P. B. (2001): Controlling Newcastle in rural

chicken. *A Field Manual.* Australian Center for International Agriculture Research Monograph No 82, Pp. 37.

ALLAN, W. H. AND GOUGH, R. E. (1974): A standard haemagglutination inhibition test for Newcastle disease (1) A comparison of macro and micro methods. *Vet. Rec*, 95: 120-123.

ALLAN, W. H., LANCASTER, J. E. AND TOTH, B. (1978): *Newcastle Disease Vaccines, Their Production and Use.* FAO Animal Production and Health. Series No. 10. Rome: Food and Agricultural Organization, Pp. 54-94.

ALEXANDER, D. J. (1988): Newcastle disease method of spread. In: *Newcastle Disease*, D. J. Alexander, Ed., Kluwer Academic Publishers, Boston, Pp. 257-272.

ALEXANDER, D. J. (1997): Newcastle disease and other avian paramyxoviridae infections. In: *Diseases of Poultry*. 10th Edition, Edited by Calnek, B. W., Barnes, H. J., Beard, C.W., McDougnald, I. R. and Saif, Y.M., Iowa State University Press, Ames, Iowa, United States of America, Pp. 541-547.

AMAKYE-ANIM J., AWUNI, J. A., COLEMAN, T. AND SEDOR, V. (2000): Ghanian trials with a rurally produced thermostable Newcastle disease vaccine (strain I2) in chickens. *26th Animal Science Symposium Ghana Animal Science Association*, Kumasi, University of Science and Technology.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMIST (AOAC) (1995): *Official methods of analysis* 16th edition. Association of Official Analytical Chemist, Washington D.C.

BENSINK, Z. AND SPRADBROW, P. B. (1999): Newcastle disease virus strain I_2, a prospective thermostable vaccine for use in developing countries. *Vet. Microbiol*, 68: 131-139.

ECHEONWU, G. O. N., IROEGBU, C. W. AND EMERUWA, A. C. (1993): Recovery of velogenic Newcastle disease virus from dead and healthy free-roaming birds in Nigeria. *Avian Path*, 22: 383-387.

ECHEONWU, G. O. N., IROEGBU, C. U., ECHEONWU, B. C., NGENE, A., OLABODE, A. O., OKEKE, O. I., NDAKO, J., PAUL, G., ONOVOH, E. M., JUNAID, S. A. AND NWANKITI (2007): Delivery of thermostable Newcastle disease (ND) vaccine to chickens with broken millet grains as the vehicle. *Afr. Journ. of Biotech.*, (23): 2694-2699.

ECHEONWU, G. O. N., IROEGBU, C.U, NGENE, A, JUNAID, S. A, NDAKO, J, ECHEONWU, I. E, OKOYE, J. O. A. (2008a): Survival of Newcastle disease virus (NDV) strain V_4- UPM coated on three grains offal and exposed to room temperature. *Afri. Journ. of Biotech.* , 15:2688-2692.

ECHEONWU, B. C., NGELE, M. B., ECHEONWU, G. O. N., JOANNIS, T. M., ONOVOH, E. M. AND PAUL G. (2008b): Response of chickens to oral

vaccination with Newcastle disease virus vaccine strain I_2 coated on maize offal. *Afr. Journ. of Biotech.,* 7 (10): 1594-1599.

HUGHES, D.H., LYNCH, P. L. AND SOMERS, G. S. (1958): Chromatographic identification of aminoacids and carbonhydrate in cultivated mushroom. *Journ. of Agric and Food Chem.,* 6:850-853.

IROEGBU, C. U. AND NCHINDA, G.W. (1999): Evaluation of cassava feed for oral delivery of Newcastle disease V4 vaccine. *Bull. Anim. Hlth and Prod. Afr.,* 47: 155-161.

JAYAWARDANE, G.W.L. AND SPRADBROW, P.B. (1995): Cell mediated immunity in chickens vaccinated with the V4 strain of Newcastle disease virus. *Vet. Microbiol*, 46:37-41.

MCFERRAN, J.B. AND MCCRAKEN, R. M. (1988): Newcastle disease. In: *Newcastle Disease*, Alexander, D. J, (ed), Kluwar Academic Publishers, Boston, MA, Pp.161-183.

MUSA, U. (2002): Effects of vaccination of chickens against Newcastle diseases with thermostable V_4 and La Sota vaccines. *MSc Thesis*. Department of Veterinary Surgery and Medicine, Ahmadu Bello University, Zaria, Nigeria.

Office International des Epizootics, (OIE) (2000): Newcastle Disease. In: *Manual of Standard for Diagnostic Tests and Vaccines*.5th edition, Paris, Pp. 104-124.

SA'IDU, L. (2006): Factors affecting the effectiveness of the control programme against Newcastle disease in Zaria, Nigeria. *PhD Dissertation*, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Pp. 1-151.

SPRADBROW, P. B. (1992a): Oral vaccine and mucosal immunity. *Australian Centre for International Agriculture Research Proceedings*, 39, Pp. 56-60.

SPRADBROW, P. B. (1992b): A review of the use of food carriers for the delivery of oral Newcastle disease vaccine. In: Spradbrow, P. B. ed. *Proceedings of an International Workshop Kaula Lumpur,* 6-10 October 1991, Canberra, Australian Center for International Agriculture Research, No 39, Pp.18-20.

SPRADBROW, P. B. (1993/94): Newcastle disease in rural chickens. *Poult. Sci. Rev.*, 5: 57-96.

WAMBURA, P. N., KAPAGA A. M. AND HYERA, J. M. K. (2000): Experimental trials with thermostable Newcastle disease virus (strain I_2) in commercial and rural chickens in Tanzania. *Prev. Vet. Med.,* 43:75-85.