



Humoral Immune Response of Chickens Following Vaccination with different Newcastle Disease Vaccines

Aliyu, H. B.¹; Sa'idu, L.¹; Abdu, P. A.² and Oladele, S. B.³

¹Veterinary Teaching Hospital, Ahmadu Bello University, Zaria; ²Department of Veterinary Medicine, Ahmadu Bello University, Zaria; ³ Department of Veterinary Pathology, Ahmadu Bello University, Zaria. *Corresponding Authors: Email: bahayatudeen@abu.edu.ng Tel No:+234 8038386953

SUMMARY

In spite of numerous vaccines and different vaccination schedules used in the control of Newcastle disease (ND), prevention and control remain a challenge. This study evaluated three different ND vaccines. A total of one hundred and twenty, day-old brown pullets obtained from a commercial hatchery in Ibadan, Nigeria were used for the experiment. The birds were randomly assigned into 4 groups in which groups A, B and C were vaccinated on days 1, 21 and 42 of age, while group D served as unvaccinated group (control). Hitchner B1 (HB1), Clone-30 and F-Strains were used as the primers for the 3 vaccinated groups respectively. Blood samples were collected from all birds in each group on vaccination day and assayed for NDV antibody by Haemagglutination-inhibition (HI) test. Twenty five chickens from each group were challenged with virulent Newcastle disease virus (Kudu 113 strain) at 3rd week after the last vaccination. The mean antibody titres of the chickens from the vaccinated groups at 3rd week post primary vaccination showed no significant difference. However, a significant difference existed following secondary vaccination with La Sota and Komarov strains at 3rd and 6th weeks of age. A good immune response and clinical resistance were observed in group of chickens vaccinated with Hitchner B1 and Clone-30 as primers than those vaccinated using F-strain. Therefore, Hitchner B1 or Clone-30 is better primer for vaccinating chickens against Newcastle disease.

Key words: Chickens, Vaccination, antibody, Newcastle disease, Challenge virus.

INTRODUCTION

Newcastle disease is a highly contagious, viral disease of domestic poultry and wild birds, characterised by gastro-intestinal, respiratory and nervous signs (Seal *et al.*, 2000; Alexander, 2003). Newcastle disease is one of the most important viral diseases of

poultry in the world. It occurs in most countries and has devastating effect on commercial poultry production. It is generally considered that the first outbreaks of velogenic Newcastle disease (vND) occurred in 1926, in Java, Indonesia

(Kraneveld, 1926), and in Newcastle-upon-Tyne, England (Doyle, 1927). The disease is caused by NDV which is an enveloped virus belonging to the family paramyxoviridae with a negative-sense, single-stranded, non-segmented RNA genome (Aldous and Alexander, 2001). Newcastle disease is also considered as one of the major threat to poultry production in Nigeria, because of its high morbidity and mortality rates. The disease also causes reduction in productivity leading to economic losses every year. The disease was first reported in 1953 in Nigeria (Hill *et al.*, 1953; Okeke and Lamorde, 1988).

The disease is kept under control by vaccination and other preventive measures. Currently practiced vaccination programme against ND includes administration of two types of live vaccines of either lentogenic (B1, F, Clone-30, LaSota strain) or mesogenic (Komarov strain) and inactivated vaccines. Despite vaccination outbreak of ND are often reported in both vaccinated and unvaccinated flocks (Halle *et al.*, 1999; Sa'idu *et al.*, 2006a; Sa'idu and Abdu, 2008; Musa *et al.*, 2010; Aliyu *et al.*, 2015). Consequently, this may be due to vaccine failure.

In Nigeria poultry farmers have been using various imported and local ND vaccines following either same or different vaccination schedules. These vaccines are assumed to be highly effective against ND, although there is paucity of information on the immune response of birds to these vaccines. However, various scientists and field Veterinarians in the country find it difficult to determine the factors responsible for these sporadic outbreaks of ND in vaccinated flocks. Therefore, the present study was undertaken to compare the antibody titres of chickens vaccinated with three different imported ND vaccines of lentogenic strain used as primers followed

by lentogenic and mesogenic strains and also evaluate the efficacy of these vaccines following challenge with virulent NDV Kudu 113 strain.

MATERIALS AND METHODS

A total of 120 apparently healthy ISA brown day-old pullets were purchased from Zartech hatchery, Ibadan and conveyed to the Poultry Research Pens of the Veterinary Teaching Hospital, Ahmadu Bello University, (ABUVTH) Zaria. The chicks were fed with commercial chick mash (Feedtech) and water *ad libitum*. The chicks were divided into 4 groups (groups A, B, and C, received primers vaccines, Hitchner B1, Clone-30 and F-strain respectively, while group D was unvaccinated control). They were housed in separate pens and attended to by separate care taker.

Newcastle Disease Vaccine

Lyophilized ND vaccines baby chick ranikhet disease vaccine (BCRDV, F-strain, lentogenic), Izovac B1 Hitchner[®] (B1 strain, lentogenic), Izovac La Sota[®] (La Sota strain, lentogenic), Indovax R₂B[®] (Mukteswar strain, mesogenic) and Izovac Komarov[®] (Komarov strain, mesogenic) were purchased from veterinary vaccines distributor in Kaduna and were used for the experiment. The vaccines were stored and diluted during use according to the manufacturer's instruction.

Vaccination Schedules

Chickens in group A were vaccinated against ND as follows: Hitchner B1, La Sota and Komarov on days 1, 21 and 42 of age, respectively as recommended by the ABUVTH Poultry clinic; groups B and C were vaccinated with clone 30 and F-Strain, respectively on day 1 and other vaccines remained the same as in group A. group D remained as unvaccinated control.

Newcastle Disease Antigen and Challenge Virus

Newcastle disease vaccine (La Sota strain) and NDV Kudu 113 strain were obtained from the National Veterinary Research Institute (NVRI), Vom, Nigeria. The La Sota strain was used as antigen for the HA and HI tests, while NDV Kudu 113 strain was used as challenge virus.

Serological test for Newcastle disease

The titre for the NDV antigen was determined by haemagglutination (HA) test, while the antibody titres in the sera were determined by Haemagglutination-inhibition (HI) test using the method described by OIE (2009).

Challenge of chickens with virulent Newcastle disease virus (kudu 113 strain)

Twenty five chickens from each group were inoculated with 0.2 ml of virulent NDV Kudu 113 strain suspension containing 10^7 EID₅₀ per ml intramuscularly 3 weeks after the last vaccination. The Chickens were monitored for clinical signs and mortality at 12 hours intervals post challenge for 14 days. Postmortem examination was conducted on any dead chicken and postmortem lesions observed were recorded.

Determination of morbidity, mortality and protection rates post challenge

Clinically sick chickens and mortality recorded in different groups after challenge with NDV Kudu 113 strain at the end of the experiment was calculated as a percentage of the initial number of the birds (Guy and Garcia, 2008). Also, the protection rate was calculated as mortality rate in unvaccinated chickens minus the mortality rate in the vaccinated chickens divided by the mortality rate of the unvaccinated (Babiker *et al.*, 2008).

Statistical Analysis

Results were analyzed by the use of statistical package of social sciences (SPSS) version 16 using Duncan's multiple range test (DMRT) following analysis of variance (ANOVA) to establish the level of significance of the immunological response of the chickens in the various groups after primary and secondary vaccination (Beri, 2005). Values of $p \leq 0.05$ were considered significant.

RESULT

The mean \pm SEM of haemagglutination inhibition (HI) titres of group A, B, C and D measured at weeks zero, 3, 6, 9, 10 and 11 respectively (table 1).

TABLE I: Mean antibody titre of chickens (vaccinated with ND vaccines and unvaccinated) pre- and post challenge with kudu 113 strain of Newcastle disease virus

Age (week)	Mean antibody titre \pm SEM log ₂			
	A	B	C	D
0	5.53 \pm 0.29 ^a	5.43 \pm 0.32 ^a	5.53 \pm 0.24 ^a	5.63 \pm 0.20 ^a
3	8.13 \pm 0.24 ^a	8.53 \pm 0.23 ^a	7.93 \pm 0.34 ^a	4.16 \pm 0.55 ^b
6	9.39 \pm 0.13 ^a	8.96 \pm 0.25 ^a	6.41 \pm 0.37 ^b	3.50 \pm 0.41 ^c
9	8.11 \pm 0.34 ^a	7.80 \pm 0.33 ^a	4.62 \pm 0.54 ^b	2.96 \pm 0.32 ^c
10	4.68 \pm 0.74 ^a	4.00 \pm 0.57 ^a	5.20 \pm 0.72 ^a	5.72 \pm 0.43 ^a
11	4.60 \pm 0.92 ^a	4.68 \pm 0.36 ^a	4.24 \pm 0.69 ^a	5.30 \pm 1.03 ^a

a, b, c =Means with the same superscript do not differ significantly
level of significance was at $p \leq 0.05$ across the rows

The maternally-derived antibody (MDA) measured on day 1 of the experiment showed uniform mean antibody titres with no statistical difference. The mean Ab titres of the chickens in groups A, B, C and D at 3 weeks of age were 8.13 ± 0.24 , 8.53 ± 0.23 , 7.93 ± 0.34 and 4.16 ± 0.55 respectively. There was no significant difference of the mean Ab titres among chickens of groups A, B and C, while that of chickens in group D was significantly lowered at week 3 post primary vaccination. At 6 weeks of age, there were mean antibody titres of 9.39 ± 0.13 , 8.96 ± 0.25 , 6.41 ± 0.37 and 3.50 ± 0.41 of the chickens in groups A, B, C and D, respectively. The higher HI titres were found in chicken of groups A and B, which differ significantly with the chickens of group C and D. The same pattern of HI titres differences were observed at week 9. The mean antibody titres of the chickens at 10 week of age following challenge with the virus were 4.68 ± 0.74 , 4.00 ± 0.57 , 5.20 ± 0.72 and 5.72 ± 0.43 for groups A, B, C and D, respectively. Likewise the mean HI titres of the birds among the groups at 11 week of age depict similar values to that of week 10. Three days post challenge some chickens begin to show clinical signs particularly in unvaccinated control group. The clinical signs manifested were: Somnolence (15/20), ruffled feathers (17/20), listlessness (15/20), diarrhoea (10/20), reduced feed and water

intake, depression (13/20), swollen head (1/20), coughing and sneezing, rales (4/20), sitting on the hock (13/20), recumbence (7/20), leg paralysis (4/20), dropped wing (3/20), torticollis (5/20), star gazing (3/20) and in coordination (2/20). The gross lesions observed on dead chickens following post-mortem examination were congested skeletal muscles (10/15), congested liver (9/15), congested heart (5/15), congested spleen (12/15), congested kidneys (8/15), congested lungs (13/15), haemorrhagic trachea (15/15), haemorrhages in the proventriculus (15/15), haemorrhages in the duodenum (12/15) with button ulcers (4/15), haemorrhages in the jejunum (10/15) with button ulcers (4/15), enlarged and haemorrhagic caecal tonsils (12/15), haemorrhagic caeca (8/15). The gross lesions for the chicken in group C, were congested skeletal muscles and haemorrhages in the proventriculus, duodenum and caecal tonsils. The morbidity, mortality and protective rates of the treatment and control groups are summarised in table II. There were zero percent morbidity and mortality rates, while 100 % protective efficacy was observed in chickens of groups A. There was 4 % morbidity rate recorded for the chickens in group B, while 80 % morbidity rate and 40 % survival rate were recorded in the chickens of the control group.

TABLE II: Morbidity, mortality and protection rates of chickens vaccinated with ND vaccines and unvaccinated chickens post challenge with NDV Kudu 113 Strain

Groups	Vaccination schedules	Morbidity rate	Mortality rate	Protective rate
A	HB1, La Sota & Komarov (live)	0% (0/25)	0% (0/25)	100%
B	Clone-30, La Sota & Komarov (live)	4% (1/25)	0% (0/25)	100%
C	F-Strain, La Sota & Komarov (live)	4% (1/25)	4% (1/25)	93.3%
D	Control	80% (20/25)	60% (15/25)	40%

DISCUSSION

The protective MDAs observed on day 1 indicate that the parent stock with which the chicks were obtained had adequate Ab titre against ND. The increase in the mean NDV Ab titres at 3 weeks of age was as a result of the first active dose of the vaccination administered on day 1. It was clearly shown that there was no significant difference within the Ab of the chickens in vaccinated groups. This could further explain that there is no difference in the immunogenicity of HB1, Clone-30 and F-strain ND vaccines when use as primers. This finding is in agreement with the finding of Ibrahim *et al.* (1983) who found no significant difference among F-strain, B1 and La Sota strains of ND vaccines. The MDA of 4.16 ± 0.55 observed in the birds of the control group may indicate that the titre remains protective up to 2 weeks of age which is in consistence with the report of Begum *et al.* (2006) who stated that MDA of chicks remains protective until 18 days of age.

However, a significant difference existed within the vaccinated groups following secondary vaccinations with La Sota and Komarov strains at 3rd and 6th weeks of age respectively. The significantly lower Ab titres recorded in the birds of group C at 3rd and 6th weeks of age respectively may be due to the higher pathogenicity index of the vaccine viral strain (F-strain) which might not have produced adequate memory cells as compared to the other strains (B1 and Clone-30).

The lower HI titres of group A and B chickens after the administration of mesogenic strain at 6 weeks of age may be related to the virulence nature of the strain and the interval between the vaccines, which may have interfered with the ongoing Ab production, as lower immune response is expected when the existing Ab titres is high and vice versa (Kouwenhoven, 1993).

The protective efficiency (lack of clinical disease and mortality) of the vaccines post

challenge correlated with the Ab production at the period of challenge of 8.11 ± 0.34 , 7.80 ± 0.34 , and 4.62 ± 0.54 for the chickens in groups A, B and C respectively. The significant decline of the HI titres of the chickens in groups A and B at 10th and 11th weeks after the challenge at week 9 could be attributed to the high Ab titres prior to challenge, which was neutralised by the challenge ND virus.

The increase HI titre for the birds in groups C and D at 1st and 2nd weeks post challenge can be associated with the low Ab titres at the period of challenge. The introduced virulent ND virus could have acted as a booster for the group C birds while as primary vaccinations for the group D chickens. Thus may accounted for the significant increased mean Ab titre found in the birds of group D a week post challenge. This finding agreed with the report of Sa'idu *et al.* (2006) who reported an increase in HI titres following challenge with NDV kudu 113 strain in naïve chickens. Therefore, it is clear that birds exposed to virulent ND virus may respond to decrease or increase Ab titres depending on immune status of the birds.

The clinico-pathologic manifestations of the disease correlate with the mean Ab level across the groups. These were similar to those seen in velogenic ND outbreaks (McFerran and McCracken, 1988; Alexander, 1993; Sa'idu *et al.*, 2006; Sa'idu and Abdu, 2008 and Musa *et al.*, 2010).

Thus, it is clearly showed that there is no significant difference among the three different vaccines strains of the lentogenic origin, with respect to the protective Ab titres. This finding agreed with the report of Ibrahim *et al.* (1983) who found no difference among F-strain as compared with B1 and La Sota strain of ND. However, in regards to Ab production, group of chickens vaccinated with Hitchner B1 and Clone-30 produced higher HI titre than that vaccinated with F-Strain.

Furthermore, among all the vaccines of different strains, it was found that after primary vaccination, LaSota strain produced good humoral immune response. This finding is in agreement with Almassy *et al.* (1979) and Westbury (1984) who reported that LaSota strain provided superior Ab production following vaccination. However, the Komarov strain might have contributed to the protection against clinical disease and mortality from ND.

CONCLUSION

It was concluded that all the three lentogenic vaccines produced satisfactory priming effect leading to protection against challenge with virulent NDV kudu 113 strain. A good immune response and clinical resistance to the disease were observed in group of chickens vaccinated with Hitchner B1 and Clone-30 as primers than those vaccinated using F-strain.

REFERENCES

- ALDOUS, E. W. and ALEXANDER, D. J. (2001). Detection and differentiation of Newcastle disease virus (avian paramyxovirus type 1). *Avian Pathology*, 30 (2): 117-128.
- ALEXANDER D.J. (1993). Newcastle disease, other avian paramyxoviruses, and pneumovirus infections. In: Y.M. Saif, (Ed), *Diseases of Poultry*, Iowa State Press, Ames, Iowa. 11: 63–80.
- ALEXANDER, D.J. (2003). Newcastle disease, other avian paramyxoviruses, and pneumovirus infections. In: Y.M. Saif, H.J. Barnes, J.R. Glisson, A.M. Fadly, L.R. McDougald, D.E. Swayne. (Eds.), *Diseases of Poultry*. Ames: Iowa State University Press, pp. 63–99.
- ALIYU, H.B. SA'IDU, L. ABDU, P.A. and OLADELE, S. B. (2015). Retrospective Analysis of Newcastle disease diagnosed at the Poultry Clinic of Ahmadu Bello University, Zaria, Nigeria. *Sokoto Journal of Veterinary Sciences*, 13(3): 42 – 48.
- ALMASSYL, K., BARHOUMA, N., EISABBGH, A., IBRAHIM, S.N., BOKTOR, N., KHASHABA, E. and GAWAD, S.A. (1979). Comparative immunization experiments with lentogenic Newcastle disease vaccine strain. *Journal of Egyptian Veterinary Medical Association*, 35: 95-104.
- BABIKER, M.A., YAHIA, I.E., NORA, K. and TAWFEEQ, E.M. (2008). Investigation on nine flocks infected with Infectious bursal disease virus in Khartoum state (Sudan). *International Journal of Poultry Science*, 7(3):285-288.
- BEGUM, K., KHAN, M.S.R., RAHMAN, M.B., KAFI, M.A., DAS, M. and MAMUN. S. A. A. (2006). Investigation on baby chick ranikhet disease vaccine administration in chicks of vaccinated and non-vaccinated origin. *Bangladesh Journal of Veterinary Medicine*, 4(2): 93-96.
- BERI, G.C. (2005). *Business Statistics*, 2nd edn, Tata Mc Graw-Hill publishing company limited, New Delhi, India.
- DOYLE, T.M. (1927). A hitherto unrecorded disease of fowls due filter passing virus. *Journal of Comparative Pathology and Therapeutics*, 40:144-169.
- GUY, J.Y. and GARCIA, M. (2008). Laryngotracheitis. In Y.M. Saif, A.M. Fadly, J.R. Glisson, L.R. McDougald, L.K. Nolan and D.E. Swayne, (Eds.). *Diseases of Poultry* 12th edn (pp. 137-152). Ames, Iowa: Blackwell Publishers.
- HALLE, P.D., UMOH, J.U., SAIDU, L. and

- ABDU P.A. (1999). Prevalence and seasonality of Newcastle disease in Zaria, Nigeria. *Tropical Veterinarian*, 17: 53–62.
- HILL, D.H., DAVIS, O.S. and WILKES, G.E.H. (1953). Newcastle disease in Nigeria. *British Veterinary Journal*, 109:385-391.
- IBRAHIM, A.L., LAI, M.C. and AINI, I. (1983). Spray vaccination with an improved F Newcastle disease vaccine. A comparison of efficacy with the B1 and LaSota vaccines. *British Veterinary Journal*, 139: 213-219.
- KOUWENHOVEN, B. (1993) Newcastle Disease. In: J.B. McFerran, and M.S. McNulty, (Eds), *Virus Infections of Birds*, (pp. 341-361) Elsevier Science, Amsterdam and New York.
- KRANEVELD, F.C. (1926) A poultry disease in the Dutch East Indies. *Nederlands-Indische Bladen voor Diergeneeskunde*, 38:448-450.
- MARANGON, S. and BUSANI, L. (2006). The use of vaccination in poultry production. Review Scientific Technology, *Office International Des Epizooties*, 26: 265-274.
- MCFERRAN, J.B. and MCCRACKEN, R.M. (1988). Newcastle disease. In: D.J. Alexander (Ed.), *Newcastle disease*, Boston, Kluwer Academic Publishers, pp. 161-183.
- MILLER, P.J., ESTEVEZ, C., YU, Q., SUAREZ, D.L. and KING, D.J. (2009). Comparison of viral shedding following vaccination with inactivated and live Newcastle disease vaccines formulated with wild-type and recombinant viruses. *Avian Diseases*, 53: 39-49.
- MUSA, I.W., ABDU, P.A., SACKKEY, A.K.B., OLADELE, S.B., SA'IDU, L. and YAKUBU, I.U. (2010). Outbreak of Velogenic Viscerotropic Newcastle disease in Broilers. *International Journal of Poultry Science*, 9 (12): 1116-1119.
- Office International Des Epizooties (OIE) (2009). *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. www.oie.int/manual.intnl.07/06/2011.
- OKEKE, C.N. and LAMORDE, A.G. (1988). Newcastle disease and its control in Nigeria, In: W.A. Olufemi and W.N. Masiga, (Eds.). *Viral Diseases of Animals in Africa*. OAU/CTA Publication. pp. 283-287.
- SA'IDU, L. and ABDU, P.A. (2008). Outbreak of Viscerotropic Velogenic form of Newcastle disease in vaccinated six weeks-old pullets. *Sokoto Journal of Veterinary Sciences*, 7(1): 37-40.
- SA'IDU, L., ABDU, P.A., TEKDEK, L.B. and UMOH, J.U. (2006a). Retrospective study of Newcastle disease cases in Zaria, Nigeria. *Nigerian Veterinary Journal*, 27: 53–62.
- SA'IDU, L., BISALLA, M. and MOUMINI, B. (2006b). Response of Local Breeds of Chicken to Challenge with Newcastle disease virus (Kudu 113 Strain). *Journal of Animal and Veterinary Advances*, 5 (11): 975-979.
- SEAL, B.S., KING, D.J. and SELLERS, H.S. (2000). The avian response to Newcastle disease virus. *Developmental and Comparative Immunology*, 24:257–268.
- WESTBURY, H.A., PARSONS, G., and ALLAN, W.H. (1984). Comparison of the immunogenicity of Newcastle disease virus strains V4, Hitchner B1 and La Sota in chickens: 2. Tests in chickens with maternal antibody to the virus. *Australian Veterinary Journal*, 61(1):10-13.