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## The Effect of Storage Conditions on the Potency of Newcastle Disease Vaccine La Sota

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**Abstract:** Thirty (30) vials of Newcastle Disease (ND) vaccine. La Sota were procured locally and tested for potency when stored under conditions of irregular power supply. The vaccines were of the same batch and were eighteen months to the expiration time. The potency of the vaccines was tested using Haemagglutination (HA) test before storage. The vaccines' immunogenicity was determined in a batch of 250, 3 weeks old white cockerels after which the vaccines were stored in the refrigerator with two-third of the storage period at 0-4°C and the remaining one third at room temperature of 26-30°C. During the study, the methods of vaccine storage were alternated frequently to reflect the frequent power outages. The humoral immune response of the vaccinated chickens was estimated by Haemagglutination Inhibition (HI) test. The potency of the vaccines was tested on days 28, 56, 84, 112 and 140 post storage. On day 140, another batch of 250, 3 weeks old white cockerels were vaccinated with a vial from the stock and the humoral immune response of these chickens was evaluated at 3 weeks post vaccination. The mean HA titre of the vaccine before storage was 128, while the mean HA titres of the vaccines after storage (i.e., day 140) was 8. The mean HI titres of the vaccinated chickens before and after storage were 1005.7 and 8.3, respectively. Student t test was used to compare the mean HI titre of the chicken vaccinated prior and at the end of vaccine storage. The GMT mean HI titre of the chickens vaccinated prior to storage was significantly ( $p < 0.05$ ) higher than that of the chicken vaccinated at the end of vaccine storage.

**Key words:** Immunity, Newcastle disease vaccine, storage conditions, Nigeria

### INTRODUCTION

Newcastle Disease (ND) is one of the most serious infectious and highly contagious viral disease of poultry and causes large economic losses in poultry production worldwide (Alexander, 2000). The causative agent, Newcastle Disease Virus (NDV), is a member of the genus *Rubulavirus* of the family *Paramyxoviridae* (Mayo, 2000). The virus possesses helical symmetry, an RNA genome and belongs to the genus *Rubulavirus* in the family *Paramyxoviridae* (White and Fenner, 1994). The disease affects birds of all ages (Al-Falluji *et al.*, 1978). The disease resulting from an NDV infection of birds varies from mild to severe with high mortality depending on virulence of the infecting strain and host susceptibility (Alexander, 2000). NDV strains can be classified as highly virulent (velogenic), intermediate (mesogenic) or non-virulent (lentogenic). This classification is based on the results of the mean death time in chicken eggs (Beard and Hanson, 1984).

The clinical signs of a highly virulent NDV infection in chickens can be extremely different depending on the strain of virus. Newcastle disease is a major problem in many countries of the world where poultry is reared (Kouwenhoven, 1993). In Nigeria and until recently, the occurrence of avian influenza, Newcastle disease was regarded as the most deadly and economically important disease of poultry. ND is widespread in

Nigeria with frequent and sporadic outbreaks of infection involving the velogenic strain being common (Onunkwo and Momoh, 1980; Adu *et al.*, 1986). Control measures involving slaughter, sanitary measures and vaccinations are best options in the fight against ND (Alexander, 2000). In developing countries like Nigeria, these policies are not well implemented. Local chickens for instance move about on a free range system and are always unvaccinated. They therefore suffer severe outbreaks of ND especially during the November to March periods with heavy contamination of the environment (Abdu *et al.*, 1992). These infections eventually spread to commercial chickens resulting in heavy economic loss (Abdu *et al.*, 1992). One important cause of vaccine failure may be the use of poor or impotent vaccine mostly due to improper storage (Rathore, 1987). Electricity or power supply in Nigeria is poor and vaccines are also handled by untrained personnel who do not know the need for cold chain system in vaccine storage. This research therefore, investigated the direct and applied effects of fluctuation in power supply on the quality of ND vaccine La Sota.

### MATERIALS AND METHODS

**Vaccine:** Thirty vials of live attenuated Newcastle disease vaccine, La Sota were procured from the distributor. All the vaccines were of the same source and

batch and were 18 months to expiration. The vaccines were in a freeze dried state and each vial contained 1000 doses.

**Chickens:** Two batches of 250 white shaver cockerels per batch were used for the experiment. Each of the batches was obtained at day old from a commercial hatchery. They were not vaccinated against any disease and were brooded under deep litter system. The first batch was procured and used at the beginning of the experiment while the second batch was procured and used at the end of the experiment. Feed and water were given *ad-libitum*. The vaccination and medication history of the parent stock was not known. At 2 weeks of age, the chickens were vaccinated against infections bursal disease and at 3 weeks of age, the chickens were used to test the potency of the vaccines.

**Storage temperatures:** The storage temperatures were designed to reflect the power situation in the Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka, Enugu State, Nigeria, where the work was carried out. The period of power supply and the temperature of the refrigerator were monitored for two months and average values were estimated. Two-third of the periods had power with the temperature of refrigerator between 0-4°C while the remaining one-third has no power and the temperature was between 26-30°C (room temperature). Therefore 2 temperature settings were used, the 1st was the 0-4°C (refrigeration temperature) and the 2nd, 26-30°C (room temperature).

**Testing and storage:** Prior to storage, a vial of the vaccine was taken from the stock and tested for potency using Haemagglutination (HA) test described by Beard (1980). The first batch of 250, 3 weeks old chicks was vaccinated with a vial of the vaccine before the storage. The vaccines were then stored in the refrigerator with power for two-third of the period and on the bench for the remaining one-third of the period. To reflect the fluctuations in power, the periods were alternated on a weekly basis. At days 28, 56, 84, 112 and 140 after storage a vial of the vaccine was taken and tested for potency using HA test. By the end of the experiment (day 140), a vial of the vaccine was also taken and tested in the second batch of 250, 3 weeks old chickens. Serum samples were collected from each batch of the chicken at 3 weeks post vaccination and used to estimate the antibody responses by Haemagglutination Inhibition (HI) test as described by Beard (1980).

## RESULTS

The potency of the vaccines as measured by HA test prior to storage was 128. This titre declined appreciably as storage progressed. The titre was 128 by day 28 after storage and by day 56, it has declined to 32. The titre

Table 1: HA titres of stored Newcastle disease vaccine, La Sota as determined at specific periods

Days (after storage)	HA titre
0	128
28	128
56	32
84	32
112	32
140	8

Table 2: Immune responses of chicks vaccinated with Newcastle disease vaccine, La Sota prior to storage and at the end of store at as estimated by antibody HI titres

S/N	HI titre (before storage)	HI titre (at the end of storage)
1	1024	16
2	512	16
3	512	8
4	1024	16
5	2048	4
6	512	0
7	512	16
8	256	8
9	1024	16
10	512	0
11	2048	0
12	1024	4
13	2048	8
Means	1005.7	8.3

remained at 32 up to day 112 and by day 140, it was 8 (Table 1). The immune responses of chicks vaccinated with ND vaccine before storage was high with a mean HI antibody titre of 1005.7 (Table 2), while the immune responses by the chicks vaccinated after the storage was low with mean HI antibody titre of 8.3 (Table 2).

## DISCUSSION

This research has been able to investigate the storage potentials and immunogenicity of a foreign brand of ND vaccine, La Sota under a condition designed to represent the storage conditions in Nigeria. Irregular power supply has been a common phenomenon in Nigeria. Individuals, household units, small and large scale businesses, Universities and Research Institutions, Medical and Veterinary establishments suffer serious setbacks because of this problem.

Vaccines are used widely in the Medical and Veterinary professions in the control and prevention of diseases. Zander *et al.* (1997) noted handling of vaccines as a great necessity in the maintenance of the quality of the vaccine. This means that poor handling as in improper storage can render vaccines useless. These vaccines are stored in the refrigerator or any cold chain system until they are used and these cold chain systems rely on regular power supply. Power supply in Nigeria is irregular and this may be withheld for hours, days and even weeks.

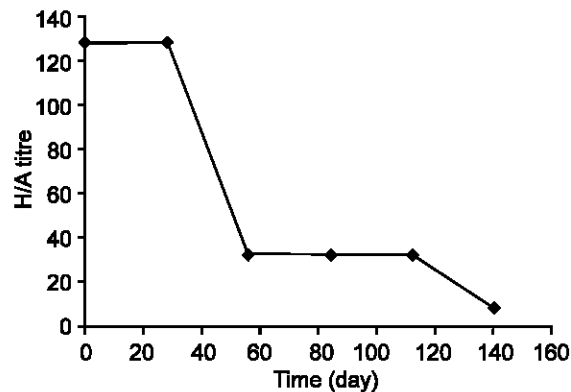


Fig. 1: HA titres of stored Newcastle disease vaccine, La Sota as determined at specific periods

In the experiment, the potency of the vaccines as measured by HA test decreased very fast during the storage period. The HA titer of 128 prior to storage declined sharply to 32 within 8 weeks of storage and to 8 within another 12 weeks of storage (Fig. 1). The HA titres obtained were a measure of the storage ability of the vaccine under the storage conditions. Allan *et al.* (1978) observed that good storage extends the storage life of vaccines while poor storage reduces the potency of vaccines appreciably. This implies that if vaccines are improperly stored, they fail in what they were produced to achieve leading to vaccine failure. As expected the vaccines were able to induce high immune responses in bird vaccinated before storage and induced a poor response in those vaccinated at the end of storage. Unfortunately, a vaccine that had up to 18 months to induce protective immunity lost this quality or attribute in less than 5 months. Serum with HI antibody titre of 8 or greater has been suggested to be positive for Newcastle disease virus antibodies and this can be protective (Report of Poultry Disease Subcommittee on Animal Health, 1971). Therefore the immunity induced in the chicks vaccinated at the end of storage may not be protective. There was also a significant difference ( $p < 0.05$ ) in the mean HI antibody titre of those vaccinated before storage and those vaccinated at the end of storage.

Outbreaks of ND in vaccinated birds in Nigeria are quite common and one may therefore conclude that improper vaccine storage under our local power supply may be contributing substantially to this. In Nigeria vaccines are handled by non trained personnel who do not understand the importance of cold chain system in vaccine preservation. Moreover, many outlets distributing vaccines do not have functional standby generators. The cost of fuelling these generators in case they are available is also high, therefore they rely on power supply from the national grid with these resultant problems.

**Conclusion:** Irregular power supply in Nigeria which continually is on the decline affects the agricultural sector and poultry in particular due to its effect on vaccine storage. Exclusive dependence on the national power supply for vaccine storage may lead to vaccine failure and its subsequent effect on poultry production. Therefore, all outlets keeping vaccines must have a power generating sets to supply power should the national power supply fails. The cost of fuelling these power generating sets should be ignored as it is more important that the quality of the vaccines being stored is maintained.

## REFERENCES

- Abdu, P.A., U.M. Mera and L. Saidu, 1992. A study of chicken mortality in Zaria, Nigeria. In: Proceeding of National Workshop on Livestock and Vet. Services, National Veterinary Research Institute Vom, pp: 51-55.
- Adu, F.D., A. Oyejide and B.O. Ikede, 1986. Characterization of Nigerian strains of Newcastle Disease virus. *Avian Dis.*, 29: 829-831.
- Alexander, D.J., 1995. World's Poultry Science Association, Barcelona. Current, Situation of Newcastle Disease Epizootiology and Control in Countries of the European Union, pp: 167-178.
- Alexander, D.J., 2000. Newcastle disease and other avian paramyxoviruses. *Rev. Sci. Technol. O.I.E.*, 19: 443-462.
- Al-Falluji, M., M.S. Saber and V.R. Kaschula, 1978. Epidemiology and Diagnosis of Newcastle Disease virus in vaccinated flocks in Iraq. *J. Egypt. Vet. Med. Ass.*, 38: 65-71.
- Allan, N.H., J.E. Lancaster and B. Toth, 1978. Newcastle disease vaccines-their production and use. FAO. Animal Production Series No 10. FAO Rome.
- Beard, C.W., 1980. Serological Procedures. Isolation and Identification of Avian Pathogens. In: Hitcher, S.B., C.U. Domermutt, H.G. Purchase and J.E. Williams (Eds.). *Am. Assoc. Avian Path. Kenneth-Square. P.A.*, pp: 129-135.
- Beard, C.W. and R.P. Hanson, 1984. Newcastle Disease. Diseases of Poultry. In: Hofstad, M.S. (Ed.). Iowa: Iowa State University Press, pp: 452-470.
- Holmes, H.C., 1979. Resistance of the respiratory tract of the chicken to Newcastle disease virus infection following vaccination: The effect of passively acquired antibody and its development. *J. Comp. Pathol.*, 89: 11-19.
- Kouwenhoven, B., 1993. Newcastle Disease. Virus Infections of Vertebrates 4: Virus infection of Birds. In: Mcferran, J.B. and M.S. McNutts (Eds.). Elsevier, Amsterdam, pp: 341-361.
- Lancaster, J.E., 1993. Newcastle disease: A review. Monograph No. 3 Can. Dep. Agric Ottawa, pp: 1926-1964.

- Lombard, M.O., 1975. Biostatistics for the Health Professions. Appleton-Centery-Crofts, New York, pp: 115-135.
- Mayo, M.A., 2000. Virus taxonomy-Houston 2002. Arch. Virol., 147: 1071-1076.
- Onunkwo, O. and M.A. Momoh, 1980. Isolation of Newcastle Disease virus from parrot (*Psittacus erythracus*) in Nigeria. Vet. Rec., 107: 179.
- Rathore, B.S., K.C. Verma, S.D. Singh and S.S. Khera, 1987. Epidemiological studies on Ranikhet disease vaccinal failures in chickens. Ind. J. Comp. Microbiol. Immunol. Infect. Dis., 8: 175-178.
- Report of Poultry Disease Subcommittee on Animal Health, 1971. Methods for the Examination of Poultry Biologics. Division of Biological and Agriculture, National Academy of Science Washington D.C.
- Timms, L. and D.J. Alexander, 1977. Cell-mediated immune responses of chickens to Newcastle disease vaccines. Avian Pathol., 6: 51-59.
- White, D.O. and F.J. Fenner, 1994. Medical Virology. 4th Edn. Academic Press. San Diego, pp: 456-474.
- Zander, D.V., A.J. Bermudez and E.T. Mallinson, 1997. Principles of Disease Prevention: Diagnosis and control. Diseases of Poultry. In: Calnek, B.W. (Ed.), pp: 3-45.