



Thermostabilization of live Newcastle disease virus (La Sota) vaccine using deuterium oxide (D₂O)

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Newcastle disease virus (NDV) infects over 200 species of birds, and this disease still ranks as one of the most serious virus diseases of poultry. The virus is having zoonotic importance as causes conjunctivitis in humans (Nelson *et al.* 1952). The thermostability of live attenuated vaccines is a very important task in vaccinations in subtropical countries where transportation under cold-chain conditions is difficult. Therefore, formulation of new vaccines to further improve stability of vaccine is of considerable interest. Recently heavy water/deuterium oxide is being used in different fields such as cosmetics, pharmaceuticals, cancer therapy, preservation of biological materials, thermostabilization of vaccines, etc. (Chauhan 2002). The potential of D₂O was demonstrated in stabilization of the oral polio and yellow fever 17D vaccines (Crainic *et al.* 1996, Adebayo 1998); the present study was conducted to explore thermostabilizing ability of D₂O by incorporating it in diluent used for reconstitution of freeze-dried NDV vaccine.

Liquid deuterium oxide (99.90%) was purchased from the Bhabha Atomic Research Center, Mumbai. Live Ranikhet disease vaccine, IP Vet. Lentogenic, 100 doses, Batch No : LA08 – 031, EID 50/dose > 10⁶ was used in the present study. Chemicals used for preparation of diluents were obtained commercially.

Reconstitution of vaccine with diluents: Diluents were prepared by dissolving 0.007 g (0.068% w/v) of KH₂PO₄ and 0.002 g (0.015% w/v) of NaOH in 10 ml of distilled water (H₂O) and 10 ml of 80% heavy water (8.01 ml D₂O + 1.09 ml H₂O) separately. The prepared diluents were got sterilized by filtration. Two vials of Lasota vaccines were reconstituted separately in 1 ml of H₂O and 80% D₂O diluent.

Haemagglutination test: Fowl blood was collected aseptically in equal volume of Alsever's solution using sterile syringe. RBCs were washed thrice using normal saline

solution (NSS) prepared in H₂O. After third washing, 0.5 ml pellet of RBCs was added in 49.5 ml NSS prepared in H₂O to yield 1% fowl RBCs. Freshly prepared RBCs were used during study. Haemagglutination (HA) test was carried out as per Anonymous (1971) and Stephen (1975) to determine HA (virus) titre. NSS in 0.025 ml quantity was dispensed into each of 10 wells of one row in V/U -bottom microtitre plate. The virus suspension in 0.025 ml quantity was added in the first well. Serial 2-fold dilutions of vaccine virus were carried out up to 10th well of plate. Virus control and fowl RBC control were added in 11th and 12th wells. Fowl RBCs (1%) in 0.025 ml quantity were added in each well including both controls. The plates were incubated for 40 min at room temperature, till RBCs in fowl RBC control got settled to form button. HA was observed to see the presence or absence of mat formation by tilting the plate. Highest dilution giving complete HA (mat formation) was taken as HA (virus) titer.

In the commercial poultry sector, several conventional vaccines are available for the control of Newcastle disease. Although this has drastically reduced the incidence of Newcastle disease in commercial poultry farms, it is not controlled completely. The major problem in NDV control has been rapid loss in potency of vaccines when exposed to ambient temperature and lack of provision of cold chain facilities in rural areas. Therefore, to improve further vaccines' stability for control of NDV outbreaks, development of thermostable vaccine which at least will not get inactivated after reconstitution during mass vaccination is today's need. In the present study, we used D₂O as thermostabilizer in the diluent for reconstitution of commercially available freeze-dried NDV vaccine for improving thermostability of vaccine.

Thermostabilization during summer (25 to 35 °C ± 2 °C): Two vials of freeze-dried Ranikhet disease vaccine were reconstituted separately with diluents prepared in water and 80% deuterium oxide respectively. HA test of vaccine virus reconstituted with H₂O and D₂O diluents was performed at different time interval up to 50 h and HA titers were determined. The results of HA test are depicted in Fig. 1. At

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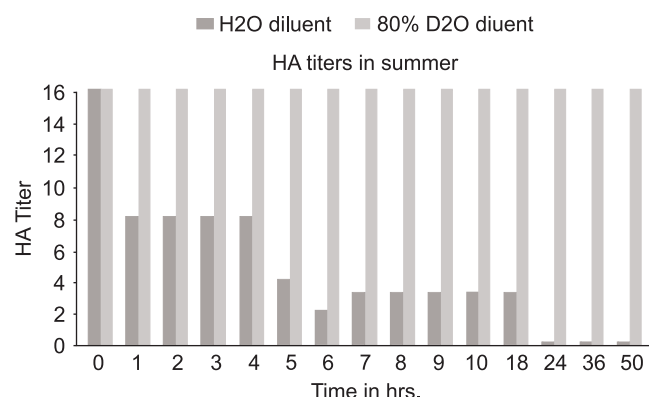


Fig. 1. Comparative HA titers of RD vaccine virus in H₂O and D₂O diluents in summer.

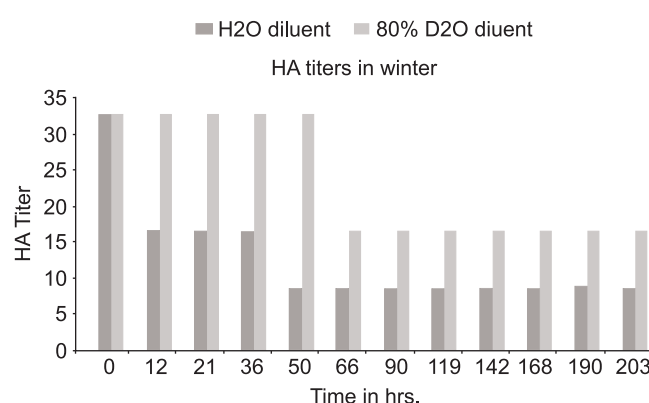


Fig. 2. Comparative HA titers of RD vaccine virus in H₂O and D₂O diluents in winter.

0 h, HA titer of both H₂O and D₂O vaccine was observed same i.e. 16. After 1 h HA titer of vaccine reconstituted with H₂O diluent was decreased to 8 and maintained same titer up to 4 h and thereafter HA titer went on decreasing as incubation/of storage time increased, finally HA titre fell down to 0 at 24 h; whereas, HA titer of vaccine reconstituted with D₂O diluent maintained constant up to 50 h.

Thermostabilization during winter (15 to 25 °C ± 2 °C): Ranikhet disease vaccine reconstituted in diluent prepared in water and in 80% deuterium oxide. HA test was carried out at different time intervals to know virus titer and the results are given in Fig. 2. Immediately after reconstitution of vaccine at 0 h, both H₂O and D₂O vaccines showed similar HA titer i.e. 32. At 12 h, HA titer of H₂O vaccine decreased to 16 and the same titer was maintained up to 36 h. Thereafter, HA titre decreased to 8 in 50 h and the same titre maintained up to 203 h. Whereas, the initial virus titer of D₂O vaccine was maintained 32 up to 50 h and thereafter decreased to 16 in 66 h and maintained the same up to 203 h.

Thermostability is a critical biophysical property of viruses that has direct applicability to the delivery of live viral vaccines. A number of stabilizers are traditionally used for live viral vaccine including magnesium chloride, sorbitol,

hydrolyzed gelatine and combination of sucrose, phosphate and glutamate (Bovarnick *et al.* 1950, Wallis and Melnick 1962). Lyophilized formulations of measles and influenza can usually be stored long term at higher temperatures, although they need to be used as soon as possible after reconstitution (Arya and Agarwal 2004, Huang *et al.* 2004). As Newcastle disease virus is an enveloped virus, which are considered more labile and susceptible to heat inactivation, therefore these are more difficult to stabilize than non-enveloped viruses. There is much more possibility of getting vaccine virus inactivated after reconstitution during mass vaccination. Crainic *et al.* (1996) and Wu *et al.* (1995) proposed deuterium oxide as a stabilizer for poliovirus vaccine.

In the present study, we used 80% deuterium oxide as thermostabilizer in the diluent of Newcastle disease vaccine and the thermostabilization studies were carried out during summer and winter. In summer, due to high ambient temperatures, original titer of virus in vaccine (16) was found lesser than that of in winter (32). In summer, HA titer of vaccine reconstituted in H₂O diluent decreased to 8 within an hour and vaccine virus got completely inactivated in 24 h. Whereas, HA titer (16) of vaccine reconstituted in D₂O diluent was maintained up to 50 h, this indicated that vaccine reconstituted with diluent prepared in D₂O had more thermostability. During winter, the fall of original virus titer from 32 to 16 in 1 h and to 8 in 203 h in H₂O diluent, whereas original virus titer was maintained up to 50 h and later fell to 16 in 203 h in vaccines reconstituted with D₂O diluent. Vaccination failure occurs in summer because of high temperature and delay in vaccination after reconstitution of vaccine. Similarly, when 17D yellow fever vaccine stability was analyzed by using 5 different reconstitution solutions, it was found that 90% D₂O yielded the best stabilizing effect under thermal temperature of 37°C up to 24 h (Adebayo 1998).

SUMMARY

The thermostability of NDV vaccine was studied by reconstituting commercially available vaccine with diluents prepared in H₂O and 80% D₂O at ambient temperature during summer and winter. The loss in vaccine potency due to inactivation of virus was studied *in vitro* using haemagglutination test at various time intervals up to 50 and 203 h in summer and winter, respectively. Diluent prepared in 80% D₂O when used for reconstitution of freeze-dried Ranikhet disease vaccine, it maintained stability of vaccine virus for more than 50 h during both summer and winter. The findings revealed that, freeze-dried NDV vaccine reconstituted in 80% D₂O diluent can be used up to 50 h or more at 35°C ± 2°C temperatures without any deterioration in potency.

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