

Full Length Research Paper

Formulation of novel trehalose flakes for storage and delivery of newcastle disease (strain I-2) vaccine to chickens

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Newcastle disease (ND) strain I-2 vaccine flakes were formulated by using an amorphous trehalose sugar as a stabilizer and evaluated for long term storage and delivery to chickens. The results showed that the I-2 virus stored in trehalose flakes maintained its infectivity titre at $10^{8.6}$ EID₅₀/0.1 mL for 4 weeks at ambient room temperature. The vaccine flakes were stable after storage for 16 weeks while maintaining the infectivity titre of $10^{7.5}$ EID₅₀/0.1 mL. The results further indicated that the infectivity titres for ND virus recovered from flakes and liquid vaccine were similar. The findings from the present study showed the vaccine flakes are easily transported and readily released into a fluid phase when mixed with water; they can be administered orally without mixing with feeds and hence suitable for individual or mass vaccination of semi-feral scavenging village chickens. Chickens vaccinated orally with the flakes developed antiviral antibodies and resisted challenge with virulent strain of ND virus. The formulation of trehalose vaccine flakes could be a useful way to store and deliver ND vaccines to village chicken flocks in rural areas, particularly in developing countries if it is optimised.

Key words: Newcastle disease, strain I-2, trehalose flakes, antibody response.

INTRODUCTION

Newcastle disease (ND) is a serious contagious disease that causes high morbidity and mortality in poultry, especially in village chickens in developing countries (Spradbrow and Copland, 1996). ND may cause up to 100% mortality thus destroying entire chicken flocks. The disease is a major hindrance to the development of village chicken industry particularly in developing countries (Spradbrow, 1992). Control of ND is therefore of paramount importance.

Vaccination has a major impact on preventing and controlling the outbreaks and spread of ND (Meulemans, 1988). However, most ND live vaccines currently in use are heat labile and require expensive cold chain, which are important limiting factors for controlling ND and consequently the productivity of village chickens (Spradbrow, 1992). The above mentioned problems can be overcome by having low cost vaccine delivery system which could be operated by chicken keepers themselves; in this case heat stable ND vaccines are highly needed (Copland, 1987; Spradbrow, 1993/4).

Strain I-2 of Newcastle disease virus (NDV) is a thermostable vaccine which is being used to control ND in village chickens in developing countries (Tu et al., 1998; Bensink and Spradbrow, 1999; Wambura et al., 2000), however, it can be thermostabilized by carbohydrates such as trehalose in order to enhance its thermostability and thus become less dependent on or independent of cold chain systems which will facilitate easy distribution of the vaccine.

Trehalose is one of the most stable and chemically non-reactive disaccharides. It has a high thermostability and a wide pH-stability range. Trehalose is not hygroscopic; however, exhibits flash solubility on hydration, a property particularly useful for dried vaccines (Colaco et al., 1995; Higashiyama, 2002). Because of its low viscosity, safety and resistant to dehydration, trehalose is ideally suited for use in eye drop formulations (Matsuo et al., 2002).

Most of dry ND vaccine formulations are in powder or pellet form (Rehmani et al., 1995; Corbanie et al., 2007); no

vaccine flakes for ND vaccine have been developed so far. A formulation of vaccine flakes may provide a medium which is inexpensive, easily prepared and is particularly useful for storage, transport and delivery of ND vaccine to village chicken flocks in remote areas where cold chain systems are inefficient or unavailable.

The objective of the present study was to develop and evaluate formulation of trehalose flakes of NDV strain I-2 vaccine for long term storage and delivery to village chickens.

MATERIALS AND METHODS

Source of the virus for vaccine production

I-2 vaccine is produced locally at Sokoine University of Agriculture (SUA). The virus was obtained from a freeze-dried master seed of strain I-2 of NDV. This was propagated in embryonated eggs to produce the vaccine. The strain I-2 has no commercial ownership and is produced and supplied by the John Francis Virus Laboratory at the University of Queensland, Australia (Spradbrow and Copland, 1996).

Source of eggs and propagation of strain I-2

All eggs used in this study were obtained from a hatchery at Department of Veterinary Microbiology, SUA. Eggs were bought when they were 9-day-old after incubation and were used the following day. The propagation of green coloured liquid I-2 vaccine was done as described by Wambura (2008a) and contained 1% gelatin as a stabiliser.

Preparation of trehalose vaccine flakes

The 2.5 g of trehalose (Hayashibara Biochemical Laboratories, Inc. Japan) were weighed in a plastic trough and 0.05 g of green dye (Maimun Supplies Ltd, Mombasa, Kenya) was added. After thorough mixing, 2.5 mL of infective allantoic fluid with I-2 strain of NDV ($10^{9.7}$ EID₅₀/mL) was added. These ingredients were mixed thoroughly until a uniform trehalose saturated solution was formed. The solution was left to dry in a plastic trough at room temperature (25-34°C) for 24 h without steam condition to produce flat pieces called flakes.

The vaccine flakes prepared above are enough for 5 chicken-dosages. Therefore to vaccinate 15 chickens 7.5 g of vaccine flakes were prepared.

Storage of vaccine flakes

The dried flakes were removed from the plastic trough and stored dried in sealed small plastic bags at room temperature (25-34°C).

Infectivity assay for vaccine virus in trehalose flakes

Seven days after storage the flakes were removed from a plastic bag and they were dissolved in 3 mL of distilled water. Thereafter 100 µL of the solution was added in 900 µL of PBS with antibiotics; it was mixed thoroughly and titrated serially at 1:10. 100 µL of each

dilution was inoculated into 5 embryonated eggs. The eggs were incubated at 37°C for 96 h. Thereafter the eggs were examined for the presence of haemagglutinin as described by Allan and Gough (1974). The infectivity titre of I-2 virus was calculated as described previously by Reed and Muench (1938) and expressed as log₁₀ EID₅₀/mL.

The same procedure was repeated at a weekly interval up to 4 weeks; thereafter it was done at a monthly interval.

Source of chickens

Rhode Island Red cross-bred one-day old pullets which were used in this study were purchased from a known ND free commercial hatchery in Dar es Salaam. They were raised in an animal house near to our laboratory. At 4 weeks of age all birds were wing-tagged and randomly assigned to two groups of 15 chickens each. Each group was separately kept in pens with concrete floors and straw litter. All chickens were clinically and serologically negative for ND.

Management of chickens

Chickens were kept on deep litter. They were fed chicken mash according to manufacturer's instructions and water was available *ad libitum*.

Vaccination of chicken with trehalose I-2 flakes

At 4 weeks of age, the chickens in group 1 were vaccinated with the green-coloured vaccine flakes whereas chickens in group 2 were not vaccinated and served as negative controls. The vaccine was administered through oral route where chickens were picking vaccine flakes from the mat where they received nominal viral dose.

Blood samples were collected from both birds in each group before starting the test and at a weekly interval for 4 weeks after vaccination in order to determine the extent of seroconversion to the vaccine.

Haemagglutination-inhibition (HI) antibody assay

Test sera were inactivated at 56°C for 10 min to destroy complement and HI inhibitors. Twenty-five milliliter of double serial diluted test sera were then added to wells containing 25 µL of four hemagglutinating units of ND virus followed by adding 50 µL of 0.5% chicken red blood cells to each well. Plates were then incubated at room temperature for 1 h. The endpoint HI titer was defined as the reciprocal of the highest serum dilution that completely inhibited hemagglutination of the chicken red blood cells (Allan and Gough, 1974).

Challenge trials

Four weeks after vaccination, all chickens in all groups were challenged with NDV virulent strain (10^3 CLD₅₀) by inoculation into the nasal sinus. After challenge, the chickens were examined daily for clinical signs consistent with ND.

Statistical analysis

Analysis of variance (ANOVA) was performed using the General linear model (GLM) procedure (SAS, 1986). The significance effect ($P < 0.05$) of geometric mean titres (GMT) of NDV antibody between treatment groups were also tested.

Table 1. Comparison of infectivity titres between liquid vaccine and dry vaccine flakes stored at room temperature (25-34 °C) for 120 days.

Days after storage	Infectivity titres (EID ₅₀ /0.1mL) ^a	
	Liquid vaccine	Dry vaccine flakes
0	9.7	9.7
7	9.7	9.7
14	8.8	9.5
21	8.7	9.5
28	8.6	8.6
60	7.5	7.5
90	7.5	7.5
120	7.5	7.5

^aEID₅₀, median embryo infectious dose.

RESULTS

Infectivity titres for vaccine flakes

The results of the present study in Table 1 showed that the I-2 virus stored in trehalose flakes maintained its infectivity titre at 10^{8.6} EID₅₀/0.1 mL for 4 weeks at ambient room temperature (25-34 °C) with the loss of 1.1 log₁₀. It is noteworthy that the titres for liquid vaccine were slightly lower than those of the dry vaccine flakes; however the difference was not statistically significant. The vaccine flakes were stable after storage for 16 weeks while maintaining the infectivity titre of 10^{7.5} EID₅₀/0.1mL (Table 1).

Safety

Moreover, there were no deaths of embryos observed in embryonated eggs inoculated with I-2 virus stored in trehalose flakes after incubation for 96 h and no adverse effects observed in vaccinated chickens.

Antibody response

All chickens tested prior to administration of vaccine were negative to HI NDV antibody. Results further indicated that 80% (12/15) of chickens vaccinated with I-2 vaccine flakes 14 days after vaccination had antibody titre of ≥3 log₂ with 2.9 GMT. However 100% (15/15) of chickens had a titre of ≥3 log₂ with GMT of 4.3 and 4.4 at 21 and 28 days after vaccination, respectively. All control (unvaccinated) chickens were negative to NDV antibody throughout the study period (Table 2).

Challenge trials

Results from the challenge trial showed that 100% of all

vaccinated chickens (n=15) survived after challenge with virulent NDV whereas all non-vaccinated chickens (n=15) died within 3 days showing clinical and post-mortem signs and virus isolation were consistent with ND.

DISCUSSION

The findings of present study showed that trehalose I-2 vaccine flakes could be stored for 16 weeks at room temperature and remained stable. These findings were comparable to the liquid vaccine which was stabilised by gelatin. The vaccine flakes were developed by using an amorphous trehalose sugar as a stabilizer (Bieganski et al., 1998; Lloyd 2000). The vaccine flakes are green coloured for easy visual identification and delivery to chickens particularly by eye drop (Wambura, 2008a).

Although the infectivity titres of dry (flakes) and liquid vaccines when stored at room temperature were not significantly different, the dry vaccine has several advantages over the latter. The vaccine flakes are easily transported and readily released into a fluid phase when mixed with water; they can be administered orally without mixing with feeds and hence suitable for individual or mass vaccination of semiferal scavenging village chickens.

Moreover, when the vaccine flakes were administered to chickens they proved to be safe and efficacious. It produced the protective antibody response in chickens against challenge with virulent NDV. The present results collaborate with the findings from the previous studies which showed that vaccination of chickens with NDV strain I-2 results in protection against subsequent ND challenge although the geometric mean titres were lower than those observed in green coloured liquid and oiled rice vaccines (Wambura, 2008a,b). The differences could possibly be attributed to increased efficiency of eye drop vaccination through the use green dye and the effect oil as an adjuvant, respectively.

The development of thermostabilized dry vaccines and delivery technologies may help to overcome the cold storage and current distribution systems and delivery methods. Dry vaccine formulations are potentially superior to liquid vaccines in their sterility and stability, and thus eliminating the need for the cold chain (LiCalsi et al., 1999; Corbanie et al., 2007). The above facts were not realized in the present study therefore the technique used for formulation of trehalose vaccine flakes need to be optimized.

The trehalose vaccine flakes could be a useful formulation in order to produce a new and affordable ways to store and deliver ND vaccines to village chicken flocks in rural areas particularly in developing countries. This technology can greatly expand the availability and the coverage of vaccination of chickens against ND in rural areas which lack basic infrastructures if it is optimized and adopted.

Table 2. Antibody response produced by chickens vaccinated with I-2 dry vaccine flakes.

Group	Vaccine dose (EID ₅₀) ^a	Antibody response at different days after vaccination				
		0 ^b	7	14	21	28
Dry vaccine flakes	10 ^{8.5}	<1 ^c	1.8±0.3	2.9±0.1	4.3±0.7	4.4±0.5
Unvaccinated	Nil	<1	<1	<1	<1	<1

^aNominal dose per chicken; ^bGeometric mean titre (GMT); ^cNumber of chickens (n=15).

In conclusion, this study demonstrated that it is feasible to produce vaccine flakes by using trehalose and avirulent thermostable strain I-2 virus of NDV. These vaccine flakes can be used for single and mass vaccination of village poultry. To the best of my knowledge this the first report of using vaccine flakes for the delivery of NDV to chickens.

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