DETERMINATION AND EVALUATION OF POTENCY OF THERMOSTABLE I-2 VACCINE AFTER LONG-TERM STORAGE BY USING CONVENTIONAL AND RAPID POTENCY TESTS

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SUMMARY

Study was conducted to determine and evaluate the potency of I-2 vaccine after a long-term (11 months) storage prior to the running of ND vaccination campaign in village chickens in Central and Southern Tanzania. Both rapid and conventional tests were employed to determine potency of I-2 vaccine. By using a rapid test the results were obtained within 12 hours and indicated that the titre of the vaccine was below the recommended titre of ≥ 10^6 EID_{50}/0.1 mL thus unsatisfactory for use in the field. When a conventional 4-day test was used vaccines from Dodoma, Mpwapwa and Mtwara had titres of 5.5, 4.8 and 5.6 EID_{50}/0.1 mL, respectively proving further that the rapid test was accurate. Although I-2 vaccine is thermostable it should be transported and stored in a cool environment (less dependent on cold chain) to extend its shelf life and hence its potency. This is the first report of using a rapid potency test to determine and evaluate the potency of I-2 vaccine in the field. Therefore continuous monitoring and assessment of conditions of storage and potency testing would contribute towards good ND vaccine strategy and vaccination campaigns.

INTRODUCTION

Village chickens play a very important role in livelihood of people living in rural and peri urban areas in developing countries. However, Newcastle disease (ND), which is highly contagious and fatal, is the main killer of village chickens (Spradbrow 1993/4). This hinders the development of local chicken industry in developing countries. In order to overcome this problem effective control of ND is necessary. Vaccination is the only feasible method used to control this disease (Meulemans, 1988; Cargill, 1999). Conventional vaccines are thermolabile and are packed in larger doses (1,000) which are not affordable to most small-scale chicken growers in rural areas. The

solution has been to use thermostable vaccines, which are packed in small doses, locally produced, cheap and effective in controlling ND (Spradbrow and Copland, 1996). I-2 is a thermostable vaccine being produced and used in some developing countries to control ND (Tu *et al.*, 1998; Wambura *et al.*, 2000).

Potency, safety and efficacy of ND I-2 vaccines are determined before release by the vaccine quality control unit at the Animal Diseases Research Institute (ADRI) Dar Es Salaam, Tanzania. However, potency has to be monitored and evaluated regularly in order to ensure that the vaccine titre is of acceptable quality for successful field use in vaccination campaigns.

Therefore before the beginning of the June 2004 ND vaccination campaigns in Central and Southern zones, the potency of the I-2 vaccine had to be determined prior to the release of the vaccine. The vaccines had been stored for 11 months with one-month grace period before the expiry date.

Like other biological products, vaccines have to be of sufficient stability under the conditions of transport and storage to maintain their potency at the point of use (Tydeman and Kirkwood, 1984). I-2 vaccine is thermostable and is stabilized by gelatin in order to suit village conditions (Bensink and Spradbrow, 1999). However the vaccine should be transported and stored in a cool environment (less dependent on cold chain) to extend its shelf life and hence its potency.

The objective of the present study was to compare the rapid and the conventional potency tests for determination of the potency of I-2 vaccine after long-term storage.

MATERIALS AND METHODS

Location

Dodoma Rural District and Mpwapwa Veterinary Investigation Centre (VIC) are located in the Central zone of Tanzania whereas Mtwara VIC is in the Southern zone.

Source of vaccine

I-2 vaccine is produced locally at ADRI Temeke. The virus was obtained from a freeze-dried master seed of strain I-2 of *Newcastle disease virus*. 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.

Five (5) vials (400 doses each) of the I-2 vaccines stored frozen (around –20 °C) in Dodoma, Mpwapwa and Mtwara were individually sampled randomly. Thereafter they were immediately transported in ice packed cool boxes to ADRI, Dar Es Salaam for testing. In order to ensure that the potency of the vaccine is not lost during shipment back to ADRI, they were transported by Air from Mtwara and reached the destination within 2 hours and those from Dodoma and Mpwapwa were transported by road and reached the destination within 6 hours.

Source of eggs

All eggs used in this study were obtained from a reputable commercial hatchery and poultry-breeding farm in Dar Es Salaam. Eggs were bought when were 9-day-old and were used the following day.

Rapid potency test

The principle behind this test is time-dose dependent. It is based on the observation that the interval between inoculation and the first detection of haemagglutinin (HA) depends on the titre of the vaccine inoculated.

The procedure described by Wambura (2003) was followed with slight modifications. Briefly, 10-day-old embryonated chicken eggs were inoculated with single chicken dose (20 µL) of each of the pooled vaccines from Dodoma, Mpwapwa and Mtwara vials, and incubated at 37 °C for 12 hours. At each 6 hourly interval, 5 eggs from each vaccine lot were tested for the presence of HA as described by Alexander (1988). The eggs from each vaccine lot were removed and placed at 2°C for at least 2 hours before testing for HA.

As a positive control I-2 vaccine immediately produced at ADRI were also titrated using the same procedures described above.

Conventional test

Ten-fold serial dilutions of the pooled I-2 vaccine from Dodoma, Mpwapwa and Mtwara were prepared in PBS with antibiotics and inoculated in 0.1 ml volume into allantoic sacs of 10-day old embryos. Five embryos were used for each dilution. The embryos were incubated and candled daily for 4 days. The viral haemagglutinating activity was measured after 4 days by HA test on allantoic fluid performed in microtitre plates as described by Alexander (1988). The infectivity titre of the virus was expressed as median embryo infectious dose (EID_{50}) and calculated as previously described by Reed and Muench (1938).

As a positive control I-2 vaccine immediately produced at ADRI were used for titration as described above.

RESULTS

Results of the present study are summarized in Table 1. The rapid potency test results showed that after 6 and 12 hours incubation of inoculated embryonated chicken eggs with I-2 vaccine no virus was detected. This indicated that the vaccines from Dodoma, Mpwapwa and Mtwara had a titre lower than 10^6 EID_{50}/0.1mL.

Conventional test results showed that the same vaccines from Dodoma, Mpwapwa and Mtwara had titres of $10^{5.5}$, $10^{4.8}$ and $10^{5.6}$ EID_{50}/0.1 mL, respectively.

As a positive control, where the vaccine was tested immediately after production using a rapid test, the virus was detected in embryonated chicken eggs 6 hours after inoculation indicating that the vaccine had a titre of above 10^9 EID_{50}/0.1 mL. The same vaccine showed a titre of $10^{9.5}$ EID_{50}/0.1 mL by using a conventional 4-day test. No virus was detected in negative control eggs.

DISCUSSION

Proper quality assurance of ND vaccines is important to avoid economic losses ensuing due to the application of unsafe or ineffective vaccines and to ensure the health and welfare of recipients of the vaccine (Thorntorn, 1988) Rapid potency test has been used to monitor I-2 vaccine for the first time after it has been developed and showed promising results by Wambura (2003). Within 12 hours it was possible to determine the titre of vaccines from Dodoma, Mpwapwa and Mtwara districts and confirm that the vaccine is unsuitable for use in vaccination of village chickens against ND. This is because the vaccine titre was lower than the recommended titre of 10^6 EID_{50}/ml (Bensink and Spradbrow, 1999). The results were later verified and confirmed by a gold standard conventional 4-day test, proving further that the rapid test was accurate

Table 1. Results after determination of potency of I-2 vaccine by using rapid and conventional tests

No	Source of vaccine	Eggs HA positive* 1[b]	6[c]	12[d]	Conv. Test (EID_{50}/0.1 mL)[e]	HA titre (log_2)
1	Dodoma Rural district	0/5	0/5	0/5	5.5	nd
2	Mpwapwa VIC	0/5	0/5	0/5	4.8	nd
3	Mtwara VIC	0/5	0/5	0/5	5.6	nd
4	ADRI Temeke	0/5	3/5	5/5	9.5	8

* Number[a] of inoculated eggs showing HA at hours after inoculation
a = Number infected/Total number inoculated
b = No virus should be detected (to check residual virus in eggs)
c = Virus detected at a titre of ≥ 10^9 EID_{50}
d = Virus detected at a titre of ≥ 10^6 EID_{50}
e = Median embryo infectious dose (EID_{50})

The results from positive control showed that the test is reliable and may be used to determine potency of the vaccine immediately after production this will help to reduce the time and cost of using conventional test (Wambura, 2003).

Vaccine potency is a useful tool to predict efficacy in the target populations. In ND vaccination it has been shown that there is a positive correlation between potency and efficacy. If the vaccine with a titre of ≥ 10^6 EID_{50} is used to vaccinate naïve chickens is likely to provoke antibody response of HI titre of ≥ log_2 2^3 those chickens. These levels of HI titres have shown to be protective to chicken against virulent ND (Bensink and Spradbrow, 1999).

A non-bird test (in ovo) test is better because is rapid, cheap and easy to undertake than in bird (in vivo) potency test (dose-response). Quality control units in many developing countries may lack functional infrastructure to assess vaccine efficacy (protection trials or dose response) with tools other than final product testing (e.g. for licensing and inspection).

It is well known that once vaccine potency has been lost, returning it

to proper storage conditions will not restore potency. Therefore careful attention to vaccine storage and handling is a critical component of a successful vaccination campaign. Continuous monitoring and assessment of conditions of storage and potency testing would contribute towards good ND vaccine strategy and vaccination campaigns.

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