Thermo stability study of Temevac® I-2 Newcastle vaccine

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SUMMARY

Newcastle is a highly contagious disease of domestic poultry and wild birds and is widely regarded as the most important avian disease. Effective Newcastle vaccines are crucial for control of the disease. The objective of this study was to establish the stability of TEMEVAC® (I-2) vaccine at three different temperatures for 204 days. The study was conducted at Tanzania Vaccine Institute laboratories for the period of April-December 2017. Two vaccine batches (Batch 0068 and 0069) were subjected at 37°C for 10 days, 22-25°C for 60 days and 2-8°C for 204 days. The vaccines were 10-fold serial diluted and then inoculated into 9-days old embryonated chicken eggs and incubated for 96 hours. A virus infectivity assay and Standard mathematical technique were applied to confirm the presence and measure the amount of live Newcastle virus in the suspension. The study revealed that TEMEVAC® can maintain potency up to more than10 days, 45 days and 197 days at 37°C, 22-45°C and 2-8°C, respectively. This study underscores the importance of using TEMEVAC® vaccine which can tolerate relatively wider range of temperatures for control of the disease in rural areas.

Keywords: local chickens, Thermotolerant vaccine, Newcastle disease

Newcastle is a highly contagious disease of domestic poultry and wild birds and is widely regarded as the most important avian disease (Young *et al.,* 2012). The best and cost effective means of controlling the disease is through vaccination (Janine *et al.,* 2013; Young *et al.,* 2012; Chakraborty *et al.,* 2014). Available heat labile vaccines are suitable in intensive system (Commercial farming) with limited usefulness in rural areas where most local poultry are kept in extensive system (Chakraborty *et al.,* 2014). The reasons that contribute to the limited use of labile heat include large dose presentation, affordability, limited stable cold chain facilities, and ignorance of the farmers (Young *et al.,* 2012; Chakraborty *et al.,* 2014). Live thermotolerant avirulent I-2 vaccine against Newcastle disease was developed to help famers with small flock sizes and extensive system with poorly established cold chain infrastructures (Young *et al.,* 2012).

Thermostability tests provide evidence of how the quality of a vaccine varies with time and different environmental conditions. The test is important for generating data that allow the establishment of the storage condition and shelf life of the vaccine (WHO 2006). The test data of I-2 vaccine produced at other laboratories are available and can serve as a guide and reference for comparison. It has been suggested that each manufacturer should perform stability test on the vaccine produced in their own laboratories for compliance with Good Manufacturing Practices (GMP) (Young *et al.,* 2012). Temperature is one of the important factors that affect vaccine stability and quality in most developing countries (WHO 2006). Thus the current study aimed to established data for the thermostability of TEMEVAC® vaccines in different environmental conditions.

MATERIAL AND METHODS

Two batches of TEMEVAC® vaccines (batch 0068 and 0069) manufactured by TVI and 9-days old chicken embryonated eggs (Certified MKUZA hatchery) were used for the current study. A total of 158 vaccine vials (droppers) from the two batches were tested by placing in the stability chamber (MEMMERT ICH 110) at temperatures 2-8˚C, 22-25˚C and 37˚C for 204, 60 and 10 days, respectively. The vaccines at 2-8˚C were collected and tested at day 60, 90, 120, 135,150, 169,180, 194,197 and 204. Vaccine samples at 22-25˚C were collected and tested at day 7, 14, 21, 30, 45 and 60 and those at 37˚C were tested at day 1, 2, 3, 4, 5, 6, 7, 8, 9, 10. Each test vaccine were10 fold serial diluted and then inoculated into 9 days old embryonated chicken eggs under sterile condition in the biosafety cabinet level two. The inoculated eggs were the incubated at 37°C for 96 hours for viral replication. Haemaglutination test was conducted to measure the ability of a virus suspension to agglutinate red blood cells according to Thayer and Beard 1998. A virus Infectivity assay and Standard mathematical formula were applied for confirming the presence and measurement of live viruses and determination of Embryo Infectivity Dose fifty (EID_{50}) as described previously (Reed and Muench 1938).

Data analysis

The obtained data were fed in the Microsoft version 2010 Spread-sheet and analyzed by Epi Info™ Version 7 (Centre for Disease Control, Atlanta, USA). The vaccine titres were compared using Chi-square test at a critical probability of $P < 0.05$.

RESULTS AND DISCUSSION

At 37˚C, the titre of the vaccine dropped from $10^{9.5}$ EID_{50}/DOSE to $10^{7.8}$ EID_{50}/DOSE and $10^{7.9}$ EID_{50}/DOSE for batch 0068 and 0069 respectively by day 10, this is above the minimum recommended field titre for I-2 vaccine ($10^{6.0}$ EID_{50}/DOSE) which correlate to other studies (Charkraborty *et al.*, 2014) where the vaccine maintained the titre above the minimum recommended filed titre up to 29 days at this temperature. These findings indicate that I-2vaccine can maintain its potency up to more than 10 days when kept at this temperature.

At 22-25˚C the titre decreased from $10^{9.5}$ EID_{50}/DOSE to $10^{6.1}$ EID_{50}/DOSE for batch 0069 and decreased from $10^{9.5}$ EID50/DOSE to $10^{6.2}EID_{50}$/DOSE for batch 0068 at day 45 which is above$10^{6.0}$ EID_{50}/DOSE, the minimum recommended field titre. The current vaccine batches maintained recommended field titre longer than vaccines observed elsewhere (Chakraborty *et al.*, 2014). On the other hand the current vaccines had shorter potency period compared with another study (Spradbow *at al.*, 2012). This difference in time between the previous study and the current study may be attributed to the nature of stabilizer (1% gelatin) that was added to the vaccine under the current study. However, by day 60 the vaccine titre for the two batches decreased to the level that is below the recommended dose. Which were $10^{5.5}$ EID_{50}/DOSE and $10^{5.0}$ EID_{50}/DOSE for batch 0068 and 0069, respectively. Therefore, the findings from this study suggested that, TEMEVAC® can retain its potency up to 45 days when kept at room temperature, hence should not be used beyond 45 days at this temperature.

At 2-8˚C the vaccine retained its infectivity titre up to 197 days where the titre dropped from $10^{9.5}$ EID_{50}/DOSE to $10^{6.3}$ EID_{50}/DOSE and $10^{7.3}$ EID_{50}/DOSE for batch 0068 and 0069, respectively. This suggest the vaccine can be viable up to 197days at 2-8˚C. The infectivity titre plunged to $10^{5.2}$ EID_{50}/DOSE and $10^{5.0}$ EID_{50}/DOSE for batch 0069 and batch 0068, respectively. This indicate that the vaccine should not be used beyond 197 days even if is stored at refrigeration.

It is concluded that the current study has found that the potency of TEMEVAC ®Vaccine can be maintained up to 10 days at 37°C and 45 days at 22-25°C and up 197 days at 2-8°C. This study underscores the importance of using TEMEVAC® vaccine which can tolerate a relatively wider range of temperatures for control of the disease in rural areas. The tolerance to environmental temperature at room temperature for 45 days is advantageous to poultry farmers in villages.

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