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SUMMARY

Newcastle disease (ND) is responsible for significant losses in chicken production worldwide. Vaccination against ND using live vaccines demands the use of viable and properly handled vaccines. The viability of the vaccine is, therefore, a critical component in the control of the disease. This study aimed at assessing the viability of ND vaccines available at the veterinary drug shops in selected areas of Tanzania. A total of 167 samples of live ND vaccine strain I-2 (TEMEVAC®) vials of six different batches were randomly collected from 42 vaccine vendors in the Southern Highlands and Eastern zones in Tanzania. The samples were tested for viability through propagation into 9-days embryonated chicken eggs followed by the Haemagglutination test. The Least Square Mean (LSM) titre of infectivity of TEMEVAC® virus was slightly higher (8.52±0.06) in the Southern Highlands zone compared to the Eastern zone (8.51±0.06) although the difference was not statistically significant ($p = 0.12$). There were statistically significant differences ($p<0.05$) in LSM infectivity titre among batches of collected TEMEVAC® vaccine, particularly when a pairwise comparison was done between Batch_1 and Batch_3 to Batch_5. Also, there were statistically significant differences ($p<0.05$) in LSM infectivity titre among batches when Batch_3 was compared to Batch_6, Batch_4 compared to Batch_6, and Batch_5 compared to Batch_6. The present study revealed that the TEMEVAC® vaccine available in veterinary drug vendors in Tanzania was viable and contained an adequate infectivity titre when used as recommended.

Keywords: Chicken, ND virus, viability, Haemagglutination, TEMEVAC®

INTRODUCTION

Poultry diseases are among the major obstacle to poultry productivity in Tanzania (Msoffe *et al.*, 2010). Newcastle disease remains a major hindrance to the poultry industry (Martinez *et al.*, 2018). However, ND can be managed with effective vaccination programs in addition to biosecurity and the culling of infected birds (Wambura *et al.*, 2006; Birhane and Fesseha, 2020). Vaccination of birds is known to provide an excellent means to decrease clinical disease caused by virulent Newcastle disease virus (NDV) (Asl Najjari *et al.*, 2017). Most commercial vaccines available are heat-labile and can easily be destroyed if the cold chain is not maintained or is

insufficient (Asl Najjari *et al.*, 2017, Habibi *et al.*, 2020). The development of live thermo-tolerant NDV strain I-2 was a breakthrough in village chicken vaccination (Alders, 2014). The strain I-2 TEMEVAC® vaccine of ND which is currently produced in Tanzania is thermo-tolerant and is very popular due to its many advantages over other thermo-labile vaccines (Campbell *et al.*, 2019). The advantages include thermo-tolerance, easy administration by various routes such as drinking in water, eye drop, and mixing with feed, and providing good protection against virulent ND viruses (Miller *et al.*, 2009, Wambura, 2009).

Despite current successful practices in poultry production and well-documented flock management programmes to maintain poultry health, many countries still suffer huge economic consequences from disease outbreaks (Martinez *et al.*, 2018). The control of disease outbreaks has not yet been achieved in many of these countries due to the lack of basic conditions to achieve successful immunization (Miller *et al.*, 2009, Martinez *et al.*, 2018). The lentogenic ND vaccine strains of low virulence are commonly used worldwide and can protect against velogenic NDV (vNDV) if the vaccines are viable, administered correctly to healthy birds, and time is allowed for an appropriate immune response to mount before exposure to NDV (Cornax *et al.*, 2012, Dortmans *et al.*, 2012, Kapczynski *et al.*, 2013). A significant drawback for ND control in developing countries is the lack of a ‘‘cold chain’’ which might be due to power supply problems or inability to access cold facilities to keep the vaccines at 4^0C. Even the best live vaccine will not induce an immune response if it is not viable due to improper storage and handling (Wambura *et al.*, 2006, Kapczynski *et al.*, 2013), particularly during the delivery process or other factors. Progress has been made with the use of a thermo-tolerant I-2 strain vaccine of NDV and has been used in some developing countries (Harrison and Alders, 2010, Kapczynski *et al.*, 2013) including Tanzania. However, this vaccine if mishandled in the field can interfere its viability (Dairo and Osizimete, 2016).

In many of these countries, the lack of basic conditions to achieve successful immunization, such as cold chain, effective vaccine application, and vaccine efficacy, makes it difficult to prevent infectious agents from causing disease (Martinez *et al.*, 2018). In Tanzania, like other developing countries there are areas where cold chain facilities to keep the vaccines at 4^0C are inaccessible due to various factors, leading to even thermo-tolerant vaccine viability can be at risk when exposed to a higher ambient temperature for prolonged periods. Thus, the assessment of NDV viability in live ND vaccines is important to predict accurately the expected immune response. To ensure that viable NDV vaccines are distributed to consumers, the present study aimed to assess the viability of the ND vaccine strain I-2 (TEMEVAC®) available in the veterinary drug suppliers in Tanzania.

MATERIALS AND METHODS

Study area and sample collection

The ethical clearance and approval to carry out this study were obtained from the Sokoine University of Agriculture (Reference number SUA/ADM/R.1/8/112). The study was conducted in two regions located in the Eastern zone (Morogoro municipality, Morogoro and Tanga city, Tanga) and two other regions located in the Southern Highlands (Iringa municipality, Iringa and Mbeya city, Mbeya). The selection of the study area was based on climatic differences between the two zones which is an important factor for the viability of vaccines. The Eastern zone area is relatively hot in terms of temperature with a mean daily temperature range between 18^0C and 34^0C (Ishengoma *et al.*, 2009, Magita and Sangeda, 2017) while southern highlands zone, the annual mean temperature ranges between 15^0C and 20^0C (Mboera *et al.*, 2008). The town and city centres and surrounding areas were the targeted areas where vaccine vendors were located. A total of 167 vial samples of ND vaccine strain I-2 (TEMEVAC®) vials from six different batches were randomly collected from 42 vaccine vendors in the southern highlands and eastern zone. The vial samples were collected between December 2018 and

October 2019. After collection samples were packed into a cool box with ice packs and transported to the Sokoine University of Agriculture (SUA) and Tanzania Vaccine Institute (TVI). The samples were stored at 4^0C before being tested for infectivity through propagation into 9 days embryonated chicken eggs and followed by haemagglutination assay (HA).

Determination of the infectivity titre of a TEMEVAC® I-2 NDV suspension

All eggs used in this study were purchased from Mkuza Chick's Limited farm, a reputable commercial hatchery and poultry-breeding farm in Kibaha, Tanzania. The propagation of the I-2 vaccine collected samples were performed as described by Spradbrow *et al* (1995) and Alders (2002). Briefly, ten-fold serial dilutions of I-2 viral suspension were prepared in Phosphate Buffered Saline (PBS) with Penicillin and Streptomycin antibiotics 0.1millilitre (ml) volume inoculated into allantoic sacs of 9-day-old embryos. Five embryos were used for each dilution (from dilution of 10^{-6} to 10^{-10}), starting from most dilute samples. The inoculated eggs were incubated and candled daily for 4 days. After four days, eggs were chilled overnight in a Refrigerator set at 2-8°C to allow the embryo to die. The viral hemagglutinating activity was measured after 4-days by HA on allantoic fluid performed in 96-well microtitre plates as described by Wambura *et al* (2007). The infectivity titre of the virus was expressed as the median embryo infectious dose (EID_{50}) and calculated as described previously by Reed and Muench (1938) and Ramakrishnan and Dhanavelu (2018). The Newcastle disease vaccine Seed Strain NDV I-2 passage 2 Production date: March 1997 produced by UQ-Veterinary Pathology, Australia/PANVAC provided by TVI employed widely for the production of a live I-2 vaccine was used here as a positive control in the determination of the infectivity titre of a suspension of TEMEVAC® I-2 NDV and PBS and blank eggs were used as a negative control. For HA, chicken RBCs used as a negative control. The infectivity titre of the TEMEVAC® I-2 vaccine vial sampled obtained used to compute the least square mean (LSM) titre using the "lsmeans" package in R studio (R Core Team, 2013, R: A language and environment for statistical computing, R Foundation for statistical computing, Vienna, Austria). The determined LSM was used to compare the viability of the ND vaccines between and among variables. The variables were zones, among batches, and duration in days after the manufacture date. The LSM titre between zones and among batches and duration in days after manufacture date were analysed for significance using One-Way ANOVA and tested for significant differences using Tukey honestly significant difference (HSD) at $P \leq 0.05$. Pairwise comparisons of the LSM for the titres were done among variables.

RESULTS

Table 1 presents the LSM for infectivity titre of I-2 NDV obtained between zones by HA after viruses propagation into Embryonated chicken eggs. The LSM virus titre was 8.52 ± 0.06 slightly higher in the southern highlands zone than to the LSM titre of 8.51 ± 0.06 observed in the eastern zone although the differences were statistically not significant ($p = 0.12$). The LSM infectivity titre observed in collected ND I-2 vaccine in different batches indicated significant statistical differences ($p<0.05$) among the batches particularly when Batch_1 was compared to Batch_3 (p=0.00007), Batch_4 (p=0.000004), Batch_5 (p=0.0003). There were no statistically significant differences when the LSM of Batch_2 compared to Batch_3 (p=0.949), Batch_4 (p=0.999), Batch 5 (p=0.988) and Batch_6 (p=0.427) (Table 2). Statistically significant differences were observed when the LSM of Batch_6 was compared to Batch_3 (p=0.005), and Batch_4 (p=0.002) versus Batch_5 (p=0.014). The viral load was high in Batch 1, 2, and Batch 3 but there was a low viral load in Batch 4, and high in viral load again in Batch 5, and a low in viral load in Batch 6 as indicated in Figure 1. LSM for ND I-2 TEMEVAC® samples from the field-tested with a minimum duration of ten days following the manufacturing date was 7.80 ± 0.22 while those tested with a maximum duration of seventy two days following the manufacturing date was 8.89 ± 0.18, the viral

load was higher compared to the accepted minimum titre for viability and potency ($10^{5.5}$ EID_{50}) recommended by OIE (2021) despite the differences in duration following manufacturing dates (Table 3).

Table 1. Comparison between zones presented as mean of log infectivity titre of I-2 NDV strain

Zone	n	[1]LSM ± SE	Lower CL	Upper CL
Eastern zone	117	8.51 ± 0.06[a]	8.39	8.63
Southern highlands zone	50	8.52 ± 0.06[a]	8.40	8.64
p-value		0.12		

LSM ± SE: Least square mean ± standard errors, *n = sample size, CL = Confidence Limits, Superscript similar letters = no statistically significance difference at p≤0.05.*

Table 2. Pairwise comparison of the log mean infectivity titre between batches of I-2 strain of NDV 9 days after inoculation of embryonated chicken eggs.

	p-value					
	Batch_1	Batch_2	Batch_3	Batch_4	Batch_5	Batch_6
Batch_1						
Batch_2	0.083					
Batch_3	0.0000686	0.949				
Batch_4	0.0000044	0.999	0.947			
Batch_5	0.000277	0.988	0.999	0.997		
Batch_6	0.850	0.427	0.00458	0.00189	0.0139	

LSM: Least square mean, One-way analysis of variance (ANOVA) with Tukey honestly significant difference (Tukey HSD)

Table 3: Mean of log infectivity titre of I-2 NDV strain levels of samples at different time points (days)

Duration in days	n	LSM ± SE	Lower CL	Upper CL
Ten	12	7.80 ± 0.22	7.57	8.43
Fourteen	19	8.93 ± 0.18	8.58	9.28
Fifteen	12	8.03 ± 0.20	7.65	8.42
Eighteen	13	7.82 ± 0.20	7.43	8.2
Twenty three	36	8.84 ± 0.17	8.5	9.18
Twenty five	11	8.88 ± 0.19	8.49	9.26
Twenty eight	4	8.05 ± 0.20	7.65	8.45
Thirty five	6	7.96 ± 0.19	7.59	8.33
Forty six	17	8.73 ± 0.16	8.43	9.04
Forty seven	4	8.75 ± 0.15	8.45	9.05
Fifty one	4	8.65 ± 0.16	8.33	8.96
Fifty six	3	8.74 ± 0.16	8.42	9.05
Fifty eight	4	8.83 ± 0.19	8.45	9.21
Fifty nine	6	8.89 ± 0.18	8.54	9.24
Sixty seven	6	8.06 ± 0.20	7.66	8.46
Sixty nine	6	8.30 ± 0.16	7.99	8.61
Seventy two	4	8.89 ± 0.18	8.53	9.24

LSM ± SE : Least square mean ± standard errors, n = sample size, CL = Confidence Limits

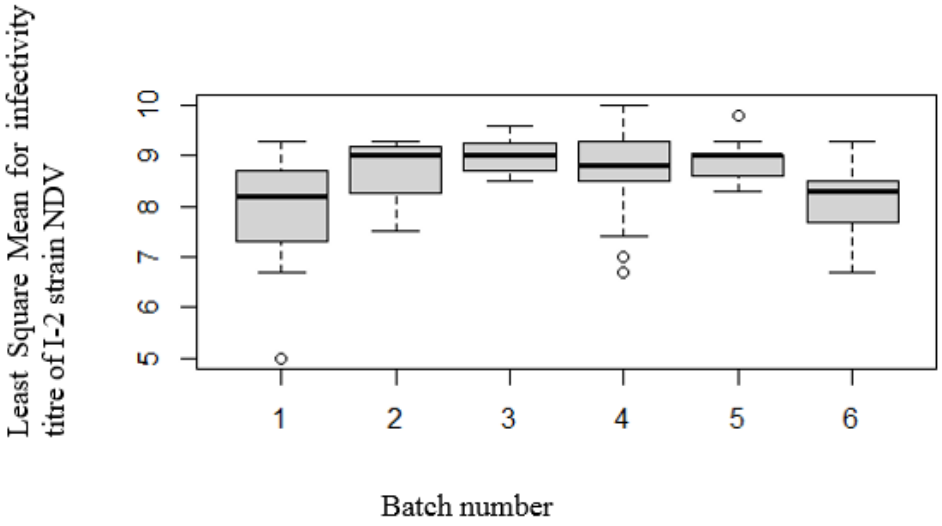

Figure 1: Box plots showing the overall least square mean (log) for infectivity titre *of six different batches of* I-2 strain of NDV after inoculation into 9-days embryonated chicken eggs.

DISCUSSION

In Tanzania like in other developing countries, despite intensive vaccination programs applied, ND is enzootic in FRLC and it has continued for years (Yongolo *et al.*, 2011, Komba *et al.*, 2012, Mwakapuja *et al.*, 2012, Alders, 2014, Asl Najjari *et al.*, 2017). Newcastle disease continues to be a major threat to the poultry industry due to the disease effects and costs related to preventive strategies. The disease is highly contagious, and without adequate control strategy, causes high morbidity and mortality in naïve or poorly vaccinated chickens, as well as drops in egg production in well-vaccinated layers (Aini *et al.*, 1990, Perozo *et al.*, 2008, Miller *et al.*, 2010, Puro and Sen, 2022). Currently, ND vaccination is applied worldwide and is effective in reducing the impact of the disease, particularly for smallholder chicken productivity in the developing world. The backyard chicken ND vaccination is an important tool for poultry health (Alders *et al.*, 2010, Asl Najjari *et al.*, 2017, Bessell *et al.*, 2020). However, easy thermal inactivation of the live virus vaccine in hot climates and distant regions can affect its efficacy (Aini *et al.*, 1990, Shahid Mahmood *et al.*, 2014, Dzogbema *et al.*, 2021) due to the prolonged duration of transportation or storage with no proper storage conditions. Reliability of the cold chain is a challenge and temperature variability outside the optimal temperature range are frequently observed during transport and storage (Matthias *et al.*, 2007, Nelson *et al.*, 2007, Osman *et al.*, 2021). Inappropriate equipment, human errors, and power shortages or interruptions are important causes of cold chain break-up (Setia *et al.*, 2002, Lloyd *et al.*, 2015, Osman *et al.*, 2021). It is estimated that roughly 50% of all lyophilized vaccines are discarded annually, and poor thermo-stability is an important contributing factor to this issue (Schlehuber *et al.*, 2011, Osman *et al.*, 2021). Therefore, outbreaks worldwide have been attributed to a multitude of causes including deficient vaccines and vaccination programs (Chumbe *et al.*, 2016, Dimitrov *et al.*, 2017). The deficient vaccine specified the need for continuous research on vaccine quality to meet the standard to elicit a protective immunological response to chickens when used as recommended. In this study, the ND virus strain I-2 (TEMEVAC®) vials were collected from veterinary drug vendors and these vials were used to assess the viability using haemagglutination (HA) assay after viruses propagation into embryonated chicken eggs. In the viral load titre obtained in different batches, the minimum least square mean was $10^{7.8}$ EID_{50} which is higher compared to the accepted minimum titre for viability and potency ($10^{5.5}$ EID_{50}) recommended by OIE (2021). This suggested that the vaccine meets accepted viability and therefore can provide full protection from overt clinical disease when vaccinated chicken challenged with virulent NDV.

There were slight variations in the viral load titre observed when compared between zones although, the differences were statistically not significant (p=0.12). The indication of the observed higher viability titre in the I-2 NDV vaccine in both eastern and southern highlands zones when compared to the accepted minimum titre for viability and the absence of statistically significant differences between the zones may be because the I-2 ND vaccine is known to be robust and a thermo-tolerant vaccine (Alders, 2002). Even though long-term storage of I-2 NDV can still require a refrigerator, it cannot deteriorate as quickly as the traditional thermo-labile ND vaccines (Alders, 2002) which were observed when exposed in a different ambient temperature of the geographical zone. However, if it is stored in direct sunlight or allowed to reach high temperatures (above 37°C) for more than a few hours it will deteriorate and be unsuitable for use as a vaccine (Alders, 2002). Also, the absence of statistically significant variation of NDV viability between eastern and southern highlands zones may be due to common practices of NDV vaccine handling along the distribution chain in both zones. However, observed statistically significant differences among batches of ND vaccine (Strain I-2) from the market need more investigation to point exactly the reason for the viral load to be higher in Batch 1, Batch 2, Batch 3 and Batch 5 than in Batch 4 and Batch 6.

The present study has revealed that the infectivity ND vaccine (Strain I-2) available in veterinary drug vendors in Tanzania had a mean titre of $10^{8.5}$ EID_{50} which is higher than the accepted minimum titre for viability and potency ($10^{5.5}$ EID_{50}) as recommended by OIE (2021). Despite the variation of the study areas in different zones, there is no statistically significant change of infectivity of Strain I-2 and therefore contained an adequate infectivity titre when used as recommended.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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