ABSTRACT

Temperature alterations due to poor cold keeping condition have consistently posed a challenge to vaccines dose EID50 titre value. The sensitivity of Newcastle disease vaccine to temperature variations due to poor cold storage condition in rural settings, led to the development of the NDVI-2 thermostable vaccine, which is known to exhibit a thermostability of 3 hours at 56 °C. In this study, three Newcastle disease vaccines (NDVI-2) with EID50 titre per dose values of log10 EID50 7.7, 7.6 and 6.75 respectively from vaccine production facility at the National Veterinary Research Institute (NVRI), Vom Nigeria, were sent for certification at African Union laboratory at Debre Zeit, Ethiopia under cold condition; using ice packs for cold chain maintenance. Tracking of the vaccine in the course of freight to the certifying laboratory revealed changes in the original transit route by the airline and delay by custom at the receiving country port. Thus, a 12 hours transit freight was delayed for over 7 days before arrival at the certifying laboratory. Though, the EID50 titre per dose values of these vaccines were originally well above recommended EID50 5.5 per dose value by log 2.2, log 2.1 and log 1.25 for NDVI-2 respectively. Comparing these vaccines EID50 values obtained from the certifying laboratory in relation to values initially obtained by the Quality Control Division of the producing Institute (NVRI), revealed loss of EID50 titre per dose value by log10 1.2, and log10 0.8 among two of the certified vaccine batches; with one of the certified vaccines retaining its original EID50 titre per dose value. This result attests that NDVI-2 thermostable vaccine produced at NVRI, Vom Nigeria are robust and of high quality. Thus, the findings of this study have further demonstrated that temperature variations, prolonged vaccine transportation under poor cold chain can affect the EID50 per dose value of a wholesome, field fit vaccine irrespective of the vaccine thermostability.

Keywords: Transit Cold Condition Alterations, EID50 per Dose Values, Thermostable Newcastle Disease Vaccine.

I. INTRODUCTION

Newcastle disease (ND) is caused by virulent strains of avian paramyxovirus type 1 (APMV-1) an envelope, single stranded RNA virus [1]. The disease is a highly contagious, rapidly spreading viral disease of domestic poultry of all ages and other wild avian species and the disease consequences is devastating in susceptible poultry flock [2]-[6]. Newcastle disease is endemic in Nigeria [7] and it is generally well-recognized by farmers in both local and exotic breed of birds [8]. Outbreak is reportable to World Organization of Animal Health [1]. The most cost-effective control strategy for Newcastle disease outbreak is by vaccination [9]. The first successful control measure against Newcastle disease in Nigeria was through the production of Newcastle disease intra ocular (NDV-i/o) and Newcastle disease Komarov (NDV-K) by the then Federal Veterinary Research Laboratory, Vom [10]; presently known as the National Veterinary Research Institute (NVRI). Biosecurity measures are possible control alternative for ND, however, its application has remains difficult or impossible to implement in rural setting due to the nomadic and free-range rearings of village poultry in Nigeria [11]. In commercial poultry production in Nigeria a regimented Newcastle disease vaccination program is widely applied for the protection of commercial poultry flocks against Newcastle disease outbreaks.

Conventional vaccination programs for Newcastle disease control in endemic regions include the use of either low-virulent live-virus vaccines or inactivated vaccines to induce protective immunity while producing minimal adverse reaction in birds [12]. Inactivated oil-emulsion vaccines are manufactured for use by individual bird injection. On the other hand, low-virulent infectious virus may be ingested or inhaled, and this is the basis for mass application of live-virus vaccines in drinking water or by large aerosol droplet [12]. Birds may also be inoculated with eye drops containing vaccines based on attenuated viruses. Vaccination using non virulent ND virus strains to protect susceptible birds against...
ND disease outbreak by eliciting the production of specific antibody response either locally, systemically or both [12].

This study was carried out to compare EID50 per dose titre values of three thermostable vaccines after production in relation to values obtained after certification; and an unnecessarily prolonged transit. The study revealed that temperature alterations, prolonged extended transit can affect a naturally thermostable vaccine EID50 per dose titre value. Thus, irrespective of a vaccine thermostability nature and its EID50 after production, temperature variations, poor cold keeping condition, can affect its EID50 per dose value.

II. MATERIALS AND METHOD

A. EID50 Values Determination

The EID50 values of a batch of ND (NDVI-2) of 50 dose and two batches of (NDVI-2) of 200 doses respectively produced by the National Veterinary Research Institute (NVRI), Vom were determined after inoculation in 10-day old embryonated chicken eggs following OIE, [13] standard protocol. The vaccine virus titres (concentration per dose) were calculated and determined according to Karber method [14].

B. Vaccine shipment tracking history

The vaccine original shipments envisaged route:
Vom to Abuja airport – 4 hours; Abuja to Adis-ababa – 6 hours; Adis-ababa airport to Debre Zeit -2 hours

The vaccine actual shipments route:
Vom to Abuja airport 4 hour; Abuja airport to Adis-ababa airport – 6 hours; Adis-ababa airport to Nairobi airport – 2 days; Nairobi airport to Adis-ababa airport – 3 days; Adis-ababa airport to Debre Zeit – 2 days.

III. RESULT

Application of the Karber method for vaccine dose assay using karber method [14] revealed EID50 titre values of log 10 EID50 7.7, 7.6 and 6.75 per dose respectively for the titrated vaccines prior to shipment. This is Log 10 2.2, Log 10 2.1 and Log 10 1.25 above the recommended reference EID50 values of log 10 5.5 per dose for NDVI-2 (Table 1).

<table>
<thead>
<tr>
<th>Vaccine type and dose (NDVI-2)</th>
<th>EID50 per dose value after production</th>
<th>Reference EID50 per dose</th>
<th>Titre per dose value EID50</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>log10 7.7 per dose</td>
<td>log 10 5.5 per dose</td>
<td>log 10 2.2,</td>
</tr>
<tr>
<td>200</td>
<td>log 10 7.6 per dose</td>
<td>log 10 5.5 per dose</td>
<td>log 10 2.1</td>
</tr>
<tr>
<td>200</td>
<td>log 10 6.75 per dose</td>
<td>log 10 5.5 per dose</td>
<td>log 10 1.25</td>
</tr>
</tbody>
</table>

However, loss of EID50 titre values in two out of the three vaccines that were sent for certification was observed. Two out of the three vaccine samples (batches) certified showed EID50 titre per dose value loss of log 1.2 and log 0.8 when the results of certifying laboratory were compared with values originally obtained by the Quality Control laboratory of the vaccine production Institute (Table II). Therefore, due to extra Log 10 EID50 values per dose in- cooperated in each of these vaccine batches originally by the producers, the vaccine dose value was still within acceptable dose per range for NDVI-2 despite the obvious titre loss or drop after certification.

<table>
<thead>
<tr>
<th>Vaccine type and dose</th>
<th>Certifying Laboratory EID50 Values</th>
<th>Reference EID50 Values</th>
<th>EID50 value Loss after transportation</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>log 10 6.5 per dose</td>
<td>log 10 5.5 per dose</td>
<td>log 10 1.2</td>
</tr>
<tr>
<td>200</td>
<td>log 10 6.8 per dose</td>
<td>log 10 5.5 per dose</td>
<td>log 10 0.8</td>
</tr>
<tr>
<td>200</td>
<td>log 10 6.75 per dose</td>
<td>log 10 5.5 per dose</td>
<td>nil</td>
</tr>
</tbody>
</table>

IV. DISCUSSION

Field vaccination control strategy for Newcastle disease outbreak in susceptible poultry flock is only achievable by the use of field fit, safe, efficacious and wholesome Newcastle disease vaccines [15], especially in endemic region. The findings of our study demonstrated that prolonged extended transportation of a naturally thermostable vaccine with poor cold condition maintenance can result in loss of EID50 titre per dose value. The three vaccines originally showed EID50 titre values of log10 7.7, 7.6 and 6.75 per dose, to a recommended reference EID50 titre value of log10 5.5 per dose. This is log 2.2, 2.1 and 1.25 above the required or recommended EID50 titre per dose for field application of NDV-2. The prolonged freight; change of freight destination and clearance delay at the point of entry were attributable factors responsible for the observed EID50 titre value loss of log 1.2, and 0.8 by two of three vaccine batches while one out of the three vaccines retained its original EID50 titre value. The NDVI-2 vaccine is thermostable by nature and can withstand weak cold conditions; the finding of this study has demonstrated NDVI-2 vaccine thermostable ability. However, this ability to withstand poor cold storage condition does not exempt a naturally thermostable NDV-2 vaccine from EID50 titre per dose loss or drop. Therefore, a thermostable vaccine should equally be handled with utmost caution and under good cold keeping conditions, bearing in mind that titre drop, or loss is absolutely very possible if the vaccine cold keeping condition is compromised.

The finding of [16] and [17] suggested that in situations where the cold chain maintenance is difficult, the only reliable alternative may be the use of thermostable ND vaccines; such as the NDVI-2, NDV4-HR and or inactivated vaccines. The findings of our study support the claims of [16], [17]; this is attributable to the robustness of NDV-2 vaccine virus to remain viable even with limited cold storage or keeping condition and extended prolonged transit, as demonstrated.

The unpredictable vaccine transit routes, temperature variations and handling in the field setting before getting to the end users are factors Newcastle disease vaccine producers should be conscious of when compounding thermostable and thermolabile Newcastle disease vaccines. The producers should as much as possible ensure that qualitative vaccine with EID50 titre value of at least log 2 or log 3 above

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recommended standard EID50 value be in-cooperated in their vaccines when compounding; to cater for possible loss of EID50 titre values, which might be the consequent impact of poor handling, temperature variations and possibly extended transportation duration with poor cold keeping condition.

Thus, in countries where ND is endemic, the use of certified, wholesome, highly efficacious, safe and field fit ND vaccine is of optimal priority to both the vaccine producers and the poultry farmers. Furthermore, Veterinary Authorities in Africa should ensure that the ND vaccines produced and used in various counties in the continent met the required safety and quality standard to ensure protection of poultry flock against the destructive and enormous economic consequences of ND disease outbreaks; since it has been established that vaccination against ND remains the major control strategy against this poultry disease [18], [19], [20] and [21]. Therefore, whether a vaccine is produced locally or imported, each batch should be tested by African Union certifying laboratory to ensure conformity of the vaccine or vaccines to required, expected and acceptable standard, prior to field application or use.

It is imperative therefore, that vaccine end users understand that a thermostable vaccine must be handled with optimal precaution accorded other vaccines or biologicals since these vaccines are equally liable and susceptible to titre loss due to poor cold keeping condition. Furthermore, vaccine cannot be exposed to sunlight or frequent changes or variations in temperature and still be expected to remain viable [22]. Therefore, the use of safe, efficacious, highly potent and wholesome vaccine for the strategic control of Newcastle disease in endemic region should be of utmost priority to regional governments and Veterinary authorities across the continent.

A thermostable vaccine reduces the challenges encountered by distributors and vaccine end users especially as it relates to poor cold chains maintenance under field situations; as it is usually encountered in developing countries, where electricity supply is inadequate. This study has shown that avoidable cold condition alterations, variations and unnecessarily prolong transport situation can alter vaccine EID50 titre value of a naturally thermostable vaccine. The extra EID50 values of 2.2, 2.1 and 1.25 above the recommended EID50 value ensured that despite the observable titre loss, these vaccines were well within expected and acceptable recommended EID50 value range for NDV1-2 intended for field use. Thus, vaccine producers should always make provision for additional EID50 value when compounding their vaccine to compensate for possible EID50 value loss these vaccines might encounter once it is beyond their care and control.

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