World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

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Study of the stability of the reconstituted vaccine against contagious bovine lumpy skin disease manufactured by the central veterinary Laboratory of Bamako (Mali)

Afou BAMBA [1], Souleymane SACKO [1], Brahima SACKO [1, 2, *], Boubacar Madio dit Aladjogo MAIGA [1] and Boubacar Ousmane DIALLO [1]

[1] *Diagnostic and Research Service of Central Veterinary Laboratory of Bamako, (Mali.).*
[2] *Department of Biology, Faculty of Science and Technology at University of Science, Technology and Technology, Bamako, Mali.*

World Journal of Biology Pharmacy and Health Sciences, 2025, 21(02), 424-431

Publication history: Received on 05 January 2025; revised on 15 February 2025; accepted on 18 February 2025

Article DOI: https://doi.org/10.30574/wjbphs.2025.21.2.0178

Abstract

The present study carried out at the Central Veterinary Laboratory of Mali (LCV) aimed to determine the stability of the DERMAPOX vaccine after its reconstitution. It focused on (03) pilot batches of vaccine against Contagious Bovine Lumpy Skin Disease (LSD) codenamed DERMAPOX. Batches of DERMAPOX Nos. Pox 001, Pox 002 and Pox 003 produced according to Good Manufacturing Practices. The production and quality control procedures of culture media, semi-finished and finished product solutions did in accordance with the production protocol and the vaccine quality control manual of the LCV in accordance with the vaccine quality control manual of the Pan African Vaccine Quality Control Center (PANVAC) and the European Pharmacopoeia monographs. Said batches of DERMAPOX N° Pox 001, N° Pox 002 and N° Pox 003 products have obtained the good quality label. The results of the quality control of the vaccine lyophilisate reconstituted with sterile physiological water were found to comply with the required sterility and titration specifications. The evaluation of the titer of the viral antigen, the standard of which is ≥102.5DICT50 per vaccine dose, made it possible to determine an average no loss or no degradation) of 0.09 log 10 ± 0.05 defining a limit of viability of use of five (05) hours after reconstitution of DERMAPOX.

Keywords: DERMAPOX Vaccine; Lumpy Skin Disease; Vaccine Lyophilisate; Desease manufactuued; Mali

1. Introduction

Contagious Bovine Lumpy Skin Disease (CLSD) is an emerging disease of livestock. It is caused by a virus belonging to the Poxviridae family. It was first reported in Zambia in 1929 [9], then in South Africa where it affected more than 8 million cattle, causing enormous economic losses. In 1957, it appeared in Kenya [11] and in 1970 broadcast in northern Sudan. In 1977, Contagious Bovine Lumpy Skin Disease reached Mauritania, Mali, Ghana and Liberia. The economic impact of the LSD is considerable. In Mali, where LSD remains endemic, in 1994 [2] it affected semi-intensive dairy cattle breeding systems. In rural areas, farmers who use draft oxen deprived of their animals at a crucial time for cultivation, since the oxen were unable to work. This has seriously compromised the food and nutritional security of the populations. However, the only means of combating this disease in developing countries is vaccination [1]. The Kenyan strain KSGP 0240 is commonly used to vaccinate ruminants against capripox infections [13, 20, 23], but the stability after reconstitution of freeze-dried vaccines is rarely studied by manufacturing laboratories. Therefore, to make our contribution to the stability of lyophilized vaccines after reconstruction, the present study carried out.

* Corresponding author: SACKO Brahima

2. Material and Methods

The methodology is essentially based on the quality control of three (3) consecutive pilot batches of DERMAPOX produced beforehand and then specifically on the titration test after reconstitution of the vaccine lyophilisate from each of said batches. 3 batches of DERMAPOX No. Pox 001, No. Pox 002 and No. Pox 003 were collected before the reconstitution session was carried out and subjected to tests for: sterility, titration, vacuum, residual humidity and safety. Each batch of DERMAPOX is inoculated on TSA, TSB, FTM media and steamed at 37°C for 14 days, followed by steaming at 37°C of the subculture. Then on GS, BS, TSB, FTM media and steaming at 25°C and 37°C for 14 days, followed by steaming at 37°C of the subculture. If no growth of microorganisms is observed in any of the broths during the incubation period and at the end of it, the examined preparation is considered sterile. To evaluate the titer of the viral antigen, trypsinizing a Vero cell culture flask and preparing a cell suspension with DMEM medium with 10% serum having a concentration of 200,000 cells/ml. All operations are carried out in a laminar flow hood in an open circuit and the samples are kept in an ice bath for the duration of the manipulation. After this operation, the cell suspension is distributed at a rate of 100 µl per well, starting with the 12th line (control cells) and ending with the first line; skip the 11th line, then add 100µl of serum-free DMEM to the 8 wells of the 12th line. Arrange 8 test tubes marked 10-1 to 10-8 on a rack placed on crushed ice for the preparation of decimal dilutions of the antigen. If the vaccine is freeze-dried, it should be dissolved with serum-free DMEM in the freeze-drying volume (1 ml). 4.5ml of serum-free DMEM is added to each test tube. Decimal dilutions from 10-1 to 10-8 are carried out in the tubes. The different dilutions of the antigen obtained are transferred into wells and then incubated the plate at 37°C in an atmosphere containing 5% CO2 for a period of 10 to 12 days. From the 3rd day, examine the microplates daily under an inverse microscope to note the evolution of the cytopathic effect in the wells infected with the antigen and the state of the cells in the wells of line 12 (control normal cells).

2.1. Data analysis

Data entered with Excel 2016 and analyzed with spss.20. The chi-square test is used to compare the different variables

3. Results

The results obtained are presented in tabular form illustrated by curves for the batches of DERMAPOX N°Pox 001, N° Pox 002 and N° Pox 003.

Table 1 translated into the form of a curve presented in Figure No. 1 indicates the values of the titer of the vaccine suspension at the different times after the reconstitution of DERMAPOX lot No. Pox 001.

Table 1 Titles of the DERMAPOX vaccine suspension N°Pox 001

Time	Title/ 1 dose
T0	$10^{3,20}$
T-1Hour	$10^{3,10}$
T-2Hour	$10^{3,10}$
T-3Hour	10^{3}
T-4Hour	10^{3}
T-5Hour	$10^{2,70}$
T-6Hour	$10^{2,40}$

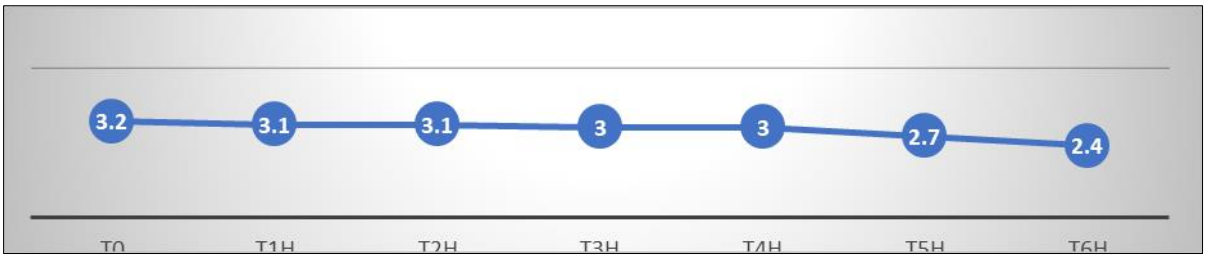

Figure 1 Maintenance of titles of the vaccine suspension of DERMAPOX lot lot N°Pox 001

It appears from the analysis of Table 1 that the titers of the vaccine suspension of DERMAPOX N°Pox 001 vary from 103.2 / dose to 102.4 / dose respectively from time T0 immediately after reconstitution of the vaccine lyophilisate to time T-6H after this. Figure No. 1 indicates the maintenance of the titer within the acceptance limits in accordance with the required specifications (≥102.5DICT50/dose) for 5 hours, while at time T-6Hour we record a titer value lower than the standard. Table 3 translated into the form of a curve presented in Figure No. 2 indicates the values of the titer of the vaccine suspension at the different times after the reconstitution of DERMAPOX batch No. Pox 002.

Table 2 Titles of the vaccine suspension of DERMAPOX lot N°Pox 002

Time	Title/ 1 dose
T0	$10^{3,40}$
T-1Hour	$10^{3,30}$
T-2Hour	$10^{3,30}$
T-3Hour	$10^{3,20}$
T-4Hour	$10^{3,20}$
T-5Hour	$10^{3,10}$
T-6Hour	$10^{2,10}$

Figure 2 Maintenance of titles of the vaccine suspension of DERMAPOX lot lot N°Pox 002

It appears from the analysis of Table 2 that the titers of the vaccine suspension of DERMAPOX N°Pox 002 vary from 103.4 / dose to 102.1 / dose respectively from time T0 immediately after reconstitution of the vaccine lyophilisate to time T-6H after this. Figure 2 indicates the maintenance of the titer within the acceptance limits in accordance with the required specifications (≥102.5 TCID50/dose) for 5 hours, while at time T-6H we record a titer value lower than the standard. Table No. 4 translated into the form of a curve presented in Figure 2 indicates the values of the titer of the vaccine suspension at the different times after the reconstitution of DERMAPOX batch No. Pox 003.

Table 3 Titles of the vaccine suspension of DERMAPOX batch N°Pox 003

Time	Title/ 1 dose
T0	$10^{3,30}$
T-1Hour	$10^{3,10}$
T-2Hour	$10^{3,10}$
T-3Hour	$10^{2,80}$
T-4Hour	$10^{2,70}$
T-5Hour	$10^{2,70}$
T-6Hour	$10^{2,40}$

Figure 3 Maintenance of titles of the vaccine suspension of DERMAPOX batch N°Pox 003

It appears from the analysis of Table No. 4 that the titers of the vaccine suspension of DERMAPOX No. Pox 003 vary from 103.3 to 102.41 / dose respectively from time T0 immediately after reconstitution of the vaccine lyophilisate to time T-6 hours after this. Figure 3 indicates the maintenance of the titer within the acceptance limits in accordance with the required specifications (≥102.5 TCID50/dose) for 5 hours, while at time T-6H we record a titer value lower than the standard. Table 5 translated in the form of a curve presented in figure 3 indicates the average titer of the dose of DERMAPOX reconstituted from time T0 to time T-6Hour.

Table 4 Average dose title of reconstituted DERMAPOX

Time	batch N°Pox 001/1dose (m001)	batch N° Pox 002/1 dose (m002)	batchN° Pox 003/1 dose (m003)	Total
T0	$10^{3,20}$	$10^{3,40}$	$10^{3,30}$	$10^{3,30}$
T-1Hour	$10^{3,10}$	$10^{3,30}$	$10^{3,10}$	$10^{3,16}$
T-2Hour	$10^{3,10}$	$10^{3,30}$	$10^{3,10}$	$10^{3,16}$
T-3Hour	10^{3}	$10^{3,20}$	$10^{2,80}$	10^{3}
T-4Hour	10^{3}	$10^{3,20}$	$10^{2,70}$	$10^{2,96}$
T-5Hour	$10^{2,70}$	$10^{3,10}$	$10^{2,70}$	$10^{2,83}$
T-6Hour	$10^{2,40}$	$10^{2,10}$	$10^{2,40}$	$10^{2,30}$

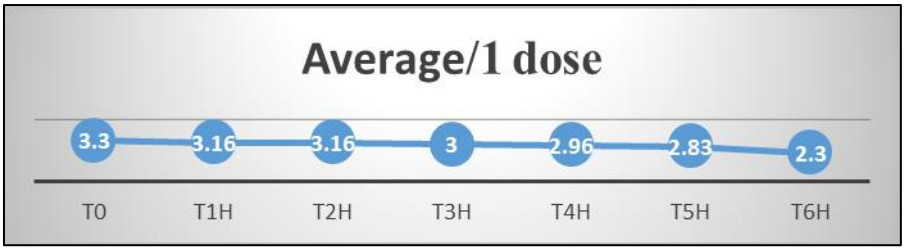

Figure 4 Maintenance of Average dose of reconstituted DERMAPOX

It emerges from the analysis of Table 4 illustrated in the curve represented by Figure No. 4, that the average titer of the vaccine suspension of batches No. Pox 001, No. Pox 002 and No. Pox 003 changed from 103.30 / dose to 102.30 / dose, from time T0 immediately after reconstitution of the vaccine lyophilisate to time T-6H after this. Figure 4 indicates the maintenance of the titer within the acceptance limits in accordance with the required specifications (≥102.5 TCID50/dose) for 5 hours, while at time T-6H we record a titer value lower than the standard

3.1. Evaluation of no loss (or no degradation) of the viability of viral particles

The results of no loss of viability of viral particles from batches of DERMAPOX No. Pox 001, No. Pox 002 and No. Pox 003 are presented in tabular form illustrated in a curve presented by a figure. Table 5. translated into the curve presented by figure 5 indicates the average step of loss of viability of the viral particles of the vaccine suspension of lot No. Pox 001 after reconstitution of the vaccine.

Table 5 The average step of loss of viral viability of the reconstituted batch N°Pox 001

Time	Title/ 1 dose	Tn1-Tn2 = PMP N°001
T0	103,20	
T-1Hour	103,10	0,10
T-2Hour	103,10	0,00
T-3Hour	103	0,10
T-4Hour	103	0,00
T-5Hour	102,70	0,30
Average		0,10

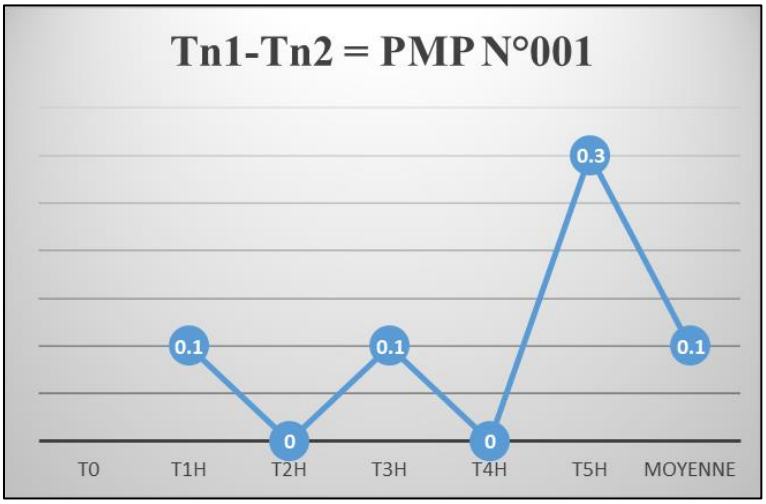

Figure 5 Maintenance of average step of loss of viral viability batch N°Pox 001 after the reconstituted

The analysis of Table 6 illustrated in Figure 5 retains an average step of loss of viral viability of the vaccine suspension of lot N Pox 001 of 0.1 log 10. Table.5 translated into the curve presented by Figure 5 indicates the average step of loss of viability of the viral particles of the vaccine suspension of lot No. Pox 002 after reconstitution of the vaccine

Table 6 The average step of loss of viral viability of the reconstituted batch N°Pox 002

Time	Title/ 1 dose	Tn1-Tn2 = PMP N°002
T0	103,40	
T-1Hour	103,30	0,10
T-2Hour	103,30	0,00
T-3Hour	103,20	0,10
T-4Hour	103,20	0,00
T-5Hour	103,10	0,10
Average		0,06

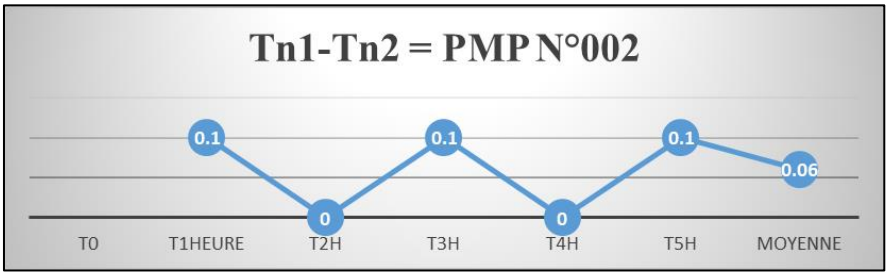

Figure 6 The average step of loss of viral viability batch N°Pox 002 after the reconstituted

The analysis of Table 6 illustrated in Figure 6. retains an average step of loss of viral viability of the vaccine suspension of lot No. Pox 002 of 0.06 log 10. Table 7. translated into the curve presented in figure 7 indicates the average step of loss of viability of the viral particles of the vaccine suspension of lot No. Pox 003 after reconstitution of the vaccine.

Table 7 the average step of loss of viral viability of the reconstituted batch No. Pox 003

Time	Title/ 1 dose	Tn1-Tn2 = PMP N°003
T0	103,30	
T-1Hour	103,10	0,20
T-2Hour	103,10	0,00
T-3Hour	102,80	0,30
T-4Hour	102,70	0,10
T-5Hour	102,70	0,00
Average		0,12

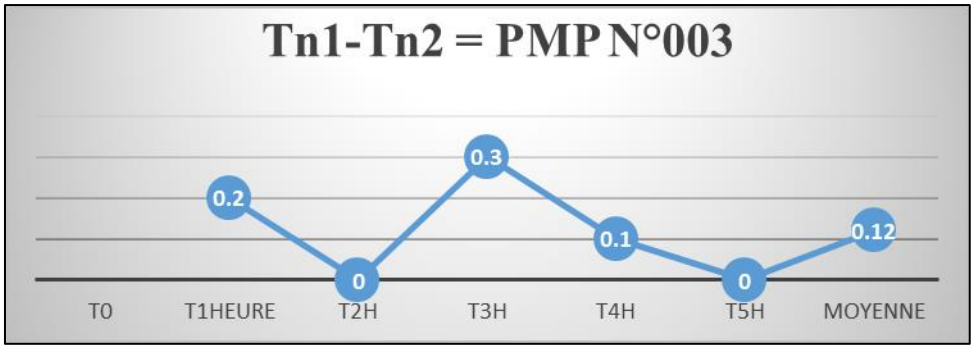

Figure 7 The average step of loss of viral viability batch No. Pox 003 after the reconstituted

The analysis of table 7 illustrated in figure N 7 retains an average step of loss of viral viability of the vaccine suspension of lot No. Pox 003 of 0.12 log 10. Table 8. translated into the curve presented in figure 7. indicates the average step of loss of viability of the viral particles of the vaccine suspension of batch No. Pox 003 after reconstitution of the vaccine. Table 8. indicates the average loss steps (MYMPM).

Table 8 Average steps of loss of viral viability

MPM N°001	MPM N°002	MPM N°003	MYMPM
0.10 Log 10	0.06 Log 10	0.12 Log 10	0,09 Log 10

The analysis of table No. 9 allows us to retain an average loss step value of 0.10 Log

4. Discussion

The present study is a first at LCV, which in its manufacturing arsenal produces the following four (04) freeze-dried live vaccines: Ovipeste, against Plague of Small Ruminants, Dermapox, against contagious bovine lumpy skin disease, cowpox and goat pox and peri-T1 SR and peri T1 44 against contagious bovine pleuropneumonia. The use of these vaccines requires reconstitution of the vaccine lyophilisate with physiological water. The results of the studies indicated in the OIE Terrestrial Manual [15, 16] only report the storage conditions of the freeze-dried vaccine. In addition, the Summary of Product Characteristics (SmPC) of the vaccine published by the manufacturing laboratories contains almost no data relating to the stability of the lyophilized vaccine after its reconstitution. The available reconstituted vaccine stability data are those held only by the competent authority which issues the MA, but they are not published, since they are confidential. To this end, the LCV commissioned the stability studies of its freeze-dried vaccines firstly to meet the UEMOA requirement for obtaining community marketing authorization, then in order to retain its customers and conquer new markets outside the UEMOA area. Thus, the study of the stability of Oviplague [14] was carried out and reported that this reconstituted vaccine maintained under ice as practiced in the field during vaccination campaigns retains its titer for four (04) hours, while the present study established the limit of use of reconstituted DERMAPOX at five (05) hours, which denotes a differential difference of one hour with Oviplague.

5. Conclusion

The results of the stability study of the three batches No. Pox 001, No. Pox 002 and No. Pox 003 revealed that DERMAPOX reconstituted in sterile physiological water and kept under ice retains its antigenic titer within the limits of the required specifications (≥102.5 TCID50/dose) for five (05) hours. They also showed that the average step of loss (or no degradation) of the viability of the viral particles in the vaccine suspension is 0.1 log 10 ± 0.05.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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