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(RESEARCH ARTICLE)

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Evaluation of the stability of the vaccine against Contagious Bovine Nodular Dermatitis, "DERMAPOX," under accelerated conditions, produced by the Central Veterinary Laboratory of Bamako (Mali)

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Abstract

Lumpy Skin Disease (LSD) still remains a major health problem in livestock farms in Mali. Among the means of combating this disease, vaccination remains the only solution envisaged for developing countries. The present study is part of the regulatory framework (Implementing Regulation N°007/2009/COM/UEMOA) of the West African Economic and Monetary Union (WAEMU), relating to the eligibility criteria for immunological medicinal products for obtaining marketing authorization (MA) community. Also the Central Veterinary Laboratory (CVL) vaccine producer needs scientific data concerning the accelerated conditions for the stability of the vaccine called DERMAPOX against LSD one of the sine qua non conditions to obtain the quality label in accordance with the World Organization for Animal Health (OMSA).

So to determine the stability and degradation of the vaccine against lumpy skin disease (LSD), three successive batches of DERMAPOX vaccine were produced by the Central Veterinary Laboratory (CVL) in Bamako and then exposed and titrated under accelerated at +37 °C +40 °C and +45 °C. The production and quality control methods and procedures for the culture media, solutions, semi-finished products, and finished products were carried out in accordance with the CVL's vaccine production protocol and quality control manual, and in compliance with the Pan-African Vaccine Quality Control Centre (PANVAC) vaccine quality control manual.

The three DERMAPOX batches were exposed to +37 °C, +40 °C, and +45 °C for two weeks. The initial titers of these vaccine batches were determined before exposure to the different temperatures. These batches of DERMAPOX have obtained the quality label compliant with sterility, vacuum, titer (≥102.5 DICT50), residual moisture content (≤ 3%), and safety standards.

The initial titers of the DERMAPOX batches before undergoing the stability study were 103.36/dose, 103.43/dose, and 103.63/dose for DERMAPOX batch No. Pox 001, DERMAPOX batch No. Pox 002, and DERMAPOX batch No. Pox 003, respectively.

At +37 °C on the 14th day of exposure, the titers of the samples from DERMAPOX batch No. Pox 001, DERMAPOX batch No. Pox 002, and DERMAPOX batch No. Pox 003 were 101.00/dose, 100.70/dose, and 101.25/dose, respectively, with titer loss rates of less than 30% for all three DERMAPOX batches from time T0 to time T-7 of exposure, beyond which the rates of Securities losses ranged from 40% to over 70%.

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At +40°C on the 12th day of exposure, the titers of the samples from batches of DERMAPOX No. Pox 001, DERMAPOX No. Pox 002, and DERMAPOX No. Pox 003 were respectively 100.90/dose, 100.90/dose, and 101.00/dose, with titer loss rates of less than 30% for all three batches of DERMAPOX from time T0 to time T-3 of exposure, beyond which, the titer loss rates varied from 35% to 100%. At +45°C on the 5th day of exposure, the titers of samples from batches DERMAPOX No. Pox 001, DERMAPOX No. Pox 002, and DERMAPOX No. Pox 003 were 101.70/dose, 101.60/dose, and 102.10/dose, respectively, with titer loss rates of less than 30% for all three DERMAPOX batches from time T0 to time T-2 of exposure beyond which, the titer loss rates varied from 34% to 100%. These data will allow for improvements to the DERMAPOX package insert and labeling, which will be included in the application for amendments to obtain Marketing Authorization (MA). They will also contribute to minimizing vaccination failures and improving disease control.

Keywords: DERMAPOX; Stability; Degradation; Titration; Titration Loss Rate

1. Introduction

Contagious Bovine Nodular Dermatitis (CBND) is an emerging livestock disease characteristic of the African continent, particularly the sub-Saharan region, with significant economic impact [8, 10, 14, 17, 19, 29]. The disease is caused by a virus belonging to the Poxviridae family, Chordopoxviridae subfamily, and Capripoxvirus genus [6, 7, 9]. It was first reported in Zambia in 1929, spreading to Botswana in 1943 [11], and then to South Africa, where it affected more than 8 million cattle, causing enormous economic losses. In 1957, it appeared in Kenya, associated with sheep pox [13]. In 1970, it spread to northern Sudan; in 1974, it extended westward to Nigeria [24], and in 1977 reached Mauritania, Mali, Ghana, and Liberia. The economic impact of NCDV is considerable. The most affected countries are those in Africa [28, 29]. In Mali, where DNCB remains endemic, it affected semi-intensive dairy cattle farming systems supplying the capital, Bamako, in 1994 [2], and reduced the city's milk supply. In rural areas, farmers who use draft oxen were deprived of their animals at a critical time for plow-based farming. This severely compromised the food and nutritional security of populations with limited self-sufficiency. However, the only means of combating this disease in developing countries is vaccination, which plays a key role in controlling the spread of the disease in endemic regions [1]. To this end, veterinary services in Mali organize an annual national livestock vaccination campaign against major animal diseases, including NCD, which is a major problem in livestock farming [3, 4, 5, 12] The Kenyan strain KSGP 0240 is commonly used to vaccinate ruminants against capripox infections [15, 25, 31], but the stability of vaccines under accelerated conditions has been studied very little by manufacturers. This study meets the requirements of the West African Economic and Monetary Union (WAEMU—Implementing Regulation No. 007/2009/COM/WAEMU), the sole body authorized to grant a Marketing Authorization (MA) contingent upon the applicant laboratory providing data, including vaccine stability under accelerated conditions. Furthermore, LCV needs to have scientific data on the stability of the vaccines it manufactures in order to retain its customers in the West African subregion and to capture new markets. Thus, knowledge of the degradation rate and stability of DERMAPOX under accelerated conditions at +37°C, +40°C, and +45°C allows for the definition of the viability limits of the viral antigen in DERMAPOX.

2. Materials and Methods

2.1. Production and testing of DERMAPOX batches

The methodology is primarily based on the quality control of three (3) consecutive pilot batches of DERMAPOX produced in advance, and specifically on the titration testing of samples taken from vials of these DERMAPOX batches subjected to accelerated stability conditions. Samples from each of the three batches are collected prior to storage under accelerated conditions at +37°C, +40°C, and +45°C for testing for sterility, titration, vacuum, residual moisture content, and safety. Thus, this study involved DERMAPOX batches No. Pox 001, No. Pox 002, and No. Pox 003, which had already received the quality label in accordance with international standards—that is, sterile, non-toxic vaccine batches containing a viral antigen with a titer ≥ 102.5 DICT50/dose. The degradation and stability profiles were determined on samples from each of the three DERMAPOX batches, and these samples were stored as follows:

Seventy (70) vials from each batch are incubated at +37 °C, and five (5) vials from each of the three batches are sampled daily through the fourteenth day, with three vials tested daily;

- Seventy (70) additional vials from each of the three lots are stored at +40°C, and five vials from each lot are sampled daily for fourteen days, with three being titrated;
- Seventy (70) additional vials from each of the three lots are stored at +45°C, and five vials from each lot are sampled daily for two weeks, with three being titrated

2.2. Description of the technique for assessing vaccine sterility

Bacterial and fungal sterility was established in accordance with the requirements set forth in the LCV Vaccine Quality Control Manual [18] and that of the Pan-African Vaccine Quality Control Center (PANVAC) [17], in accordance with the manual of the World Organisation for Animal Health (formerly OIE, now renamed OIE) and the European Pharmacopoeia 2.6.12 and 2.6.13 [19].

2.3. Description of the technique for evaluating viral antigen titer

This involves evaluating the concentration of a viral antigenic preparation by using, in parallel, a reference preparation with a known titer, in accordance with the procedures manual of the Pan-African Centre for Veterinary Vaccine Quality Control (PANVAC) [17].

2.4. Appréciation des paramètres de contrôles

Cinq (05) paramètres (Stérilité, Vide, Humidité résiduelle, Titre, innocuité) ont été testés avec différentes méthodes (Culture, PCR, Testeur de vide, titrage, thermogravimétrie, inoculation aux animaux) comme l'indique le tableau N°1

Tableau N°1 : Résumé tabulaire des paramètres de contrôle de la qualité des lots de DERMAPOX N° Pox 001, N° Pox 002 et N° Pox 003 avant le stockage des échantillons dans les conditions accélérées.

2.5. Data Analysis

The data were recorded and organized in a Microsoft Excel 10 spreadsheet. The statistical tests used were comparisons of logarithmic means by vaccine batch, taking into account the fixed effect of exposure temperatures (+37°C, +40°C, and +45°C) using analysis of variance (ANOVA) with Stata 12.0 software, and the comparison of viability rates using the chi-square (χ^2) test with R software (R i386 4.0.3). A significance level of 0.05 was used for comparing the different parameters. Repeatability analysis was used to verify the reliability of experimental data, such as those in this study, where a series of repetitions of vaccine titration were conducted at different exposure temperatures over varying durations.

Table 1 Quality control parameters for DERMAPOX batches prior to storing samples under accelerated conditions

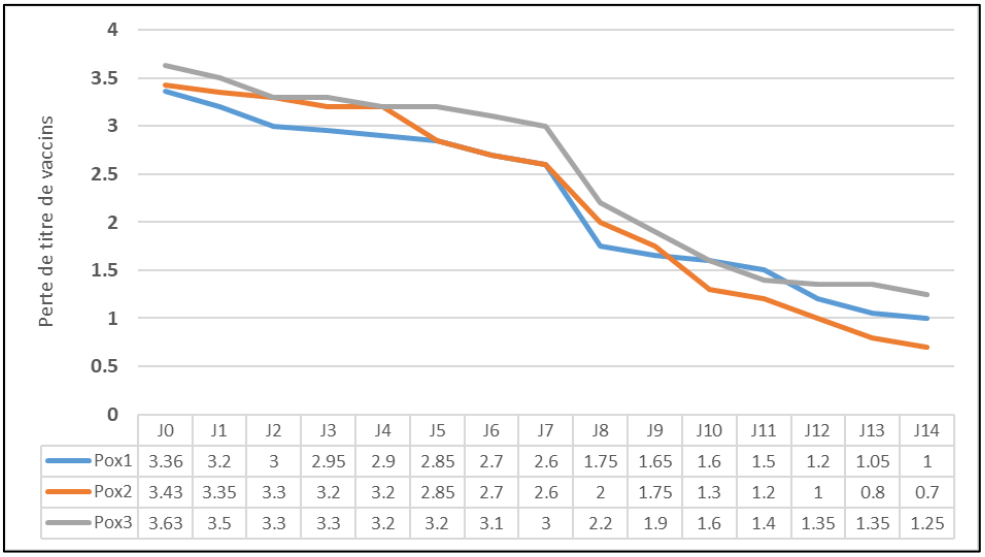
Test		**Methods**	**Reference value**	**Interpretation**
infertility	Bacterial and fungal	Culture medium	Lack of growth	Accepted
	Mycoplasm.	PCR	No matching tape	Accepted
	BVD	RT-PCR	No matching tape	Accepted
Vide		Vacum tester	Presence of violet light	Accepted
residual moisture		Thermogravimetry	≤ 3%	Accepted
Title		Vero cell titration	$\geq 10^{2.5}$ $DICT_{50}$/dose	Accepted
5. safety		01 subcutaneous dose in laboratory animals and target animals	No adverse reactions	Accepted
		10 subcutaneous doses in laboratory animals and target animals	No adverse reactions	Accepted

Accordingly, the stability testing under accelerated conditions of DERMAPOX batches No. Pox 001, No. Pox 002, and No. Pox 003 was conducted in accordance with the specifications and routine tests for sterility, residual moisture, and potency.

3. Results

3.1. Assessment of the concentration of samples from DERMAPOX batches No. Pox 001, No. Pox 002, and No. Pox 003 subjected to accelerated stability testing at +37 °C

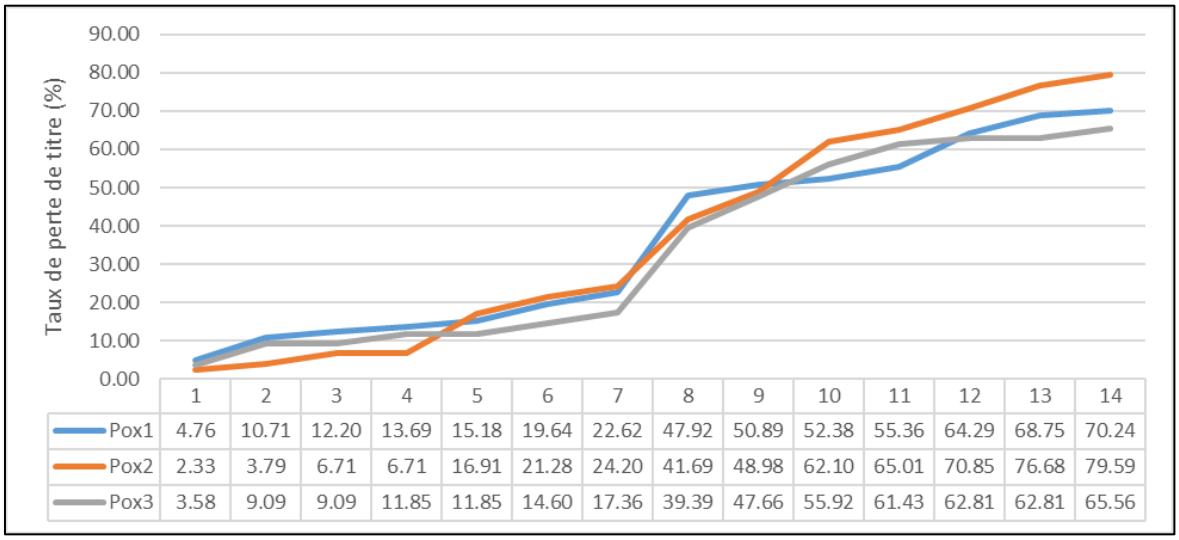
On average, the titers of DERMAPOX batches No. Pox 001, No. Pox 002, and No. Pox 003 ranged from 103.47/dose to 100.98/dose, respectively, from time T0 to time T-14 days. For individual lots, the titers of samples from the DERMAPOX Lot No. Pox 001 batch subjected to accelerated stability conditions at +37 °C ranged from 103.36/dose to 101.00/dose, respectively, from time T0 to time T-14 days. Those of the samples from DERMAPOX batch No. Pox 002 ranged from 103.43/dose to 100.70/dose, and those of the samples from DERMAPOX batch No. Pox 003 ranged from 103.63/dose to 101.25/dose. Figure 1 illustrates the different variations in titers of the samples from the three DERMAPOX lots subjected to the accelerated stability conditions at +37 °C described above



	J0	J1	J2	J3	J4	J5	J6	J7	J8	J9	J10	J11	J12	J13	J14
Pox1	3.36	3.2	3	2.95	2.9	2.85	2.7	2.6	1.75	1.65	1.6	1.5	1.2	1.05	1
Pox2	3.43	3.35	3.3	3.2	3.2	2.85	2.7	2.6	2	1.75	1.3	1.2	1	0.8	0.7
Pox3	3.63	3.5	3.3	3.3	3.2	3.2	3.1	3	2.2	1.9	1.6	1.4	1.35	1.35	1.25

Figure 1 Average loss (in log10) of the titers of 3 batches of DERMAPOX exposed to +37°C for 14 days

Thus, the potency of the three batches of DERMAPOX remained within the acceptance limits specified in the requirements (≥102.5 DICT50/dose) for seven (7) days; beyond that period, values below the standard were recorded. Analysis of the data, as shown in Figure 2, reveals a titer loss rate of less than 30% for all three (03) DERMAPOX batches from time T0 to time T-7 days of exposure at +37°C; beyond this point, viability loss rates ranged from approximately 40% to over 70%.

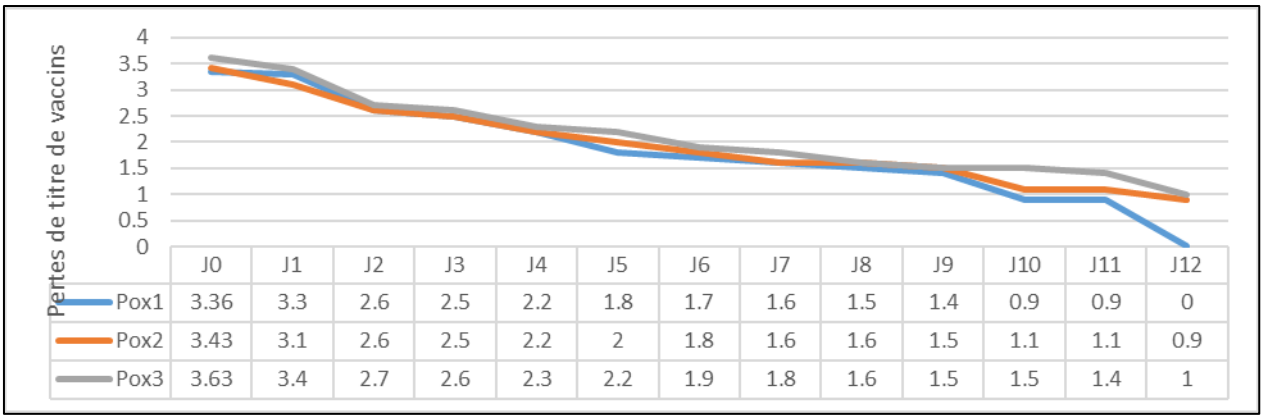


	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Pox1	4.76	10.71	12.20	13.69	15.18	19.64	22.62	47.92	50.89	52.38	55.36	64.29	68.75	70.24
Pox2	2.33	3.79	6.71	6.71	16.91	21.28	24.20	41.69	48.98	62.10	65.01	70.85	76.68	79.59
Pox3	3.58	9.09	9.09	11.85	11.85	14.60	17.36	39.39	47.66	55.92	61.43	62.81	62.81	65.56

Figure 2 Loss rate (%) of the samples from 3 batches of DERMAPOX exposed to +37°C for 14 days

3.2. Assessment of the concentration of samples from DERMAPOX batches No. Pox 001, No. Pox 002, and No. Pox 003 subjected to accelerated testing at +40 °C

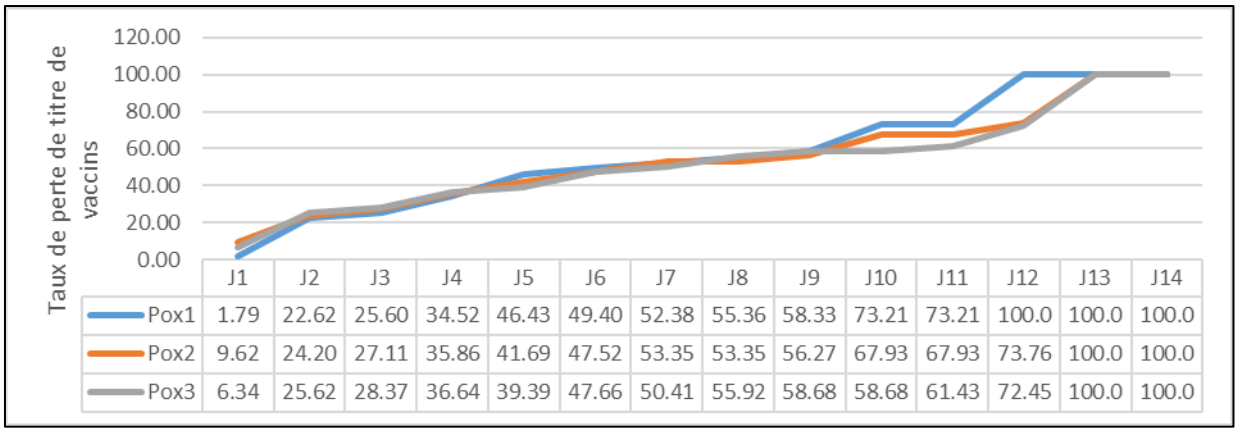
On average, the titers of DERMAPOX batches No. Pox 001, No. Pox 002, and No. Pox 003 ranged from 103.47/dose to 100.95/dose, respectively, from time T0 to time T-12 days. For individual DERMAPOX batches, the titers of samples from the DERMAPOX batch No. Pox 001 subjected to accelerated conditions at +40°C ranged from 103.36/dose to 100.90/dose, respectively, from time T0 to time T-11 days. The titers on the 12th, 13th, and 14th days proved unusable, as they were illegible. The titers of the samples from DERMAPOX batch No. Pox 002 subjected to accelerated conditions at +40°C ranged from 103.43/dose to 100.90/dose, respectively, from time T0 to time T-12th day. The titers for the 13th and 14th days of this batch were found to be unusable, as they were illegible. As for the titers of the samples from DERMAPOX batch No. Pox 003 subjected to accelerated conditions at +40°C, these ranged from 103.63/dose to 101.00/dose, respectively, from time T0 to time T-12th day. The titers on the 13th and 14th days of this batch



	J0	J1	J2	J3	J4	J5	J6	J7	J8	J9	J10	J11	J12
Pox1	3.36	3.3	2.6	2.5	2.2	1.8	1.7	1.6	1.5	1.4	0.9	0.9	0
Pox2	3.43	3.1	2.6	2.5	2.2	2	1.8	1.6	1.6	1.5	1.1	1.1	0.9
Pox3	3.63	3.4	2.7	2.6	2.3	2.2	1.9	1.8	1.6	1.5	1.5	1.4	1

Figure 3 Titre loss (in log10) of vaccines exposed to +40°C. Average titre loss (in log10) of three batches of vaccines against bovine nodular dermatosis (Dermapox) during 14 days of exposure

Thus, the potency of the three batches of DERMAPOX remained within the acceptance limits specified in the requirements (≥102.5 DICT50/dose) for three (3) days; beyond that period, values below the standard were recorded. Analysis of the data, as shown in Figure 4, indicates a loss in titer of at least 30% for all three (03) DERMAPOX batches from time T0 to time T-3 days of exposure at +40°C. Starting on the 4th day, viability loss rates ranged from 35% to less than 80% by the 11th day, after which 100% loss was recorded.



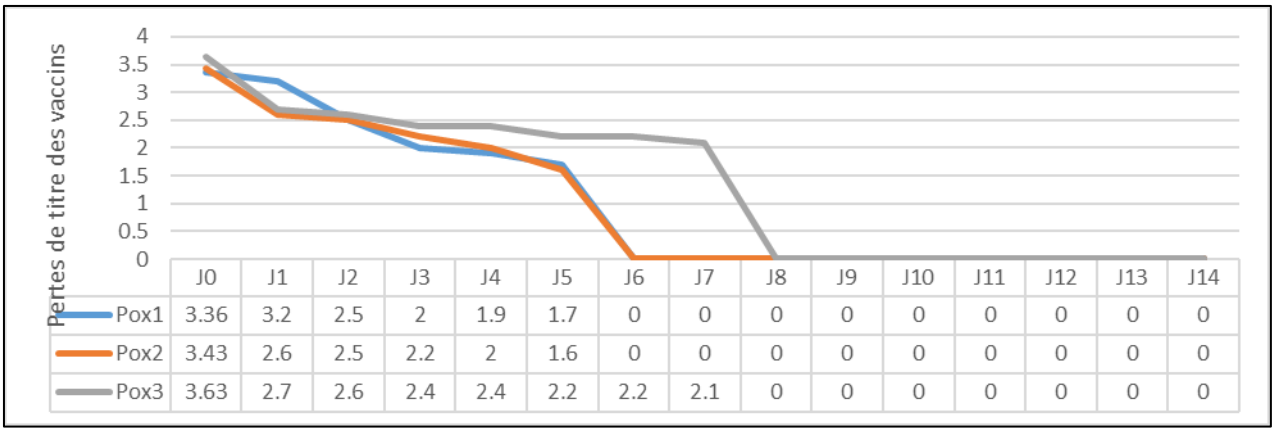
	J1	J2	J3	J4	J5	J6	J7	J8	J9	J10	J11	J12	J13	J14
Pox1	1.79	22.62	25.60	34.52	46.43	49.40	52.38	55.36	58.33	73.21	73.21	100.0	100.0	100.0
Pox2	9.62	24.20	27.11	35.86	41.69	47.52	53.35	53.35	56.27	67.93	67.93	73.76	100.0	100.0
Pox3	6.34	25.62	28.37	36.64	39.39	47.66	50.41	55.92	58.68	58.68	61.43	72.45	100.0	100.0

Figure 4 Loss rate (%) of the samples from the three Dermapox batches exposed to +40°C for 14 days

3.3. Assessment of the concentration of samples from DERMAPOX lots No. Pox 001, No. Pox 002, and No. Pox 003 subjected to accelerated testing at +45°C

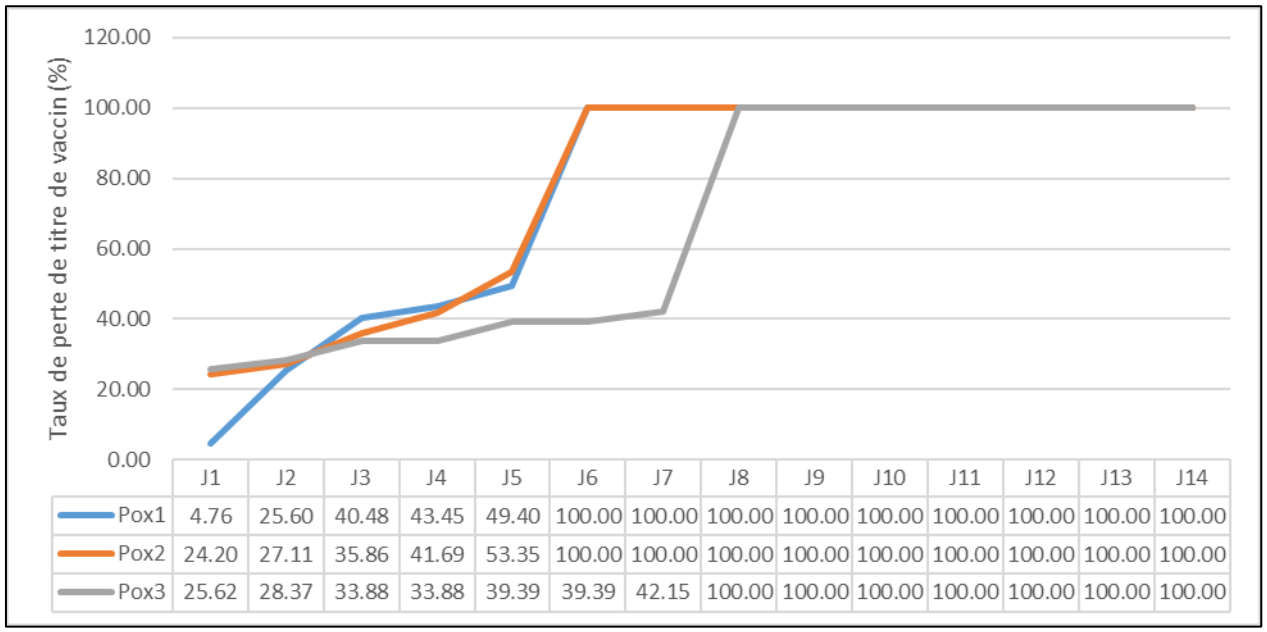
On average, the daily titers of the samples from DERMAPOX batches No. Pox 001, No. Pox 002, and No. Pox 003 subjected to accelerated conditions at +45°C ranged from 103.47/dose to 101.35/dose, respectively, from time T0 to time T-5 days. Those from the 6th, 7th, 8th, 9th, 10th, 11th, 12th, 13th, and 14th days proved unusable, as they were illegible. For individual DERMAPOX lots, the titers of samples from DERMAPOX Lot No. Pox 001 subjected to accelerated conditions

at 45°C ranged from 103.36/dose to 101.70/dose, respectively, from time T0 to time T-5 days. The titers on the 6th, 7th, 8th, 9th, 10th, 11th, 12th, 13th, and 14th days of this batch proved unusable, as they were illegible. The titers of the samples from the DERMAPOX batch No. Pox 002 subjected to accelerated conditions at +45°C ranged from 103.43/dose to 101.60/dose, respectively, from time T0 to time T-5 days. The titers on the 6th, 7th, 8th, 9th, 10th, 11th, 12th, 13th, and 14th days proved unusable, as they were illegible. The titers of the samples. Figure 5 illustrates the different variations in the sample designations for the three batches of DERMAPOX subjected to the accelerated stability conditions at +45°C described above



	J0	J1	J2	J3	J4	J5	J6	J7	J8	J9	J10	J11	J12	J13	J14
Pox1	3.36	3.2	2.5	2	1.9	1.7	0	0	0	0	0	0	0	0	0
Pox2	3.43	2.6	2.5	2.2	2	1.6	0	0	0	0	0	0	0	0	0
Pox3	3.63	2.7	2.6	2.4	2.4	2.2	2.2	2.1	0	0	0	0	0	0	0

Figure 5 Log-scale loss of vaccine titers following exposure to +45°C. Average log-scale loss of titer for three batches of bovine nodular dermatitis (Dermapox) vaccine over 14 days of exposure. Thus, at +45°C, the titer remained within the acceptance limits in accordance with the required specifications (≥102.5 DICT50/dose) for two (02) days, after which values below the standard were recorded. Analysis of the data, as shown in Figure 6, indicates a titer loss rate of less than 30% for all three (03) DERMAPOX batches from time T0 to time T-2 days of exposure at +45°C. From the 3rd to the 5th day, DERMAPOX lots No. Pox 001 and No. Pox 002 exhibited a loss rate of approximately 36% to over 50%, while lot No. Pox 003 recorded a loss rate of approximately 34% to over 40% from the 3rd to the 7th day



	J1	J2	J3	J4	J5	J6	J7	J8	J9	J10	J11	J12	J13	J14
Pox1	4.76	25.60	40.48	43.45	49.40	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Pox2	24.20	27.11	35.86	41.69	53.35	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Pox3	25.62	28.37	33.88	33.88	39.39	39.39	42.15	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Figure 6 Loss rate (%) of titers for the three batches of Dermapox exposed to +45°C for 14 days

Note that the value 100% represents samples whose titers were found to be illegible and therefore unusable

4. Discussion

This study marks a first for LCV, which currently produces and distributes the following four (04) freeze-dried live vaccines:

- Ovipeste, against small ruminant plague;
- Dermapox, against contagious bovine nodular dermatitis, lumpy skin disease, and goatpox;
- Péri-T1 SR and Péri T1 44 against contagious bovine pleuropneumonia

The use of these vaccines requires reconstitution of the lyophilized vaccine with saline solution. The results of the studies cited in the OIE (now OIE) Terrestrial Manual [19,20] report only the storage conditions for the lyophilized vaccine. Furthermore, the Summary of Product Characteristics (SPC) for the vaccine published by the manufacturing laboratories contains little data regarding the stability of the lyophilized vaccine under accelerated conditions (+37°C, +40°C, +45°C). The results of KANTAO O. 2017 [18], when compared to the content of the OIE (now OIE) terrestrial manual and the data from the study by DIAWARA et al. 2012 [16], allow us to conclude that Ovipeste maintains an acceptable titer for three (03) days at +37°C and one (01) day at +45°C. The experimental batches from DIAWARA S. 2012 [16] were processed using the Xerovac method, which provides greater heat stability to the vaccine, whereas the batches from the study by KANTAO O. 2017 [18] were subjected to the conventional freeze-drying cycle. Statistical analysis of the concentrations of the three batches of DERMAPOX at +37°C, +40°C, and +45°C did not reveal a significant difference: chi-square = 0.36; p = 0.37. L.C. LEFEVRE [26] reports the production of a freeze-dried live-virus vaccine against bovine nodular dermatitis at the Farcha laboratory (Chad). In this article, the production technique, the stability of the lyophilized product, and that of the reconstituted product are studied. According to the author, lyophilization does not result in any decrease in titer regardless of the lyophilization medium used. However, the neopeptone medium provides better thermal protection for the lyophilized product. It should be noted that the vaccine against bovine nodular skin disease produced by L.C. LEFEVRE [26] differs significantly from the LCV's DERMAPOX in that: The strain used as the active ingredient, which is not standardized like the LCV strain recommended by the WHO;

- The nature of the stabilizers used;
- The lack of an assessment of the rate of titre loss;

The data obtained by L.C. LEVFEVRE [26] as well as the results of the present study lead to the same conclusion, namely that the vaccine remains stable against DNCB, regardless of the nature of the freeze-drying medium.

5. Conclusion

The results of the stability study under accelerated conditions for the three batches of DERMAPOX (No. Pox 001, No. Pox 002, and No. Pox 003) lead to the following conclusions:

- The stability parameters of three (03) consecutive batches of DERMAPOX were determined under accelerated conditions at +37 °C, +40 °C, and +45 °C;
- The viability limit for the viral antigen, in accordance with the international standard (titer ≥102.5 DICT50/dose), was established at:

Seven (07) days, during which the loss of titer was less than 30% for all three batches of DERMAPOX from time T0 to time T-7 days after exposure at +37 °C. Beyond this time, the loss of viability ranged from 40% to over 70%;

  - Three (03) days, with a titer loss rate of at least 30% for all three DERMAPOX lots from time T0 to time T-3 days of exposure at +40 °C. Beyond this period, viability loss rates range from 35% to less than 80%;
  - Two (02) days showing a loss in titer of at least 30% for all three DERMAPOX lots from time T0 to time T-2 days of exposure at +45 °C. From the 3rd to the 5th day, the DERMAPOX batches No. Pox 001 and No. Pox 002 have a loss rate of approximately 36% to over 50%, while batch No. Pox 003 records a loss rate of approximately 34% to over 40% from the 3rd to the 7th day.

This data will help minimize vaccination failures and improve disease control.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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