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Optimization and Scaling-Up of Peste des Petits Ruminants (PPR) Vaccine Production Using Roller Bottle Platform

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Abstract

Peste des Petits ruminants (PPR) is considered as one of the major constraints to the productivity of small ruminants in Sudan. Presently, control measures for PPR are primarily reliant on vaccination using an attenuated PPR strain Nigeria 75/1 that has been produced in monolayers of Vero cells grown in static flasks. This study investigates the potential for scaling up PPR vaccine production using roller bottle technology, a more advanced method. A live, homologous vaccine against PPR in sheep and goats was successfully produced on a large scale in roller culture bottles, with DMEM supplemented with ten percent fetal bovine serum serving as the growth medium. The cells were infected with a multiplicity of infection of 0.01, and the vaccine was harvested when the cytopathic effect reached 80%. The vaccine was then freeze-dried to preserve its stability. A series of tests were conducted to ensure the safety and quality of the vaccine. Using PCR, the identity of vaccine was confirmed. It was found to be safe in both single and 100-times dose inoculations in sheep, with the produced batches showing a high titre of 6.4 ± 0.11 $\log_{10}$ $TCID_{50}$/ml. All batches met the criteria of sterility, passing tests for bacteria, fungi, and mycoplasma. Furthermore, the vaccine proved effective in small ruminants, with antibodies persisting for over a year post-vaccination. The residual moisture content remained below 2.5%, and the vaccine successfully passed vacuum testing. Stability tests indicated that the vaccine has a shelf-life of at least one year when stored at temperatures of 2˚C - 8˚C and −20˚C. These results demonstrate the potential for applying roller bottle culture technology to PPR vaccine production, significantly streamlining the existing process and enhancing its efficiency. Further research is warranted to address the economic analyses of adopting roller bottle technology with existing PPR control program.

Keywords

Large-Scale Production, Pest des Petits Ruminants, Roller Bottle Technology, Vaccination, Vero Cell

1. Introduction

Peste des petits ruminants (PPR) is a highly infectious, transboundary disease that is caused by the Peste des petits ruminants virus (PPRV), which belongs to the morbillivirus genus and the *Paramyxoviridae* family [1]. It is now officially named Small Ruminant Morbillivirus (SRMV) [2]. In the acute form of infection, morbidity and case fatality rates may remain high (up to 100%) [3] [4]. PPR was first described as a rinderpest (RP)-like disease of small domestic ruminants. However, in the recent past PPRV has been reported to infect not only goats and sheep, but also camels [5] [6], cattle [7], and wild animals [8] [9]. However, the factors that influence disease expression in wild and atypical hosts, and the role these hosts play in the circulation and maintenance of PPRV, remain poorly understood and may vary across various ecosystems [10]-[12]. More than 68% of sheep and goats and 2.5 billion small ruminants reside in countries affected by PPRV, according to FAO data from 2018 [13]. The annual cost Control and Eradication PPR Southern-Africa-Region of PPR-associated sheep and goat deaths for worldwide infected countries is estimated between 794 million and 2.7 billion US dollars [14].

PPR eradication efforts rely mainly on massive vaccination campaigns. In 1989, Diallo and colleagues successfully developed the first PPR vaccine, Nigeria 75/1, through serial passages of the PPR virus on Vero cells [15]. Several studies have demonstrated the efficacy of this vaccine on more than 98,000 sheep and goats in the field between 1989 and 1996. During those trials, no adverse effects were seen [16]. Additionally, vaccinated sheep and goats resisted the challenge of the virulent virus and did not transmit the virus to in-contact susceptible animals. The protective antibodies persisted for more than 3 years in the vaccinated animals. This vaccine is used worldwide for protection against all genetically defined lineages of PPRV. subsequently, Sungri/96 a lineage IV origin vaccine [17] was developed and is extensively used to control the disease in the Indian subcontinent, Middle East and South Asia [18]. Local production of a homologous live attenuated vaccine (Nigeria 75/1) against PPR was established in Sudan in 2004 in stationary flasks [19].

Vaccine manufacturers can choose from diverse formats supporting attached or suspension cell culture [20]. T-flasks are the most commonly used plastic consumables for early-stage cell expansion, while conveniently economical, these flasks are labor-intensive and become cost-inefficient when expanding cells beyond bench scale, mainly because of their high footprint [21]. Multi-Layered Flasks is a useful device for scaling up at bench scale, there are concerns regarding the cell quality and the associated labor intensity. For instance, there might be a heterogeneous availability and distribution of nutrients and gasses between the

different layers of the flask [22]. Moreover, simple operations like cell seeding, media change, and cell detachment/harvest become challenging due to their size and weight. In this regard, system automation would greatly enhance day-to-day operations [21]. The roller bottle technique is extensively employed in both research laboratories and industrial settings for cultivating large numbers of cells, particularly for viral vaccine production [23]-[25]. This method, which involves growing cells in a rotating culture, was developed by George Gey in 1933 at Johns Hopkins University to facilitate the cultivation of larger quantities of attachment-dependent cells. Roller bottles offer several advantages over static cultures, including increased surface area, prevention of gradients in cell culture media, and enhanced gas exchange due to thinner cell culture medium overlay layers [26] [27]. Challenges with the roller bottle technology include difficulties in controlling culture parameters such as pH, dissolved oxygen, and nutrient amount during a process [20]. Roller bottles are widely used to produce vaccines (e.g., chickenpox and herpes zoster [20] and remain in significant use for legacy processes [26]. Spinner flasks can generate high cell numbers, provide a better aeration system, a more homogeneous nutrient supply, a longer culture period, and reduce costs. Microcarriers can be added to spinner flasks mainly to do preliminary tests before moving to larger bioreactors [28].

Given the urgent need for a safe and effective vaccine in the context of global efforts to control and eradicate PPR, this study aims to establish a robust roller bottle platform for cultivating PPR vaccine strains. This aims to enhance production efficiency, scalability, and overall vaccine quality.

2. Materials and Methods

2.1. Cells and Virus

The study utilized Vero cells, originally from the American Type Culture Collection (Vero ATCC CCL-81), and a PPR vaccine strain Nigeria 75/1, kindly provided by the African Union Panafrican Veterinary Vaccines Centre (AU-PANVAC), Diberzit, Ethiopia, and stored in the Viral Vaccine Production Department at Central Veterinary Research Laboratory, Sudan.

2.2. Cell Culture Media

DMEM powder (Sigma, Cat # D5523) was dissolved in pure distilled water following the manufacturer's instructions, with modifications to the sodium bicarbonate (Sigma Cat #S5761) concentration (from 3.7/L to 1.5/L) and the addition of 25mM Fructose (Sigma Cat #3510). The media was then filtered through a 0.22 μm Millipore filter. Before use, the media was supplemented with antibiotics (penicillin and streptomycin at final concentrations of 100 IU [International Units]/ml and 100 μg/ml).

2.3. Culture Bottles

T Flasks (175 cm^2) (Greiner Cat # 660160) and roller bottles (Corning Cat

#CLS430851, 850 cm^2) were used for the propagation of cells and final vaccine production respectively.

2.4. Propagation of Vero Cell

For passaging Vero cells from T175 cm^2 static flask to roller bottle, the protocol described by Ammerman *et al.* 2008 [29] was followed, it began with the removal of the growth medium from T175 Cm^2 confluent monolayer flask of Vero cells, followed by a wash with 10 mL of 1X Dulbecco's Phosphate Buffered Saline(Sigma Cat #D8537) Subsequently, 5 mL of 1X trypsin-EDTA solution was added, and the cells were incubated at 37˚C for 2 - 3 minutes until detachment was observed, facilitated by gentle tapping, 5 mL of DMEM with 10% FBS was introduced. After washing the cells with media and gently pipetting to break up clumps, the cell suspension was transferred to a sterile 15 mL conical tube. Following centrifugation at 200 × g for 5 minutes at room temperature, the supernatant was discarded, and the cells were resuspended in 10 mL of DMEM with 10% FBS. The cell was counted using hemacytometer [30]. Finally, the desired cell dilution was prepared in 20 mL of DMEM with 10% FBS and added to roller bottle flasks with vented caps for further cultivation and vaccine production.

2.5. Production of PPRV

Vero cells were seeded at a density of 1×10^7 cells/mL in 850 cm^2 roller bottle flasks and incubated at 37˚C in a roller apparatus at 20 revolutions/hour. After 24 hours, the media was discarded, and the flasks were infected with the PPR vaccine at an MOI of 0.01 [31]. The flasks were then incubated for 30 minutes, after which DMEM supplemented with 2% fetal bovine serum was added to achieve a final culture volume of 250 mL per flask. The cultures were checked daily, with one flask used to assess for cytopathic effect (CPE) development, and harvested when CPE reached 30% - 40%. The final harvest occurred when CPE reached 70% - 80% [31].

2.6. Lyophilization

Using a Telstar LyoBeta freeze-dryer, the vaccine batches were lyophilized in sterile 5 mL capacity vials. Equal volumes of the vaccine and stabilizer (Lactalbumin hydrolysate 5% and sucrose 10%) were mixed. One milliliter was dispensed in vaccine vials and partially sealed with vented rubber stoppers. The vaccine vials were first chilled to −45˚C for one and a half hours and maintained at that temperature for another 3 hours, the condenser was prepared for ten minutes and the vacuum chamber was set to 400 μbar. Primary drying was conducted at −20˚C shelf temperature and 300 μbar pressure for 3 hours. The shelf temperature subsequently increased to −10˚C for 5 hours. The shelf temperature was maintained at 0˚C and for another 6 hours. This was followed by ramping the shelf temperature to 20˚C for 30 min and maintaining at 20˚C for another 5 hours.

2.7. Quality Control

2.7.1. Sterility

Sterility testing was conducted on the working seed, chilled viruses, freeze-dried vaccines, and all other ingredients employed in vaccine production. The tests were performed using fluid thioglycolate medium at 30˚C - 35˚C and soybean casein digest medium at 30˚ - 35˚C and 20˚C - 25˚C. Observation of the broths was carried out over 14 days [30]. Direct inoculation of fluid thioglycolate medium (FTM) and soybean casein digest medium (SCDM) was executed, employing a sterile pipette to aseptically transfer 0.1 ml of all substances used in vaccine production, including the final product vaccine chosen at random [32].

2.7.2. Mycoplasma

Batches of live viral vaccine, a lot of master seed virus (MSV), a lot of primary and master cell stock (MCS), and all ingredients of animal origin not steam-sterilized used in the production of PPR vaccine were tested for the absence of mycoplasmas. Culture examination for mycoplasmas was performed in mycoplasma broth and agar according to WOAH Terrestrial Manual [32] under both anaerobic and microaerobic conditions. Briefly, 1 ml of cell or virus seed was inoculated into 9 ml of the liquid medium and 100 µl onto solid mycoplasma agar. The liquid medium was incubated at 37˚C in 5% - 10% CO_2 and 100 µl of broth were sub-cultured on to agar at days 7, 14 and 21. The agar plates are incubated at 37˚C in 5% - 10% CO_2 for no fewer than 14 days, except those corresponding to day 21 subculture, which were incubated for 7 days. An un-inoculated mycoplasma broth and agar plate were incubated as negative controls [32].

2.7.3. Identity

1) RNA extraction

The viral RNA was extracted from four randomly selected lyophilized vaccines from two batches by reconstituting the vials in one ml DPBS. Subsequently, a commercial Qiamp Mini Kit (Qiagen, Germany) was used for RNA extraction according to the manufacturer's instructions. Briefly, 560 µL of lysis buffer and 700 µL of 70% ethanol were added to 140 µL of diluted vaccine and spun in a spin-filtered column for one minute. For washing, wash buffer and buffer RPE were added and spun. To elute the extracted RNA, 40 µL of RNA-free water was used. The extracted RNA was stored at −20˚C until needed.

2) One-step RT-PCR

The test was carried out according to WOAH 2022 [31] based on the initial protocol described by Couacy-Hymann *et al.* (2002) [33] using primer kindly brought from the Joint FAO/IAEA Centre of Neuclear Techniques in Food and Agriculture. This primer sets directed to the conserved partial sequence of the N gene: (NP3) (forward: 5-TCTCGGAAATCGCCTCACAGACTG-3) and (NP4) (reverse: 5-CCTCCTCCTGGTCCTCCAGAATCT-3). The assay was carried out in a 50 µl reaction mixture containing, 3 µl of each primer, 10 µl of Q solution, 10 µl of the 5X one-step RT-PCR buffer (Qiagen, Germany), 2 µl dNTPs, 2 µl of One

Step RT-PCR enzyme mix (Qiagen, Germany), 15 µl distilled water and 5 µl of the extracted RNA. RT-PCR for the N gene was carried out at 50˚C for 30 min to activate the transcriptase enzyme. Initial denaturation was performed at 95˚C for 15 min, followed by 40 cycles of 95˚C for 30 s, 60˚C for 30 s, and 72˚C for 1 min for PCR amplification of cDNA, and a final extension step was performed at 72˚C for 5 min. The amplified PCR products were electrophoresed through a 1.5% agarose gel in 1X TBE buffer at 90 V for 80 minutes and visualized using ultraviolet illumination.

2.7.4. Safety and Immunogenicity

The present research study was conducted at the Central Veterinary Research Laboratory's quarantine animal facility in Sudan, where male and female sheep aged 12 months were kept. Before commencing the trial, the animals were pre-screened for the presence of PPRV antibodies using a competitive ELISA method (ID screen RPPR competition, IDvet Genetics, Grabels, France). Before the trial, the animals were dewormed, and their rectal temperature was measured. The animals were divided into three groups: Group A comprised of Ten seronegative Sheep, including six males and four females, two of whom were pregnant ewes, and received one field dose (2.5 $\log_{10}$ $TCID_{50}$/ml) of live attenuated PPRV Nigeria 75/1. Group B comprised ten seronegative sheep, including six males and four females, two of whom were pregnant ewes, and were vaccinated with 100 field doses of live attenuated PPRV Nigeria 75/1. Group C, the control group, consisted of five seronegative sheep. All vaccinated sheep were observed daily, and their rectal temperature was recorded for 21 days post-vaccination (DPV). Groups A and C were observed for one year, during which the sheep were sampled (blood taken via jugular vein puncture) at one-month, six-month, and one-year intervals and screened for PPRV antibodies [31].

2.7.5. Batch Potency

The potency of the PPR vaccine was determined by virus titration ($TCID_{50}$) in Vero cell culture in 96 well microtitre plates using standard cell culture procedures [31]. Briefly, 10-fold serial dilution down to 10^8 of samples in GMEM without serum were prepared. For each dilution, 12 replicates were done with 100 µL virus suspension and 100 µL cell suspension (30,000 cells per well) per well. All the test plates were incubated at 37˚C. The cytopathic effect was checked on day 10 of titration. End points ($TCID_{50}$/mL) were calculated according to Spearman-Kärber methods [34].

2.7.6. Vacuum Test

The vacuum inside the freeze-dried vials was tested using high voltage leak detection Spark tester [31].

2.7.7. Measurement of Residual Moisture

According to WOAH 2022 requirements [31], the residual moisture content of the lyophilized vaccine was determined using a KERN moisture analyzer,

employing the thermogravimetric method.

2.7.8. Stability

Vaccine vials from the batch were randomly selected and stored at temperatures of +4˚C and −20˚C for 360 days. At intervals of 1, 90, 180, and 360 days, five vials were retrieved from each temperature condition for titration.

3. Results and Discussions

The increasing global and national demand for PPR vaccines underscores the need for efficient and scalable production methods to address the challenges posed by infectious diseases. The biopharmaceutical industry has identified the roller bottle platform as a versatile and effective technology for cultivating adherent cells essential for vaccine manufacturing. This study aims to evaluate the roller bottle platform as an alternative to static T-flask culture for producing attenuated PPR vaccines, focusing on optimizing conditions to enhance scalability and meet the growing demand for effective immunization strategies. The initial step in scaling up production typically involves a shift from stationary flasks to roller bottles, which is a widely adopted practice for many products. Roller bottles can have a surface area of up to 1750 cm^2, use 350 mL of medium, and have a volume of 2.5 [27], representing a ninefold increase in surface area, but only a threefold increase in medium and total volume. This is because the cells use the total internal surface area for growth, and more efficient aeration occurs because the cells move in and out of the culture fluid [25]. Vaccine demand is influenced not only by the number of doses produced in campaigns but also by the time window available for manufacturing [35]. The roller bottle (RB) expansion strategy entails a sequential increase in the number of roller bottles to optimize the production of Peste des Petits Ruminants (PPR) vaccine. Starting with one T 175 cm^2 flask expanded to 2 roller bottle 850 cm^2, then the system can be expanded to 6 bottles, further scaled to approximately 18 - 25, and ultimately reaching a total of 50 roller bottles. The expansion process from 2 RB to 50 rollers is projected to take approximately 16 days. The vaccine harvest within these roller bottles is estimated to yield 500 ml of virus harvest (2 × 250 ml). Consequently, this 500 ml equates to a substantial range of 50,000 to 100,000 antigen doses. Scaling up to 50 roller bottles is anticipated to yield 2.5 to 5 million antigen doses. To achieve a target of 100 million doses, an extended antigen production period of 20 to 25 weeks is foreseen, highlighting the significance of the roller bottle platform in the context of PPR vaccine manufacturing.

3.1. Vaccine Harvest

The control cells exhibited typical fibroblast-like morphology, growing in a monolayer until reaching confluence, characterized by elongated shapes and minimal cytoplasmic granulation. This observation aligns with the findings reported by Genari and Wada in 1995 [36] (**Figure 1(a)**).

Upon inoculation with the PPR Nigeria 75/1 vaccine virus, the cells remained healthy and comparable to the control cells until the third-day post-inoculation. However, on the fourth day, the initiation of cytopathic effect was evident, characterized by cell rounding (**Figure 1(b)**). This effect progressed gradually, leading to the aggregation of cells and the formation of syncytia (**Figure 1(e)**). The induction of syncytia by PPRV Nigeria 75/1 in Vero cells has been previously described by Lefevre and Diallo in 1990 [37], Seth and Shaila in 2001 [38], and Mahapatra *et al.* in 2006 [39]. Notably, this phenomenon was not observed in infected Vero-Montpellier cell cultures as reported by Osman *et al.* in 2019 [40].

The first harvest was conducted when the cytopathic effect (CPE) reached 40% - 50% on day 6 post-seeding (**Figure 1(e)**). The final harvest occurred on day 8 post-seeding, when CPE reached 80%. According to WOAH 2022 [31]. Subsequently, the culture flasks were frozen at −70˚C. All virus suspensions collected underwent two freeze-thaw cycles, followed by sterility testing. These suspensions were then pooled to create a single batch. The virus suspension was clarified by low-speed centrifugation (5 minutes at 1250 g). The titer obtained before lyophilization was 6.5 ± 0.25 $\log_{10}$ $TCID_{50}$/ml (refer to **Table 1**).

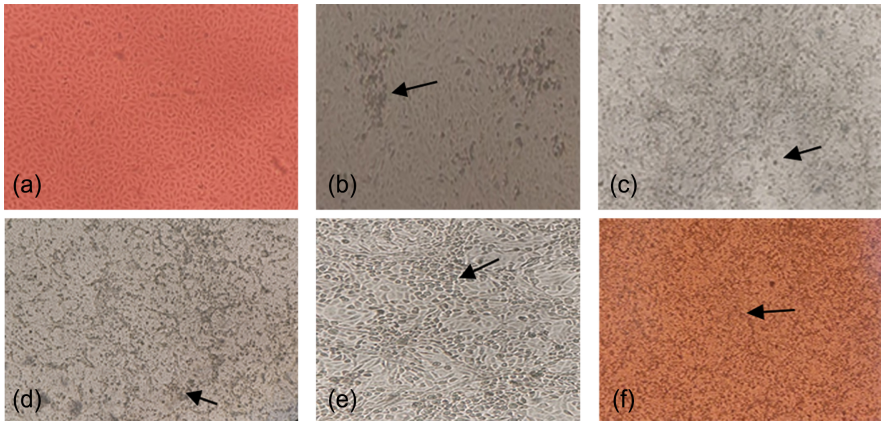

Figure 1. Progressive cytopathic effects in Vero cell culture following PPR Nigeria 75/1 inoculation. (a) Confluent monolayer of control Vero cell culture. (b) Cell rounding and clustering of cells. (c) Cytopathic effect on 5 days. (d) Cytopathic effect on 6 days. (e) Cytopathic effect on 7 days. (f) Cytopathic effect on 8 days/cells showing 80% CPE characterized by syncytial formation and eventual detachment of cells on day 8 post infection.

3.2. Identity

In this study, the identity of two batches of locally produced PPR vaccines was conformed as per the World (WOAH 2022) [31] guidelines and Couacy-Hymann *et al.*, 2002 [33] using RT-PCR technique that based on the amplification of fragments of N-protein yield an amplicon of the expected size of 350 bp (**Figure 2**).

3.3. Sterility

PPR-produced vaccine batch passed all the sterility tests performed, that is, it did not show any growth in the inoculated media. The culture media used in the

sterility test are fluid thioglycollate medium (FTM) and soybean-casein digest medium (SCDM). FTM is used to detect aerobic and anaerobic microorganisms, and SCDM is used to detect aerobic bacteria and fungi. The incubation period of both media is 14 days, and each medium has a specific incubation temperature [32]. The sterility testing method is described in chapter 1.1.9. of WAHO Terrestrial Manual 2023 [32] based on the observation of turbidity in liquid culture media due to the growth of potential contaminants.

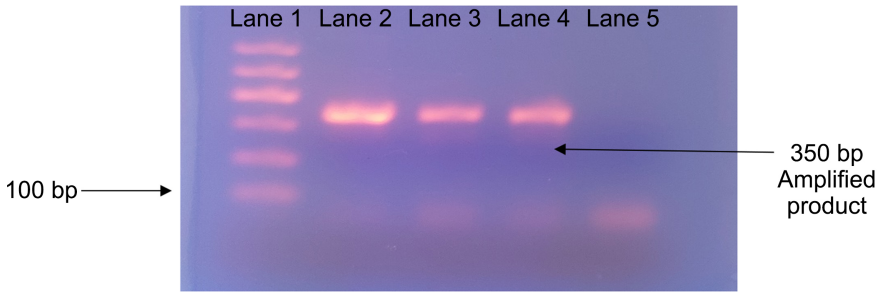

Figure 2. Amplification of 350 bp fragment of N gene by RT-PCR (from left - right). First lane, DNA ladder of 100 bp. Lanes 2 - 3 vaccine batch, Lane 4: Positive control, Lane 5: Negative control.

3.4. Mycoplasma Test

The three batches were subjected to testing for potential contamination with mycoplasma and were found to be devoid of any mycoplasma species. While conventional culture methods are sensitive, they are not ideal for routine assessment of cell line contaminations due to their time-consuming nature. Cultures were maintained for up to 4 weeks, as recommended by previous studies, allowing for four subcultures before drawing a negative conclusion [32]. A developed PCR method for mycoplasma detection [41] [42] was utilized as an alternative. This method offers fast, sensitive, and specific detection of mycoplasma contaminations, making it suitable for routine mycoplasma detection in cell cultures and bovine sera.

3.5. Safety

In our study, safety tests were conducted on sheep, administered both field doses and 100-time doses. No signs of swelling or redness were observed at the injection sites, and the animals exhibited normal body temperature and appetite throughout the study period. Additionally, no cases of abortion were recorded in pregnant animals. Previous research has also demonstrated the efficacy of this vaccine on over 98,000 sheep and goats in field trials conducted between 1989 and 1996, during which no adverse effects were reported [16]. As outlined in Chapter 1.1.8 of the Principles of Veterinary Vaccine Production in the WOAH Terrestrial Manual 2022 [43], regulatory authorities may choose to waive the target animal batch safety test (TABST) if a sufficient number of production batches have been produced under a seed lot system and have consistently complied with the test requirements. However, some regulatory bodies may still mandate safety tests for

each batch release.

3.6. Efficacy

Efficacy is determined by the titre of live virus in the vaccine batch (batch potency); whereas the control experiment with roller bottles yielded a virus titre range of 6.4 ± 0.11 $\log_{10}TCID_{50}$/ml in the three produced batches (**Table 1**). In the context of the attenuated PPR Nigeria 75/1 vaccine, the minimum titer per dose required has been established as $10^{2.5}$ $TCID_{50}$/ml [31]. Vaccination with the locally produced PPR vaccine (Nigeria 75/1) produced an antibody response in 100% (10/10) of negative sheep, as detected by cELISA (IDvet, Grabels, France). The commercially available cELISA is indicated by the WOAH (2022) [31] to assess antibody responses following PPR vaccination or infection and is among the most commonly used tests for this purpose [44]. A 100% seroconversion in experimental animals (mainly goats) vaccinated with the Nigeria 75/1 vaccine was reported in laboratory studies [44]-[46]. The pre-vaccination ELISA results of all animals in the three groups were found to be diagnostically negative (S/N over 50%), the animals in group A which received the normal recommended dose of PPR vaccine produced an antibody response in 100% (10/10) of negative sheep, as detected by cELISA (IDvet, Grabels, France) Thereafter, a steady decline was observed in the subsequent samplings at defined intervals. Significant antibodies were observed even at the end of the observation period *i.e.* 12 months post vaccination (**Figure 3**). This study demonstrated that immunization with locally produced PPR vaccine persisted the antibody for up to one year at the end of the study. Notably, the PPR vaccine strain Nigeria 75/1 demonstrated efficacy in conferring protection against PPR for at least 3 years [15].

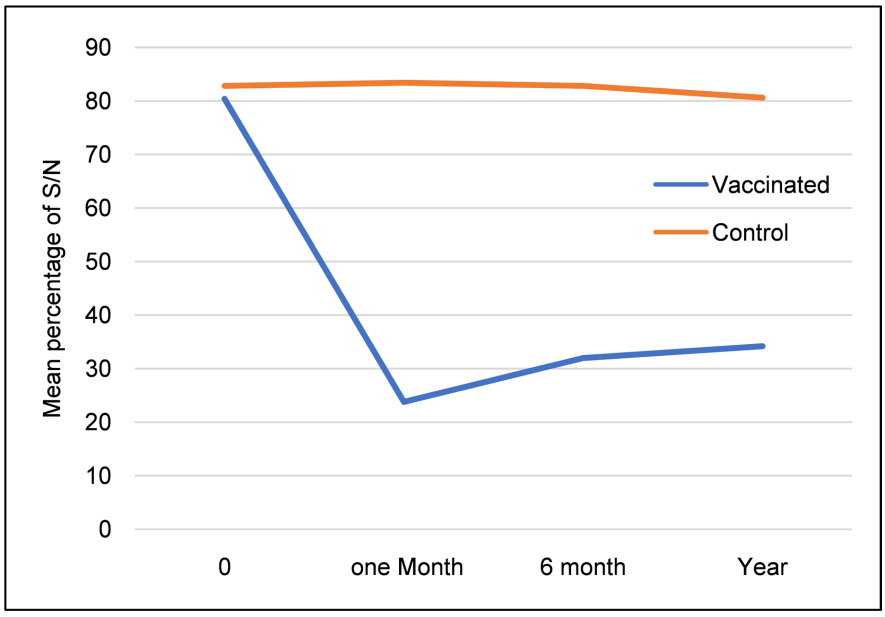

Figure 3. Mean percent inhibition (PI) values of PPR c-ELISA in animals among three treatment groups during 360 days of experiment.

3.6.1. Vacuum Test

The vacuum pressure and residual moisture content of the three batches of vaccines were measured after freeze-drying. The vacuum degree was deemed satisfactory. the freeze-dried PPR vaccine exhibits a visually appealing uniform and elegant "cake" appearance, which dissolves promptly upon dilution. The vaccine sample is transparent and free of aggregation. Cake appearance is an important attribute of freeze-dried products [47].

3.6.2. Residual Moisture

The residual moisture content was less than 2.5%, in line with AU-PANVAC standards. The outcomes indicate that the containing 5%(w/v) LAH and 10% sucrose stabilizers were successful in preserving the vaccine's integrity during the freeze-drying process. Significantly improved stability at temperatures above 4˚C with this cryoprotectant was reported [48] [49].

3.6.3. Stability

Real-time stability testing was conducted by titrating the vaccine stored under recommended conditions (4˚C and −20˚C) at regular intervals over one year (**Table 1**). The results confirmed that the vaccine remained stable for at least one year when stored at 2˚C - 8˚C or −20˚C. This finding is consistent with previous studies, which reported significantly improved vaccine stability at temperatures above 4˚C when a freeze-drying medium containing 5% (w/v) LAH and 10% sucrose was used [48] [49]. The consistency of titre across batches and time points can indicate the robustness of the lyophilized vaccine formulation and its suitability for storage and distribution.

Table 1. Titre of the freeze-dried PPR vaccine after keeping at 4˚C and −20˚C for one year at different periods intervals.

Batch No	Pre-lypholization Titre*	Post-lypholization Titre*	3 Month		6 months		12 months	
			4˚C	−20˚C	4˚C	−20˚C	4˚C	−20˚C
B1	6.5	6.5	6.5	6.5	6.5	6.5	6.3	5.9
B2	6.3	6.3	6.1	6.3	6.1	6.3	5.9	6.3
B3	6.8	6.5	6.5	6.5	6.3	6.3	6.3	6.5
Mean ± SD	6.5 ± 0.25	6.4 ± 0.11	6.3 ± 0.17	6.4 ± 0.19	6.2 ± 0.14	6.4 ± 0.16	6.16 ± 0.21	6.43 ± 0.23

*$\log_{10}TCID_{50}$/ml.

4. Conclusion

These results provide further insights into the feasibility of applying Roller bottle culture technology to produce PPR vaccine in Vero cells significantly simplifying the existing production process. The successful implementation of a roller bottle platform for PPR vaccine production in Sudan holds the potential to contribute significantly to the control and eradication of PPR in the region. Future studies should include economic analyses to evaluate the feasibility and potential benefits

of incorporating roller bottle technology into existing PPR vaccination strategies.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Acknowledgments

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Authers' Contributions

Conceptualization: Algezoli OA, Nour TAM, Abdalla MA. Algezoli OA: designed the study, performed the laboratory work, wrote the protocol, and wrote the original draft of manuscript. Muzdalifa AA: Laboratory work and experimental procedures. Nour TAM and Abdalla MA: Reviewed, edited the manuscript, and supervised. All authors read and approved the final manuscript.

Ethical Approval

This study rigorously follows institutional, national, and international guidelines, including the Basel Declaration, ensuring ethical standards, animal welfare, and scientific integrity. Approval was obtained from the Central Veterinary Research Laboratory/academic committee No. 021/092-8004365/2. Sample collection prioritized animal welfare. The study adheres to ARRIVE guidelines, ensuring transparency, rigor, and reproducibility for critical evaluation and replication of findings.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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