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Shelf Life and Accelerated Stability Studies of Porvac®, a Marker Subunit Vaccine Against Classical Swine Fever

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***Abstract*—** Porvac® subunit vaccine, based on the chimeric E2-CD14 antigen, induces a very early protective immune response against classical swine fever. It is an attractive alternative for modified live vaccines (MLV) in endemic areas. One of the main advantages of subunit vaccines over MLV, especially for low-income settings, is their better thermal stability. This investigation aimed to assess both, shelf life, and accelerated stability of Porvac®. Three batches of Porvac® were stored at 2 - 8 °C for 36 months and their organoleptic properties, rheology, droplet size, the mechanical and thermal stability of the emulsion, immunogenicity, and capacity to confer protection in piglets were evaluated at different time points. A short-term accelerated stability study was also conducted by incubating three different batches at 37 °C for 7 days. Finally, a one-month accelerated stability experiment was conducted with a single vaccine batch. The vaccine maintained its organoleptic properties and droplet size up to at least 36 and 24 months, respectively. The emulsion was mechanically and thermally stable up to at least 9 months, and its rheological parameters remained below the acceptance limits for up to at least 24 months. All vaccinated swine developed neutralizing antibody titers ⩾ 1:1000 at 28 days post-vaccination and were fully protected from the viral challenge at all the time points evaluated. In summary, Porvac® retains the capacity to induce protective neutralizing antibodies after 36 months of shell storage at 2 - 8 °C, and one month at 37 °C.

Keywords— NPLA; classical swine fever; virus, vaccine, stability, Montanide

I. INTRODUCTION

Classical swine fever (CSF) is an enzootic infection in Southeast Asia, Eastern Europe, Central and South America, and the Caribbean [1]. This disease causes considerable losses in the pig industry, mainly in developing countries [2], [3], therefore, it is a priority for the veterinary authorities worldwide. Continuous surveillance, linked to stamping out policies and vaccination, are the main tools for the regulation of CSF worldwide [1]. Multiple CSF virus (CSFV) outbursts have been described in Cuba, where the disease is endemic. This disease has a very negative economic impact on the Cuban economy [4], [5].

Immunization of swine with different types of vaccines has been conducted for decades and it is accepted as the most economic and viable procedure for CSFV control [5], [6]. The most effective CSF vaccines are Modified Live Vaccines (MLV), which can confer an early onset of protection [7], [8], [9].

Unfortunately, these vaccines are sensitive to high temperatures, and therefore, have to be preserved between 2-8°C [10]. This continuous cooled supply sequence is known as the "cold chain", and any interruption can provoke the administration of inefficacious vaccines. The disruption of the cold chain is one of the principal reasons for the failure of MLV vaccines. This issue is particularly severe in remote areas of the global south, which frequently lack the necessary infrastructure to maintain the cold chain [11].

Thermostability studies constitute a fundamental stage in the development of biotechnological products. The establishment of the range of thermal stability of the products during storage and distribution is a factor of great importance to ensure their effectiveness, avoiding gaps in any health system [12].

II. RELATED WORK

Porvac® is a CSF subunit vaccine recently registered for commercialization in Cuba. The antigen included in this vaccine is the hybrid polypeptide E2-CD154, which contains the extracellular moiety of E2 glycoprotein of CSFV fused to the extracellular region of the swine CD154 receptor. E2-CD154 is formulated in Montanide TM ISA50 V2 (SEPPIC, City, France). Previous results with Porvac® described full protection as early as 5 days after a single immunization [13]. The vaccine has also proved to be effective against vertical transmission in pregnant sows [14] and confers long-lasting immunity [15]. However, the stability of this vaccine has not been reported yet. Hence, this study aimed to assess the shelf stability of Porvac®, stored for up to 36 months at the standard temperature range of 2°C - 8°C, and the thermal stability of the vaccine at 37°C.

III. METHODOLOGY

Vaccine

Four GMP quality batches of Porvac® (P-51031; P-61011; P-61021 and P-81061-1) were used. All were produced at the facilities of the Centre for Genetic Engineering and Biotechnology of Camagüey. E2-CD154 protein was produced by growing the recombinant HEK 293 cell line (ATCC CRL1573) in a serum-free medium. After a very rapid and economic down string process, the E2-CD154 solution was mixed with Montanide^{TM} ISA50 V2 (SEPPIC, Paris, France) following a 60/40 ratio aqueous/oil phases [13]. The ‘‘water in oil” emulsion was generated with a mechanical homogenizer; model SD-41 (IKA, Germany). The final concentration of the E2-CD154 in the vaccine was 25 µg or protein/mL.

Experimental Swine

Non-vaccinated, nine weeks old hybrid Duroc/Yorkshire pigs (between 25 and 30 kg of weight) were used. The animals were obtained from a CSF-free herd at the Central Laboratory Unit for Animal Health (Havana, Cuba). The pigs were identified in the paddocks with notches. They were given 2 Kg of commercial forage per animal per day (ALYco CENPALAB, Cuba), and a continuous supply of water.

Sample Collection

Blood samples were taken from the animals by puncturing the ophthalmic venous sinus with sterile Pasteur pipettes, and placed for 2 h at room temperature, and for 12 h at 4 ^{0}C. The sera were collected by centrifugation for 10 min at 5000 x g and preserved at -20 ^{0}C.

Shelf life at 2-8°C

Samples from three batches of Porvac® (P-51031; P-61011 and P-61021) were stored at 2-8°C. Batches P-51031 and P-61021 were studied for up to 30 months; batch P-61011 was evaluated for up to 36 months. The following parameters were considered:

Organoleptic characteristics

The organoleptic properties of the vaccine formulation were visually evaluated every 6 months and up to 30 months. The vaccine is described to be a viscous, white bright, homogeneous emulsion.

Droplet size

The vaccine was 1:50 diluted in 10% Montanide^{TM} ISA50 V2 and examined by optical microscopy. The photography area was adjusted using the Periplan 12.5 x /20 eyepieces, which have a square in the centre of the circle (photography area) engraved on its lens. For phase contrasting, the image was observed with the 10/0.25 CP-A CHROMAT objectives and the 100/1.25 oil DPlan 100. A representative area was chosen to count the number of drops in the following ranges: A: less than 1 µm; B: from 1 µm to 2 µm; C: from 2 µm to 3 µm; D: from 3 µm to 4 µm; E: from 4 µm to 5 µm and F: greater than 5 µm. The following formula was used to calculate the percent of drops below the acceptance limits (drop size ≤ 5 µm). H = (A + B + C + D + E) / (A + B + C + D + E + F) * 100. Where: H: is the percent of drops ≤ 5 µm. This parameter was measured every 6 months and until 36 months. A minimum of 100 droplets were counted for each sample.

Rheological properties

The viscosity of the vaccine was determined using a Brookfield DV-III ultra-rheometer, coupled to a cryostat to allow control of the temperature of the sample. The rheometer was calibrated using the Brookfield 100 mPas-sec, and Brookfield 1000 mPas-sec reference materials, with rotor speeds of 60 and 250 rpm, respectively. After the cryostat reached a temperature of 20 °C, the content of the vaccine was placed into the reservoir designed for this purpose. The rotor speed was initially set at 60 rpm and gradually increased by 10 rpm up to 250 rpm. Three independent replicates of the viscosity readings were made for each sample. The acceptance limits for viscosity are v ≤ 1500 mPa-sec and the flow index, n <1. These parameters were measured at time 0 and after 6, 9, 12, 18, and 24 months of shell storage.

Mechanical Stability

Ten millilitres of the vaccine were dispensed into a 15 mL centrifuge tube. The initial height (Ho) of the emulsion was measured with a graduated ruler. The tubes were centrifuged for 1 hour at 3000 rpm (Hitachi SCT-5B centrifuge, 30 cm rotor radius). After this, the final height of the column (Hu) was determined. The mechanical stability was calculated through the Hu/Ho quotient for each replicate of the samples. According to the quality specifications of the product, the formulation passes the test if the average Hu/Ho ratio of 3 replicates was equal to or higher than 0.80. This ratio was calculated at time 0 for the three batches, at 6 months for batches P-61011 and P-61021 and at 9 months for batch P-51031.

Thermal Stability

To evaluate the thermal stability of the emulsion a sample of 10 millilitres was loaded into a 15 mL centrifuge tube

and the initial height (Ho) was measured. Subsequently, the tubes were placed in the humid chamber at 37 °C in a vertical position on a rack for 15 days. After this time the height of the column was measured again (Hu). The Hu/Ho quotient was determined for each replicate. According to the quality specifications of the product, the formulation passes the test if the average Hu/Ho of 3 replicates was equal to or higher than 0.90. This ratio was calculated at time 0 for the three batches; at 6 months for batches P-61011 and P-61021 and at 9 months for batch P-51031.

Immunogenicity and protective capacity

Five pigs were immunized with each batch at time 0 and every 6 months, until month 36. Each animal received two doses of 2 mL of Porvac® by the intramuscular route in the neck on days 0 and 21. Five non-vaccinated pigs were the negative controls of the viral challenge. Serum samples were taken at 0, 21, and 28 days post-vaccination (dpv), and the Neutralizing Peroxidase-Linked Assay (NPLA) was used to determine the NAb titres. Animals were intramuscularly inoculated with 10^5 LD_{50} of the virulent "Margarita" strain of CSFV, one week after the second immunization (28 dpv). The temperature of the vaccinated animals was measured 1 hour before, 1 hour after, and 4 days after each immunization.

One-week accelerated stability studies (thermal stress) at 37 °C

Three batches of Porvac® (P-51031; P-61011 and P-61021) were incubated for 7 days at 37 °C and immediately used to vaccinate pigs. Five animals were included in each group, and five unvaccinated pigs were used as negative controls. The immunization schedule was similar to the one described above. Serum samples were taken at 0, 21, and 28 dpv, and the NPLA was used to measure the NAb titres. Animals were challenged with 10^5 LD_{50} of the highly virulent "Margarita" strain of CSFV. A positive control group of five animals was immunized with the Porvac® vaccine conserved between 2 and 8 °C. The temperature of the vaccinated animals was measured 1 hour before, 1 hour after, and 4 days after each immunization.

One-month accelerated stability studies at 30 °C and 37 °C

One batch of Porvac® vaccine (P-81061-1) was stored at 30 °C or 37 °C for 30 days and immediately used to vaccinate pigs. Five animals were immunized for each experimental group, and five unvaccinated pigs were used as negative controls. The immunization schedule was similar to the one described above. Blood was collected at 0, 21, and 28 days post-vaccination (dpv), and the NAb titres were studied by the NPLA method. Animals were confronted with 10^5 LD_{50} of the "Margarita" strain of CSFV. A positive control group of five animals was immunized with the vaccine conserved between 2 and 8°C. The temperature of the animals was recorded 1 hour before and 1 hour after each immunization, and once a day during the 4 subsequent days.

Neutralizing antibodies detection

Serum samples were tested for the presence of neutralizing antibodies against the Margarita CSFV strain using the NPLA technique, as described elsewhere [16]. The assay was revealed with the horseradish peroxidase-conjugated monoclonal antibody CBSSE2.3 (CIGB-SS, Cuba), followed by 3-amino-9-ethyl carbazole (AEC), and H_2O_2. The presence of stained viral proteins in the cells was visualised under the optical microscope. The last dilution of the serum without any signal of the virus was regarded as the NAb titre. The geometric mean (GM) of the NAb titters plus the 95% confidence intervals were calculated for each experimental group.

Viral challenge

The challenge was conducted by intramuscular inoculation of 10^5 LD50 of the CSFV ¨Margarita¨ strain [17], according to the protocol referred by the Central Laboratory Unit for Animal Health (PNO VM0003/2010).

Clinical observation

After the viral challenge, the animals were observed daily looking for CSF-related clinical signs such as prostration, inappetence, and appreciable changes in respiratory rate. Rectal temperature was recorded daily. Clinical signs were scored for 21 days after the challenge according to Mittelholzer et al., [18] with minor modifications. Three parameters were eliminated (breathing, body shape, and body tension, and the rectal temperature was included.

Viral Isolation and Detection

Blood samples were collected from vaccinated animals in heparin-containing tubes at 21 days post-challenge (dpc). For the control group (non-vaccinated animals), samples were taken at 7 dpc. In addition, spleens, ileum, and tonsils were also extracted at sacrifice. A piece of 1 cm^3 of each organ was macerated in 1 mL of DMEM (Sigma, St Louis, USA) containing penicillin (100 IU), streptomycin (100 mg), and 5 % foetal calf serum The extracts were suspended in 4 mL of DMEM and incubated for 1 hour at room temperature. Samples were then centrifuged for 15 min at 1200 rpm, and the supernatant was stored at -70 °C. Viral isolation was conducted in PK15 cells following the protocol recommended by the World Organization for Animal Health (OIE 2019). Culture supernatants were passed twice consecutively in 24 wells microplates and a third passage was made onto 96 wells plates. Six replicates for each sample were tested. The plates were revealed with the horseradish peroxidase-conjugated monoclonal antibody CBSSE2.3, specific for the E2 protein (CIGB-SS, Cuba), followed by diaminobenzidine and H_2O_2.

Statistical Analysis

The normality of the data was evaluated with D'Agostino-Pearson tests. Kruskal-Wallis test was applied for the general comparison of the antibody titres among groups, followed by Dunn's multiple comparisons test to evaluate differences between individual groups. The GraphPad Prism 6 software was used for all the analysis (Prism 6 for

Windows, Version 6.01, GraphPad Software, Inc., La Jolla, USA). A $p<0.05$ was indicative of statistical significance.

IV. RESULTS

Shelf life of Porvac® at 2-8°C

The first study was designed to study the shelf life of Porvac® at the range of temperature recommended by the manufacturers for storage. The following parameters were studied:

Organoleptic characteristics

All vaccine vials were seen as viscous, white bright, homogeneous emulsions throughout the study. Batches P-51031 and P-61021 were observed for up to 30 months, and batch P-61011 for up to 36 months.

Droplet size

The distribution of the droplet size complied with the acceptance criteria throughout the study. The 100% of the droplets were ≤ 5µm in the 3 batches analysed up to 30 months of incubation.

Rheological properties

The time course of V and n values are summarized in table 1. Although some slight increases were observed in time, both rheological parameters remained well below the acceptance criteria for the 3 batches up to 24 months of study (v ≤ 1500 mPa-sec and the flow index, n <1).

Table 1. Rheological parameters of 3 batches of Porvac® with 24 months of follow-up

		Time (months)					
Batches		**0**	**6**	**9**	**12**	**18**	**24**
P51031	V	650	-	633	649	682	656
	n	0.768	-	0.767	0.769	0.763	0.765
P61011	V	643	676	-	672	668	752
	n	0.756	0.748	-	0.746	0.741	0.739
P61021	V	878.6	910	961.2	1114.4	1114.8	1168.8
	n	0.716	0.711	0.703	0.692	0.684	0.678

Mechanical and thermal stability of the emulsion

Table 2 shows the Hu/Ho quotients for both mechanical and thermal stability of the 3 batches of Porvac® studied for up to 6 or 9 months. All values met the acceptance criteria for these tests; therefore, the emulsion passed both tests after 6 months of shelf storage, or even 9 months in the case of batch P51031.

Table 2. Mechanical and thermal stability of the emulsion of 3 batches of Porvac® with 6 or 9 months of follow-up

	Mechanical stability			**Thermal stability**		
	Time (months)					
Batches	**0**	**6**	**9**	**0**	**6**	**9**
P51031	0,99	-	0.98	0.98		0.96
P61011	0,99	0.97	-	0.97	0.98	-
P61021	0,97	0.98	-	0.98	0.99	-

Safety and Immunogenicity

A total of 100 vaccinated pigs were followed up after vaccination, and no local reactions or behavioural changes were documented. All immunized pigs developed GM NAb titres above 1:100 after the first dose. All three vaccine batches stored at 2 to 8°C for up to 30 months were able to induce a NAb response, with geometric mean titres higher than 1:2000 after two immunizations. In addition, two of these batches were also shown to be immunogenic at 36 months (figure 1). Although NAb titres fluctuated between 1:2000 and 1:12000 within batches at different time points, no significant statistical differences were documented among the different time points (Kruskal-Wallis test, $p<0.05$). Additionally, no significant statistical differences ($p<0.05$) in the GM NAb titres among the three batches were documented for every experimental point studied.

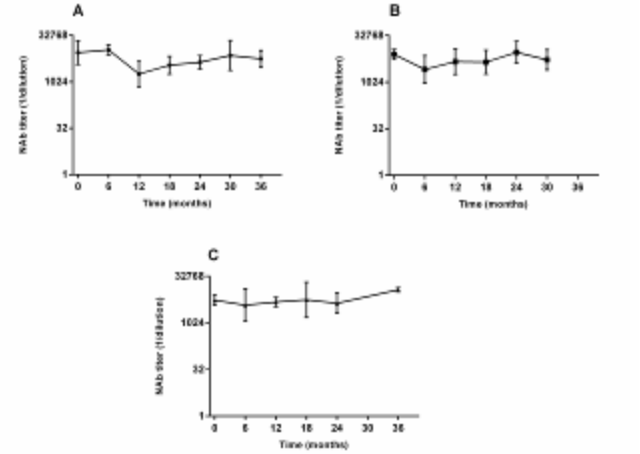

Figure 1. Time course of the neutralizing antibody response in piglets immunized with three batches of Porvac® stored at 2-8°C. Vaccine samples were evaluated every 6 months. A: batch P51031; B: batch P61011; C: batch P61021. The animals were vaccinated on days 0 and 21, and NAb titers were measured on day 28. Data are expressed as the GM of the NAb titers per group and the 95% confidence intervals.

Protection versus lethal CSFV challenge

All vaccinated animals were challenged at 28 days post-vaccination with the ¨Margarita¨ strain. For all batches and times studied, the immunized animals did not show clinical signs of CSFV after the confrontation. No pathological injuries were detected at 21 dpc. On the contrary, control animals developed fever with temperature values above 40.3 °C from 3 dpc. At 6 dpc other clinical signs were observed, such as conjunctivitis, diarrhoea, severe prostration, respiratory disorders, and nervous symptoms. In this branch, the pigs were euthanized at 8 dpc, with a clinical score of over 10 points. Specific lesions of CSFV infection were found at necropsy (mostly, marginal splenic infarction, suppurative tonsillitis, and extensive bleeding). No virus was isolated from the blood and organ samples of vaccinated animals, while 100% of the unvaccinated animals were positive for viral isolation.

One-week accelerated stability study

The animals immunized with the vaccine incubated for 7 days at 37°C developed NAb titres higher than 1:430 at 21

dpv. An anamnestic response was evident after the booster when the geometric mean NAb titres increase to 1:5700. No differences in the NAb titres were observed between the animals inoculated with Porvac® batches just after release, or after 7 days at 37°C (figure 2). All vaccinated animals in the study were protected from the viral challenge, showing no clinical signs, pathological lesions, or virus isolation. On the other hand, control animals developed clinical signs of CSF from the 3 dpc and had to be euthanized by 7 dpc.

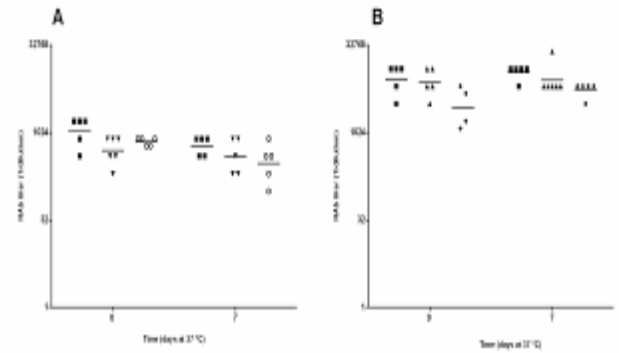

Figure 2: NAb response in pigs immunized with three batches of Porvac® immediately after batch production (time 0) and after 7 days at 37°C (day 7). Pigs were immunized at days 0 and 21, and NAb titres were measured at 21 dpv (A) and 28 dpv (B). Squares: bath P51031; triangles: batch P61011; circles: bath P61021. Dpv: days post-vaccination. Individual values of the NAb titres are plotted and the horizontal line represents the GM of the NAb titres for each experimental group.

One-month accelerated stability study

The vaccine batch P-81061-1, incubated for 30 days at either 30 °C or 37 °C, was as well tolerated as the same batch of vaccine stored at 4°C. No clinical signs or local reactions were documented after vaccination. No significant differences in the rectal temperature were observed among the four experimental groups after vaccination (Kruskal Wallis tests, p>0.05) (figure 3). In all cases, the rectal temperature values fluctuated within the physiological ranges described for the species.

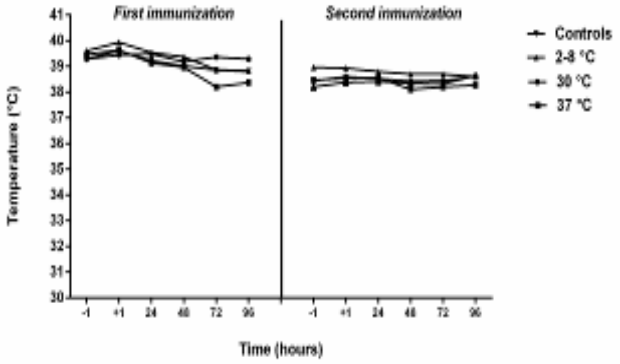

Figure 3: Rectal temperature of pigs after vaccination with Porvac®. Vaccine batch P-81061-1 was used. Left panel: temperatures after the prime immunization; right panel: temperatures after the second immunization. Groups: (A) non-vaccinated controls; (B) vaccine stored at 2-8 °C for 1 month; (C) vaccine stored at 30 °C for 1 month; (D) vaccine stored at 37 °C for 1 month.

On the other hand, all vaccinated pigs developed protective NAb titres 21 days after the first immunization. The first group (animals vaccinated with Porvac® incubated at 30 ± 3 °C for 30 days) showed GM NAb titres of 1:193 at 21 dpv. A similar outcome was observed in the piglets vaccinated with Porvac® incubated at 37 ± 3 °C for 30 days. NAb titres reached geometric mean values of 1:182 on day 21 after the first administration of the vaccine. Animals in group 3, immunized with Porvac® stored for 1 month at 4 °C, exhibited a higher GM NAb titre of 1:577. The differences among groups were statistically significant (Kruskal-Wallis test, p= 0.0348), although individual differences between groups did not reach statistical significance (Dunn test, p> 0.05) (figure 4).

Seven days after the booster, NAb titres increased in all groups, and, although group 2 exhibited the lowest GM NAb titres, no statistical differences were found among the 3 conditions studied (G NAb 1: 6941 in group 1; 1: 3663 in group 2, and 1:7620 group C (Kruskal-Wallis test, p=0.058) (figure 4).

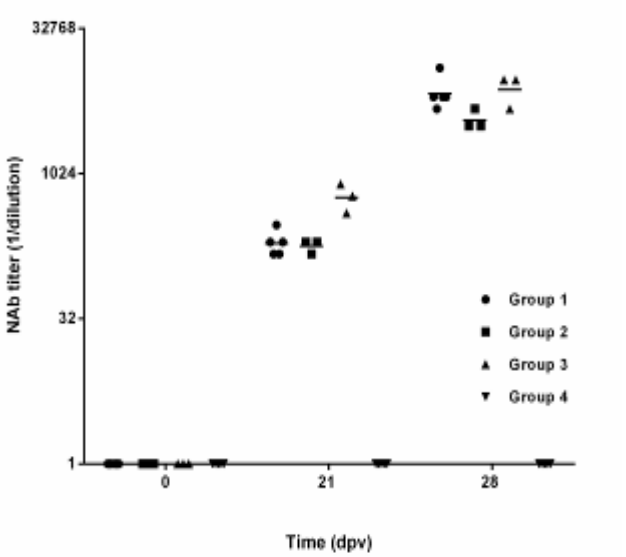

Figure 4: Neutralizing antibodies response in animals vaccinated with Porvac® after thermal stress. Batch P-81061-1 was used. Dpv: days post-vaccination. Group 1: (1 month at 30 °C); group 2: 1 month at 37 °C); group 3: 1 month at 4 °C; group 4: non-vaccinated controls.

After the viral challenge, all non-vaccinated control animals developed fever (> 40.4 °C) by 5 dpc (figure 5), together with several clinical signs of CSF. CSFV was readily isolated from those pigs that were sacrificed at 8 dpc. In contrast, none of the vaccinated animals developed fever (figure 5); showed neither clinical signs of the disease nor pathological lesions. The virus could not be isolated from the tissues of any of the vaccinated pigs.

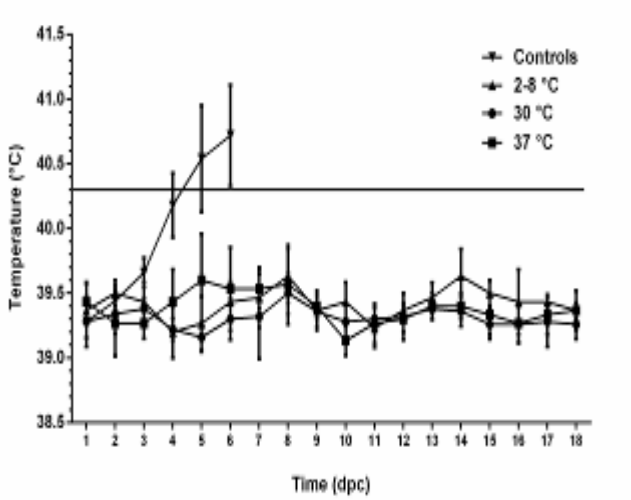

Figure 5. Rectal temperature after the viral challenge of pigs vaccinated with Porvac® after thermal stress. Batch P-81061-1 was used. (A): non-vaccinated controls; (B): vaccine stored at 4 °C for 1 month; C: vaccine stored at 30 °C for 1 month; (D): vaccine stored at 37 °C for 1 month.

V. DISCUSSION

Three types of vaccines have been used worldwide to regulate the spread of the CSFV: modified live virus (MLV), chimeric live recombinant viral vectors, and subunit vaccines based on the E2 viral protein [19]. Subunit vaccines have several advantages over live virus vaccines; for instance, they have a better safety profile and higher production capacities [20].

Another feature in which subunits vaccines are superior to MLV is in their thermal stability. Active virus vaccines may lose potency or even be completely damaged after exposure to temperatures outside the recommended range [10], [21]. The fact that almost all commercially available CSFV vaccines are thermally labile and must be kept at temperatures between 2–8 °C from manufacturing to dispensation, is an important financial and logistical load for vaccination campaigns [11], [21]. Expensive packaging conditions are required to preserve the required temperature during the vaccine distribution [11].

In particular, MLV vaccines are very susceptible to environmental conditions. Its efficacy can be significantly hampered if they are not stored in a temperature range of 2–8 °C. The issue is very sensitive for the global south, which usually lacks the optimal infrastructure for these operations [22].

Therefore, the production of stable vaccines can greatly contribute to reducing this obstacle and, in consequence, increase vaccine availability, especially in poor settings [11]. Subunit vaccines are usually less sensitive to high temperatures and, therefore, less dependable on a cold chain for distribution. Recombinant proteins are generally stable to temperature changes. Additionally, oil-based adjuvants are known to increase the shelf life of protein antigens [23], [24].

Porvac® is a subunit vaccine formulated as water in oil emulsion with Montanide™ ISA50 V2. This vaccine provides very quick protection against CSFV challenges and arrests both, horizontal and vertical transmission of the virus. The extension phase of Porvac® requires the support of appropriate data to demonstrate that the vaccine remains efficacious throughout the in-use shelf-life.

The results of this study showed that Porvac®, stored at 2-8°C, preserved its organoleptic and rheological properties for at least 24 months. Additionally, the mechanical and thermal stability of the emulsion were intact after 9 months of incubation at this temperature. These last parameters were only evaluated for 6 or 9 months according to the specification of the product. The mechanical and thermal stability tests are accelerated stability studies *per se* since the sample is centrifuged or incubated for 15 days at 37 °C, respectively. These accelerated studies can predict the stability of the emulsion at 2-4°C. It is generally accepted that if the emulsion past these tests it would be stable for at least 24 months at 2-8°C. In this particular case, since the vaccine passed the test 6 or 9 months after production, the predicted shelf life at 2-8°C would be at least 30 months.

The droplet size is another important parameter of water in oil emulsions. Small droplets increase the stability of the emulsion and allow more efficient dissemination of the antigen towards the lymphatic tissue [25]. The droplet size of Porvac® remained below the 5 µm limit throughout all the 30 months of the study.

Even more relevant is the fact that Porvac® preserved its immunogenicity and efficacy against a viral challenge with a highly virulent strain of CSFV for at least 36 months at 2°C - 8°C. Although the neutralizing antibody titres fluctuated during the different time points in the study, they were always well above the threshold for protection against CSFV infection which has been established as 1:50 by previous studies [26], [27]. None of the challenged piglets were infected or showed clinical signs of CSV after the challenge.

Porvac® stability outranges the one reported for another E2-based subunit vaccine, expressed in insect cells and formulated as double water/oil/water emulsion [28]. In the latter, the immunogenicity and protection were evaluated only for 18 months of shelf-life.

In addition to the stability at the recommended storage temperature of 2-8°C, Porvac® conserved its high protective capacity when exposed to thermal stress. The vaccine can be stored at 37°C for up to one month, without substantial loss of its protective capacity. Moreover, it also retains its safety profile.

WHO has classified the vaccines into four groups according to their thermal stability at 37°C: high (30 days); medium (14 days), moderate (7 days), and least stable (2

days) [29]. According to this classification, Porvac® can be considered a highly stable vaccine.

The one-month stability found in this study for Porvac® at 37 °C is considerably longer than the 1 week previously reported for another recombinant vaccine against CSF formulated in Montanide 888 [30]. A recombinant vaccine against cattle tick was stable for 14 days at 37 °C, although this study was conducted in punctured vaccine vials [31]. In contrast, seven proteins formulated in Montanide ISA 720, and incubated for one week at 37°C, experienced post-formulation modifications and reduced immunogenicity [32]. Therefore, the stability of each new vaccine must be carefully evaluated since it does not depend solely on the stability of the emulsion but also on the antigens included.

These results support previous considerations from experts in the field, that Porvac® is an attractive option for CSF control and eradication in endemic areas [33], [34], especially in the global south, where the harsh field conditions require a robust vaccine, lacking the shortcomings related to the thermal sensitivity of traditional MLV [35].

VI. Conclusion and Future Scope

The Porvac® vaccine against CSFV is stable for at least 36 months in shelf conditions (2- 8 °C) and up to one month at 37°C. The mechanical properties of the oil and water emulsion, and the immunogenicity and protective capacity of the vaccine are well preserved during these periods. These properties encourage the use of Porvac® in CSFV endemic countries in the global south.

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