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Classical swine fever virus vaccine stability in Lao PDR

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Abstract

Classical swine fever (CSF) virus is a highly contagious but vaccine-preventable disease of swine. A locally produced lapinised C-strain vaccine is used to control CSF in Lao PDR; however, vaccine failure has been reported. The CSF vaccine is produced at the National Vaccine Production Centre (NVPC) as a freeze-dried rabbit spleen homogenate in a rubber stoppered glass vial and stored at \(-20\ ^\circ\text{C}\) with a recommended shelf life of 1 year. This paper describes two studies to (i) determine the stability of the locally produced vaccine when stored at \(4\ ^\circ\text{C}\) and \(-20\ ^\circ\text{C}\) and (ii) determine if the vaccine elicits a protective immune response when delivered to village pigs under good transport conditions. The vaccine was found to be stable for only 4 months when stored at \(-20\ ^\circ\text{C}\) and for less than 3 months when stored at \(4\ ^\circ\text{C}\). Under field conditions, vaccine stored at \(-20\ ^\circ\text{C}\) for 2 months and transported at temperatures less than \(1\ ^\circ\text{C}\) elicited an immune response in 89\% of vaccinated pigs by day 35 and 100\% of pigs by day 70 post vaccination.

Introduction

The control and management of classical swine fever (CSF) virus in an endemic country is reliant on rapid disease recognition and the use of an effective vaccine. Commercially available vaccines for CSF are widely available, the most common being live attenuated virus vaccines or subunit vaccines of the immunodominant E2 protein. The most effective live attenuated vaccines are based on wild-type virus strains and include the C-strain vaccine, the Japanese guinea-pig-exaltation-negative (GPE--) strain derived from the virulent ALD strain, the cell culture adapted Thiverval strain derived from the virulent Alfort strain (de Smit 2000; van Oirschot 2003) and the PAV-250 strain (de Smit 2000). Traditionally, C-strain vaccine was produced from the organs of rabbits inoculated with a working seed (Terpstra et al. 1990). The C-strain virus has subsequently been adapted to cell culture systems for large-scale production of vaccine using the swine kidney cell line SK-6 (Terpstra et al. 1990) or minipig kidney (MPK) cells (Ferrari 1992; Rivero et al. 1988).

In Lao PDR the CSF vaccine is produced from the live attenuated C-strain virus from homogenised rabbit spleen and mesenteric lymph nodes freeze dried in the presence of stabilisers in a rubber stoppered vial (Khounsy et al. 2007). Only a small proportion of pigs are vaccinated in Lao PDR and vaccine failure has previously been documented and attributed to one or more of the following: (i) vaccine not viable at manufacture, (ii) inactivation due to incorrect storage and transport, (iii) incorrect administration of the vaccine or (iv) the presence of
maternally derived antibodies. The research presented in this paper specifically addresses the second factor, vaccine stability during transport and storage. The ‘cold chain’ in Lao PDR is perhaps the most important factor limiting the successful delivery of temperature-sensitive live virus vaccine that requires storage at –20 °C. Figure 1 represents a descriptive model of the cold-chain limitations under ideal conditions, where several temperature fluctuations occur and storage at provincial and district levels is inadequate according to the manufacturer’s recommendations.

To determine if the temperature fluctuations could be eliminated from the transport equation by storing vaccine at 4 °C instead of –20 °C, the long-term stability of the vaccine was assessed at these two temperatures. Secondly, the ability of the vaccine to elicit a protective immune response in village pigs was assessed.

Vaccine stability

Materials and method

A single batch (05/2004) of the lapinised CSF C-strain vaccine was procured from the National Vaccine Production Centre (NVPC) and transported on ice to the National Animal Health Centre (NAHC), Vientiane, Lao PDR. The vaccine batch was assessed by vaccinating four pigs that had been brought into the laboratory pens, allowed to acclimatise and treated with ivermectin and antibiotics to eliminate any infection. Two pigs were included as non-vaccinated controls.

One month after procuring the vaccine, an adequate volume of vaccine was stored at 4 °C and the remainder kept at –20 °C. Temperature was monitored for both lots of vaccine using a temperature logger (Thermocron, OnSolution, Australia) at 20-minute intervals. After 3 months’ storage at 4 °C, one group of four pigs was vaccinated with vaccine stored at 4 °C and a second group with vaccine stored at –20 °C for 4 months, and one unvaccinated pig was included as a control. As above, pigs were treated with ivermectin and antibiotics to eliminate infection prior to vaccination. After 4 months’ storage at 4 °C, the above protocol was repeated. Pigs were bled on days 0, 10, 14, 21 and 28 post vaccination (pv) to monitor neutralising antibody titre by the neutralising peroxidase linked assay (NPLA) (OIE 2004).

Results

Vaccine stored frozen during the experiment was held at an average temperature of –18.28 °C with a standard deviation of 1.28. Vaccine stored in the refrigerator was held at an average temperature of 4.34 °C with a standard deviation of 0.73.

All pigs vaccinated during the pre-trial assessment of batch number 05/2004 were CSF antibody negative on day 0 and all four vaccinated pigs were

![Figure 1. Conceptual model of CSF vaccine delivery in Lao PDR. The blue line represents adequate temperature and the red line represents substandard storage temperatures. PAFO = Provincial Agriculture and Forestry Office; DAFO = District Agriculture and Forestry Office. Storage times at PAFO and DAFO can be variable, depending on demand for vaccine.](image-url)
positive for the presence of CSF virus neutralising antibodies on day 35 pv, with a median titre of 1:44 (range 1:32–1:44). The median antibody titre on day 28 pv was 1:32 (range 1:22–1:32). Antibodies were first detected in one pig on day 10 pv, three pigs on day 14 pv and all pigs on day 21 pv.

For the pigs vaccinated with vaccine stored at 4 °C for 3 months, the median neutralising antibody titre was 1:4 (n=4; range 1:4–1:32); no significant difference could be demonstrated in comparison to the pre-trial assessment (Fisher Exact Test, p=0.07) due to the small sample number. However, storage at 4 °C for 3 months was four times more likely to result in vaccine failure than new vaccine (risk ratio=4, 95%CI: 0.73–21.84). For the pigs vaccinated with vaccine stored at 4 °C for 4 months, the median neutralising antibody titre was 1:4 (n=4; range 0–1:8), a significant difference when compared to new vaccine (Fisher Exact Test, p=0.01).

For the pigs vaccinated with vaccine stored for 4 months at –20 °C, the median neutralising antibody titre was 1:36 (n=4; range 0–1:352); no significant difference could be demonstrated in comparison to the pre-trial assessment (Fisher Exact Test, p=0.5) and the risk of failure after storage for 4 months at –20 °C was low (risk ratio=1.33; 95%CI: 0.76–2.35). For the pigs vaccinated with vaccine stored at –20 °C for 5 months, the median neutralising antibody titre was 1:4 (n=3; range 0–1:8), a significant difference when compared to new vaccine (Fisher Exact Test, p=0.01). One of the four pigs in this final group died during the experiment and no CSF virus antigen was detected in its organs.

Discussion

The cold chain for the delivery of frozen vaccine in Lao PDR is poor; as a result, the delivery of frozen vaccine to village pigs requires several freeze–thaw cycles and substandard storage temperatures. It is well recognised that CSF virus is adversely affected by temperature fluctuations such as repeated freezing and thawing. The principal aim of this research was, therefore, to determine if the locally produced CSF vaccine could be stored at 4 °C for prolonged periods of time and remain immunogenic. An added component of this research was one of quality assurance—determining an estimate of vaccine shelf life when stored at –20 °C.

The results of this study show that, for this batch at least, the vaccine cannot be stored at 4 °C for extended periods of time and remain viable. After 3 months’ storage at 4 °C at the NAHC with no temperature fluctuations, the vaccine was not able to elicit a good immune response in test animals. Somewhat surprisingly, this study found that the vaccine is not stable for at least as long as the manufacturer recommended. The vaccine was still viable after 4 months’ storage at –20 °C but was unable to elicit a good immune response in test animals when stored for 5 months. This experiment was conducted under ideal conditions of storage and transport; it is anticipated that under field conditions the vaccine stability would be even less. Many provincial and district vaccine storage freezers are unable to maintain temperatures in the range of –15 °C to –20 °C. To navigate through the constraints of delivering a quality CSF vaccine to a village pig, a great deal of planning will be required on the part of the Lao animal health service. More research and investment is required to address the quality of CSF vaccine produced at the NVPC and its subsequent delivery to village farms.

Vaccine delivery at the village level

Material and methods

Two villages in Bolikhān district, Bolikhāmxaဿ province, were selected for this study. Thirty CSF vaccine vials (300 doses) were procured from the NVPC (batch number: 03/2006) and stored frozen at the NAHC for approximately 2 months. The vaccine was transported in an ice box to the selected villages and the temperature was monitored throughout with a temperature logger (Thermocron, OnSolution, Australia) at 20-minute intervals.

All pigs in the villages were vaccinated (excluding pregnant sows and piglets <1 week of age). Blood samples for serology were collected from 10 pigs in each village prior to vaccination and again 35 and 70 days post vaccination. Sera were tested in the complex trapping blocking (CTB)-ELISA at the NAHC, and a portion of the samples were also sent to the CSIRO Australian Animal Health Laboratory (AAHL), Geelong, Australia, for testing by the NPLA and Ceditest (CEDI Diagnostics, the Netherlands) for CSF antibody.

Results

The average storage temperature at the NAHC over the 2 months prior to vaccination was –21.2 °C (standard deviation: 1.8). During transport to the village, the temperature within the icebox was main-
tained at or below 0 °C, with logger readings gradually increasing from –14.5 °C to 0 °C.

By NPLA, one pig was weakly positive for antibodies to CSF virus on day 0 pv (titre=1:8) and the remaining 18 pigs were negative for neutralising antibodies. One serum sample could not be tested by NPLA due to cell toxicity. By day 35 pv, only 19 pigs remained in the cohort and 17/19 (89%) were positive for the presence of neutralising antibodies; however, only 6/19 (32%) pigs had antibody titres ≥1:32. On day 70 pv, 18 pigs remained in the cohort and all were positive for the presence of neutralising antibodies, with 17/18 (94%) having an antibody titre ≥1:32. The Ceditest had very strong agreement with the NPLA results (kappa=0.88) and diagnostic specificity and sensitivity were 90% and 97%, respectively. The CTB-ELISA, on the other hand, had very poor agreement with the NPLA results (kappa=0.32) and diagnostic specificity and sensitivity were 100% and 39%, respectively.

Discussion

This study has clearly demonstrated that, under ideal storage and transport conditions, a relatively new batch of vaccine can elicit a protective immunity in village pigs. Seventy days after vaccination, 94% of pigs had an antibody titre ≥1:32, which, for epidemiological purposes, affords complete protection and prevents virus shedding (Terpstra and Wensvoort 1988). Post-vaccinal antibody titres can continue to increase for up to 12 weeks (Dahle and Liess 1995; Terpstra et al. 1990; Terzic et al. 2003), and this was observed during this study. The NVPC recommends that the vaccine be stored at –20 °C for up to 1 year; however, as demonstrated above, the vaccine loses viability after 4 months’ storage under recommended conditions. The capacity of rural agricultural offices to hold vaccine at –20 °C is low regardless of the timeframe it can be stored for; therefore, the critical issues of vaccine stability and delivery remain. Future strategies for the improvement of vaccine delivery at the village level need to be put in place if farmers are expected to embrace this technology.

This study has also highlighted the critical issue of having the capacity to monitor vaccination success. The only antibody detection test routinely available for CSF in Lao PDR is the CTB-ELISA. However, this test was unable to reliably detect vaccinated positive animals, with a sensitivity of just 39% and a very low level of agreement with the NPLA (kappa=0.32). Additional work is required to increase the capacity of the laboratory to enable the detection of vaccine-related serological responses. The Ceditest and NPLA are expensive tests in comparison to the CTB-ELISA, and could not be introduced into mainstream laboratory testing without the continued support of foreign donors. Research is required to develop a simple and inexpensive alternative to the CTB-ELISA that is capable of sensitive and specific detection of vaccinated positive animals.

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