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Preparation and heat resistance study of porcine reproductive and respiratory syndrome virus sugar glass vaccine

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ABSTRACT

To improve the preservation period without cold-chain of the live attenuated vaccine of porcine reproductive and respiratory syndrome (PRRS), a set of thermostable formulations composed of trehalose, tryptone and other protectants were dried by vacuum foam drying (VFD) along with PRRSV solutions. In the 37 °C and 45 °C resistance ageing test, the dried foam vaccine showed significant thermostability, and the virus titer lost 0.8 Log_{10} at 37 °C for 4 months, 1.0 Log_{10} at 45 °C for 25 days. Furthermore, the foam vaccine could be stored at 25 °C for at least one year. Besides, the vaccine preserved in 37 °C, 25 °C and 4 °C for 3 months were inoculated on 20-days old piglet, and the serum titer was monitoring by ELISA kit. Inoculated two weeks later, the ELISA titer were all qualified and had the similar level compared to the commercial vaccines of the lyophilization dosage.

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is a highly contagious disease, which cause acute reproductive failure in sows and respiratory disease in pigs of all ages. The pathogen is the porcine reproductive and respiratory syndrome virus (PRRSV). Vaccination is one of the important prevention and control measures of the disease. Live attenuated vaccine immunization can stimulate humoral immunity and cellular immunity in pigs, bring broader protection against heterologous PRRSV strains. At present, three additional commercial Highly pathogenic PRRS modified live virus vaccines (HP PRRS MLV), JXA-1R [1–3], TJM-F92 and HuN4-F112, were all introduced into the Chinese swine industry, all providing adequate protection of pigs to HP-PRRSV infection [4,5].

Currently, live attenuated vaccines are mostly lyophilized products. Although relatively heat resistant, freeze-dried vaccines would still need to be preserved in cold-chain, such as −20 °C and 37 °C [6,7]. The titer of PRRS freeze-drying vaccine lost 1.0Lg $TCID_{50}$ at 2–8 °C for 7–14 days. Therefore, there is a desire for increased heat stability, especially on transport and storage.

The vacuum foam drying (VFD) is a scalable technology for preservation of sensitive biotherapeutics in the dry state. In 1998, Bronshtein reported therapeutic biomolecules stable at moderate temperatures and pressures, such as enzymes and vaccines, can be stabilized in the presence of common protectants by VFD [8,9]. The process which based on the principle of evaporation under vacuum at low temperatures [10], can be performed using the commonly available lyophilizers [11,12]. The solutions of biologically-active material is transformed into foam by boiling under vacuum, for 60 to 90 min, at a temperature initially no greater than 37 °C [13] and not to fall to 0 °C. The foam consists of thin films, because water removed at an elevated temperature efficiently [10]. The VFD vaccine possessed a high glass transition temperature, which could stable preserved for a long time.

In 2000, Worrallhad dried Rinderpest and the Pese des petits ruminants attenuated vaccine (PPRV) strain by VFD and the virus titer was no loss during the drying process; the PPRV vaccine could be stored at 37 °C for 7 days and 25 °C for 14 days [14]. Vu Truong-Le prepared live attenuated influenza virus vaccine by the formulation of 40% sucrose, 5% gelatin and 25 mM 7.2 pH KPO_4 buffer, which could be stored at 37 °C for 146 days and 25 °C for two years (2006, 2007) [15,16]. Satoshi Ohtake reported that Salmonella

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Ty21a bacterial vaccine appeared translucent, foamy structure, and the foam product could be stored at 37 °C and 25 °C for 12 weeks (2011) [17]. In 2015, live attenuated rabies virus (RABV) was preserved by vaporization in a dry, stable foam, which remains viable for at least 23 months at 22 °C [18].

The purpose of this research was to develop heat-resistant PRRSV vaccine by VFD. We had screened foaming agents and optimized process parameters of VFD for PRRSV. The PRRSV VFD vaccine was more thermostable than frozen dried product and had good immunogenicity.

2. Materials and methods

2.1. Materials

The Highly Pathogenic Porcine Reproductive and Respiratory Syndrome attenuated vaccine (JXA1-R strain) was purchased from Guangdong Wens Dahuanong Biotechnology Co.,Ltd. Marc-145 cells (ATCC CRL-12231) were cultured in modified Eagle's medium (MEM) with 10% newborn calf serum as described previously [19]. The virus was blindly passaged in MARC-145 cells for three times and the progeny viruses were served as the working virus stock.

Trehalose dehydrate (Tre), Ethylene Diamine Tetraacetic Acid (EDTA), Tryptone (Try), Sucrose (Suc), Tris, Dextran-40 (Dex), and Glycerol (Gly) were purchased from Sigma–Aldrich (St. Louis, MO).

Porcine Reproductive and Respiratory Syndrome Virus Antibody Test Kit (PRRS X3 Ab) was purchased from IDEXX.

2.2. Single factor design of fomulation

We had screen a group formulation T1 before, which contain 17.5% (w/v) Tre, 25 mM KPO_4, and pH7.2 (With phosphoric acid, potassium hydroxide as a pH adjustment). Based on T1, the single component experiment was designed in Table 1. Fifteen-group T1 series of heat-resistant protective agent were prepared.

2.3. Vacuum foam drying of PRRSV

The PRRS virus solution and formulations were mixed as 1:1 volume rate, packed in Vials, gradient foam vacuum dried using Virtis Ultra freeze-dryers (SP Scientific, Warminster, PA, USA). The shelf temperature was 25 °C and the condenser temperature was set below −40 °C. Vials were then placed on the shelves at 25 °C for 15 min. The initial pressure of the dryer chamber was 60,000 Pa, and then decrease in a stepwise manner to 300 Pa for 1 h. Afterwards the shelf temperature was raised to 28–30 °C, and the vacuum was decreased to 10 Pa for 12 h. Lastly the pressure of the chamber was decreased to 1.0 Pa for 18–24 h. The whole dry process of total is 30–42 h.

2.4. Appearance, foaming success rate, residual moisture analysis (RM) and Scanning electron micrograph (SEM) of the VFD vaccine

After drying, we counted the foam vials, calculated the foaming success rate, and detected the foam height and RM of VFD vaccine [20] (The foaming success rate% was defined as following equation: Foam succeed vials/All vials × 100%). The micro-appearance of VFD samples was analyzed for product morphology using a scanning electron microscope (s-3400n II, Japan Hitachi).

2.5. Measurements by DSC

DSC studies were performed to determine the glass transition temperature (Tg) of the VFD samples to investigate their stability by using a DSC (PerKinElmer, Pyris1). Samples in the range of 100–200 mg were analyzed in crimped, vented aluminum pans under a dry nitrogen purge with an automated liquid nitrogen-cooling accessory. Samples were heated from −50 to 150 °C with a scanning rate of 10 °C/min.

Table 1
Screening results of thermostable protectants.

Formulation	$TCID_{50}$/ml of products		The lost of PRRSV titer Log_{10}	Residual moisture (RM) %
	Zero time	Storage in 37 °C after 10 days		
T1	6.60	6.17	0.43	3.66
T2	6.38	5.62	0.76	>4
T3	6.80	5.83	0.97	3.98
T4	6.50	6.20	0.3	3.03
T5	6.76	6.32	0.44	3.12
T6	6.76	5.93	0.83	3.75
T7	6.67	6.29	0.38	3.05
T8	6.67	5.90	0.77	>4
T9	6.67	6.01	0.66	>4
T10	6.76	5.20	1.56	>4
T11	6.76	5.30	1.46	>4
T12	6.83	5.50	1.33	>4
T13	6.67	5.67	1.00	>4
T14	6.67	5.75	0.92	>4
T15	6.67	5.43	1.24	>4

T1: contain 17.5% (w/v) Tre, 25 mM KPO_4, pH7.2; T2: contain T1 and 1% EDTA; T3: contain T1 and 2% EDTA; T4: contain T1 and 1.5% Try; T5: contain T1 and 3% Try; T6: contain T1 and 0.025 M Tris; T7: contain T1 and 0.05 M Tris; T8: contain T1 and 3% Dex; T9: contain T1 and 5% Dex; T10: contain T1 and 1.5% Suc; T11: contain T1 and 3% Suc; T12: contain T1 and 6% Suc; T13: contain T1 and 0.5% Gly; T14: contain T1 and 1.0% Gly; T15: contain T1 and 2.0% Gly.

2.6. Thermal stability of foam vaccine and freeze drying vaccine

The dried products were placed in 25 °C, 37 °C and 45 °C. The $TCID_{50}$ was measured at different time points, and the end point was determined by the decrease of 1.0 Log_{10} of the virus titer.

2.7. Immunogenicity study

20 day-old healthy piglets were inoculated by VFD and FD vaccine, and 10 pigs of each group. Group A, B and C were vaccine by VFD vaccine of 3 months preserved at 37 °C, 25 °C and 4 °C respectively, while Group D was FD vaccine. The potion was 10^6 $TCID_{50}$ virus per piglet. The blood antibody in 2 weeks, 3 weeks, 4 weeks after inoculating was measured by ELISA.

3. Results

3.1. Screening of heat resistant protective agents

The $TCID_{50}$/ml of the products was detected (Table 1). After stored at 37 °C for 10 days, T1, T4, T5 and T7 lost only 0.43 Log_{10}, 0.3 Log_{10}, 0.44 Log_{10} and 0.38 Log_{10} in 15 formulations, and RM were no more than 4%. The results showed that the heat resistance of dry vaccine is a little better after adding 0.05 M Tris buffer or 1.5% Tryptone to the T1. In addition, the RM of T4 was the smallest, and its titer lost lowest. It was concluded that the RM was less, the heat resistance of the vaccine was better. There was greater correlation of stability with moisture content. The VFD vaccine which RM nearly 3% (T4, T5 and T7), were placed at 37 °C for longer time, and T1 was same treated as control fomulation.

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