Veterinary Microbiology 174 (2014) 86–92

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

The spray-drying process is sufficient to inactivate infectious porcine epidemic diarrhea virus in plasma

Priscilla F. Gerber [a], Chao-Ting Xiao [b], Qi Chen [b], Jianqiang Zhang [b], Patrick G. Halbur [b], Tanja Opriessnig [a,b,*]

[a] The Roslin Institute and The Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Midlothian EH25 9RG Scotland, UK
[b] Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA

ARTICLE INFO

Article history:
Received 20 June 2014
Received in revised form 10 September 2014
Accepted 12 September 2014

Keywords:
Porcine epidemic diarrhea virus (PEDV)
Spray-dried porcine plasma (SDPP)
Transmission, RT-PCR
Inactivation

ABSTRACT

Porcine epidemic diarrhea virus (PEDV) is considered an emergent pathogen associated with high economic losses in many pig rearing areas. Recently it has been suggested that PEDV could be transmitted to naïve pig populations through inclusion of spray-dried porcine plasma (SDPP) into the nursery diet which led to a ban of SDPP in several areas in North America and Europe. To determine the effect of spray-drying on PEDV infectivity, 3-week-old pigs were intragastrically inoculated with (1) raw porcine plasma spiked with PEDV (RAW-PEDV-CONTROL), (2) porcine plasma spiked with PEDV and then spray dried (SD-PEDV-CONTROL), (3) raw plasma from PEDV infected pigs (RAW-SICK), (4) spray-dried plasma from PEDV infected pigs (SD-SICK), or (5) spray-dried plasma from PEDV negative pigs (SD-NEG-CONTROL). For the spray-drying process, a tabletop spray-dryer with industry-like settings for inlet and outlet temperatures was used. In the RAW-PEDV-CONTROL group, PEDV RNA was present in feces at day post infection (dpi) 3 and the pigs seroconverted by dpi 14. In contrast, PEDV RNA in feces was not detected in any of the pigs in the other groups including the SD-PEDV-CONTROL group and none of the pigs had seroconverted by termination of the project at dpi 28. This work provides direct evidence that the experimental spray-drying process used in this study was effective in inactivating infectious PEDV in the plasma. Additionally, plasma collected from PEDV infected pigs at peak disease did not contain infectious PEDV. These findings suggest that the risk for PEDV transmission through commercially produced SDPP is minimal.

1. Introduction

Spray-dried porcine plasma (SDPP) is used widely in diets for weanling pigs to improve their growth rate and feed intake, and to reduce diarrhea (Peace et al., 2011; Pettigrew, 2006; Van Dijk et al., 2001; Zhao et al., 2007). The plasma utilized for production of SDPP is collected at veterinary-inspected abattoirs from animals designated as fit for human consumption. Specifically, blood is collected into containers with anticoagulant and the erythrocytes are removed by centrifugation. The plasma obtained is subsequently spray-dried and used for the production of food, feed and for industrial applications. It has been shown that specific spray-drying conditions substantially reduce the number of viable microorganisms (Ananta et al., 2005; Polo et al., 2005; Pujols et al., 2007). The blood is

* Corresponding author at: The Roslin Institute, University of Edinburgh, Easter Bush, Midlothian EH25 9RG Scotland, UK. Tel.: +44 (0)131 651 9177
E-mail addresses: tanja.opriessnig@roslin.ed.ac.uk, tanjaopr@iastate.edu (T. Opriessnig).

http://dx.doi.org/10.1016/j.vetmic.2014.09.008
0378-1135/

pooled from 6000 to 10,000 animals slaughtered on the same day, resulting in a mixture of antibodies against various pathogens that have a neutralizing effect on potential pathogens present in the plasma and can be considered as an effective biosafety step in the manufacturing process of SDPP (Polo et al., 2013).

Porcine epidemic diarrhea virus (PEDV), an enveloped single-stranded, positive-sense RNA virus which belongs to the family *Coronaviridae*, was first reported in feeder and fattening pigs in the UK in 1971 (Wood, 1977). PEDV causes an acute, highly contagious diarrhea in pigs of all ages with up to 90–95% mortality in suckling pigs in naïve breeding herds (Stevenson et al., 2013; Sun et al., 2012; Temeeyasen et al., 2013; Wang et al., 2013). Outbreaks of PEDV have occurred in all pig producing areas in Europe (Song and Park, 2012) followed by introduction into Asia in the early 1990s (Kusanagi et al., 1992). More recently, PEDV was introduced to North America and rapidly spread across farms and states causing large-scale outbreaks with high rates of morbidity and mortality (Huang et al., 2013; Mole, 2013; Stevenson et al., 2013). Thus far, there is no effective vaccine or specific treatment available, and the only measures to control the disease are those directed at preventing the introduction of the virus to farms (Park et al., 2011; Song and Park, 2012; Temeeyasen et al., 2013).

The source of introduction and routes of transmission of PEDV in North America are being investigated. Potential routes of PEDV transmission include direct and indirect contact with feces of infected animals (Jung et al., 2014; Saif et al., 2012), contaminated transport vehicles (Lowe et al., 2014), and milk from infected sows (Sun et al., 2012). Recently, SDPP products were implicated to transmit PEDV in the USA and Canada (Anonymous, 2014). Analytical results released by the Ontario Ministry of Agriculture and Food indicated the presence of PEDV RNA in commercial SDPP; bioassay tests confirmed infectious PEDV in the SDPP but not in the associated feed in which it was used as an ingredient (Pasick et al., 2014).

To investigate the potential of plasma obtained from PEDV infected pigs to contain infectious PEDV and to investigate the infectivity of PEDV after the spray-drying process, plasma samples derived from PEDV positive piglets or plasma samples spiked with cell culture propagated PEDV were tested in a swine bioassay, either untreated (raw) or after spray-drying. The objectives of this study were to (1) evaluate whether plasma from PEDV infected pigs at the peak of disease contains infectious PEDV and (2) if the spray-drying process is efficient to inactivate PEDV.

2. Material and methods

2.1. Ethical statement

The experimental protocol in this study was approved by the Iowa State University Institutional Animal Care and Use Committee (Approval no. 2-14-7742-S; approved on 5-Mar-14).

2.2. Animals and housing

Colostrum-fed, crossbred, specific-pathogen-free, 2-week-old pigs were purchased from a PEDV-free herd. Sixteen pigs obtained from six litters were transported to biosafety level 2 research facilities at Iowa State University, Ames, IA, USA. Upon arrival, the pigs were ear-tagged, randomly divided into groups of two-to-four pigs and housed in five separate rooms. Each room had 18 m^2 of solid concrete floor space, separate ventilation systems, and one nipple drinker. All groups were fed *ad libitum* a balanced, pelleted, complete feed ration free of animal proteins (Heartland Coop, Prairie City, IA, USA).

2.3. Experimental design and treatments

The experimental design is summarized in Table 1. Treatments included spray-dried plasma from PEDV negative pigs (SD-NEG-CONTROL), spray-dried plasma from pigs experimentally infected with PEDV (SD-SICK), PEDV negative plasma spiked with PEDV and then spray dried (SD-PEDV-CONTROL), liquid (raw) PEDV negative plasma spiked with PEDV (RAW-PEDV-CONTROL), and liquid (raw) plasma from pigs experimentally infected with PEDV (RAW-SICK). Experimental inoculation was done after a one week acclimation period when the pigs were 3 weeks old. Fecal swabs were collected using polyester swabs two days prior to inoculation, and at days post-inoculation (dpi) 3, 5, 7, 11, 14, 21 and 28 and stored in 5 ml plastic tubes containing 1 ml of sterile saline solution (Fisher Scientific, Inc.). Blood samples were collected at dpi 2, and at dpi 7, 14, 21 and 28. The blood was collected in 8.5 ml serum separator tubes (Fisher Scientific, Inc.), immediately centrifuged at $3000 \times g$ for 10 min at 4 °C, separated, and stored at −80 °C until use. Following challenge, the pigs were individually monitored daily. Pigs were weighed at dpi 0 and 28.

2.4. Plasma sources and processing

Plasma was obtained by collecting blood from pigs euthanized with an overdose of pentobarbital (Fatal Plus, Vortech Pharmaceutical, Dearborn, MI, USA) in jars containing 12,000 USP heparin units (Hospira Inc., Lake Forest, IL, USA) per liter of blood. The plasma was immediately centrifuged at $2000 \times g$ for 10 min at 4 °C in 50 ml centrifuge tubes and stored at 4 °C until use.

2.4.1. PEDV-negative plasma

PEDV RNA and antibody negative plasma was obtained from five 15-week-old crossbred PEDV naïve pigs as part of another study (T. Opriessnig, unpublished data). One liter of the PEDV-negative plasma was spray-dried two hours after plasma collection and served as sham-inoculum for the NEG-CONTROL group.

2.4.2. PEDV-spiked plasma

Ten milliliter of the PEDV-negative plasma described under "PEDV-negative plasma" was spiked with a cell culture propagated PEDV strain ISU13-19338E (Chen et al., 2014) to a final concentration of 5×10^2 50% tissue culture