Veterinary Microbiology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Veterinary Microbiology

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

Use of processing fluids and serum samples to characterize porcine reproductive and respiratory syndrome virus dynamics in 3 day-old pigs

Carles Vilalta[a,*], Juan Sanhueza[a], Julio Alvarez[b,c], Deb Murray[d], Montserrat Torremorell[a], Cesar Corzo[a], Robert Morrison[a,1]

[a] Veterinary Population Medicine Department, College of Veterinary Medicine, University of Minnesota, 1365 Gortner Ave., St Paul, MN 55108, United States
[b] VISAVET Health Surveillance Center, Universidad Complutense, Avda Puerta de Hierro S/N, Madrid, 28040, Spain
[c] Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad Complutense, Avda Puerta de Hierro S/N, Madrid, 28040, Spain
[d] New Fashion Pork, Jackson, MN, United States

ARTICLE INFO

Keywords:
PRRSV
Monitoring
Processing fluids

ABSTRACT

Collection of serum samples of pigs at weaning to monitor for porcine reproductive and respiratory syndrome virus (PRRSV) has become a common practice to determine PRRSV herd infection status. Diagnostic sensitivity of this practice is low in herds undergoing PRRSV elimination once prevalence of infection is near zero. Thus, the goal of this study was to characterize the dynamics of PRRSV infection in 3 day-old pigs overtime using serum and serosanguineous fluids obtained as part of castration and tail docking practices (processing fluids (PF)). Secondary goal was to estimate sensitivity and specificity of PF in the 3 day old population. A 6000 breed-to-wean sow herd was monitored every three weeks for 23 weeks after a PRRSV outbreak by collecting both PF and individual serum samples from all pigs in the selected litters. Out of the 77 litters tested, 23 (29.8%) were identified as positive using the PF and the serum samples, with a Cohen's kappa statistic of 0.81 (95% CI: 0.59–1) between the results obtained in each sample type. The sensitivity and specificity of the PF relative to the results in serum was 87% (95% CI: 66%–97%) and 94% (95% CI: 85%–99%) respectively. The percentage of PRRSV positive litters decreased over time and litters from gilts were more likely to test positive than those from older sows. Overall, the study demonstrates that PF can be a convenient and reliable specimen to monitor PRRSV infection in breeding herds.

1. Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) continues to generate economic losses to producers (Holtkamp et al., 2013). In the last two decades, methods to eliminate PRRSV have been developed and optimized (Corzo et al., 2010; Torremorell et al., 2003). Despite the success to eliminate the virus through herd closure strategies, there are still unanswered questions on duration of closure protocols and risk of newborn pigs as factors for prolonged infections in breeding herds (Allerson et al., 2014; Linhares et al., 2014). PRRSV infections during the third gestation trimester lead to the birth of viremic pigs which contribute to the dissemination of the virus during the suckling period (Cano et al., 2009). Cross-fostering of pigs, movement of contaminated fomites (i.e. needles and boots) and poor internal biosecurity can also contribute to endemic PRRSV infections in piglets (Otake et al., 2002a,b). Elimination of PRRSV from infected breeding herds is possible when PRRSV-free litters are born consistently and there are internal biosecurity measures in place to stop within-farrowing barn viral transmission.

In recent years, molecular diagnostic tests have become an important tool to declare herds as stable (i.e. consistently weaning PRRSV-negative piglets) (Holtkamp et al., 2011). Traditionally, these tests have been used in due-to-wean pig serum samples to generate evidence of absence of viral replication and transmission in pigs prior to wean. The recommended sampling protocol consists of bleeding at least 30 due-to-wean pigs 30 days apart for a minimum of 90 days and testing them by RT-PCR in pools of 5 (Holtkamp et al., 2011). Pooling allows to test a larger number of animals, thus increasing herd-level sensitivity while keeping the diagnostic costs low (Rovira et al., 2007). However, cost-effective sampling approaches that can be integrated in the farm routine procedures are needed.

In pig production there are management practices (i.e. tail docking

* Corresponding author.
E-mail addresses: cvilalta@umn.edu, carlesvilalta@yahoo.es (C. Vilalta).
[1] Deceased on May 2, 2017.

https://doi.org/10.1016/j.vetmic.2018.09.006
Received 10 July 2018; Received in revised form 12 September 2018; Accepted 13 September 2018

and castration) performed during the first week (3–5 days) of life that can facilitate the collection of samples that can be used to monitor infection status without representing a major inconvenient. Tail docking is done to prevent caudophagy and castration aims to prevent boar taint in pork carcasses. Tools used for tail docking and castration in PRRSV endemic farms have tested positive in prior studies (O'Connor et al., 2014; Thompson et al., 2012) and litters handled after processing PRRSV infected litters have a higher risk of testing RT-PCR positive (Thompson et al., 2012), suggesting that these procedures are associated with PRRSV dissemination. In farms that practice tail docking and castration is a common practice to place the tails and testicles obtained as part of processing in a pail to avoid the spread of bloodborne pathogens within the farrowing room. Thus serosanguineous fluids originating from these tissues which are known as processing fluids (PF), accumulate at the bottom of the pail and can be used as a sensitive sample to determine the presence of PRRSV in processed pigs (Lopez et al., 2018). However, there is scarce data on the use of PF to determine herd-level sensitivity and PRRSV infection dynamics in approximately 3 day old pigs. Therefore, the objectives of this study were to characterize the dynamics of PRRSV infection in pigs at processing overtime and estimate the sensitivity and specificity of using PF to detect PRRSV infection in approximately 3 day-old pigs.

2. Materials and methods

2.1. Study population and PRRSV history

The study was conducted in a commercial 6000-sow breed-to-wean farm located in Illinois, US. The herd became infected with PRRSV in April 2015 and it was not until June 2016 that pigs prior to wean tested PRRSV RT-PCR negative, at which time the herd was considered stable (Holtkamp et al., 2011). A PRRSV elimination plan was put in place and the herd was considered provisionally negative in January 2017 (Holtkamp et al., 2011). In February 22nd 2017, the herd experienced reproductive clinical signs compatible with PRRSV infection and samples were submitted to the diagnostic laboratory which confirmed a PRRSV infection with the same strain. A new PRRSV immunity homogenization plan was initiated by ensuring exposure of PRRSV to all breeding animals and incoming gilts eight weeks after the outbreak following industry standard protocols (Torremorell et al., 2003). However, during the time of the study it was decided to not perform herd closure due to remodeling of the facilities.

2.2. Sampling protocol and testing

Sampling started 10 days after the outbreak had been confirmed with diagnostics and sampling repeated every three weeks for a total of eight sampling events, representing 24 weeks since infection was confirmed. The sampling protocol consisted of bleeding all the pigs within a litter and collecting all the tails and testicles of the castrated piglets for each litter. At each sampling event, 10 litters prior to cross-fostering were conveniently selected at processing (approximately 3 days of age). Such sample size was set to obtain an 80% chance (power) of detecting a difference of 20% in discordant pairs between results obtained by the gold standard (RT-PCR on individual samples) and the alternative approach (RT-PCR on PF) with 95% confidence (Connor, 1987).

Every pig was bled using a new sterile needle. Processing tissues (i.e. tails and testicles) were collected for each litter and placed in 4×6 inch Minigrip® Red Line reclosable Zip bags (Minigrip, Alpharetta, GA). The tissues remained in the bags for at least three hours before removing the fluids with a sterile pipette and place them in sterile sera tubes. Both individual serum samples and PF were centrifuged at the farm and transported to the laboratory while refrigerated. Pig gender was recorded to assess, quantify and evaluate its association with litter PRRSV status (positive/negative).

Gloves were changed between litters when bleeding. However, no specific instructions to change gloves were given to farm employees during tail docking and castration as this study wanted to represent field conditions.

Serum and PF samples were individually tested at the University of Minnesota Veterinary Diagnostic Laboratory for PRRSV by RT-PCR (Rovira et al., 2007). A sample was considered positive if the cycle threshold (Ct) value was ≤ 35 or ≤ 37 for a serum or PF, respectively as a result of the receiver operating characteristic (ROC) curve analysis conducted as part of this study.

Three PF with the lowest Ct values were tested for infectious PRRSV by virus isolation using both porcine alveolar macrophages (PAM) and MARC-145 cells. Also those same PF samples were also sequenced (open reading frame (ORF) 5) using Sanger technology.

2.3. Data analysis

The proportion of positive pigs and litters by gender, parity and week post-outbreak was compared using chi-square and Fisher's exact tests. Ct values at the individual level were compared between males and females using the Mann-Whitney test. The distribution of PF and serum RT-PCR Ct values was assessed visually using scatter plots and a receiver operating characteristic (ROC) curve was used to assess the optimal cut-off values for PRRSV RT-PCR interpretation. Sensitivity (Se), specificity (Sp), and positive and negative predictive values (PPV, NPV), and total percentage of agreement were estimated using the results from all pig sera in the litter as the gold standard: a litter was considered positive when at least one pig had a serum RT-PCR Ct value ≤ 35.

Agreement between PF and serum RT-PCR results at the selected optimal cut-offs was assessed at the litter level using the Cohen's kappa statistic (Thrusfield, 2007). Correlated positive proportions of both sample types were also assessed using McNemar's test.

The association between the number of positive pigs in a litter (0, 1, 2, 3–6 or > 6) and the mean Ct values of the serum samples on the outcome of the PF test was assessed using logistic regression models. For litters in which serum samples were negatives a Ct value of 40 was assigned. Analyses were conducted using R software, version 3.2.5 (R Core Team, 2016).

3. Results

A total of 78 litters with 945 piglets (480 males and 465 females) were sampled. One litter which had six pigs and was sampled on week 11 post outbreak (PO) was removed from the analysis due to loss of the associated PF. Of the remaining 77 litters, 23 (29.8%) litters and 100 out of 939 (10.6%) pigs tested PRRSV RT-PCR positive. There were also 23 out of 77 (29.8%) PF samples that yielded PRRSV RT-PCR positive results.

The proportion of positive pigs and litters changed over time with more samples testing positive earlier in the outbreak. However, a transitory increase was seen in week 8 post-outbreak (PO), coinciding with the virus inoculation with the same outbreak strain that took place as an intervention strategy in breeding females (Table 1). The proportion of PF RT-PCR positive samples followed a similar pattern as the one observed with the serum samples. At least one positive litter was detected in each sampling event during the whole study regardless of sample type tested except in week 11 PO for PFs (Table 2).

The parity distribution of the sows included in the study followed the expected parity distribution in a commercial farm, with 23 (29.9%), 14 (18.2%), 14 (18.2%) 10 (13.0%), and 16 (20.8%) of litters born to first, second, third, fourth and fifth-to-ninth parity sows, respectively.

The percentage of RT-PCR positive PF samples was higher in lower parities. It was not possible to detect any positive pigs in parities above 5 although some of the PF were RT-PCR positive. There were significant differences between the proportion of positive litters from parity 1 and 2 sows, 78.3% (18/23), compared to litters from parity 3 or higher,