

SWINE HEALTH

Title: Epidemiology of porcine pestivirus and Torque teno virus in wean-to-finish pigs - **NPB #09-134**

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ABSTRACT

The purpose of this study was to describe the circulation of TTV and/or porcine pestivirus in commercial wean-to-finish populations. Oral fluid samples were collected from 10 wean-to-finish sites at 2-week intervals from placement (3 weeks of age) to close-out. Testing for porcine pestivirus and TTV using PCR-based assays was performed on these samples. Among the 600 oral fluid samples tested, one tested positive for porcine pestivirus. In contrast, 155 (26%) tested positive for TTV1; 483 (80%) tested positive for TTV2; and 134 (22%) tested positive for both TTV1 and TTV2. Thus, the results indicated that TTV infection was common in the 10 commercial wean-to-finish cohorts monitored in this survey.

INTRODUCTION

In recent years, several previously unrecognized viruses have been described in commercial swine populations in the context of clinical disease. The purpose of this study is to describe the epidemiology of two of these newly described viruses, i.e., porcine pestivirus (PPEV) and torque teno virus (TTV), in wean-to-finish pigs.

New porcine pestiviruses (PPEV) In Australia, a postulated pestivirus (Bungowannah virus) was recently described in association with reproductive failure (stillbirths) and sudden death in 3 to 4-week-old piglets (Kirkland et al., 2007). This virus has not been detected in the U.S. swine population (Murtaugh et al., 2008), but its association with “porcine reproductive and neurologic syndrome” has been postulated.

Torque teno virus (TTV) TTV is a small, non-enveloped, single-stranded, negative-sense, DNA virus belonging to genus Anellovirus in family Circoviridae (Okamoto et al., 2002). TTV infections have been identified in swine, chickens, cows, sheep, cats, and dogs, but TTV's potential to cause disease largely remains undetermined. TTV has been found in swine showing clinical signs of porcine circovirus associated disease (PCVAD), but it is also found in clinically healthy animals (Kekarainen et al., 2006). Disease has not been reproduced in any species and its role in pathogenesis is unknown.

The genome of the swine TTV is approximately 2.8 kb and two distinct genogroups (genogroup1 and genogroup 2) have been identified (Niel et al., 2005, Okamoto et al., 2002). The prevalence of TTV virus in swine in Europe and the U.S. ranges from 33-97% (Kekarainen et al., 2006, McKeown et al., 2004), The disease-causing role of this virus in swine unknown at present. TTV has been found in swine showing clinical

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signs of porcine circovirus associated disease (PCVAD), but it is also found in clinically healthy animals (Kerarainen et al., 2006). Disease has not been reproduced in any species and its role in pathogenesis is unknown.

PROJECT OBJECTIVES

The objective of this study was to describe the epidemiology of two newly described viruses [porcine pestivirus (PPeV) and Torque teno virus (TTV)] in wean-to-finish pigs.

MATERIALS AND METHODS

On each of the 10 commercial swine production sites, oral fluid samples were collected from 6 pens in one barn at 2-week intervals from placement to slaughter in the summer of 2007, i.e., 60 pens total every 2 weeks. Pigs were sourced from Colorado, Illinois, Oklahoma, and Utah (Hoffmann et al., 2008). Oral fluids were collected by suspending a length of cotton rope in a location accessible to the pigs in the pen. Cotton rope was used because it is highly absorbent. We use 1/2" rope for nursery pigs; 5/8" rope for grow-finish pigs.

The rope was placed at shoulder height to the pigs (hang the rope, then cut to length), then left in place for 20-30 minutes. during which time the pigs played with the rope, depositing oral fluids in the process.

To recover the sample, the bottom 6-8" of the rope was inserted into a plastic bag with the rope still hanging from the bracket. Holding the rope using the outside of the bag, the rope was pulled taut, then cut above the bag. With the wet rope in the bag, the rope could be squeezed so that fluids accumulated in the corner of the bag. The corner of the bag was then cut and the contents poured into a plastic snap-cap tube (Falcon 2054 or equivalent). Volumes of 5 – 20 ml can be recovered using this procedure.

The 600 samples from this study were previously tested by PCR for PRRSV, SIV, and PCV2. For PCR testing of PeV and TTV, RNA or DNA was extracted using the KingFisher instrument, (Thermo Fisher Scientific Inc., Waltham, MA) and MagAttract Virus Mini M48 Kit (Qiagen, Valencia, CA). The PCR for TTV and RT-PCR for pestiviruses was performed using sequence-specific oligonucleotide primers described elsewhere (Hoffmann, et al., 2006; Kekarainen et al., 2006). Gel-based PCR was performed as described earlier (Kekarainen et al., 2006). Positive products were sequenced using an automated sequencer at the Purdue University genomic core facilities and the Veterinary Research Institute. The sequences of the untranslated region were compared between the varieties of swine TTVs. DNASTAR 8.0 software (Lasergene), Clustal W method was used for comparison of viruses.

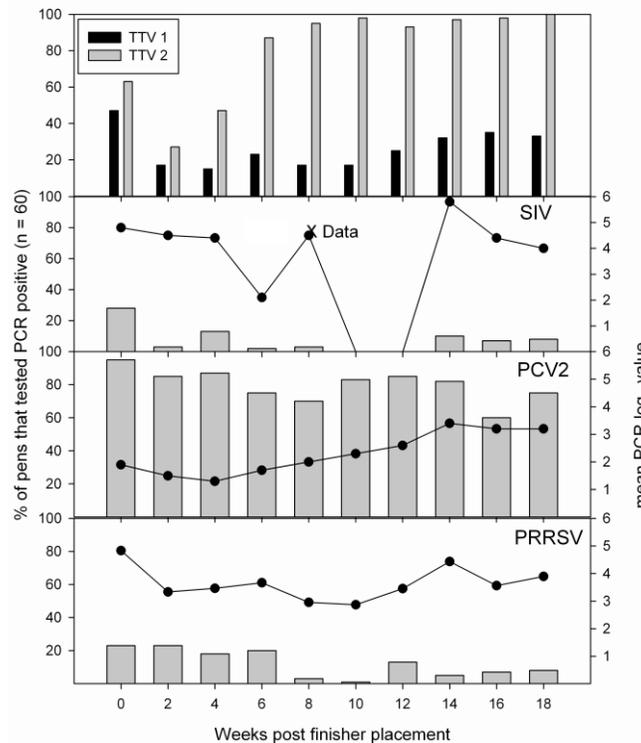


Figure 1. Circulation of TTV1, TTV2, SIV, PCV2, and PRRSV in oral fluid samples collected from commercial swine finishers.

RESULTS

Oral fluid samples (n = 600) were tested by PCR for PRRSV, SIV, PCV2, TTV1, and TTV2. As shown in Figure 1, the results showed that the circulation of pathogens in growing pig populations could be tracked using oral fluid samples. Likewise, these results corroborated previous reports of the detection of diseases of swine in oral fluid.

Only 1 of 600 oral fluid samples in this study tested positive for PPeV. This singleton reactor should be considered a false positive reaction.

Among the 600 oral fluid samples tested for TTV, 155 (26%) tested positive for TTV1, 483 (80%) tested positive for TTV2, and 134 (22%) tested positive for both TTV1 and TTV2.

Although the level of homology is about 80-90% between the two groups, sequence homology clearly differentiated between TTV1 and TTV2 (Figures 2 and 3). No unique sequence information was identified during this experiment. That is, the viruses analyzed by comparing nucleotides at the UTR region were found to be similar or that same as types previously reported in US and Europe. Importantly, we found that conducting sequencing analysis from oral fluids collected in the field was possible for TTV and may be applicable to other viruses as well.

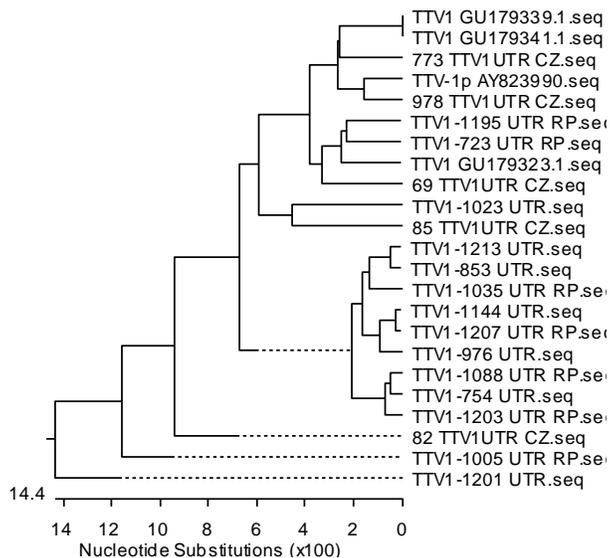


Figure 2. Phylogenetic tree TTV1 UTR

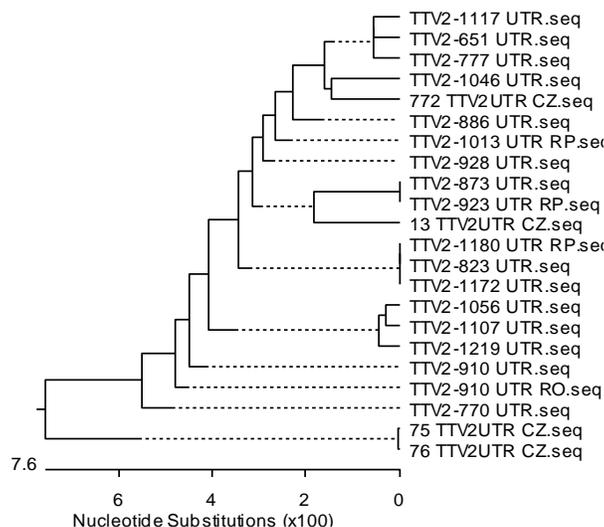


Figure 3. Phylogenetic tree TTV2 UTR

DISCUSSION

The results of this study corroborate earlier work demonstrating the utility of oral fluids for the surveillance of infectious agents of swine. As additional pathogens are demonstrated in swine oral fluid and improvements in assay optimization are achieved, this approach will be increasingly used for the surveillance of other endemic and foreign animal pathogens. The use of oral fluids in conjunction with on-farm rapid diagnostics will revolutionize our approach to control and / or elimination of infectious diseases of swine.

LAY INTERPRETATION

Oral fluids are inexpensive to collect. Each pen sample requires a length of cotton rope, a plastic bag, and a snap-cap plastic tube. Labor costs include the time required to place and recover the ropes, plus time to process the sample. Additional expenses include shipping and laboratory (PCR) costs.

We found that the use of oral fluids worked very well in conjunction with PCR-based diagnostics for PRRSV, PCV2, SIV, and TTV. We believe that it will also be possible to develop antibody-based assays that use oral

fluids in the future. In addition, research currently under way will eventually result in rapid on-farm diagnostics that use oral fluids.

Based on our experience, we are currently recommending sampling at 3-4 week intervals for surveillance of PRRSV and/or PCV2. The number of samples to collect per site or building depends on the specific objective, the site, and facility design. Precise estimates of the number of samples necessary for effective surveillance of buildings, sites, or populations will require additional research. In our experience, circulation of PRRSV and PCV2 in grow-finishers barns was detected using 6 samples per building (~15% of pens).

This work was published in the following proceedings

McNutt A, Prickett J, Pogranichniy R, Main R, Clark A, Zimmerman J. March 2010. Torque teno virus (TTV) infection in commercial wean-to-finish population concurrently infected with PRRSV, PCV2, and influenza virus. Proceedings of the American Association of Swine Veterinarians. Omaha, Nebraska, pp. 325.

Prickett J, Pogranichniy R, Main R, Clark A, Zimmerman J. December 2009. Torque teno virus (TTV) in commercial wean-to-finish populations concurrently infected with PRRSV. Proc 90th Ann Meet Conference of Research Workers in Animal Diseases, Abstr #137.

Prickett J, Pogranichniy R, Main R, Clark A, Zimmerman J. December 8, 2009. Torque teno virus (TTV) in commercial wean-to-finish populations concurrently infected with PRRSV. Proc 2009 International PRRS Symposium. Chicago, Illinois, p 68.

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