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Detection of porcine reproductive and respiratory syndrome virus (PRRSV)-specific IgM-IgA in oral fluid samples reveals PRRSV infection in the presence of maternal antibody

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ABSTRACT

The ontogeny of PRRSV antibody in oral fluids has been described using isotype-specific ELISAs. Mirroring the serum response, IgM appears in oral fluid by 7 days post inoculation (DPI), IgA after 7 DPI, and IgG by 9 to 10 DPI. Commercial PRRSV ELISAs target the detection of IgG because the higher concentration of IgG relative to other isotypes provides the best diagnostic discrimination. Oral fluids are increasingly used for PRRSV surveillance in commercial herds, but in younger pigs, a positive ELISA result may be due either to maternal antibody or to antibody produced by the pigs in response to infection. To address this issue, a combined IgM-IgA PRRSV oral fluid ELISA was developed and evaluated for its capacity to detect pig-derived PRRSV antibody in the presence of maternal antibody. Two longitudinal studies were conducted. In Study 1 (modified-live PRRS vaccinated pigs), testing of individual pig oral fluid samples by isotype-specific ELISAs demonstrated that the combined IgM-IgA PRRSV ELISA provided better discrimination than individual IgM or IgA ELISAs. In Study 2 (field data), testing of pen-based oral fluid samples confirmed the findings in Study 1 and established that the IgM-IgA ELISA was able to detect antibody produced by pigs in response to wild-type PRRSV infection, despite the presence of maternal IgG. Overall, the combined PRRSV IgM-IgA oral fluid ELISA described in this study is a potential tool for PRRSV surveillance, particularly in populations of growing pigs originating from PRRSV-positive or vaccinated breeding herds.

1. Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) was first identified in 1991 (Wensvoort et al., 1991). Assays for the detection of PRRSV serum antibody became widely available shortly thereafter, including immunoperoxidase monolayer (Wensvoort et al., 1992), immunofluorescence (Benfield et al., 1992), serum-virus neutralization (Benfield et al., 1992), and ELISA (Albina et al., 1992). The detection of PRRSV serum antibody using fluorescent microsphere-based assays has been reported under experimental conditions (Langenhorst et al., 2012). At the present time, the ELISA is the most common format for PRRSV antibody detection and commercial PRRSV antibody ELISA kits are widely available for serum and swine oral fluid specimens (Pejsak et al., 2017).

The ontogeny of PRRSV antibody in serum and oral fluids has been described using isotype-specific ELISAs. Kittawornrat et al. (2013), using paired samples collected over time post inoculation, showed that the temporal appearance of antibody isotypes in serum and oral fluid was essentially identical in animals inoculated with viable, replicating PRRSV. That is, IgM was detectable by 7 days post inoculation (DPI), IgA after 7 DPI, and IgG by 9 to 10 DPI. Because of the higher concentration of IgG relative to other isotypes, commercial ELISA kits usually target the detection of IgG, although detection of IgM and IgA has been used in human diagnostic medicine.

In addition to antibody produced in response to PRRSV infection or vaccination, younger animals may also have ELISA-detectable PRRSV-specific passive antibody, primarily IgG, in serum and oral fluid (Biernacka et al., 2016; Goyal, 1993; Ramirez et al., 2011). In oral fluid-based testing, maternally-derived antibody creates a challenge in discerning whether a positive ELISA is the result of infection, vaccination, or maternal antibody. Therefore, the goal of this study was to develop a PRRSV combined IgM-IgA oral fluid ELISA and evaluate its performance under experimental and field conditions.

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2. Materials and methods

2.1. Experimental design

Two longitudinal studies were conducted to evaluate PRRSV oral fluid antibody ontogeny using isotype-specific ELISAs (IgM, IgA, IgG, IgM-IgA). In Study 1 (experimental data), oral fluid antibody isotype responses were evaluated in individual pigs following administration of a modified-live PRRSV vaccine. In Study 2 (field data), PRRSV antibody isotype responses were monitored in oral fluid samples collected from PRRS unvaccinated, group-housed pigs in commercial wean-to-finish farms in Iowa USA. In Study 2, wild-type PRRSV infection was determined by PRRSV real-time reverse-transcriptase PCR (RT-rtPCR) testing and sequencing. Studies were conducted with the authorization of the Iowa State University Office for Responsible Research and the permission of the producers.

2.2. Animals and animal care and PRRSV status

Study 1 was an experimental study conducted in a biosafety level 2 research facility located at Iowa State University and accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). The facility was designed with a single-pass, non-recirculating ventilation system, i.e., unidirectional flow from low contamination areas to high contamination areas. Each room was ventilated separately and humidity and temperature was strictly controlled. Zones of negative pressure prevented airborne contamination from area-to-area or room-to-room. Pigs were housed in individual pens (1.52 m × 1.83 m) in one room.

Partitions with evenly-spaced vertical bars allowed interactions between pigs in adjacent pens. Animal care, housing, and feeding were under the supervision of the research facility staff.

Pigs (n = 12; 50 kg) were sourced from a PRRSV-naïve commercial herd. To confirm PRRSV-naïve status, pigs were tested for PRRSV serum antibody at 19 and 11 days prior to arrival at the research facility and again prior to vaccination. Pigs were acclimated in the facilities for 5 days and then vaccinated with a modified-live PRRS vaccine on Day 0 of the study (Ingelvac PRRS® MLV, Boehringer Ingelheim Vetmedica Inc., St. Joseph Missouri). Individual oral fluid samples were collected twice daily from each of the 12 pigs from Day −7 through Day 42 using a protocol described elsewhere (Prickett et al., 2008a,b). In brief, one 100% cotton rope was hung in each pen for 30 min, during which time the pigs interacted (chewed on) the rope. Thereafter, the wet portion of the rope was inserted into a plastic bag and severed from the remainder of the rope. Oral fluid was extracted by passing the wet rope, still within the bag, through a portable towel wringer (Dynajet, Nürtingen, Germany). Samples were decanted into 50 mL centrifuge tubes and placed on crushed ice. The morning and afternoon oral fluid samples from each day were combined, aliquoted into 5 mL cryovial tubes and stored at −80 °C.

Study 2 was a field study conducted on three separate farms in one production system. Each farm (A, B, C) consisted of three curtain-sided, wean-to-finish barns (1, 2, 3) sited parallel to each other and spaced 10 m apart. Barns (13.4 m × 61.0 m) were designed with split-zone ventilation, independent control of curtains, and ridge ventilation by zone. Manure was collected in shallow pits beneath each barn and moved to an outdoor above-ground slurry storage tank via a scraper system. All farms were managed on an all-in-all-out basis, with buildings cleaned and disinfected between groups. Animal housing, handling, feeding, and health care were implemented by producers and with the assistance of production system veterinarians. For the purpose of implementing this study, producers and veterinarians did not vaccinate or move pigs between pens or barns during the 2-month sampling period.

Each barn contained 40 pens arranged as 20 pens on either side of a central walkway. On Farm A, pens (3 m × 6 m) were built with solid concrete walls and partial slats. On Farm B and C, pens (3 m × 6 m) were built with gated walls and partial slats. During the collection period, all occupied pens held ~25 pigs. Barns were populated with weaned pigs (~21 days of age) sourced from one PRRSV-endemic breeding herd over the course of 7 to 14 days, but each farm's pigs came from a different sow herd. For all breeding herds, commercial modified-live PRRS vaccines were administered to replacement gilts during quarantine, but not to sows or pigs in other phases of production.

Oral fluid samples were collected weekly from every occupied pen (n = 36) in every barn (n = 3) on each farm (n = 3) using the procedure described above. Samples were decanted into 50 mL centrifuge tubes and placed on crushed ice for transport to the laboratory. Samples were aliquoted into 4 mL cryovials in the laboratory and stored at −20 °C.

After weekly oral fluid collection, blood samples were collected from 20 pigs in each barn by sampling 10 pigs from each of two pens. Pens selected were approximately 1/4 of the distance from each end of the barn on opposite sides of the walkway. The same pens were sampled each week, but not necessarily the same pigs (convenience sampling). Blood samples were collected using a single-use vacutainer system with 10 mL serum separation tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Blood samples were placed on ice for transport to the laboratory. In the laboratory, samples were centrifuged (1000*g* for 10 min), aliquoted into 4 mL cryovials, and stored at −20 °C.

2.3. PRRSV ELISAs

Serum samples were tested for PRRSV antibodies using a commercial PRRSV ELISA (IDEXX PRRS ×3 Ab Test, IDEXX Laboratories, Inc., Westbrook ME USA) following the instructions provided by the manufacturer. Sample-to-positive (S/P) results ≥0.4 were considered positive for PRRSV antibody.

Oral fluid samples were tested for PRRSV antibodies using a commercial PRRSV oral fluid antibody (IgG) ELISA (IDEXX PRRS OF Ab Test, IDEXX Laboratories, Inc.) following the instructions provided by the manufacturer. S/P results ≥0.4 were considered positive for PRRSV antibody. IgM, IgA, and IgM-IgA oral fluid ELISAs were performed as instructed by the manufacturer for the PRRSV OF Ab ELISA (IgG) with the following exceptions: the kit IgG conjugate was replaced with goat anti-pig IgM (A100-100P Bethyl Laboratories) diluted 1/5000 in IDEXX conjugate diluent; or goat anti-pig IgA (A100-102P Bethyl Laboratories) diluted 1/3000 in IDEXX conjugate diluent; or dual mixture of IgM (1/5000)-IgA (1/3000). Plate positive controls for the IgM, IgA, or IgM-IgA ELISAs were based on oral fluid samples of known positive PRRSV status diluted in kit sample diluent to produce optical density (OD) values between of 0.6 and 0.7. Tests were performed as recommended by the manufacturer and results reported as S/P ratios. Cutoffs for the IgM, IgA, and IgM-IgA oral fluid ELISAs were determined by receiver operator characteristic curve (ROC) analysis, as described in Section 2.6 (statistical analysis).

2.4. PRRSV RT-rtPCR

All samples were tested for PRRSV RNA at the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) using standard protocols. Systematic bias was addressed by randomizing samples order prior to submission.

In Study 2 (field data), serum samples from the same pen were pooled by five and tested by PRRSV RT-rtPCR. Nucleic acid extraction was performed using the MagMAX™ viral RNA isolation kit (Life Technologies, Carlsbad, CA, USA) and a Kingfisher 96 magnetic particle processor (Thermo-Fisher Scientific, Waltham, MA, USA) using a standard lysis procedure. A lysis/binding solution was prepared with 65 µL lysis/binding solution, 1 µL carrier RNA, 65 µL isopropanol and 2 µL Xeno™ RNA template at 10,000 copies/µL. At the lysis step, 130 µL of