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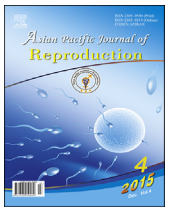

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Serological profile of offspring on an intensive pig farm affected by porcine reproductive and respiratory syndrome

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ABSTRACT

Objective: To evaluate/predict the offspring serum profile of antibodies against Porcine reproductive and respiratory syndrome virus (PRRSV) in an affected intensive herd, whereas sows are vaccinated, and determine the better sample time using pigs as sentinels.

Methods: Serum samples were collected from a total of 66 offspring pigs from a PRRSV endemic herd, whereas only sows were vaccinated. Six animals per grouped age were randomly selected with 0 (at perinatal time), 1, 2, 3, 6, 9, 12, 15, 18, 21 and 24 weeks of age. Individual and three pooled samples were tested by ELISA and PCR, respectively.

Results: The proportion of seropositive animals was 75.0% (18/24), 33.3% (6/18) and 95.8% (23/24) at farrowing (0–3 weeks), nursery (6–12 weeks) and growing/finishing (15–24 weeks) phases, respectively. It was 46 times more likely ($P < 0.01$) to observe seropositive pigs on the growing/finishing than on nursery phase. European PRSSV strain was detected on serum of 6, 9 and 15-weeks-old pigs. Polynomial fit degree 4 regression between sample-to-positive (S/P) ratios and age of pigs ($r = 0.78$; $R^2 = 0.60$; $P < 0.001$) estimated a quickly decrease of maternal antibodies, above 0.4 S/P value (cut off), until the 6th week following by a progressive increment of humoral immunity until 21st week of age, when it declines.

Conclusions: The results indicate that the piglets can present viremia early at nursery, and the 18th – 21st weeks are the best time to sample offspring pigs, as sentinel, from PRRSV contaminated intensive herds.

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is a swine disease which appeared simultaneously in Europe (early 1990s) and North America (late 1980s) [1,2] and continues to be one of the major diseases that affect the swine industry worldwide, being responsible for losses of $664 million annually in U.S.A national breeding and growing pig herds [3,4].

PRRS is caused by a virus (PRRSV), which belongs to the family *Arteriviridae*, genus *Arterivirus* [5]. Generally, PRRSV is divided into two major genotypes, the type I (European) and type II (North American), which are antigenically, genetically and clinically different [1,6,7].

PRRS is characterized by respiratory problems, like cough and dyspnea, in growing and finishing pigs and reproductive failure in sows, with late term abortion, stillbirth and increased preweaning mortality [8]. The diagnosis is obtained by virus isolation or by the identification of antibodies, antigens or nucleic acid in infected samples. However, there are limitations on the PRRS diagnosis. Serological tests for PRRSV normally detect serum antibody response after 14–21 days post-infection, and do not allow the distinction between infected and vaccinated animals [9]. Virus isolation and identification has also limits due the wide diversity of PRRSV strains, being selective between cell cultures [8,10].

The PRRS surveillance in swine populations is not easy to be done. Several studies evaluated the use of oral fluids as samples for diagnostics of swine diseases, including PRRS [11–13] for

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easier and less cost effective sample collection. However, there is only few information about the quantity of virus-specific antibodies in oral fluids of infected pigs [14] and the development of oral fluid-based assays and the PRRS evaluation of their results has only started recently [15]. So, at present time, blood samples collection remains a precious tool for PRRS surveillance and virus circulation, even if clinical form rate decrease. Nevertheless, the offspring evaluation of serum profiles on affected herds, whereas vaccinated sows, was not sufficiently reported.

The main objectives of the present study were to evaluate the offspring serum profile of antibodies against PRRSV in an affected intensive herd in Portugal and determine the better sample time using pigs as sentinels.

2. Material and methods

2.1. Animals and general features of the herd

The herd is located in the Center region of Portugal, at latitude 39.649478 and longitude −8.307067. The herd structure is constituted by three buildings: two "grow-finishing" buildings and one with the gestation room, the farrowing rooms and the nurseries. It is operated as a closed herd which comprises approximately 480 sows, 1500 weaned piglets and 2000 growing-finishing pigs. The gilt reposition has external origin. The commercial pigs were based on Large white (X Landrace) X Pietrain breeds.

The herd management was based in weekly artificial insemination of approximately 25 sows. At the farrowing rooms, piglets are weaned with 4 weeks of age, being transferred to the nursery. Around 10–12 weeks of age, piglets are again transferred to the "grow-finishing" building.

The PRRSV vaccine used in the prophylactic plan was Porcilis® PRRS (Intervet International BV, Boxmeer, Netherlands), a European live attenuated PRRS virus strain DV, administered every 4 months to sows on the multiplication unit building. The primovaccination of all gilts was performed on the quarantine facilities. The vaccination plan also included the Aujeszky's disease virus and Circovirus. The offspring pigs were only vaccinated against Circovirus. The farm used this vaccination plan at least during the last 3 years.

The PRRS was previously (last 3 years) diagnosed on the herd by ELISA and PCR techniques, similar to the reported on the present paper. Reproductive and respiratory symptoms were also present. The farm simultaneously monitored serum antibodies profiles concerning Circovírus (PCV K2- IgM and IgG), *Actinobacillus pleuropneumoniae* (APP ApxII/TBp 2), Influenza and Aujeszky disease virus (ADV gB and ADV gE) from sentinels on each production cycle, i.e., the same 4 to 6 pigs were periodically prospected at different times.

2.2. Sample collections

Blood samples were collected, on November 2014, from a total of 66 offspring pigs of different ages and all rooms/pens were considered. Six animals per grouped age were randomly selected with 0 (at perinatal time), 1, 2, 3, 6, 9, 12, 15, 18, 21 and 24 weeks of age. Blood was collected by vena caval puncture. After collection, tubes were refrigerated at 4 °C and sent to laboratory. The serum was removed from whole blood by centrifugation and stored at −20 °C until use.

2.3. Enzyme-linked immunosorbent assay of PRRSV specific antibody

The samples were analyzed individually, according to the manufacturer instructions, by the enzyme-linked immunosorbent assay (ELISA) with a commercial kit – IDEXX PRRS X3 (IDEXX Laboratory Inc., Liebefeld-Bern, Switzerland), which detects both strains – type I (European) and type II (North American).

The cut off of sample-to-positive ratios (S/P values), i.e., the threshold level for positive samples, was 0.4.

2.4. Quantification of PRRSV by real-time RT-PCR and amplicon analysis

Due to economic reasons, RT-PCR was performed using a pooling strategy. Serum of three samples from pigs with the same age was previously mixed (total of 22 pools) and submitted to analysis.

Viral RNA was isolated using the BioSprint® 96 One-For-All Vet Kit (Qiagen S.A., Hilden, Germany).

The RNA viral amplification was developed using the QuantiTect™ SYBR® Green RT-PCR kit (Qiagen S.A., Hilden, Germany). Real-time PCRs were run on the thermocyclers Rotor Gene Q (Qiagen S.A., Hilden, Germany).

All these steps were performed following the manufacturer's instructions manual.

The genotyping was obtained by sequencing the fragment ORF7, which encode the nucleocapsid protein, using Sanger method. The sequences were then analysed by BLAST (available at http://www.ncbi.nlm.nih.gov/), comparing to the European, American and Chinese reference strains, allowing to assign which of three strains is closer.

2.5. Statistical analysis

The odds ratio according nursery and "growing/finishing" phases was calculated using univariate logistic regression. Wald test was considered.

Polynomial fit degree 4 regressions between S/P values and age of pigs were used for model prediction assessment.

The JMP® 7 [16] software statistical package was used.

3. Results

The proportion of seropositive animals at farrowing (weeks 0, 1, 2 and 3), nursery (weeks 6, 9 and 12) and "growing/finishing" phases (weeks 15, 18, 21 and 24) were 75.0% (18/24), 33.3% (6/18) and 95.8% (23/24), respectively.

It was 46 more likely to observe seropositive pigs on the growing/finishing phase than on nursery phase (95% interval of confidence of odds ratios from 5.0 to 427.4; $P < 0.01$).

A correlation $r = 0.78$ ($P < 0.001$) between S/P values and the pig age, considering all positive and negative animals to PRRSV antibodies, was observed (Figure 1). This correlation remained significant ($r = 0.69$; $P < 0.001$) even if only positive pigs to S/P values were considered (Figure 2).

A positive field sample (pool) from 6-weeks-old pigs was closer to Europan Lelystad AY588319.1 [M96262.2 (gi 51094507)] strain. The nucleotide sequence of the amplified fragment was: