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Oral fluid *versus* blood sampling in group-housed sows and finishing pigs: Feasibility and performance of antibody detection for porcine reproductive and respiratory syndrome virus (PRRSV)

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ABSTRACT

The feasibility of using individual and pen-based oral fluid samples to detect PRRSV antibodies in growing-finishing pigs and group-housed sows was investigated. The diagnostic performances of a commercial oral fluid ELISA (OF-ELISA) and a serum ELISA (SER-ELISA) performed on individual or pooled samples from 5 or 10 pigs and sows was evaluated. The performance of the OF-ELISA was also assessed for pen-based oral fluids. Eight hundred and thirty-four pigs and 1598 sows from 42 PRRSV-infected and 3 PRRSV-negative herds were oral fluid sampled and bled. PRRSV antibodies were detected by an OF-ELISA performed at individual, pool (5 or 10 samples) and pen levels. Serum samples were tested by a SER-ELISA at individual and pool levels. The sensitivity and specificity of ELISAs for individual samples were assessed by Bayesian analysis. The relative diagnostic performance for the pools was calculated by taking individual samples as the gold standard. SER-ELISA and individual OF-ELISA results were used as references for estimating OF-ELISA performance for pen-based samples. Individual oral fluid collection was feasible in all kinds of pigs, whereas pen-based samples were unsuccessful in 40% of the group-housed sow pens. High levels of sensitivity comparable to those of the SER-ELISA were found for the OF-ELISA when performed on individual, 5-sample pool or pen-based samples from pigs or sows. The OF-ELISA lacked specificity for individual samples from sows. Pooling 5 individual oral fluid samples or using pen-based samples increased test specificity.

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is considered to be one of the most costly diseases disrupting the health and welfare of pigs worldwide and affecting all pig-producing countries since its emergence. The disease is caused by a virus of the *Arteriviridae* family known as PRRSV and has become enzootic in most swine production areas (Zimmerman et al., 2012). PRRSV is responsible for reproductive disorders in sows and respiratory problems in pigs. It is also a key pathogen involved in other economically important diseases such as the porcine respiratory disease complex (PRDC) (Done et al., 1996; Fablet et al., 2012) or zoonoses (*Salmonella*, Hepatitis E) (Beloeil et al., 2004; Salines et al., 2015). Consequently, PRRSV monitoring and eradication programmes have been designed and some are already being implemented (Corzo et al., 2010). Successful infectious disease surveillance and control programmes require fast, convenient and simple sampling procedures and laboratory tests. These methods need to be accurate, cost-effective, animal welfare friendly and user friendly. The need for efficient, cost-effective and easy-to-use sampling tools and laboratory tests is therefore particularly true for PRRSV monitoring and elimination owing to its widespread distribution and economic impact on the swine industry.

Traditionally in veterinary medicine the most frequent sampling method used in the field for monitoring or control purposes is labour-intensive, i.e. blood collection from individual animals. Taking blood samples by venepuncture from pigs is a tedious and, under certain circumstances, dangerous procedure for the samplers (e.g. with boars, sows or group-housed animals) and is stressful for the pigs. As a result, blood sampling based routine herd surveillance is cost-prohibitive and generally under-used on a large scale. At the laboratory, the pooling of

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samples from several individuals for a single test has long been used to reduce the cost and effort of diagnostic testing (Boulard and Villejoubert, 1991; Rodake et al., 1997). However, diagnostic test properties assessed on individual samples may be affected when several samples are mixed together, thus requiring them to be reassessed for pooled samples (Hutet et al., 2003; OIE, 2013). It is rare to find an estimation of the sensitivity and specificity of pooled samples (of various sizes) in current routine serological tests for PRRSV infection (Rovira et al., 2008). A more thorough knowledge of the diagnostic reliability of pooled samples would help stakeholders choose knowledgeably when addressing the issue of sampling design for monitoring or surveillance programmes.

In the last decade, an alternative collection method has raised growing interest as a diagnostic matrix for various infections, including PRRSV infection. This relatively new method is based on the sampling of oral fluids (Prickett and Zimmerman, 2010). Oral fluid is collected by allowing the pigs to chew on an absorptive device, commonly a cotton rope or swab (Atkinson et al., 1993; Kittawornrat et al., 2010; Olsen et al., 2013; Decorte et al., 2014; Sattler et al., 2015). Collecting oral fluid has several advantages over traditional blood sampling. It is simple, non-intrusive, incurs few labour costs, and is quick to set up and carry out. It is thus being acknowledged as a more animal welfare friendly method than blood sampling. Besides, oral fluid may be collected by the animal caretakers, i.e. on-site farm personnel, hence reducing biosecurity risks.

Oral fluid specimens may be collected at both the individual or group (pen-based) level. The feasibility of pen-based oral fluid has been assessed for growing pigs (Kittawornrat et al., 2012) and recently for a type of group-housed sows (Pierdon et al., 2016). Oral fluid sampling has also been evaluated at the individual level for boars (in boar studs) and growing pigs (Gerber et al., 2014; Decorte et al., 2015; Pepin et al., 2015). Even though the sow breeding herd may represent a non-negligible proportion of the population in a pig herd (particularly in farrow-to-finish herds), it needs to be tested to understand within-herd infection dynamics. Up to now, data on the effectiveness of oral fluid sampling in sows are scarce and limited to individuals (Pepin et al., 2015) or large dynamic groups (Pierdon et al., 2016). However, pen-based oral fluid sampling may be of interest in several types of group-housed sows, a mandatory practice in the European swine farming system.

Besides PCR-based assays for detecting PRRSV circulation, antibody-based tests inform on herd immunity and the history of prior infection. When monitoring infection and immunity in sows or pigs, it is common practice to test for the presence of antibodies against PRRSV with a commercial ELISA. In the last few years, the ELISA has been adapted to the oral fluid matrix to detect antibodies against PRRSV (Kittawornrat et al., 2012; Gerber et al., 2014). Nevertheless, the diagnostic performance of this oral fluid ELISA for individual or pooled samples has not been investigated to date, whether for finishing pigs or sows. The objectives of the current study were therefore 1/to test the feasibility of oral fluid collection at individual and pen levels in finishing pigs and group-housed sows and compare this to blood sampling and 2/to assess the diagnostic performance of a commercial oral fluid ELISA and a commercial serum ELISA on individual, pooled or pen-based samples for PRRSV antibody detection in finishing pigs and sows.

2. Material and methods

The study was performed in accordance with current legislation on ethical and welfare recommendations. ANSES-Ploufragan is certified for animal experimentation and is registered under certification number C-22-745-1 delivered by the official French veterinary services.

2.1. Study design

The study was carried out over two periods. Firstly, 10 herds were selected for collecting blood and oral fluid samples from growing-finishing pigs (from November 2013 to February 2014). Secondly, 35 breeding herds were selected for collecting the same type of samples from group-housed sows (February 2014 to June 2015). Each herd was selected on a voluntary basis according to the farmers' willingness to participate and was proposed by swine veterinarians. The 10 growing-finishing pig herds were known to be PRRSV-infected, as were 32 of the 35 breeding herds. On the other hand, 3 breeding herds were known to be PRRSV-negative (based on routine serological and PCR analyses). All 45 herds were located in Brittany, western France.

In each of the 10 herds selected for the growing-finishing pig study, 2 batches were selected, one of 16-week-old pigs and the other of at least 22 weeks old. Biological samples were taken in three pens selected at random per batch. In every breeding herd included in the sow study population, at least 30 pregnant group-housed sows located in at least three pens were sampled at random. To avoid reproduction failure due to blood sampling, the selected sows were all at between 4 and 14 weeks of gestation.

2.2. On-site samples collection and recordings

In all the herds, individual paired oral fluid and blood samples were taken from each animal housed in the selected pens. From these pens, pen-based oral fluid samples were then collected on a chewing device provided for 45 min. Individual oral fluid samples were taken at first and were followed by pen-sized oral fluid samples. Finally, blood was collected. The sampling collection was the same whatever the type of animal (sow or growing-finishing pig) as described below. Before starting the sampling procedures, the pigs had a numbered written on their back so as to be able to match the samples throughout the sampling process.

2.2.1. Individual oral fluid sampling

An individual oral fluid sample was first taken from each of the growing-finishing pigs or sows in the selected pens. The oral fluid was collected by a single operator allowing the animal to chew on dry swabs (Swab cloth, Sodibox®, Nevez, France) until they were thoroughly moistened. The dry swab was held in the mouth of the pig with a surgical clamp and the oral fluid was allowed to soak into the swab (Fig. 1). It was not necessary to restrain the pigs. The swabs were stored in a 50 ml Falcon™ tube at +4 °C until transfer prior to analysis of the oral fluid at the laboratory. The time taken for the whole oral fluid sampling procedure was recorded, from the moment a pig was selected to chew on a swab to the time when a sufficient amount of oral fluid was considered to be taken. The surgical clamp was washed in 70% alcohol, rinsed and dried with a gauze compress between each pig sampling operation to prevent cross-contamination. Operators wore personal protective equipment and changed gloves between each new sampling operation. At the laboratory, the swab was transferred from the Falcon™ tube and compressed in a 10–20 ml disposable syringe to squeeze the oral fluid from the swab into a sterile tube. The samples were then stored at −20 °C until analysis.

2.2.2. Pen-based oral fluid sampling

Pen-based oral fluid was collected by suspending a chewing device from a bracket fixed to the side of each pen for 45 min. The pigs were therefore allowed to interact with and chew on the sampling device throughout this time, depositing oral fluid in the process. Pigs were observed chewing on the sampling device from the time the devices were put in the pens until they were removed. In order to optimize the number of pigs interacting with the oral fluid sampling device, the number of sampling devices placed in the pen was adapted to the total number of pigs in the pen, with a ratio of one sampling device for 15