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Novel ELISAs for differentiated detection of antibodies against either PRRSV EU or US in oral fluid.

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Background: In the Danish SPF system PRRSV surveillance is based on the ability to differentiate between the American (US, Type 2) and the European (EU, Type 1) strain of PRRSV. The blocking ELISAs used in this SPF surveillance are only validated for serum (Sørensen et al., 1998). Based on the same antigens, indirect ELISAs for PRRSV EU and US were developed for analysis of oral fluid (OF) samples.

Materials: Samples for the validation were obtained from PRRSV positive and negative Danish herds. 281 OF pen pools were collected by hanging a rope in selected pens. From same pens blood was drawn from all pigs in each OF samples, resulting in 2551 sera in total. The selected pools were collected by hanging a rope in selected pens of PRRSV positive and negative Danish herds. 281 OF pen samples from each herd were diluted in 1:10 and these results were the gold standard.

Methods: The sera were tested in the PRRS blocking ELISA used in the surveillance (Sørensen et al., 1998), and these results were the gold standard for the novel OF ELISAS: A PRRSV-positive pen was defined as a pen with at least 50% pigs positive in the blocking ELISA.

Results: As shown i Fig 1, there was an obvious clustering into three populations, when testing expectedly positive and negative OF samples in the two OF ELISAS. This indicates that the tests can differentiate between EU and US positive samples.

In the novel US OF ELISA, choosing a pen specificity of 0.97, leading to a cut off value of 84 (calibrated OD value), the herd sensitivity with 10 pens sampled and a within herd pen prevalence of 0.2 would be 0.83. Likewise in the EU OF ELISA, with a pen specificity of 0.97 and a cut off value of 219 (calibrated OD value), herd sensitivity would be 0.78 with the chosen pen specificity and sampling 10 pens in a herd, the herd specificity will be 0.74 for both ELISAS.

As expected, a slight cross reactivity was found between the EU ELISA and the US ELISA (Fig 2). However, use of the abovementioned cut offs results in a reasonable specificity towards the heterologous strain in the two ELISAS. Thus specificity to the US strain in the EU-positive herds, is 74% and specificity to EU in the US herds, is 90%.

Discussion: The herd specificities may appear low – but when calculating these an assumption is made that all samples taken in one herd are independent on each other. This is naturally not the case in reality, therefore the herd specificity is expected to turn out to be higher in practice. We are going to test more paired OF/serum samples, to get to know the test even better. Further work is also to be done concerning description of guidelines for choosing sample size and performing safe diagnostics. Contrary to serum, OF is a highly variable material due to natural variation, risk of contamination and dilution (Fig 3). Collection should thus be as standardized as possible. Switching from serum to OF is a way to intensify the sampling routine within a surveillance, without an excessive rise in analysis costs.

Conclusion: Based on these data the intention is to continue the validation of this test system for differentiated detection of PRRS antibodies in oral fluid. OF-diagnostics will be a useful supplementary tool to the otherwise serum based surveillance of PRRSV EU and US in Danish swine herds.

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