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NOVEL ELISAS FOR DIFFERENTIATED DETECTION OF ANTIBODIES AGAINST EITHER PRRSV EU OR US IN ORAL FLUID

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INTRODUCTION:
In the Danish SPF system PRRSV surveillance is based upon the ability to differentiate between the American (US, Type 2) and the European (EU, Type 1) strain of PRRSV. Danish swine herds are declared either free from PRRSV or positive to either PRRSV EU or PRRSV US – or both strains. The blocking ELISAs used in this surveillance are only validated for serum (Sørensen et al. 1998). Based on the same antigens (supplied by B. Strandbygaard and A. Bøtner, National Veterinary Institute, Denmark) as in the blocking ELISAs, indirect ELISAs for PRRSV EU and US were optimized for analysis of oral fluid (OF) samples.

MATERIALS AND METHODS:
Samples for validation were obtained from PRRSV positive and negative Danish herds in collaboration with Practitioners from Odder Svinepraksis. OF pen pools were collected by hanging a rope in selected pens. For comparison, blood was drawn from all pigs in each OF-sampled pen. A total of 2551 sera and 281 OF pools were sampled, representing pigs from 15-100 kg. All sera were tested in the PRRS blocking ELISAs used in the SPF surveillance, and these results were used as a gold standard for the novel OF indirect ELISA: A PRRSV-positive pen was defined as a pen with at least 50% pigs positive in the blocking ELISA.

RESULTS:
Performance of the OF tests is plotted in Fig. 1. In the novel US OF ELISA, choosing a pen specificity of 0,97, and 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. This implies that if you take 10 rope samples, i.e. sample 10 pens, in one 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. 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%.

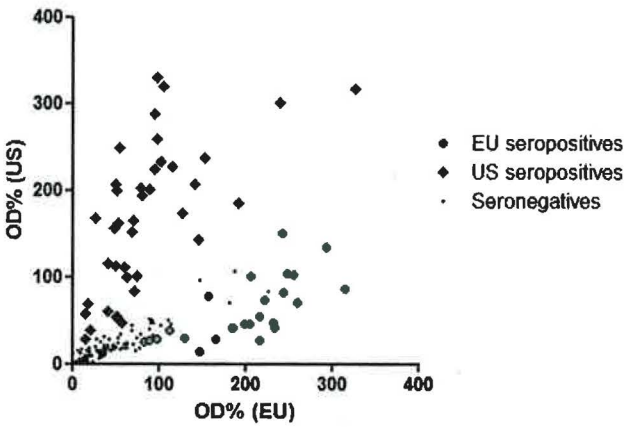

Fig. 1: Plot showing oral fluid samples tested in the new OF ELISAs. Results from the US-ELISA plotted against results from the EU-ELISA on the same samples. Symbols are referring to the serum gold standard. EU seropositives : 100% of serum samples from the pen were EU-positive in the blocking ELISA (rings). US seropositives : 100% of serum samples from pen were US-positive in the blocking ELISA (squares). Seronegatives : 100% of serum samples from the pen were negative in blocking ELISA (dots).

DISCUSSION AND CONCLUSIONS:
Based on these data we will continue developing a test system for OF, that can be used as a supplement for the serum based surveillance of PRRSV EU and US in Danish swine herds.

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Sørensen KJ, Strandbygaard B, Bøtner A, Madsen ES, Nielsen J, Have P. 1998. "Blocking ELISA's for the distinction between antibodies against European and American strains of porcine reproductive and respiratory syndrome virus." Vet Microbiol. 60, pp169-77.