COMPARISON OF TWO ELISAS FOR DETECTION OF ANTIBODIES AGAINST PRRSV WITH SPECIAL RESPECT TO FALSE POSITIVE OUTLIERS

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Introduction and Objectives

Porcine reproductive and respiratory syndrome (PRRS) is characterized by late-term abortions, stillbirths and respiratory disorder. The causative agent is a positive-stranded RNA virus of the genus *Arteriviridae* (1). Control programs for PRRS have been developed, because the disease is spreading more easily due to an increase of animal transports on account of specialisation in pig production. The most used serological diagnostic tool for PRRS monitoring is the enzyme-linked immunosorbent assay (ELISA). The purpose of this study was to evaluate the performance of two commercial tests available on the European market for the detection of antibodies against PRRSV to determine the best tool for characterization of herd immunity. The parameters that have been investigated were prevalence data and Spearman's coefficient of correlation (ρ). As the IDEXX-ELISA is mainly used for the monitoring of Austrian farms, the reproducibility of this system in different laboratories was also checked.

Material and methods

465 pig sera were analysed via two different ELISA test systems, the IDEXX HerdCheck® Porcine Reproductive and Respiratory Syndrome Virus Antibody Test Kit (IDEXX Laboratories, Wörrstadt, Germany) and the Ingezim PRRS Universal® (Ingenasa, Madrid, Spain) (Table 1) according to the manufacturers' specifications. 236 of these serum samples had been collected during routine diagnostics of 55 farms, 33 % could be defined as "outliers" with the IDEXX-ELISA. An "outlier" was defined as possible false positive sample (single positive samples of randomly tested herds, in which all other analysed samples were negative and showed a S/P ratio of < 0.2 in the IDEXX-ELISA). 129 samples were collected during a PRRSV-challenge.

Table 1. Number of serum samples analysed in various test repetitions

Analysis 1 (IDEXX)1	Laboratory 1	465 sera
Analysis 2 (Ingenasa)	Laboratory 2	465 sera
Analysis 3 (IDEXX)2	Laboratory 2	454 sera

[1]Lot 09418-DB147, 2Different Lot-numbers between 2004 and 2006

Results

The analysis 1 (IDEXX) showed a seroprevalence of 51.8 %, analysis 2 (INGENASA) of 59.1 %, and analysis 3 (IDEXX) of 60.8 %. The correlations of the different test systems and sample sets were expressed as Spearman's coefficient of correlation ρ (Tables 2-4). When results from the three different tests were compared, it was found that 42.7 % (194 samples) were positive in all three tests (true positive) and 26.4 % (120 samples) were negative in all tests (true negative). 30.8 % (140 samples) showed no corresponding result. 17.9 % of these non-corresponding results were outliers. Also the results of the two compared IDEXX-ELISA sample series showed only a percentage of corresponding results of 83.0 % (377 sera).

The positive-controls were quite different within tests. The optical density (OD) values of the IDEXX Herdcheck® PRRS had an arithmetic mean of 0.431 (0.385-0.477) for the analysis 1, and 1.075 (0.43-1.72) for test analysis 3. The positive controls of the Ingezim PRRS Universal® (analysis 2) resulted in an average of 2.27 (1.88-2.66), but these data are not directly comparable to the IDEXX-positive controls.

Table 2. Correlation of the complete sample sets expressed as Spearman's coefficient of correlation?

	Analysis 1	Analysis 2
Analysis 2	0.771	-
Analysis 3	0.828	0.662

Table 3. Correlation of the outliers expressed as Spearman's coefficient of correlation?

	Analysis 1	Analysis 2
Analysis 2	0.294	-
Analysis 3	0.694	0.338

Table 4. Correlation of the sample sets excluding the outliers expressed as Spearman's coefficient of correlation?

	Analysis 1	Analysis 2
Analysis 2	0.862	-
Analysis 3	0.857	0.742

Discussion and Conclusions

The analysis of ρ of the S/P-Ratios of the complete sample sets showed differences between the different sets, which were even more distinctive comparing only the outliers. In contrast, the results improved essentially, when outliers were excluded from the calculation. The correlation of IDEXX HerdCheck® and Ingenasa Ingezim PRRS Universal® was high (0,862) when outliers were excluded from the calculation. The reproducibility of results (analysis 1 vs. analysis 3) was about 83 %.

The reason for the occurence of false-positive results could be based on several presumptions (2). A PRRSV infection is not only the origin for the generation of IgG, but causes also the creation of IgG-containing immune complexes, which are able to bind to the coated ELISA microtiter plates. Especially, sera of older sows possessed high levels of IgGs and of ELISA plate-binding immune complexes, in spite of being PRRSV infection negative by all criteria presently available.

ELISAs are the most used serological tool for the detection of PRRSV antibodies. Although the manufacturers specify a sensitivity of 97.4 and a specifity of 99.6 %, interpretation of results has to be carried out with caution. Antibodies can be detected via ELISA 10 to 12 days post infection (p.i.) and remain detectable for about 4 months. In some secretions and excretions, antigen can be detected even before day 10 and longer than 4 months p.i. This means that, even if PRRSV antibodies are not detectable during one single analysis, there is no guarantee for the farm for being completely free from PRRSV.

The use of serological methods as the ELISA for farm monitoring within the scope of surveillance programs demands great reliability of the respective method. The eradication of a certain agent requires a high specifity of the used analytical method, because positive animals must be definitely identified to guarantee that the stock is free. One false result can put a risk to the whole surveillance program. In this context it is worth mentioning, that the outliers that were analysed in this study derived to a bigger part from a monitoring program (3). As long as there are no more reliable methods available, false-positive results have to be considered interpreting diagnostic findings.
The use combined of different assays could improve the accuracy of the results and could minimize the presence of outliers. Particulary when they are based on different principles.

References

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