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Comparison of four ELISAs for detection of antibodies against different genetic subtypes of European genotype PRRSV

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) strains belong to the two genotypes which are antigenically different. The genetic diversity in the European genotype (EU-PRRSV) is much higher than in the North American genotype (NA-PRRSV) (3). The evidence of the restricted serological cross reactivity between the genetic subtypes exists (1, 3) but it is unknown whether it impacts the sensitivity or specificity of the ELISA diagnostic tests methods. To address this problem 175 sera from farms where different genotypes and genetic subtypes circulated were tested with three commercial and one in house ELISA tests.

Materials and Methods

Serum samples were obtained from 3 Polish, 4 Belarusian and 1 Ukrainian farms, where different genotypes and subtypes circulated as it was determined by PCR and DNA sequencing. In 2 of 3 Polish farms subtype 1 of EU-PRRSV circulated. In one farm the NA-PRRSV strain was present. In Belarusian and Ukrainian farms diverse East European subtypes circulated (3). Additionally, 31 samples from pigs from farms free from PRRSV were used. They included 17 sera from Sweden that earlier were found to react false positive in HerdChek PRRS 2XR (IDEXX) ELISA and negative in IPMA test. The samples were tested with HerdChek 2XR and 3X (IDEXX), Ingezim PRRS DR (Ingenasa) and the in house test produced in our laboratory. The Ingezim test is specific for EU-PRRSV while the remaining tests are specific for both genotypes. Results were compared for each pair of ELISA tests separately using McNemar's test.

Results

All ELISAs detected PRRSV specific antibodies in sera from all farms were EU-PRRSV infection was present. Both IDEXX tests and the in house ELISA detected antibodies to NA-PRRSV in all sera from the Polish farm were this genotype circulated while Ingenasa test detected seroconversion in only 5 of 12 of the samples. In case of samples from one Ukrainian farm infected by EU-PRRSV subtype 2 the Ingezim test appeared to be significantly more sensitive than PIWet and HerdChek X3 tests. The analysis of the specificity proved it to be highest in case of the HerdChek X3 test. The HerdChek 2XR test gave false positive results with most of the Swedish sera as it was found before.

Discussion

Very high genetic diversity of PRRSV poses a serious problem for PCR diagnosis, especially for the detection of the European genotype. However, our preliminary data show that it does not influence serological diagnosis by ELISA. All tests used in the study were sensitive in detection of antibodies induced by different, diverse genetic subtypes of EU-PRRSV. However, the 3X test seemed to be slightly less sensitive than the older 2XR test and the Ingezim DR. The 3X test was the most specific test of all. Further studies are necessary to fully evaluate the impact of the antigenic diversity of the EU-PRRSV on sensitivity and the specificity of the serological methods of PRRS diagnosis.

Acknowledgements

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Farm and genotype	Number of samples	PIWet % of positive samples	HerdChek 2XR	HerdChek X3	Ingezim DR
PL1 (EU sub 1)	10	80.0	90.0	80.0	100.0
BY1 (EU)	6	100.0	100.0	100.0	100.0
BY2 (EU sub 3)	9	100.0	100.0	100.0	100.0
PL2 (EU sub 1)	24	95.8	91.7	95.8	87.5
BY3 (EU)	8	62.5	87.5	50.0	100.0
BY4 (EU sub 3)	28	67.9	75.0	57.1	67.9
PL (NA)	12	100.0	100.0	100.0	41.7
UA (EU sub 2)	16	56.3	75.0	62.5	100.0
BY (EU)	31	90.3	96.8	96.8	96.8

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Comparison of four ELISAs for the detection of antibodies against PRRSV with special respect to false positive outliers

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Introduction

The points of interest of PRRS control programs are an early detection of disease, a rapid laboratory confirmation and a reliable confirmation of a negative herd status. The most used serological diagnostic tool for PRRS monitoring is the ELISA. A new product, the Ingezim PRRS DR (Ingenasa, Madrid, Spain) was developed, which is able to detect not only IgG but also IgM antibodies, which occur earlier in the course of infection. Furthermore, the high specificity of this test should minimize the risk of false positive results in negative herds. The purpose of this study was to evaluate the performance of four European commercial tests.

Materials and Methods

In total, 478 pig sera were analysed via four different ELISA test systems (Ingezim PRRS DR (ING-DR) and Ingezim PRRS Universal® (ING-U), Ingenasa, Madrid, Spain, IDEXX HerdCheck® Porcine Reproductive and Respiratory Syndrome Virus Antibody Test Kit (ID), IDEXX Laboratories, Wörrstadt, Germany, Civtest suis PRRS E/S (CIV), Laboratorios HIPRA, S.A., Amer, Spain) according to the manufacturers' specifications. 353 of these serum samples had been collected during routine diagnostic of 148 farms, 16.4 % could be defined as "outliers". An "outlier" was defined as possible false positive sample, which means single samples of randomly tested herds that were positive, while all other analysed samples were negative and showed a S/P (sample/positive control) ratio < 0.4 in the IDEXX ELISA. 125 sera were collected during a PRRSV challenge.

Results

The analysis via ING-DR showed a seroprevalence of 67.6 %, via ING-U of 56.3 %, via ID of 59.2 % and via CIV of 29.9 %. The correlations of the different test systems and sample sets were expressed as Spearman's coefficient of correlation ρ (Table 1). When results from the four different tests were compared, it was found that 24.1 % were positive in all four tests and 21.6 % were negative in all tests. 45.1 % showed no corresponding result. 16.1 % of these non-corresponding results were outliers.

Table 1: *Spearman's coefficient of correlation ρ of the positive/negative decisions of the complete sample set (a), outliers (b), sample set excluding the outliers (c) and challenge samples (d) (*level of significance < 0.010)*

		ING-DR	ING-U	ID
(a)	ING-U	0.678*	—	—
(a)	ID	0.425*	0.513*	—
(a)	CIV	0.414*	0.392*	0.421*
(b)	ING-U	0.487*	—	—
(b)	ID	-0.344*	-0.123	—
(b)	CIV	0.589*	0.553*	-0.019
(c)	ING-U	0.705*	—	—
(c)	ID	0.525*	0.608*	—
(c)	CIV	0.390*	0.375*	0.469*
(d)	ING-U	0.917*	—	—
(d)	ID	0.824*	0.869*	—
(d)	CIV	0.444*	0.492*	0.532*

Discussion

This study underlines the complexity of PRRSV serology. The analysis of the coefficient of correlation of the complete sample set showed differences between the ELISAs, which were even more distinctive comparing only the outliers. In contrast, the results improved essentially, when outliers were excluded of the calculation. The best correlation could be found evaluating the samples of the challenge, which were defined positive or negative. The ING-DR resulted in the highest seroprevalence because of its ability to detect IgM and IgG, but nevertheless its specificity of 97.3 % should be sufficient to minimize the risk of false positive results. The different answers to different ELISAs could also be related to significant field strain differences, to the phase of the infection or other factors (1).

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Performance of an immunoenzimatic assay based on the double recognition ELISA for early detection of PRRS virus infection

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Introduction

Routine diagnosis of PRRS is generally done by means of antibodies detection against the virus, most often using indirect or blocking ELISA. With those tests seroconversions are usually detected at +14 post-inoculation (1,2). In other instances, RT-PCR is chosen as diagnostic test for PRRS. RT-PCR usually allows and earlier detection of infected animals compared to serology. Early diagnosis of PRRS is particularly crucial in two circumstances: entry of gilts in PRRS-free farms and testing of boars. INGENASA has recently developed a new ELISA based on the concept of double recognition (DR) of the antigen (3). In the Ingezim PRRS DR (11.PRS.K0) (Ingenasa) for PRRS, plates are coated with the viral protein N. Serum and protein N conjugated to horseradish peroxidase are added to the coated plates in one-step incubation. If PRRS virus (PRRSV)-specific antibodies were bound to the plate, the free binding sites of those antibodies will be able to bind the conjugated N-protein and thus, an increased sensitivity will be obtained after revealing the reaction with a colorimetric substrate. The aim of the present study was to evaluate the performance of the DR for early detection of anti-PRRSV antibodies raised against different genotype-I strains in experimental or field conditions. Comparison with HerdChek 2XR® (Idexx Laboratories) was also performed.

Material and Methods

ELISAs. Two different ELISAs were used in the present study. In all cases, sera were examined by Ingezim PRRS DR (11.PRS.K0) (INGENASA) and for particular comparisons HerdChek 2XR® (Idexx Laboratories) was used. All tests were used as recommended by the manufacturer. Results were expressed as a ratio of the optical density (OD) of a given sample over the OD of the positive control provided by the test (S/P ratio).

Sera from experimental infections. Sera (n=38) obtained from five experimental infections (days 0 to 28 post-inoculation) of piglets (4 week old) with different PRRSV genotype I strains (S1-S5) were used. Selected strains shared from 90.6% to 96.0% of similarity in protein N. In all cases, animals were intranasally inoculated with $\geq 1x10^{5.0}$ $TCID_{50}$/ml. All sera were analyzed using DR. Samples for strains S4 and S5 were also examined by the HerdChek ELISA®.

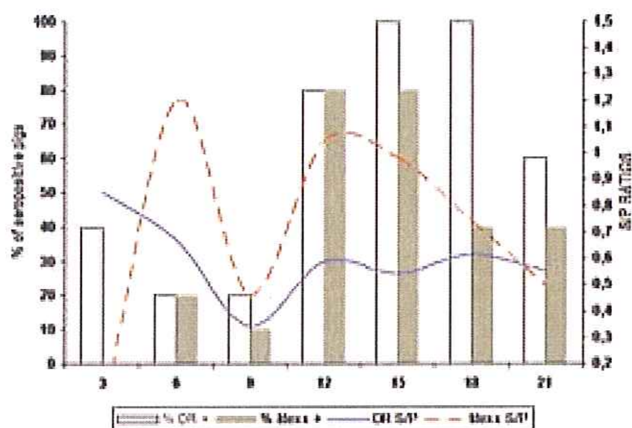
Cross-sectional profiling of an endemic farm. A cross-sectional serological profiling was comparatively performed in an endemic farm (pigs of 3, 6, 9, 12, 15 18 and 21 weeks of age) using both ELISAs.

Results

Sera from experimental infections. At day +7 PI, DR detected 29/38 sera as positive. Interestingly, for strains S4 and S5 HerdChek® did not detect any positive pig by day +7 PI while DR recognized all animals infected with S4 (8/8) and one pig in the group infected with S5 (1/8). By day +14 PI, all sera were positive in both HerdChek® and DR tests.

Cross-sectional profiling. Both DR and HerdChek® showed that PRRSV did spread explosively around 9-12 weeks of age as indicated by the seroprofile but DR had an increased sensitivity for detecting low levels of maternal antibodies or early infections as seen in 3-week-old piglets and in the higher proportion of seropositive pigs from week 15 afterwards (Figure 1).

Figure 1. *Cross-sectional profiling of a PRRS endemic farm using DR-ELISA and HerdChek®.*



Discussion

DR-ELISA allowed an earlier detection of experimentally infected pigs (76% at 7 PI). On the basis of the results in field conditions, DR also showed some enhanced sensitivity related to HerdChek®. Present results indicate the suitability of the DR-ELISA for early detection of PRRSV infection.

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Risk assessment of PRRS outbreak in endemic farms according to productivity and health management variables

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) is a devastating disease in intensive pig production and is an endemic disease in most farms in the world, but triggering factors for outbreaks should be studied for each scenario.

The objective of this study was to assess the factors linked with PRRS outbreak presentation in field conditions.

Material and Methods

Forty-three Spanish farms, belonging to the same company and with a census between 100 and 5,250 sows and gilts (mean±SD: 643±1,012) were surveyed during a minimum period of 3 years (and a maximum of 10 years) (total of 397 farm-years) in a prospective observational study.

Twelve variables related to farm facilities, stocking management, health status and strategies were studied.

Four different strategies for PRRS control were used in these farms: S1: PRRS-Positive Gilts, Live Vaccine and No Infection with Piglets; S2: PRRS-Positive Gilts, Live Vaccine and Infection with Piglets; S3: PRRS-Positive Gilts, Killed Vaccine and Infection with Piglets; and S4: PRRS-Negative Gilts, Killed Vaccine and Infection with Piglets. Live vaccine was AMERVAC®-PRRS (HIPRA) and killed vaccine was PROGRESSIS® (MERIAL). They were applied according to producer specifications. A commercial indirect-ELISA test was used to confirm PRRS diagnosis (Ingezim PRRS K1, Ingenasa).

In this study, a PRRS outbreak was defined as the sudden occurrence of reproductive problems associated to an abrupt increase of seroprevalence to this disease. Comparison of outbreak frequencies was performed with Likelihood Ratio test (for more than 2 frequencies) or Fisher's exact test (for 2 frequencies). Relative Risk and 95% confidence interval were calculated for significant associated variables. Error α was established at 0.05. All statistical analyses were carried out with SPSS® 15.0 for WINDOWS®.

Results and Discussion

During the study period only 7 PRRS acute outbreak were recorded. The only variable significantly associated with outbreak risk was the stock variation (see Table 1), so an increase of census led to a higher probability of PRRS outbreak occurrence (40%) *versus* a probability of 1.3% when no changes in census; that corresponded to a significant Relative Risk of 31.2 (95%CI: 7.8 – 124.3). It means, that there is 31 2 times more risk for PRRS outbreak to occur in farms that undergo a relevant increase of population size, than in farms with constant census.

Other variables had not been associated with PRRS outbreak (Table 1). In this study, we didn't find any significant impact of the type of vaccine used (live or killed) on the outbreak risk.

However several variables could be related to outbreak risk, but the very low number of outbreaks did not allow us to get significant results (i.e. existence of infected piglets in post-weaning, use of outsource gilts and presence of in-farm AI station).

Table 1: *Relationship between productivity & health management variables and PRRS acute outbreak*

Variable	Category	N	Outbreak	p
Fattening unit	Absence	328	1.8%	>0.999[2]
	Presence	69	1.4%	
Post-weaning unit	Absence	10	0.0%	>0.999[2]
	Presence	387	1.8%	
In-farm AI station	Absence	377	1.9%	>0.999[2]
	Presence	20	0.0%	
In-farm gilts rearing	No	358	1.7%	0.518[2]
	Yes	39	2.6%	
Stock variation	Decrease	2	0.0%	<0.001[1]
	No changes	390	1.3%	
	Increase	5	40.0%	
Gilt source	Out	363	1.9%	>0.999[2]
	In	34	0.0%	
PRRS status of gilts	Negative	178	2.2%	0.705[2]
	Positive	219	1.4%	
Gilt infection with piglets	No	69	1.4%	>0.999[2]
	Yes	328	1.8%	
Type of vaccine for gilts	Live	164	1.2%	0.705[2]
	Killed	233	2.1%	
Killed vaccine use	Only gilts	357	1.4%	0.150[2]
	All breeders	40	5.0%	
Infected piglets in post-weaning	No	26	0.0%	0.289[2]
	Yes	46	8.7%	
Health strategy for gilts	S1	67	1.5%	0.896[1]
	S2	96	1.0%	
	S3	54	1.9%	
	S4	177	2.3%	
Total		397	1.8%	

[1] Likelihood Ratio test p-value; [2] Fisher's exact test p-value

Conclusion

Increase of the population size is a critical risk factor for acute outbreak in farms with endemic PRRS independently of implemented health strategies.

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Comparative analysis of different strategies for PRRS control

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Introduction

PRRS is a devastating disease for intensive pig production. Seroprevalence studies show that it is an endemic disease in most farms. Several control measures are currently used at farm level with variable results (1).

The objective of this study was to assess, under field conditions, the effectiveness of different health management strategies for PRRS control.

Material and Methods

Forty-three Spanish farms belonging to the same company and with a census between 100 and 5,250 sows and gilts (mean±SD: 643±1,012), were surveyed during a minimum period of 3 years (maximum of 10 years) (total of 397 farm-years) in a prospective observational study.

Four different strategies for PRSS control were used in these farms: S1: PRRS-Positive Gilts, Live Vaccine and No Infection with Piglets; S2: PRRS-Positive Gilts, Live Vaccine and back passage (infection) with viremic piglets; S3: PRRS-Positive Gilts, Killed Vaccine and Infection with Piglets; and S4: PRRS-Negative Gilts, Killed Vaccine and Infection with Piglets.

Live vaccine (AMERVAC®-PRRS (Hipra, Spain)) and killed vaccine (PROGRESSIS® (MERIAL)) were applied according to producer specifications in gilts in quarantine, except in some farms where adult sows were also vaccinated with PROGRESSIS.

In this study, a PRRS outbreak was defined as the sudden occurrence of reproductive problems associated to an abrupt increase of seroprevalence to this disease.

A commercial indirect-ELISA test was used for PRRS diagnosis (Ingezim PRRS K1, Ingenasa). Seroprevalences were calculated every year from min. 20 samples (max. 370) depending on farm size (48.3 ± 35.1).

Comparison of average year seroprevalences was performed with ANOVA or Student t-test (normality was previously checked with Kolmorogov-Smirnov test). Error α was established at 0.05. Statistical analyses were performed with SPSS® 15.0 (WINDOWS®).

Comparison between outbreak prevalence was tested with classical percentage comparison.

Results

Using live vaccine and introducing PRRS-positive gilts (S1, starting point) gave us an average seroprevalence of 52% (minimum 11%). Further infection of gilts with positive piglets (S2) produced a significant increment of seroprevalence (71.9%, minimum 28.6%). A change from live to killed vaccine (S3) led to a significant decrease of seroprevalence (63.4%, minimum 2%). Finally, introducing PRRS-negative gilts (S4) led to a further reduction of seroprevalence (47.4%, with some farms becoming totally seronegative) in spite of the infection of gilts with piglets (Table 1).

However, some farms under S4 strategy still showed high seroprevalence as a probable result of PRRS virus circulation. Only a very low number of outbreaks occurred whatever the strategy (Table 2).

Table 1: *Average seroprevalences depending on control strategy*

		Seroprevalence (%)		
Strategy	N	Mean±SD*	Min	Max
Strategy 1	67	52.0±26.6[a]	11.0	100
Strategy 2	96	71.9±17.8[b]	28.6	100
Strategy 3	54	63.4±24.3[c]	2.0	100
Strategy 4	177	27.5±30.2[d]	0.0	100
Total	**394**	**47.4±32.4**	**0.0**	**100**

*ANOVA p-value<0.001 (different superscripts indicate significant differences among seroprevalences applying Duncan's post-hoc test)

Table 2: *Relationship between seroprevalence and PRRS outbreak prevalence during the subsequent year*

Use of a killed vaccine	PRRS outbreak			Seroprevalence (%)	
		N	%	Mean±SD	p*
Only gilts	No	351	1.71[a]	45.1±31.8	
	Yes	6		16.4±13.8	0.003
All breeders	No	39	2.56[a]	69.6±28.6	
	Yes	1		95.0	0.386
Total	**No**	**390**	**1.79**	**47.6±32.3**	
	Yes	**7**		**27.6±32.3**	**0.106**

* Student t-test p-value; [a] non significantly different

Discussion and Conclusion

It is well known that ELISA antibodies are more of a proof that pigs have been in contact with the virus than related to protection (2) and that ELISA antibody response to PROGRESSIS vaccination are variable (3). The use of killed vaccine in seronegative gilts with infected piglets did lead to a significant reduction of PRRS-seroprevalence in most farms due to no new virus brought through the incoming gilts and through the control of existing infection. This was confirmed by the low overall number of outbreaks occurring during the study. The PRRS status reached in most of the farms could eventually let us consider an eradication program.

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