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Using commercial ELISAs to assess humoral response in sows repeatedly vaccinated with modified live porcine reproductive and respiratory syndrome virus

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Abstract

Background Sows in breeding herds are often mass vaccinated against porcine reproductive and respiratory syndrome (PRRS) every few months using modified live vaccines (MLV). Field veterinarians repeatedly report that multiple vaccinated sows test negative in ELISA. Obviously, this creates uncertainty when assessing the compliance of vaccination and the status of sows.

Methods In the present study, four commercial ELISAs were used to assess the serological PRRS status in gilts and sows of three farms that were PRRS MLV vaccinated every four months. Animals were tested before vaccination (BV) and postvaccination (PV). Total and neutralising antibodies and cell-mediated responses were also measured in animals that yielded negative results in all ELISAs.

Results The proportion of seronegative animals BV varied depending on the farm and the ELISA used. When samples were analysed using only one ELISA, a substantial number of negative results obtained BV remained as negative afterwards. Five animals were negative BV and PV with all the examined ELISAs. Those animals also yielded negative results in all the other immunological assays.

Conclusion Our findings suggest that the use of ELISA for monitoring multiple PRRS MLV vaccinated sows is very limited due to the variability of the humoral responses and the moderate agreement between tests.

Introduction

Since the emergence of the disease in the middle of the 1980s, porcine reproductive and respiratory syndrome (PRRS) has been considered one of the most challenging and costly diseases for the pig industry.¹ The main tools for controlling PRRS are vaccination, management of the pig flow and biosecurity measures, as well as the monitoring of the herd either serologically or by PCR.^{2 3} Both inactivated and modified live vaccines (MLV) against PRRS virus (PRRSV) are commercially available, with MLV being preferable for the primary immunisation.⁴ Current vaccines induce only partial immunity against the heterologous challenge,^{5–7} but they are effective in reducing the frequency of abortions, stillbirths and other reproductive disorders related to PRRSV infection in sows.⁸ One of the most common PRRSV vaccination protocols in breeding herds is based on the application of a blanket vaccination approach with recall MLV doses every 3–4 months. In principle, compliance to vaccination or simple monitoring of the breeding herd could be achieved by testing sows using an ELISA assay, since all vaccinated or infected animals should have tested positive beforehand. Nevertheless, swine practitioners often report that some multiply MLV-vaccinated sows give negative results in ELISA. This creates uncertainty about the real status of the sows, as well as the performance of the ELISAs. The aim of the present study was to assess the presence of PRRSV ELISA-negative sows in multiply vaccinated herds and to examine the possible causes of that phenomenon. Four different commercial ELISAs were used to determine the serological status of gilts and sows before vaccination (BV) and postvaccination (PV). When animals yielded negative results in all the ELISAs BV and PV, total and neutralising antibodies, as well as the cell-mediated response against PRRSV, were also analysed to evaluate the immune status of the seronegative sows.

Materials and methods

Farms

The study consisted of three farrow-to-finish farms (designated as F1, F2 and F3), each one housing around 300 breeding sows. In F1 and F2, female breeders were Landrace x Largewhite x Meishan breedcrosses, while in F3 were Landrace x Largewhite. Semen from Pietrian males were used in F1 and F3 and from Duroc ones in F2. In all those farms, the veterinary services reported that a high proportion of sows usually tested negative in ELISA (IDEXX PRRS X3; Idexx Laboratories, Westbrook, Maine, USA), in spite of being multiply vaccinated with a PRRS MLV. In all three farms, the immunisation scheme against PRRSV included the acclimatisation of gilts by exposure to the wild-type PRRSV1 present in the farm, with further recall immunisation of sows. This was achieved by means of blanket vaccination with a PRRSV1 MLV every 4 months (three doses per year).

Experimental design

The initial sampling (at least n=56, gilts and sows) was designed to detect the presence of seronegative sows if their frequency was at least 5% of the breeding stock (95% CI). Eventually, 171 animals were used: F1 (n=59), F2 (n=60); in F3, only 52 animals could be bled. In order to account for differences in the proportion of seronegative sows by parity, the total number of samples was distributed as much as possible according to the distribution of parities in the sows' stock. After that first bleeding, animals were immediately vaccinated intramuscularly as usual using a commercial PRRSV1 MLV (Porcilis PRRS; MSD Animal Health). Twenty-one days PV, the same animals were bled again. Animals yielding negative results in ELISA BV and PV (n=5) and other randomly chosen ones (n=55) were bled again into heparinised tubes (28 days PV) in order to analyse the cell-immune responses. Consent from the animal's owners was also obtained.

ELISA tests

All sera collected BV and PV were tested for antibodies against PRRSV using four commercial indirect ELISAs as follows: E1 (IDEXX PRRS X3 Ab ELISA), E2 (Pigtype PRRSV Ab; Qiagen, Leipzig, Germany), E3 (Ingezim PRRS universal; Inmunologia y Genetica Aplicada SA, Madrid, Spain) and E4 (Civtest suis PRRS E/S; Laboratorios Hipra, Amer, Spain). According to the manufacturers, all tests are developed to detect IgG antibodies against PRRSV. E1, E2 and E3 are based on the nucleocapsid as antigen, whereas E4 is coated with nucleocapsid and a glycoprotein-rich extract obtained by solubilising whole PRRSV particles. All tests were performed according to the manufacturer's instructions.

For E1, E2 and E3, results were expressed as a ratio of the optical density (OD) of a given sample over the OD of the positive control provided (sample-to-positive (S/P) ratio), whereas a relative index \times 100 (IRPC) using the formula described in the manufacturer's instructions was calculated for E4. The positive S/P threshold value was ≥ 0.4 for E1 and E2; cut-off was $0.15 \times OD$ of positive control for E2 and IRPC greater than 20 for E4. In order to minimise the biases, all samples from a given farm were tested using the same batch of a given ELISA kit. All samples yielding negative or doubtful results in a given ELISA were retested to discard any potential error attributable to the laboratory processing.

For those animals that yielded negative PRRS ELISA results BV and PV, total antibodies against Aujeszky's disease virus (ADV) were also measured using ELISA (Civtest suis ADV; Laboratorios Hipra, Amer, Spain). ADV ELISA was performed according to the manufacturer's instructions. Several additional PRRS ELISA positive animals were randomly chosen from each farm, and included as controls (n=20).

Immunofluorescence antibody and viral neutralisation tests

When an animal was negative by all the ELISAs used BV and PV, postvaccination sera were tested in MARC-145 cell cultures by the immunofluorescence antibody test (IFAT) and by the viral neutralisation test (VNT) in order to be able to detect antibodies other than those directed at the ELISA antigens (mainly nucleocapsid protein). For the IFAT test, MARC-145 monolayers were infected with the vaccine virus for 72 hours and fixed with ethanol at -20°C . After washing the plates, samples to be tested were serially diluted from 1:5 to 1:160 and added to the plates

(45 min 37°C). The plates were then washed and a goat anti-Pig IgG H+L FITC- labelled antibody (Abcam, Cambridge, UK) was used to reveal the presence of antibodies against PRRSV vaccine strain. Known positive and negative pig sera from other experiments were used as controls. Several ELISA-positive and ELISA-negative sera randomly chosen from each farm were also included (n=50). The procedure for the VNT was done as previously described⁹ with minor modifications. In this latter case, sera were serially diluted from 1:2 to 1:128, with the sera dilutions then being mixed with the PRRSV vaccine strain, and final assessment of the neutralisation was made by the addition of an anti-PRRSV antibody (ICH5, Ingenasa, Madrid, Spain) and a fluorescein-labelled anti-mouse IgG antibody (Jackson ImmunoResearch Laboratories, West Grove, USA). Neutralisation titres of at least 1:4 were considered of biological significance. Samples were run in duplicate in both IFAT and VNT assays. Furthermore, known positive and negative sera from other studies and several ELISA-positive and ELISA-negative sera randomly chosen from each farm were also included.

Interferon- γ enzyme-linked immune absorbent spot (ELISPOT)

Sows that tested negative in all ELISAs BV and PV were bled again into heparinised tubes (n=5). Peripheral blood mononuclear cells (PBMCs) were isolated, and the interferon- γ (IFN- γ) ELISPOT for PRRSV was performed as described previously.^{10 11} The vaccine strain was used to stimulate PBMCs at a multiplicity of infection of 0.1. Several ELISA-positive animals were randomly chosen from each farm and included as controls (n=55).

Quantitative reverse transcription (qRT)-PCR

The sera of animals testing negative in all ELISAs BV and PV were analysed by qRT-PCR¹¹ in order to assess the persistence of viral replication. Viral RNA was extracted using the BioSprint 96 One-For-All Vet kit (Qiagen, Leipzig, Germany) and the BioSprint 96 workstation (Qiagen) according to the manufacturer's instructions. A plasmid containing an ORF7 amplicon was used as a positive control and for quantification purposes (from 100 to 10⁹ genomic copies/tube). Positive and negative controls for the RNA extraction were also included in each analysis. Several randomly chosen sera from other ELISA-positive and ELISA-negative animals from all three farms were also included for comparative purposes (n=55).

Statistical analyses

When needed, Excel Software 2013 was used to perform the random selection from the set of animals. Descriptive statistics, box-plot representations and inferential statistics, including calculation of the correlation coefficient (r^2) between S/P values of the ELISAs E1, E2 and E3, comparison of the proportions of animals in each category (χ^2) and the non-parametric Mann-Whitney U test were performed using Statsdirect V.3.0.167. GraphPad Software was used to calculate the agreement between all ELISAs using Cohen's Kappa coefficients.

Results

ELISAs

Distribution of samples according to their S/P or IRPC values is shown in [figure 1a-d](#). In general, S/P values were widely scattered within a farm both BV and PV, but the mean S/P or IRPC of the population significantly increased PV for all four ELISAs ($p < 0.05$) ([figure 1a](#)). However, a more detailed examination revealed that the increase was not observed in F3 for any ELISA, or in F1 for E3, and in any farm for E4 ([figure 1b-1d](#)).

The proportion of seronegative animals BV varied with the farm, as well as with the ELISA kit used ([table 1](#)). Thus, for F1, seronegative animals BV ranged from 1.7% to 6.8%; in F2, from 26.7% to 45% and for F3, negative animals accounted from 15.4% to 30.8%, depending on the ELISA. The percentage of seronegative animals was reduced PV, except in F1 (5%-10% in F1 compared with 3.3%-18.0% in F2 and 9.6%-19.2% in F3). Overall, five animals—one in F1 (parity 7), one in F2 (parity 3) and three in F3 (parities 3, 5, and 6)—were negative BV and PV with all the examined ELISAs.

The animals that yielded positive results BV and PV in a given ELISA were categorised according to the magnitude of the changes in the S/P ratios (E1, E2, and E3) or the IRPC (E4) BV and PV as follows: (1) the ratio of ELISA values PV and BV was up to 0.5; (2) the ratio

between the BV and PV values was between 0.5 and 2.0 and (3) the ratio was ≥ 2.0 (table 2 and online supplementary data table 1). For most animals in all farms, the ratio of S/P or IRPC values was between 0.5 and 2.0, with no significant differences between ELISAs. This ratio was ≥ 2.0 (depending on the ELISA) in only between 13% and 22.7% of the cases. Animals that did not seroconvert PV or became negative did not significantly accumulate in older parities.

As an example, when the distribution of S/P values was examined in detail for one of the most used ELISA to detect PRRSV-antibodies (E1), animals that increased the S/P value ≥ 2 PV had, on average, a lower S/P BV than the ones that did not (0.82 ± 0.35 vs 1.35 ± 0.7 ; $p < 0.05$). Nevertheless, some animals with low S/P BV also did not increase PV (figure 2).

Correlation and agreement between tests

Correlation coefficients between ELISA S/P values for each pair of tests (E1, E2 and E3) are shown in table 3 and in online supplementary data figure 1a-1c. Overall, correlation coefficients PV were always higher compared with those calculated BV (0.48 vs 0.82 for E1-E2, 0.62 vs 0.80 for E1-E3 and 0.51 vs 0.71 for E2-E3). To further evaluate the diagnostic agreement between the different ELISAs, kappa (κ) values were calculated for all samples, and by farm, using the Cohen's Kappa coefficients; table 4 summarises kappa values according to all samples and to samples BV and PV, separately. In online supplementary data, tables 2-4 summarise the agreements for each farm separately, and online supplementary table 5 shows the distribution of positive and negative results according to the ELISAs. Overall, kappa values for all samples could be considered from moderate to good, as they ranged from 0.50 (E3-E4) to 0.72 (E2-E3). Similar results were obtained when BV and PV periods were individually analysed. Thus, during the BV period, the lowest kappa value was obtained for E3-E4 (0.54) and the highest for E2-E3 (0.73), while during PV period, the lowest corresponded to E3-E4 (0.43) and the highest to E1-E4 (0.73).

IFAT, VNT, ELISPOT and additional tests for ELISA-negative animals

Sera from animals yielding negative results BV and PV in all ELISAs (five animals) were further analysed by the IFAT and VNT, yielding negative results in both assays. In contrast, randomly selected animals with positive ELISA status were positive for IFAT and VNT. For one animal that was positive BV, but became negative in all ELISAs PV, VNT was positive at 1:8.

The five all-negative sows were then bled again in order to measure the cell-mediated immunity against PRRSV using the IFN- γ ELISPOT. All were negative, whereas randomly selected animals with different positive ELISA status were positive, with IFN- γ -secreting cell frequencies between 25 and $600/10^6$ PBMCs.

Since the results for the five mentioned animals might suggest a lack of immune response against the virus, sera of the pigs were tested by qRT-PCR PV in order to evaluate the possible persistence of the vaccine virus. None of them tested positive 21 days PV, and of all the other animals, only three tested positive: one in F1 ($C_q = 36.99$, seropositive BV and PV) and two in F3 ($C_q = 36.90$ and 38.84 of which one was negative BV and positive PV for all ELISAs and the other one was positive for three ELISAs BV and positive for two ELISAs PV, respectively).

During the period of study, animals were also vaccinated against ADV as a part of the routine vaccination scheme of the farm. All five animals that yielded negative results in PRRS ELISA BV and PV were positive in the ADV ELISA at day 0 and 21 and, as a matter of fact, the mean IRPC increased on average from 77 BV to 199.4 PV (cut-off IRPC more than 12.0).

Discussion

The origin of the present study were the frequent reports from field veterinarians indicating that sows multiple vaccinated with different PRRS MLV became negative in ELISA, a fact that created uncertainty about the real immunological status of vaccinated sows, and complicated the monitoring of the sow herd. At present, when a considerable proportion of sows test negative in ELISA, it is difficult to say if the cause is the lack of compliance of vaccine administration, a matter of the ELISA used, a problem related to the immunological response of individuals or to other causes. The present study dealt with the investigation of this problem.

Most commercial PRRS ELISAs use the nucleocapsid protein (N) as the diagnostic antigen in indirect ELISA. Antibodies against this protein appear as early as 7-14 days after infection or

vaccination and are thought to persist for months, and some epitopes in N are shared between PRRSV1 and PRRSV2.¹²⁻¹⁵ However, for PRRSV, it is unclear if there is a classical anamnestic humoral response after a re-encounter with the viral antigens,^{16 17} and thus it is difficult to interpret results of the ELISA in multiple vaccinated animals.

The first point that deserves to be mentioned is the scattering of S/P values obtained with all ELISAs, except E3. This can be the result of the different cut-offs of each ELISA, in combination with a larger or narrower dynamic range of the ELISA.

The approach of the present study permitted to observe whether or not individual sows had an increase in the S/P ratios or IRPC values PV. Interestingly, animals that had the highest increase in the S/P ratios PV had, on average, lower S/P values compared with animals that did not increase the S/P. This was not related to parity and, in consequence, with the number of vaccine doses received during life. Given that animals were vaccinated every 3-4 months, the interval of time between vaccinations would be enough to avoid overlapping with the response to the previous vaccination. This would permit an increase in the response if a plateau phase had not been reached. In principle, the lack of increase in the S/P can be interpreted as a saturation of the specific response, and this seemed to be related more to the individual idiosyncrasy of each sow than to the number of times that a sow had been vaccinated, suggesting that the genetic background of the pig plays a role.

Several studies have shown that contact with PRRSV induces antibodies against N protein, which for most animals last about 24-32 weeks in either vaccination or infection,^{14 18} although a certain individual variability exists. Certainly, animals negative in ELISA BV could be either truly PRRSV-negative pigs or just animals becoming negative for N protein antibodies, but still positive to other viral proteins not tested in this study. PV, some sows became positive to all ELISAs, others became positive to only some of the tests and finally, five sows remained negative to all serological tests. It was evident that the test had an influence on the result.

Interestingly enough, some previously seropositive animals in a given ELISA became negative after a recall immunisation. All of them but one still tested positive in at least two other ELISAs and can thus be considered false negative results. The negative one yielded positive results in both VNT and ELISPOT, demonstrating that the animal had developed immunity against PRRSV. This result suggests that for vaccinated animals testing negative in a given ELISA does not necessarily mean the lack of immunity.

As regards the five animals that remained negative to all tests PV (including IPMA, VNT and ELISPOT), compliance was not considered an issue, since vaccination was performed under supervision. Interestingly, these animals were healthy and distributed in parities more than 3. Therefore, they had been already vaccinated several times before the beginning of the present study. In that case, pre-existing immunity could play a role in the immune response in multiply vaccinated sows; however, for these particular five sows, neutralising antibodies were not detected BV, and therefore the interference of pre-existing immunity seems unlikely for this case. These five animals were positive in ELISA against ADV, an antigen against which they were also vaccinated, and therefore, the lack of detectable immunity against PRRSV was not due to a physiological impossibility (eg, a B immunodeficiency), suggesting that, for some reason, those animals did not develop an immune response detectable by the usual means used in PRRS testing. Several studies have demonstrated that a single shot of MLV induces a weak and delayed response in terms of neutralising antibodies and cell-mediated immunity; however, naive animals seroconvert and antibodies can be detected for several months using commercial ELISAs.^{6 14 19} By contrast, other authors have reported that repeated homologous immunisation with PRRSV field strains or MLV can induce very limited responses and that the immune responses in terms of both humoral and cellular responses cannot be continuously improved by this schedule.²⁰⁻²³ Moreover, some authors claimed that repeated MLV immunisation could provoke a reduction in the proliferation of effectors and memory T cells,²⁰ and it has even been hypothesised that repeated immunisation with MLV may induce a state of anergy.²⁴ However, vaccination did not result in a long viraemia in those negative animals, although the strain used is considered to replicate poorly in vivo.²⁵ In the present study, just one vaccine was evaluated (one of the most sold in Europe). That the use of a different vaccine could have resulted in slightly different outcome cannot completely deny; however, the little literature published on this subject does not seem to point to such a thing.

Most gilts in F2 were negative BV, probably indicating a failure in the initial exposure to the field strain, since all those gilts were shown to seroconvert in at least one ELISA PV.

Nevertheless, for a given farm, the number of negative animals, both BV and PV, varied depending on the ELISA. Also, for a given ELISA, results varied among farms. As regards farms, the causes for this can be diverse including particularities related to management procedures, strain(s) that were circulating or idiosyncratic responses related to the genetics. As for the ELISAs, the causes could include the particular antigens used in each ELISA (although in principle only E4 used an antigen other than the N protein), the potential antigenic diversity of the virus and the characteristics of each kit (reagents used, etc).

As the present study shows, the usefulness of ELISA results is very limited in multiple PRRS-vaccinated sows, because of the variable humoral response of vaccinated animals and the limited agreement between tests. Apart from this, it is worth noting that some multiple vaccinated sows may apparently be anergic. This deserves further investigation in order to determine the causes and the potential impact of these animals in the epidemiology of the infection. The need for improving the monitoring of PRRS-vaccinated herds is becoming increasingly evident.

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Competing interests

None declared.

Ethics approval

The present study has been approved by ethics committee for animal experimentation from the Universitat Autònoma de Barcelona (Id. 5796) and the Animal experimental commission from the local government (Departament de Medi Ambient i Habitatge from the Generalitat de Catalunya).

Data availability statement

All data relevant to the study are included in the article.

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Table 1 Distribution of seronegative animals. Seronegative animals in each farm by parity for four commercial ELISAs (E1, E2, E3, and E4) before and after PRRS MLV vaccination (BV and PV, respectively), and the number of them that seroconverted (Neg → Pos), remained negative (Neg → Neg) or became seronegative (Pos → Neg) after vaccination.

FARM 1 (n=59)									
Parity	N° of sows	E1		E2		E3		E4	
		BV	PV	BV	PV	BV	PV	BV	PV
0	8	-	-	-	-	-	-	-	-
1	19	-	-	-	-	-	-	-	-
2	8	-	-	-	-	-	-	-	-
3	5	1	1	-	-	-	-	1	1
4	2	-	-	-	-	-	-	-	-
5	3	-	1	-	-	-	1	-	-
6	4	-	-	-	-	1	1	-	-
7	8	2	3	1	3	1	4	2	3
8	2	-	-	-	-	-	-	1	1
TOTAL		3	5	1	3	2	6	4	5
Neg → Pos		0		0		0		1	
Neg → Neg		3		1		2		3	
Pos → Neg		2		2		4		2	

FARM 2 (n=60)									
Parity	N° of sows	E1		E2		E3		E4	
		BV	PV	BV	PV	BV	PV	BV	PV
0	16	12	4	8	2	12	1	10	3
1	12	2	-	2	-	2	-	-	1
2	11	2	1	-	-	3	-	-	1
3	8	3	1	1	-	2	-	1	2
4	8	5	3	3	-	4	-	3	4
5	3	1	-	-	-	-	-	1	-
6	1	1	1	1	-	1	-	-	-
7	1	1	1	1	-	1	-	1	1
TOTAL		27	11	16	2	25	1	16	12
Neg → Pos		16		14		24		10	
Neg → Neg		11		2		1		6	
Pos → Neg		0		0		0		6	

FARM 3 (n=52)									
Parity	N° of sows	E1		E2		E3		E4	
		BV	PV	BV	PV	BV	PV	BV	PV
3	1	1	1	1	1	1	1	1	1
4	11	4	2	1	2	2	2	-	1
5	24	7	3	5	1	6	3	7	2
6	16	4	4	1	1	1	2	2	1
TOTAL		16	10	8	5	10	8	10	5
Neg → Pos		7		4		3		6	
Neg → Neg		9		4		7		4	
Pos → Neg		1		1		1		1	

Table 2 Percentage of distribution of seropositive animals from all farms. Animals yielded positive results before and after PRRS MLV vaccination were classified in three categories according to the changes in the S/P ratios (ELISAs E1, E2 and E3) or in the IRPC (ELISA E4): a) $\leq x0.5$ in the after vaccination value compared with the before vaccination one, b) animals with a change in the ELISAs values between $>x0.5$ and $<x2.0$, and c) an increase $\geq x2.0$ comparing before and after vaccination.

Change of S/P or IRPC (after/before vaccination)	E1	E2	E3	E4
$\leq x0.5^a$	1%	0.7%	0.8%	0.7%
$>x0.5-<x2.0^b$	86%	76.6%	83%	86%
$\geq x2.0^c$	13%	22.7%	16.2%	13%

Table 3 Correlation coefficients. Correlation coefficients and 95% confidence intervals for the comparison of S/P ratios obtained with ELISAs E1, E2 and E3 considering all sera before PRRS MLV vaccination (BV) and 21 days post-vaccination, and in total. All coefficients were statistically significant ($P<0.05$).

Correlation coefficient r^2 (95% confidence interval)			
ELISAs	E1-E2	E1-E3	E2-E3
BV	0.48 (0.36-0.58)	0.62 (0.52-0.70)	0.51 (0.40-0.61)
PV	0.82 (0.78-0.86)	0.80 (0.74-0.85)	0.71 (0.62-0.77)
TOTAL	0.68 (0.61-0.72)	0.71 (0.64-0.76)	0.61 (0.53-0.66)

Table 4 Agreements. Kappa values and 95% confidence intervals for the results of samples taken before and post-PRRS MLV vaccination (BV and PV, respectively) and in total for the four ELISAs used in the study.

Kappa-value and 95% confidence interval				
BV	E1	E2	E3	E4
E1	-	0.63 (0.50-0.77)	0.67 (0.54-0.80)	0.60 (0.46-0.74)
E2	-	-	0.73 (0.60-0.86)	0.68 (0.52-0.83)
E3	-	-	-	0.54 (0.38-0.70)
E4	-	-	-	-
PV	E1	E2	E3	E4
E1	-	0.51 (0.32-0.71)	0.48 (0.28-0.67)	0.73 (0.58-0.88)
E2	-	-	0.70 (0.49-0.91)	0.52 (0.31-0.74)
E3	-	-	-	0.43 (0.21-0.64)
E4	-	-	-	-
TOTAL	E1	E2	E3	E4
E1	-	0.60 (0.49-0.71)	0.61 (0.50-0.71)	0.65 (0.55-0.71)
E2	-	-	0.72 (0.61-0.83)	0.62 (0.49-0.74)
E3	-	-	-	0.50 (0.37-0.63)
E4	-	-	-	-

Figs. 1a-1d Boxplots. Boxplots showing S/P (ELISA E1 green, ELISA E2 yellow and ELISA E3 orange) or IRPC (ELISA E4 blue) values –mean, median, quartiles and range (min, 25, 50, 75, max) - considering all samples (Figure 1a) and for each farm (Figure 1b: F1; figure 1c: F2; figure 1d: F3), before PRRS MLV vaccination (BV) and 21 days post-vaccination (PV).

Black rhombus: median.

X: mean.

Dotted red line: cut-off of the ELISAs.

* $P < 0.05$ for the comparison of results BV and PV for a given ELISA.

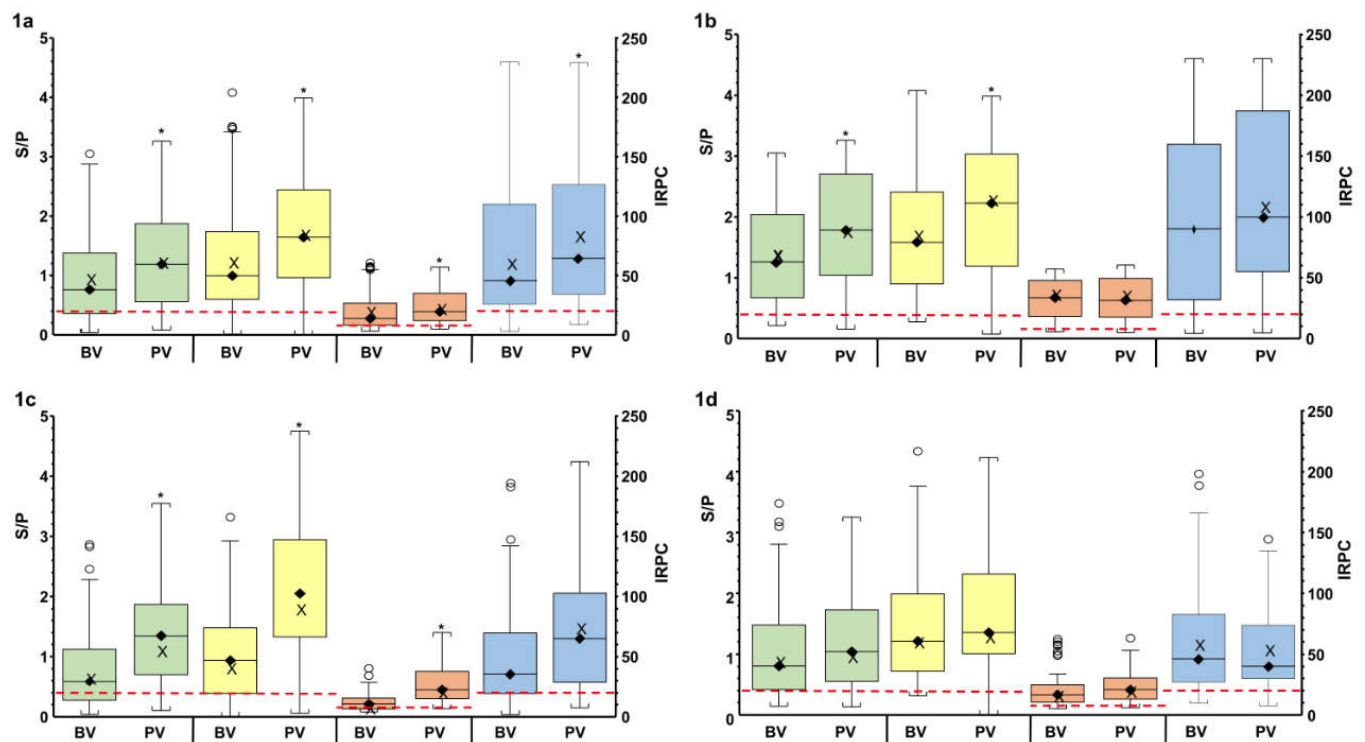


Fig. 2 Distribution of S/P ratios for seropositive animals (ELISA E1). According to the magnitude of the increases in their S/P ratios animals that yielded positive results using ELISA E1 were individually distributed in two categories: increase ≥ 2 (red bars) or increase < 2 (green bars) comparing the value obtained after vaccination to the value obtained before vaccination.

