

## **PRRS DIAGNOSIS OBTAINED BY ELISA METHOD IN PROFFESIONAL AND HOUSEHOLD BREEDING UNITS FROM BRAILA**

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### **Abstract**

*Porcine Reproductive and Respiratory Syndrome (PRRS) is an infectious disease produced by a virus and it is characteristic to swine. The disease produces important economic damages by reproduction and breathing problemes and with expensive cost by prevention and control of spreading (Rotaru, 2005).*

*This paper shows the results obtained using serological examination by ELISA method in big proffesional breeding swine units compared with household breeding swine units from Braila.*

*In the blood samples taken from different categories was observed a high incidence of antibodies PRRS to the swine growth in proffesional units compared with the results from swine growth in household units where the antibodies against PRRS were not presents.*

*In literature antibodies and virus detection may be a surprise because the clinical signs are not always present (Perianu, 2012).*

**Key words:** *ELISA test, PRRS, swine.*

### **INTRODUCTION**

The disease was initially confused with other diseases. At first it was called mystery swine disease (MDS) or infertility and respiratory syndrome (SIRS), blue ear disease (BEPD), epidemic abortion and respiratory syndrome of pigs (Pears). Currently run under three important names: in U.S.-infertility and respiratory syndrome (SIRS); in European countries - respiratory and reproductive syndrome of pigs (PRRS) porcine epidemic abortion and respiratory syndrome (Pears) (Zimmerman et al., 2006).

The virus that causes this syndrome was isolated and studied for the first time (1991), the Institute Lelystad in the Netherlands, for which it received initially name of Lelystad's virus. It is now included in the family Arterividae, like Arterivirus, along with other three pathogenic viruses to animals (Rotaru Elena, 2005; Zimmerman et al., 2006).

The clinical evolution could be acute or chronic marked by two main components respiratory and / or breeding (Rotaru Elena, 2005). Respiratory disorders are present in all types of animals and manifests by: loss of appetite, moderate hyperthermia, cough and dyspnea (OIE, 2012). Reproductive disorders are expressed by: late abortions, premature births or abnormal prolongation of gestation period, high neonatal mortality. At sows are reported: anestrus, agalactia and anorexia (OIE, 2012).

## **MATERIALS AND METHODS**

Serum samples were collected from two commercial pigs units, from the following categories of pigs: piglets, young pigs, boars and sows, as well as from non- professional units in the surroundings.

Holdings are located at a distance of 30 km from each other and will be called in this paper farm A and farm B.

In the farm A, the disease was suspected based on clinical signs and lesions. The first clinical signs appeared in the maternity ward where the highest percentage of disease was reported in newborn piglets, weaned piglets and less to fatty pigs.

When an outbreak occurs serological examination was performed in alive and the dead animals.

From farm A were collected 50 samples of serums as follows: from piglets -10 samples; young pig-10 samples, fat pigs-10 samples, sows- 10 samples; boars-10 samples.

From same farm were collected 18 thoracic fluid samples from bodies as follows: 9 piglets bodies, 3 bodies of dead piglets, 3 bodies from young pigs and 3 bodies fat pigs.

In the farm B disease appeared after the introduction of newly purchased lots of animals.

From this unit were tested a total of 25 blood samples as follows: piglets - 5 samples, young pigs-5 sample, fat pigs-5 samples; sows- 5 samples; boars -5 samples.

From farm B were collected 14 thoracic fluid samples from bodies as follows: 4 bodies piglets, 5 bodies of dead piglets, 3 bodies from young pigs and 2 bodies fat pigs.

In this study were analyzed blood samples collected from non-professional growth units (households), at a distance of 1 km around the professional farms.

For detection of serum antibodies anti-virus PRRS was used immunoassay test (ELISA). PRRS virus antibodies can be detected by enzyme immunoassay in 7-14 days after infection and levels of antibody titers reached maximum at 30-50 days. Some pigs may become serumnegative within 3-6 months, but others remain serumpositive for longer. The maternal antibody can generally be detected until 4-10 weeks after birth, depending on the sows antibody titre (Yoon, 2002).

The HerdChek PRRS X3 test kit is an enzyme immunoassay for the detection of IgG antibody to porcine reproductive and respiratory syndrome virus (PRRSV) in swine serum and plasma samples. A microtitration format has been configured by coating recombinant PRRSV antigens on the plate.. Studies were made using IDEXX PRRS ELISA kit (IDEXX Laboratories HerdCheck Switzerland AG.).

IDEXX PRRS ELISA is generally considered to have good specificity and sensitivity (OIE, 2012)

The kit components are as following:

- 5 microtiter plates with 96 strips each lined with PRRS antigen virus,
- Sample diluent, phosphate buffer with protein stabilizers and sodium azide as a preservative,
- PRRSV-positive control, anti-PRRS antibody in phosphate buffer with protein stabilizers,
- PRRSV-negative control, not reacting to swine serum PRRS in phosphate buffer with protein stabilizers preserved with sodium azide,
- Anti-Porcine IgG conjugate labeled with peroxidase (HRPO),
- TMB substrate solution,
- Stop-solution (SDS),
- Wash-concentrate solution (10X).

After washing steps, unbound materials in these complexes are removed, then an anti-swine conjugate labeled with peroxidase is added and it will be bind to the antibodies wich are bind to antigens in the wells. Unbound conjugate is removed by washing stage and the next stage will add TMB substrate. The color that developed is directly proportional to the amount of PRRS specific antibodies present in the sample.

The first step was reprezent by sample preparation.

The sample is diluted 1:40 with sample diluent (e.g. by diluting 5 µl of sample with 195 µl of sample diluent). Do not dilute controls.

Washing solution

The wash concentrate should be brought to room temperature (18-25°C) and mixed to ensure dissolution of any precipitated salts. The Wash Concentrate

must be diluted 10 fold (1/10) with distilled/ deionized water before use (e.g. 30 ml of concentrate plus 270 ml of water per plate to be assayed). All reagents must be allowed to come to room temperature (18-25°C) before use.



Figure 1. ELISA kit

Dispense 100 µl of undiluted Negative Control in the first two wells (A1, B1). Then it will be add 100 ml undiluted Positive Control in the next two wells (C1, D1).

Dispense 100 µl of diluted sample into two wells of the assay plate Incubate for 30 min at 18-25°C. The plate was washed 3-5 times with 300 ml of washing solution, then beat on a filter paper to remove traces of liquid wich could be on well.

Add 100 ml each of swine anti IgG conjugated with peroxidase labeled (HRPO) to each well. Incubate at 18-25 °C, 30 minutes.

The plate will be wash 3-5 times with 300 ml of washing solution. Microtiter plate will be gently shake on a filter paper to remove traces of liquid wich could be on well. Next will be add 100 ml of TMB substrate solution into each well. Incubate 15 minutes at 18-25 °C.

Add 100 ml of stop solution to each well to stop the reaction. Measure and record the wavelength of A (650) samples and controls.



Figure 2. ELISA results

#### Validation test.

The test could be valid if the following specifications are found:

The positive control mean minus the mean of the negative control must be greater than or equal to 0.150. In addition the negative control mean (NCX) must be less than or equal to 0.150. For invalid assays, technique may be suspect and the assay should be repeated. The presence or absence of antibody to PRRSV is determined by calculating the sample to positive (S/P) ratio (Yoon, 2002).

#### Calculations

Calculation of negative control mean (NCX)

$$NCx = \frac{NC1 (A650) + NC2 (650)}{2}$$

Calculating of positive control mean (PCX)

$$PCx = \frac{PC1 A (650) + PC2 (650)}{2}$$

#### Interpretation of results

The presence or absence of antibody to PRRV is determined by calculating the S/P ratio for each sample. If the S/P is less than 0.40 the sample is considered Negative for PRRSV antibodies. If the S/P is greater than or

equal to 0.40 then the sample is considered Positive for PRRSV antibodies (Yoon, 2002).

## RESULTS AND DISCUSSION

Of the 50 serum samples collected from pigs breed in farm A were obtained the following results presents in table 1.

Table 1. ELISA test results on serum of pigs from farm A

Category	No. samples	No. (%) samples		
		positive	dubioase	negative
suckling	10	10 (100%)	0	0
young pigs	10	5 (50%)	0	5 (50%)
fat pigs	10	3 (30%)	0	7 (70%)
sows	10	7 (70%)	0	3 (30%)
boars	10	4 (40%)	0	6 (60%)
Total	50	29 (58%)	0	21 (42%)

Following bloking ELISA immunoassay test at the 18 samples taken from the bodies, results were presents in table 2.

Table 2. ELISA test results of thoracic fluid from cadavers of pigs from farm A

Category	No. samples	No. (%) samples		
		positive	dubioase	negative
piglets farrowed dead	3	3 (100%)	0	0
suckling	9	9 (100%)	0	0
young pigs	3	0	0	3 (100%)
fat pigs	3	0	0	3 (100%)
Total	18	12 (66,66%)	0	6 (33,33%)

In farm B were obtained the following results presents in table 3.

Table 3. ELISA test results on serum of pigs from farm B

Category	No.samples	No. (%) samples		
		positive	dubioase	negative
suckling	5	4 (80%)	0	1(20%)
young pigs	5	5 (100%)	0	0
fat pigs	5	5 (100%)	0	0
sows	5	5 (100%)	0	0
boars	5	5 (100%)	0	0
Total	25	24 (96%)	0	1 (4%)

Following bloking ELISA immunoassay test for the 14 samples taken from the bodies, results were as follows in table 4.

Table 4. ELISA test results of thoracic fluid from cadavers of pigs from farm B

Category	No. samples	No. (%) samples		
		positive	dubioase	negative
piglets farrowed dead	4	4 (100%)	0	0
piglets bodies	5	5 (100%)	0	0
young pigs	3	3 (100%)	0	0
fat pigs	2	0	0	2 (100%)
Total	14	12 (85,7%)	0	2 (14,3%)

Samples were taken from non- professional breeding units were also made by enzyme immunoassay ELISA and the result was negative in all samples examined.

## **CONCLUSIONS**

The presence of PRRS antibodies suggests the circulation of virus in the studied farms.

The risk of becoming an infected unit is directly proportional to the size of the flock, with the quarantine absence and frequency of introducing new animals in farm.

PRRS is a disease that occurs in the intensive system of breeding pigs due to growth technology: the introduction of new effective to cohabitation with the existing one, artificial insemination, etc.

In the farms studied, the biggest losses were recorded at piglets because the passive immunity transferred from sows to piglets is short.

ELISA test can detect antibody against PRRS virus and is very important to know which is the serum status.

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