Tropical and Subtropical Agroecosystems

SHORT NOTE [NOTA CORTA]

PREVALENCE AND RISK FACTORS ASSOCIATED WITH THE PRRS VIRUS IN SEMEN OF BOARS IN PIG FARMS OF YUCATAN

[PREVALENCIA Y FACTORES DE RIESGO ASOCIADOS CON EL VIRUS DEL PRRS EN SEMEN DE VERRACOS EN GRANJAS PORCINAS DE YUCATÁN]

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SUMMARY

The objectives of the present study were to estimate the prevalence of and to determine the risk factors associated with the porcine reproductive and respiratory syndrome virus (PRRSV, American strain) in semen of boars in pig herds of Yucatan, Mexico. Ninety two boars from 26 herds were ejaculated once. Semen samples were processed by the RT-nPCR test using the ORF7 primer to detect the PRRS virus. The true prevalence estimated was 10.1% (95% CI = 4.1-16.1%). Significance of risk factors was determined by Fisher-exact test. The odds of detecting genetic material of the PRRSV was greater (OR = 9.2) in semen of boars used under natural mating than those used in artificial insemination. In herds where boar's acclimatization was not practiced the odds of a positive boar was 4.3. Another risk factor ($P < 0.05$) was the origin of the animals. In conclusion, the prevalence of the PRRSV in boar semen was smaller to the notified in the literature and determinate in blood serum. Management practices, such as the use of the artificial insemination and acclimatization of the boar, could be useful in reducing the prevalence of the PRRS virus in the pig farms.

Key words: Prevalence; risk factors; PRRS; boar; semen; RT-nPCR.

RESUMEN

Se estimó la prevalencia y determinó los factores de riesgo asociados con el síndrome reproductivo y respiratorio porcino (PRRS, cepa americana) en semen de verracos en granjas porcinas de Yucatán, México. Se obtuvo el semen de una sola eyaculación proveniente de 92 verracos de 26 hatos durante una ocasión. Las muestras de semen fueron procesadas mediante la prueba RT-nPCR usando el cebador ORF7 para detectar al virus del PRRS. La prevalencia verdadera estimada fue 10.1% (95% IC = 4.1-16.1%). La significancia de los factores de riesgo fue determinada mediante la prueba exacta de Fisher. La razón de probabilidad (RP) de detectar material genético del virus de PRRS fue mayor (RP = 9.2) en semen de verracos durante la monta natural, en comparación con aquellos usados en inseminación artificial. La RP en los hatos donde no se practicaba la aclimatación del verraco fue 4.3. Otro factor de riesgo ($P< 0.05$) fue el origen de los animales. En conclusión, la prevalencia de PRRS en semen de verracos fue menor a la notificada en la literatura y determinada en suero sanguíneo. Prácticas de manejo, tales como el uso de la inseminación artificial y la aclimatación del verraco, podrían ser de utilidad en la reducción de la prevalencia de PRRS en las granjas porcinas.

Palabras clave: Prevalencia; factores de riesgo; PRRS; verraco; semen; RT-nPCR.

INTRODUCTION

The Porcine Reproductive and Respiratory Syndrome (PRRS) cause great losses to the pig production industry both at the national and international level. This disease affects pigs in all the stages of production, and causes reproductive (in adult pigs) or respiratory (in growing pigs) alterations; it is associated with other diseases by the presence of opportunistic pathogens (Robles *et al*., 2004). PRRS virus (PRRSV) characterizes itself by its ample genetic and antigenic variability, as well as by its immune suppressor properties and capacity to induce persistent clinical infections that complicates the diagnosis and

control of the disease (Lager *et al*., 1996; Dee, 1997; Goldberg *et al*., 2000).

The economic impact of PRRS is due to the increase in the repetition of heats, delayed abortions, diminution of fertility and litter size, increase in the number of mummified animals, increase of the number of dead or weak pigs been born by litter and in the percentage of mortality during lactation (Collins *et al.*, 1992). In boars it causes anorexia, somnolence, fever, as well as diminution of sexual desire; poor seminal quality expressed in reduced volume, motility and sperm concentration below the standards and increase of abnormalities in the spermatozoa, which harms the reproductive potential of the boars (Hooper *et al*., 1992; Done and Paton, 1995). The severity of PRRS depends on factors like poor hygienic conditions in the farm, poor management of the animals and the virulence of the involved strain (Goldberg *et. al.*, 2000).

Actually, in the international plane the pig industry is making changes in order to increase the levels of production, to improve the excellence of its products, to offer better prices and to generate greater gains; for example, the greater use of the artificial insemination (AI); however, the presence of the PRRS continues being a risk for the production process. The IA improves the levels of production and the use of genetically superior animal; nevertheless, the risk of the PRRSV being transmitted by means of semen is high (Robles *et al*., 2004). It is certain that infected boars that shed the PRRSV via semen constitute a potential transmitting source of the disease; in addition to which this disease could extend through the commercialization of semen infected used for the AI (Cristopher-Hennings *et al*., 1995). The transmission of the virus by means of semen is notified as the second via in importance, after the introduction of pigs infected to the farm (Weimersheimer-Ruby *et al*., 1997; Zimmerman, 2003; Wasilk *et al*., 2004).

In Mexico the PRRSV control programs available consider the closing of farms and the inclusion of replacement females. It also has been recommended the use of modified alive vaccines and inóculos from the same farm; nevertheless, epidemics of PRRS still appear, the ironic thing is that many of them happen after the depopulation and repopulation of the farm in order to eliminate the virus (Carvajal, 2004).

In Yucatan there are 234 commercial pig farms, where feeding is based on commercial feed, weaning is realized to the 21 days of age, and the disinfection and cleaning of the farms are partial. In most of the farms the personnel is not exclusive of an area. Vaccination and medication are applied according to the diseases present in the farm and the reproduction of herd is made by means of AI, natural mating (NM) or both, and boars are replaced approximately at three years of age.

At the national level there are PRRS seroprevalence studies of PRRSV measure in blood (Barroso, *et al*., 2002; Rovelo, 2008), but no of prevalence in semen; therefore, the objectives of this study was to estimate the prevalence and associated PRRSV (American strain) risk factors in boar semen in pig farms of the state of Yucatan, Mexico.

MATERIAL AND METHODS

The study was carried out in Yucatan, Mexico between the coordinates 19º 30’ and 21º 35’ North latitude and 87º 30' and 90º 34' west longitude of the meridian of Greenwich (INEGI, 2004), with subhumid tropical climate (Aw_0) and rain regime in summer (May to October), annual pluvial precipitation of 997 mm with ranks of 700-2000 mm and average temperature of 26.5ºC (rank of 7-42ºC), relative humidity between 61%-87% and predominant winds of the North and Southeastern (INEGI, 2004).

A cross-sectional study with a cluster sampling design in one stage was carried out, including 26 farms willing to participate in the survey. According to a previous study (Rovelo, 2008), the studied farms were positive to the PRRSV (they had at least one seropositive pig). The criterion of boar inclusion was that they had at least one month in the farm.

Semen samples collection and processing (ejaculates)

Semen was collected by hand independently if the boars were used for AI or NM. From the total volume of each ejaculate, 10 ml were used, which were drained in an assay tube. Each sample was identified with the number or name of the boar, and the date and the name of the farm. Afterwards, the ejaculates were transported, in a cool box to the laboratory of the Faculty of Veterinary Medicine and Animal Science of the Autonomous University of Yucatan.

The ejaculates were centrifuged to 500 g during 15 minutes and the plasma was eliminated, conserving the packed sperm volume. Later the packed sperm volumes were conserved in a freezer to - 70ºC until viral extraction. The extraction of the PRRSV was made by means of a commercial kit, following the manufacturer instructions (Quigen laboratories Inc., France). Identification of the PRRSV was made by the reverse transcription – polymerase chain reaction (RT-PCR) test and the reverse transcription nested polymerase chain reaction (RT-nPCR) test using the specific sequence of the open reading frame ORF7

(with 236 pair bases) of the American PRRSV-strain (ATTC VR-2332) (Collins *et al*., 1992; Horter *et al*., 2002); these tests have widely been used to detect the PRRSV in semen samples (Cristopher-Hennings *et al.*, 1995; Wasilk *et al*., 2004). The amplification products were visualized in 2% agar dyed gels with etidio bromide. The RT-PCR and RT-nPCR tests were carried out using commercial kits and following the instructions of the manufacturer (Quiagen QIA amp® Viral RNA mini kit handbook, France; Quiagen® One Step RT-PCR and Hot Star Taq DNA Polymerase, France). The test is 97% sensitive and 100% specific.

Statistical analysis

The apparent prevalence (AP) was obtained as a proportion of the number of positive ejaculates divided by the total number of ejaculates, which was adjusted by the sensibility (SE) and specificity (SP) of the test to obtain the true prevalence (TP). The TP and its confidence interval (CI) were calculated according to the following formula:

$$TP = \frac{AP + SP - 1}{SE + SP - 1} \qquad CI = TP \pm t\sqrt{\frac{TP(1-TP)}{N}}$$

Where:
N = Total number of ejaculates.
t = Table value of the t distribution with 95% confidence level.

The information on the risk factors was obtained by means of a questionnaire. The evaluated risk factors were: type of service (NM, IA, both), uses of the boar as teaser (no, yes), make tests before introducing the boar (no, yes), acclimatization of the boar (no, yes), origin of the boar (local, other). The association of the risk factors with the presence of the PRRSV in semen was determined by the Fisher exact test using the Statistix software (1996). Also, the odd ratios (OR) and their confidence intervals were calculated, by means of the logarithmic approach, using the WinEpiscope software (Thrusfield, *et al*., 2001).

RESULTS

Seven of the 26 of the sampled farms had at least one boar with a positive ejaculate to the RT-nPCR test; of the 92 ejaculates, nine were positive. The apparent and true prevalences were 9.8% and 10.1% (95% CI = 4.1%-16.1%), respectively.

The risk factors, prevalences and their OR are shown in Table 1. The probability of detecting genetic material of the American strain PRRSV was greater (OR = 9.2) in semen of boars that were used in natural mating in comparison with the boars used in AI. Also, in the farms where the acclimatization of the boars was not practice the OR was 4.3 times greater in comparison with those farms that did it. Another risk factor associated with the presence of the PRRSV (OR = 4.8) was the boar origin ($P < 0.05$).

DISCUSSION

Prevalence

The true prevalence (10.1%) of the PRRSV found in this study is smaller than the notified in other seroprevalence studies in the United States (NAHMS, 1995; 59%), Denmark (Botner, 2003; 60%), Spain (Segalés, 2003; 62%) and Mexico (Morilla *et al*., 2003; 83%). In Yucatan, Mexico, seroprevalences of 56% have been notified (Barroso *et al*., 2002). The high seroprevalences notified in the literature in comparison with the prevalence in semen here found, might be due the fact that the serologic tests detect the exposition to the virus, without differentiating vaccine antibodies from field virus (Zimmerman *et al*., 1998), whereas the RT-nPCR test detects the viral ARN indicating the presence of the virus in semen (Benson *et al*., 2002; Horter *et al*., 2002). Viremia in the adult boars is of short duration and usually it finishes before the elimination of the PRRSV via semen concludes. In the initial phase of the infection, the serologic results can be negative although the virus still begins to be eliminated via semen. Also, the possibility exists that boars that remain seropositive during long time no longer are eliminating the PRRSV via semen (Prieto and Castro, 2005). PRRSV can be eliminated via semen from 50 days postinfection until six months; very few persistently infected boars shed the virus by long time (Bouma, 2000); in addition, the shedding of the virus via semen is intermittent and there are differences in the duration of elimination (Christopher-Hennings *et al*., 2001).

Risk Factors

The greater probability of detecting the virus of PRRS in semen of boars used in NM (OR = 9.2) in comparison with the ones used in AI could be explained by the fact that boars used in NM commonly make contact with infected sows and during the process of mating they get infected (Benfield, 2004; Le Potier *et al*., 1997). IA is a habitual practice in the modern pig industry; in this context, the use of contaminated semen could be a risk of introduction and dissemination of the PRRSV in the farms (Rovira and Munoz-Zanzi, 2008). When AI is used, one semen dose could transmit the PRRSV to between 75 to 100 sows, so the transmission risk would be greater if those doses were commercialized, which would allow that the PRRSV arrives and infects animals of other farms, inclusive a great distances (Wasilk *et al*., 2004.

Table 1. Prevalence of PRRS on boar semen and odd ratios (OR) by risk factor in pig farms in Yucatan, Mexico.

	N	+	p (%)	OR	95% CI
Service type					
Insemination	59	4	6.8	1	----
Natural mating	5	2	40.0	9.17	1.17 - 71.7
Both	28	3	10.7	1.65	0.34 - 7.93
Use of boar as teaser					
Yes	74	7	9.5	1	----
No	18	2	11.1	0.84	0.16 - 4.41
Tests before introducing the boar					
No	53	5	9.4	1	----
Yes	39	4	10.2	1.10	0.28 - 4.38
Boar acclimatization					
No	75	5	6.7	4.31	1.02-18.2
Yes	17	4	23.5	1	----
Boar origin					
Local	22	5	22.8	4.85	1.17 - 20.0
Other	70	4	5.7	1	----

N = Number of positive ejaculates; + = Positive ejaculate, p = Prevalence; CI = Confidence interval

It has been demonstrated that sows artificially inseminated with semen, diluted or without being diluted, from boars experimentally infected with PRRSV, become clinically ill and seroconvert with a ≥200000 $TCID_{50}$/50 ml dose (Benfield, 2004). On the contrary, the IA of sows with diluted semen (dose of 20000 $TCID_{50}$/50 ml) of PRRSV experimentally infected boars, do not cause seroconversion. The factors probably involved in the transmission by IA are the route of exhibition and the dose of the virus (Swenson *et al*., 1995). Consequently, it is advised the use of IA with doses of semen free of PRRSV.

In the farms where boar acclimatization (quarantine) was not practiced the OR was 4.3 times greater in comparison with the farms in where acclimatization was practiced. The latter is partially explained by the fact that during the acclimatization process a series of actions are made, such as the controlled exposition of the boar, serologic monitoring for antibodies detection, the use of viricidas, among others, with the objective to reduce the risk of introducing infected animals to the reproductive herd that could shed the virus via semen (Morrow and Roberts, 1999).

The risk of infection by the introduction of an animal from non-local farms was 4.8 times smaller than for an animal bought in another region (Rodriguez *et al*., 2002); perhaps due to the producers common practice of asking that the animals bought outside Yucatan are free of diseases, such as Aujeszky disease, classic pig fever (eradicated in the State) and PRRS, among others; what would reduce the risk of introducing an infected animal (Guérin and Pozzi, 2005).

CONCLUSIONS

The prevalence of the PRRSV in boar semen was smaller to the notified in the literature and determinate in blood serum. Management practices, such as the use of the artificial insemination and acclimatization of the boar, could be useful in the control and circulation of the PRRS virus in the pig farms.

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