

Identification of *Escherichia coli* and *Staphylococcus aureus* in the prepuce, semen, and vulvar secretions of swine

Identificação de *Escherichia coli* e *Staphylococcus aureus* no prepúcio, sêmen e secreções vulvares de suínos

Fabiana Moreira¹; Carine Dahl Corcini^{2*}; Grazieli Rodrigues³; Eugênio Gonçalves de Araújo⁴; Fabio Leivas Leite²; Thomaz Lucia Júnior²

Abstract

In successful artificial insemination (AI) programs, it is critical that AI studs produce high-quality semen samples. Samples presenting bacterial contamination reduce *in vitro* sperm quality and also act as vehicles for disease transmission. The objective of this study was to identify the presence of bacteria contaminants in the vulvar secretion of inseminated sows and in semen and preputial secretion of the boars used as semen donors and to relate such presence with clinical findings. Three crossbred F1 boars and 15 sows from a commercial swine farm were evaluated. The microorganism isolated from the vulva, prepuce and sperm were submitted to biochemical and stain characterization. One of the evaluated boars presented clinical signs of genital infection. From the five sows inseminated with semen from such boar, three returned to estrus after AI and two presented purulent vulvar discharge. *Escherichia coli* and *Staphylococcus aureus* were the agents isolated from the samples. Simultaneous contamination with both agents was observed in two boars and in 20% of the sows. The presence of *E. coli* as a single contaminant was only observed in females (13%), whereas the presence *S. aureus* alone was observed in both sexes. The presence of pathogens in the examined samples suggests that it might be responsible for the low reproductive performance in the herd.

Key words: Bacterial contamination, boar, semen, sow

Resumo

Para alcançar o sucesso dos programas de inseminação artificial (IA), é fundamental que a produção de doses de sêmen de alta qualidade para IA. Amostras que apresentam contaminação bacteriana reduzem a qualidade do ejaculado *in vitro* e também atuam como veículo para a transmissão de doenças. O objetivo deste estudo foi identificar a presença de bactérias contaminantes na secreção vulvar de porcas inseminadas e no sêmen e a secreção prepucial de machos reprodutores utilizados como doadores de sêmen e relacionar esta presença de contaminantes com os achados clínicos. Foram avaliados três machos e 15 fêmeas suínas da linhagem F1 provenientes de uma criação comercial. Os microorganismos isolados da vulva, prepúcio e ejaculados foram enviados para a realização de testes biquímicos e caracterização das amostras. Um dos reprodutores avaliado apresentou sinais clínicos de infecção genital. Das cinco porcas inseminadas com sêmen destes machos, três retornaram ao estro após a IA e duas apresentaram descarga vulvar purulenta. Os agentes isolados das amostras foram *Escherichia coli* e *Staphylococcus*

¹ Pesquisador, Pós-Graduação em Veterinária, Universidade Federal de Pelotas, UFPel, Pelotas, RS. E-mail: fabimorvet@gmail.com

² Profs. da Faculdade de Veterinária, UFPel, Pelotas, RS. E-mail: corcinicd@gmail.com; fabio_leite@ufpel.edu.br; tomjr2004@yahoo.com.br

³ Discente, Faculdade de Veterinária, UFPel, Pelotas, RS. E-mail: grazieli_fr@hotmail.com

⁴ Prof. da Faculdade de Veterinária de Goiás, Universidade Federal de Goiás, UFG, Goiânia, GO. E-mail: earaujo@vet.ufg.br

* Author for correspondence

aureus. A contaminação simultânea de ambos os agentes foi observada em dois cachaços e em 20% das porcas. A presença de *E. coli* como único agente contaminante foi observada somente nas fêmeas (13%), enquanto que a presença de *S. aureus* como único contaminante foi observado em ambos os sexos. O baixo desempenho reprodutivo da criação pode ser determinado pela presença dos agentes patogênicos nas amostras examinadas.

Palavras-chave: Contaminação bacteriana, reprodutor, sêmen, porca

Introduction

The production of high quality semen is critical for the reproductive performance of commercial swine herds (SMITAL, 2008), especially because the semen of one single boar can be used to inseminate many females. For this reason, the evaluation of the ejaculate quality is the first step in semen processing to ensure efficiency in artificial insemination (AI) (ROZEBOOM, 2000). Semen collection is a process that often presents bacterial contamination, mainly by Gram negative bacteria from the *Enterobacteriace* family (MAES et al., 2008). According to some studies (FERNANDÈZ et al., 2001; ALTHOUSE; LU, 2005), among the several microorganisms detected as contaminants of boar semen, *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), were those identified more often.

The spermicidal effect of *E. coli*, when in high concentrations ($>2 \times 10^7$ CFU/mL), decreases sperm motility and increases the sperm agglutination in human seminal plasma (DIEMER et al., 1996). The presence of *S. aureus* can lead to decrease in the number of spermatozoa, the suppression of their motility, changes in their morphology and fertilizing capacity (EMOKPAE; UADIA; SADIQ, 2009). Semen contamination may impair female's performance, causing return to estrus and vulvar discharges, and reduce the number of born piglets (ALTHOUSE et al., 2000). Therefore, it is necessary to characterize the association of the bacterial agents present in the animals secretions to the clinical signs, besides the observation of these microorganisms presence in both boars used as semen donors and inseminated sows, considering some agents belong to the bacterial flore usually present in the semen (ALTHOUSE; LU, 2005).

According to the International Office of Epizootics (OIE, 2001) the maximum number of colony-forming unities (CFU) allowed to be present in semen samples without negative effects in boar semen quality is 5×10^3 per ml.

The objective of this study was to identify the presence of contaminating bacteria in the vulvar secretion of inseminated sows and in preputial secretion and semen of boars used as semen donors in a commercial herd in intensive production and to relate such presence with clinical findings.

Materials and Methods

The study involved 15 randomly selected females in production and with parity from 1-8 and three crossbreeding F1 (Landrace X Large White) boars, from a complete cycle rearing located in Pelotas-RS region. The animal production system was intensive and in complete intensive production, with approximately 200 sows in production. All females (100%) were submitted to AI. For this study, all boars (n=3) from the swine farm AI routine were used. Pregnancy was diagnosed by the non return to estrus in the presence of a sexually mature boar and by also transabdominal ultrasound (Anser Vet 485 Pie Medical®) with a 5,0 MHz convex probe, 25 days after the last AI. Semen samples from each boar were used to inseminate five sows (n=15). Secretions from the internal region of the prepuce of the three males and from the vulva of the 15 females were collected by sterile swabs (COPAN Innovation, Brescia, Italy). Samples from the sow's vulva secretions were collected before and after AI, after cleaning the vulva with a dry paper towel. Before collecting the samples, the prepuce was washed with water

and subsequently dried with a paper towel. Semen samples were collected from each boar through the gloved-hand method (HANCOCK; HOVELL, 1959). From each sample, two aliquots of 20 mL (one undiluted and other diluted) were collected for subsequent bacteriologic analyses directly sown in the specific means, as described below. The basic medium used for sperm extension was Beltsville thawing solution (BTS, composed of 205 mM glucose, 20.39 mM NaCl, 5.4 mM KCl, 15.01 mM NaHCO₃, and 3.35 mM EDTA, pH 7.2 and 290±5mOsmol/kg, (PURSEL; JOHNSON, 1975), containing gentamicin (50µg/mL).

Bacteriological exam

The collected samples were processed at the microbiology laboratory of the Biology Institute of UFPEl, and they were cultivated in brain heart infuse (BHI, Difco), solidified with Agar at 1%, enriched with ovine blood at 8%, and incubated at 37°C in aerobiosis for 48 hours. After incubation, the colonies were isolated and cultivated for 48 hours at 37°C, in three other means of culture: McConkey agar and Endo agar plates were used for the specific isolation of *E. coli* from those samples that were positive on brilliant green agar. McConkey and Endo agar were prepared according to the instructions of the manufacturer (Oxoid), and Chapman (Difco) means, selective for *Staphylococcus*, and differential for *S. aureus*.

Samples were submitted to serial dilutions up to 10⁻⁸, plated in duplicates in BHI agar (Acumdia®, Neogen, São Paulo, Brazil) and incubated at 37°C for 48 h. Concentration was expressed in Colony Forming Units per millilitre (CFU/ml). The CFU were counted in plates that contained 30 to 300 colonies, regardless of color, size and form. Gram staining was carried out for each sample after isolation.

These tests were performed according to procedures described in literature (YORK et al., 2004). Colonies that show typical *E. coli* characteristics were further characterized using the

Gram stain and biochemical tests that allow the identification of this bacterium, i.e. the oxidase and the urease (YORK et al., 2004).

Antibiogram

According (NCCLS, 2003) with some modifications the antibiograms were conducted in Agar Mueller-Hinton (Acumedia®, Neogen, São Paulo, Brazil) to test the susceptibility *in vitro* to the antibiotics included in the extender. Filter paper discs were impregnated with 90 µg gentamicin, 10 µg penicillin and enrofloxacin (at both 200 µg and 400 µg). After incubation of the plates at 37°C for 24 h, reading was done by measuring the halos of microorganism's growth inhibition (NCCLS, 2003).

Semen evaluation

Sperm progressive motility was evaluated in a 10 µL semen sample in a slide covered with a coverslip, both previously heated at 37°C, using a phase contrast microscopy (Olympus BX 40, America Inc., Sapporo, Japan) at 200x magnification (HANCOCK; HOVELL, 1959). Sperm morphology was evaluated, after dilution, by phase contrast microscopy at 100x magnification, after counting 200 cells, which were classified as described by Hancock and Hovell (1959). Membrane integrity was evaluated with an epifluorescent microscope (Olympus BX51), in each slide, 200 cells were counted and classified as intact (green fluorescence) or not intact (red fluorescence) (HARRISON; VICKERS, 1990).

Results and Discussion

The frequency of animals that presented *E. coli* and *S. aureus* bacteria in the semen and in the prepuce and vulvar secretions are described in Table 1. The samples result revealed that 100% of the boars were colonized by *E. coli* or *S. aureus* or by both agents.

Table 1. Presence (%) and CFU/ml (x103) of *Escherichia coli* and *Staphylococcus aureus* in the semen and prepuce secretion of swine males (n=3), and in the vulvar secretion of swine females (n=15) from a commercial farm in Pelotas-RS region.

		<i>E.coli</i>	<i>S. aureus</i>	<i>E. coli</i> + <i>S. aureus</i>	Without increase
Prepuce	Presence	-	33.0 (1)	67.0 (2)	-
	CFU/mL	-	5.7	5.90	-
Semen <i>in natura</i>	Presence	-	33.0 (1)	67.0 (2)	-
	CFU/ml	-	5.3	4.7	-
Semen after dilution	Presence	-	40 (6)	60 (9)	-
	CFU/ml	-	4.8	3.9	-
Vulvar secretion before AI	Presence	13.3 (2)	20.0 (3)	20.0 (3)	46.7 (7)
	CFU/ml	4.6	5.7	5.7	-
Vulvar secretion after AI	Presence	13.3 (2)	20.0 (3)	20.0 (3)	46.7 (7)
	CFU/ml	3.8	5.7	5.0	-

AI: artificial insemination

Source: Elaboration of the authors.

These results differ from other study that observed the presence of a single bacterial contaminant was obtained from 66% of the submitted samples (n=37 doses) and 34% contained two or more different bacterial genera (ALTHOUSE et al., 2000). One of the boar presented preputial mucosa with red coloration and an increase of prepuce region volume, which are suggestive clinical signs of genital infection. From the five sows inseminated with such boar's semen, three returned to estrus after AI, and two presented purulent vulvar discharge, suggesting that these problems might be caused by the transmission of bacteria from the boar to the sows.

Simultaneous contamination with both agents was observed in two boars and in 20% of the sows. The presence of *E. coli* as a single contaminant was only observed in females (13%), whereas the presence *S. aureus* alone was observed in both sexes. The presence of *E. coli* as a single contaminant was also not observed in the fresh and diluted semen samples, although it *E. coli* was observed in some semen samples, in association with *s. aureus*. In a study by Martín et al. (2010), 12 semen samples presented contamination by only one bacteria strain, 45 samples presented two bacteria strains and 24 samples were contaminated by three bacteria strains,

whereas *E. coli* was the bacteria most frequently found, being positively associated with sperm agglutination, which may have negative effect on the subsequent litter size.

In the present study, the values of CFU/ml observed for *S. aureus* and *E. coli* in fresh and diluted sperm samples were 4.7×10^3 and 3.9×10^3 respectively (table 1). Martín et al. (2010) reported that litter size was reduced when the counts of *E. coli* in semen exceeded 3.5×10^3 CFU/ml.

Nevertheless, there are studies confirming the presence of both agents as cause of potential infections of the genital and urinary tract in male human individuals (BERKTAS et al., 2008), represented by low density semen and progressive decrease of sperm motility, besides contributing for the infertility process (SANOCKA-MACIEJEWSKA; CIUNPINSKA; KURPISZ, 2005). Progressive motility is an important parameter in the evaluation of sperm because it is indicative of the vitality of the cells, which is directly correlated to viability. Motility is considered as optimal when at least 60% of sperm cells are motile (FLOWERS, 1996). In our semen samples, the motility of the sperm cells *in natura* ranged between 68 and 77%. Progressive motility after dilution ranged between 50 and 70%. The mean of normal sperm morphology was 90%.

Membrane integrity of the sperm cells ranged between 49 and 82%. According to Yaníz et al. (2010), diluted semen samples stored at 15°C having pure *E. coli* culture presented drastic reduction in sperm motility, velocity and viability, whereas such reductions were less intense in the presence of *S. aureus* e *S. epidermidis*.

There was bacterial contamination in vulvar secretion, indicating the presence of the isolated bacteria (*E. coli* and *S. aureus*), in 53% of the samples collected from the sows. Out of a total of 15 females submissions, 7 (46.7%) were negative for bacterial contaminants. From the total of sows involved in this study, three (20%) returned to estrus and two (13%) presented purulent vulvar discharge before the sample's collection and after AI, which can be considered common when the females are inseminated by semen contaminated by *E. coli* and *S. aureus* (ALTHOUSE et al., 2000). The presence of agents in the semen increases the risk of infection. However, the risk is even higher with the increase of the number of pathogenic agents, the number of inseminated sows by the contaminated semen, and the lack of protective immunity in the females from the herd (MAES et al., 2008). The two sows that presented purulent secretion at the 20th day after mating besides *E. coli* and *S. aureus*, were diagnosed with endometritis. The same agents were identified in the secretions and semen of the boars used in the AI of these females. The pipette introduction and the infusion of the insemination dose carried out without the appropriate hygiene may introduce bacterial agents, predisposing to the appearance of endometritis, whose most visible symptom is vulvar discharge (FLOWERS, 1996).

The contamination of semen in boars and of the vulva in sows was positive for pathogenic bacteria, identified as *E. coli* and *S. aureus*. In the antibiogram, the isolated bacteria (*E. coli* and *S. aureus*) presented resistance against penicillin, gentamicin and 200 µg enrofloxacin, however were sensible only to 400 µg enrofloxacin.

The findings of this study suggest that the boars used for semen collection are responsible for the contamination of the sows, warning for the importance of semen microbiological control, in order to minimize reproductive failures and consequently economic losses for the herd. The data also suggest that the microbiological exam of the semen and of the genital tract is recommended before the reproduction period.

References

- ALTHOUSE, G. C.; LU, K. G. Bacteriospermia in extended porcine semen. *Theriogenology*, Philadelphia, v. 63, n. 2, p. 573-84, 2005.
- ALTHOUSE, G. C.; KUSTER, C. E.; CLARK, S. G.; WEISINGER, R. M. Fields investigations of bacterial contaminants and their effects extended porcine semen. *Theriogenology*, Philadelphia, v. 53, n. 2, p. 1167-76, 2000.
- BERKTAS, M.; AYDIN, S.; YILMAZ, Y.; CECEN, K.; BOZKURT, H. Sperm motility changes after coincubation with various uropathogenic microorganisms: an in vitro experimental study. *Int Urol Nephrol*, v. 40, n. 2, p. 383-89, 2008.
- DIEMER, T.; WEIDNER, W.; MICHELMANN, H. W.; SCHIEFER, H-G.; ROVAN, E.; MAYER, F. Influence of *Escherichia coli* on motility parameters of human spermatozoa in vitro. *Int J Andro*, v. 19, n. 5, p. 271-77, 1996.
- EMOKPAE, M. A.; UADIA, P. O. SADIQ, N. M. Contribution of bacterial infection to male infertility in Nigerians. *J Health Allied Sci.*, 2009. Available at: <<http://openmed.nic.in/3277/01/2009-1-6.pdf>>. Accessed at: 16. aug. 2012.
- FERNANDÈZ, E.; CRUZ, A.; LAZO, L.; ARREDONDO, C.; BRITO, A. Estudio bacteriológico del semen de porcino. valoración preliminar del efecto de la lectina de *Escherichia Coli* en la aglutinación espermática. *Revista Salud Animal*, v. 23, n. 2, p. 73-79, 2001.
- FLOWERS, W. L. *Common issues associated with on-farm A.I. use*. Proc. Allen D. Lemman Swine Conference. University of Minnesota, US, 1996. p. 69-73.
- HANCOCK, J. L.; HOVELL, G. J. R. The collection of boar semen. *Veterinary Records*, London, v. 71, p. 664-665, 1959.

- HARRISON, R. A. P.; VICKERS, S. E. Use of fluorescent probes to assess membrane integrity in mammalian spermatozoa. *J. Reprod. Fertil.*, v. 88, n. 1, p. 343-352, 1990.
- MAES, D.; NAUWYNCK, H.; RIJSSELAERE, T.; MATEUSEN, B.; VYT, P.; DE KRUIF, A.; VAN, S. O. O. M. A. Diseases in swine transmitted by insemination artificial: An overview. *Theriogenology*, Philadelphia, v. 70, n. 8, p. 1337-1345, 2008.
- MARTÍN, L. O. M.; MUÑOZ, E. C.; DE CUPERE, F.; VAN DRIESSCHE, E.; ECHEMENDIA-BLANCO, D.; RODRÍGUEZ, J. M. M.; BEECKMANS, S. Bacterial contamination of boar semen affects the litter size. *Animal Reproduction Science*, Amsterdam, v. 120, n. 1-4, p. 95-104, 2010.
- NCCLS. *Performance standards for antimicrobial disk susceptibility tests; approved standard*. 8. ed. Wayne, Pennsylvania, USA: NCCLS, 2003. 81 p.
- OIE. Sêmen de ruminantes e suínos. Código Zoosanitário Internacional. Parte 3. 2001.
- PURSEL, V. G.; JONSON, L. A. Freezing of boar spermatozoa: fertilizing capacity with concentrated semen and a new thawing procedure. *Journal of Animal Science*, Champaign, v. 40, n. 1, p. 99-102, 1975.
- ROZEBOOM, K. J. Evaluating boar semen quality. *Animal science facts*. 2000. p. 7. Publication number ANS00-8125. Available at: <<http://mark.asci.ncsu.edu/Publications/factsheets/812s.htm>>. Accessed at: 16. aug. 2012.
- SANOCKA-MACIEJEWSKA, D.; CIUNPINSKA, M.; KURPISZ, M. Bacterial infection and semen quality. *Journal Reproductive Immunology*, v. 67, n. 1-2, p. 51-56, 2005.
- SMITAL, J. Effects influencing boar semen. *Animal Reproduction Science*, Praga, v. 110, n. 3-4, p. 335-346, 2008.
- YANÍZ, J. L.; MARCO-AGUADO, M. A.; MATEOS, J. A.; SANTOLARIA, P. Bacterial contamination of ram semen, antibiotic sensitivities, and effects on sperm quality during storage at 15 °C. *Animal Reproduction Science*, Amsterdam, v. 122, n. 1-2, p. 142-149, 2010.
- YORK, M. K.; TRAYLOR, M. M.; HARDY, J.; HENR, Y. M. 2 Biochemical tests for the identification of aerobic bacteria. In: ISENBERG, H. D. (Ed.). *Clinical microbiology procedures handbook*. 2nd ed. Washington, D.C: American Society for Microbiology, 2004. v. 1, 2, 3. p. 68-75.