Revista MVZ Córdoba
ISSN: 0122-0268
ISSN: 1909-0544
revistamvz@gmail.com
Universidad de Córdoba
Colombia

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Revista MVZ Córdoba, vol. 23, no. 2, 2018
Universidad de Córdoba, Colombia
Available in: http://www.redalyc.org/articulo.oa?id=69356053007
DOI: https://doi.org/10.21897/rmvz.1338

Original

Bacterial Contaminants and Antimicrobial Susceptibility Profile of Boar Semen in Southern Brazil Studs

Contaminantes bacterianos y perfil de susceptibilidad del semen porcino en centros de recogida em Brasil

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DOI: https://doi.org/10.21897/rmvz.1338
Redalyc: http://www.redalyc.org/articulo.oa?id=69356053007

Received: 03 April 2017
Accepted: 02 October 2017

Abstract:

Objective. To assess the microbiological profile in seven Boar Studs (BS) in Southern Brazil, as well as evaluate antimicrobial susceptibility response to most commonly found microorganisms in BS. **Material and methods.** Bacteriologic analysis was carried out in samples from the water purification system, semen extender, raw and stored semen, lab benches, and other working surfaces. **Results.** Growth of a mixed bacterial population was observed in water samples from all but one BS. Approximately 85% of the BS had significant contamination on their working surfaces with at least one bacterial contaminant. A total of 86% of raw semen samples were contaminated with one or more different bacteria, while 100% of the boar studs provided contaminated samples. Bacterial susceptibility to antimicrobial agents varied from over 80% for gentamycin, neomycin and ceftiofur to 40% or less for penicillin and lincomycin. **Conclusions.** The identification of the critical points provides necessary support to devise better strategies to minimize contamination in BS. Also, assessing the level of antimicrobial drug resistance offers accurate information to formulate more efficient antibacterial protocols that closely observe the rational use of antibiotics.

Keywords: Boar semen, bacterial contamination, antimicrobial susceptibility.

Resumen:

Objectivo. Evaluar el perfil microbiológico de siete centros de recogida de semen porcino (BS) en el sur de Brasil, así como evaluar la susceptibilidad antimicrobiana a la mayoría de los microorganismos encontrados en los BS. **Material y métodos.** El análisis bacteriológico se realizó en muestras del sistema de purificación de agua, diluyentes de semen, semen crudo y almacenado, bancos de laboratorio y otras superficies de trabajo. **Resultados.** El crecimiento de una población bacteriana mixta se observó en muestras de agua de todas las BS excepto una. Aproximadamente el 85% de la BS tenía contaminación significativa en sus superficies de trabajo con al menos un contaminante bacteriano. Un total de 86% de muestras de semen crudo fueron contaminadas con una

o más bacterias diferentes, mientras que el 100% de BS proporcionaron muestras contaminadas. La susceptibilidad bacteriana a agentes antimicrobianos varió en más del 80% para gentamicina, neomicina y ceftiofur a 40% o menos para penicilina y lincomicina. **Conclusiones.** La identificación de los puntos críticos proporciona el apoyo necesario para idear mejores estrategias para minimizar la contaminación en CRSP. Además, la evaluación del nivel de resistencia a los antimicrobianos ofrece información precisa para formular protocolos antibacterianos más eficientes que tengan en cuenta el uso racional de los antibióticos.

Palabras clave: Semen porcino, contaminación bacteriana, Susceptibilidad antimicrobiana.

Introduction

Artificial insemination (AI) has been widely used in swine production mostly because of rapid distribution of genes from genetically superior males (1). Approximately 66% of the commercial Brazilian herd is artificially inseminated (2); however, there is empirical evidence that this percentage could reach over 90%, demanding an annual production of 9.5 million high quality semen doses (SD).

Bacterial contamination of the ejaculate is one of the factors affecting sperm viability due to production of microbial metabolites (3,4), changes in pH, competition for substrate (3,4), and promotion of cell membrane injury (3). Ejaculate contamination is virtually impossible to avoid, nevertheless it can be significantly reduced if proper hygiene procedures take place throughout all the steps of semen processing. The quality of water, extender, and materials that enter in contact with the semen, in addition to quality of lab environment are factors that might hinder overall SD quality.

High concentration of bacteria in the SD contributes to low motility and increased rates of agglutination of sperm cells, in addition to a higher proportion of abnormal sperm cells (5). The negative impact of semen contamination exacerbates over time because it takes from 36 to 48 h of storage for the undesirable effects to become evident (4, 6). Schultze (7) demonstrated that the low quality of the SD was responsible for a 17 % decrease in the pregnancy rate, and a reduction of 1.2 piglets per litter.

Because it is virtually impossible to obtain bacteria-free semen samples, hygienic semen collection and proper processing techniques with stringent laboratory procedures are the first and primary lines of defense to successfully reduce contamination. In addition, antibiotics are added to semen extenders as a preventive measure to reduce bacterial contamination (4,5,6). However, antimicrobial resistance to the limited number of classes of antibiotics commonly used as preservative in commercial porcine semen extenders has been observed among isolates from boar semen (8). Furthermore antibiotic resistance of bacteria in extended boar semen is increasing worldwide (9); therefore, the identification of types and sources of contaminations may strength the bases for the use of boar semen free of antibiotics, a current trending research line.

Current data on quality control of SD are scarce and do not define a predetermined standard for a quality SD, especially regarding the microbiological status and susceptibility profile to antibiotics. Thus, the goals of this work were to determine a microbiological profile of seven AI boar studs (BS) in Southern Brazil as well as assess the microbial agent susceptibility response to the most commonly used antibiotics.

Materials and Methods

Sample Collection. All experimental procedures described in this experiment were conducted under experimental license (Project number 014/2016) from the Institutional Animal Care and Use Committee (CEUA-UNOESC).

This study was carried out in seven AI studs with at least 60 mature boars (ranging from 60-100 boars). Semen was collected once a week from nine to 20-month-old boars. Six ejaculates from 8 boars from each BS were used in this study (n=336). Based on Schulze et al (10), critical points for the control of the production flow were determined to define possible sources of semen contamination (10). Therefore, we collected samples from water (reverse osmosis system in all BS), semen extender, raw semen, SD stored

at 15-18°C for up to 96 h, and swabs from working surfaces (water storage tank, extender container, lab benches, water line, and valves for the water purification system). The samples were collected in triplicates and processed in PCA (Plate Count Agar, HiMedia, India) and VRB (Violet Red Bile Lactose, HiMedia, India) culture media. Therefore, a swab was used for each sampled site, which was stored in tubes with sterile saline solution for further dilution and bacterial counting on plates. All samples were transported at 5°C in an isothermal container and processed within 12-18 hour after collection.

Colony-Forming Units. The samples were diluted up to 10^{-4} in a 0.85% (w/v) sterile saline solution and inoculated in duplicates in PCA medium (HiMedia Lab., India) by plate streaking in duplicate. Following distribution, the Petri dishes were incubated in aerobiosis at 37°C for up to 48 h. Next, the colonies in each plate were counted, according to OIE (1998). Plates containing between 30 and 300 colony forming units (CFU) were counted, and all others with >300 CFU were attributed a grade "+300 UFC". The number of CFU per mL (CFU mL-1) for each sample was calculated by multiplying the average number of colonies counted in duplicates by the inverse of the higher dilution, considering a 100 μL inoculum per plate.

Consistent with methodology used by Gaczarzewicz (11), all colonies that grew in Petri dishes containing PCA medium were counted, while only typical total coliform colonies were considered for culture using VRB (1-2 mm in diameter, red with a pinkish precipitation halo).

Identification of Microorganisms and Susceptibility to Antibiotics. The most prevalent colonies in each BS were inoculated in Blood Agar Base supplemented with 5% sheep blood (Kasvi, Brazil), McConkey and Sabouraud Agar (HiMedia Lab., India). The plates were incubated at 36°C for 24 to 48 h. The microorganisms were identified according to Markey et al (12).

Following strain isolation and identification, the Kirby-Bauer disk diffusion method for antibiotic susceptibility according to the CLSI guideline M23 for Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters (13). This test was used to determine the susceptibility of microorganisms to gentamicin (10 μg), neomycin (30 μg), ceftiofur (30 μg), penicillin (10 IU), and lincomycin (2 μg). Bacteria was considered resistant to neomycin when the inhibition halo was equal or less than 12 mm. For the remainder antibiotics the inhibition halo was considered according to the CLSI guidelines (14).

During this work, all standard operating procedures in the boar stud, including semen collection and processing, were not disturbed.

Statistical Analysis. Total bacterial counts were log transformed to adjust for data dispersion and then submitted to an ANOVA test using proc GLM (SAS v. 9.1, SAS Institute, Inc, Cary, NC). The means were compared using Tukey-Kramer test for multiple comparisons. The most prevalent bacteria, as well as the correspondent percentages of each stud were analyzed using proc MEANS and proc FREQ (SAS v. 8.0, SAS Institute, Inc, Cary, NC) and presented as descriptive statistics. Differences were considered to be significant if $p<0.05$.

RESULTS

Total bacterial count for aerobic mesophiles in raw ejaculates averaged from $1x10^1$ to $3x10^5$ (ranging from $0–10^5$) CFU mL $^{-1}$. A total of 86 % of the semen samples (raw semen) tested positive for different genera of bacteria. There was bacterial growth in more than one of the samples collected from all boar studs (Table 1), so that five bacterial genera were common to 71.4 % of the studs (Table 2).

TABLE 1

Table 1. Profile of the microbial agents found in each Boar Stud (BS).

Agent	BS 1 (%)	BS 2 (%)	BS 3 (%)	BS 4 (%)	BS 5 (%)	BS 6 (%)	BS 7 (%)
S. hyicus	5.5	15.9	40	-	15	42.9	20
S. aureus	16.7	15.9	3.4	8.8	-	-	-
S. epidermidis	-	-	20.0	34.7	-	-	20
S. intermedius	-	-	6.6	-	10	-	-
Klebsiella sp.	-	15.9	10	4.3	-	-	-
A. faecalis	27.8	7.7	3.4	4.3	5	-	-
Acinetobacter sp.	22.3	15.9	6.6	13.1	25	14.3	-
E. coli	11.1	15.9	-	8.8	20	14.3	-
Serratia sp.	-	7.7	-	4.3	-	-	-
Pseudomonas sp.	11.1	7.7	-	4.3	5	-	20
Aeromonas sp.	5.5	-	-	4.3	5	-	10
Yersinia sp.	-	-	-	4.3	15.0	-	10
Enterococcus	-	-	-	-	-	25.8	-
Bacillus sp.	-	-	-	-	-	-	20
Yeast	-	-	10.0	8.8	-	-	-

TABLE 2.
Table 2. Most prevalent microbial agents in boar studs (BS) according to source of contamination.

Location	BS 1	BS 2	BS 3	BS 4	BS 5	BS 6	BS 7
Water	S. aureus	S. aureus	A. faecalis	Aeromonas sp.	Aeromonas sp.	NG	Yersinia sp.
	A. faecalis	S. hyicus	Yeast	Klebsiella sp.	A. faecalis		
	Acinetobacter sp.	Serratia sp.	S. epidermidis	Yeast	Yersinia sp.		
		Klebsiella sp.	S. aureus	Yersinia sp.			
		E. coli	S. hyicus	Acinetobacter sp.			
		Acinetobacter sp.		S. epidermidis			
		Pseudomonas sp.					
Surface Swab	S. aureus	S. aureus	S. hyiccus	E. coli	Acinetobacter	S. hyicus	S. hyicus
	E. coli	A. faecalis	Yeast	S. epidermidis			S. epidermidis
		E. coli					Bacillus sp.
Raw Semen	Pseudomonas sp.	Pseudomonas sp.	Klebsiella sp.	S. aureus	S. hyiccus	Enterocuccus	Bacillus sp.
	S. hyicus	Klebsiella sp.	S. hyicus	A. faecallis	Yersinia sp.	S. hyicus	S. epidermidis
	A. faecalis	E. coli	S. epidermidis	S. epidermidis	Acinetobacter	Acinetobacter	Aeromonas
	Acinetobacter sp.		Acinetobacter	Acinetobacter sp.	E. coli		
				Pseudomonas sp.			
Extended Semen	E. coli	Acinetobacter sp.	S. hyicus	S. epidermidis	S. intermedius	E. coli	Pseudomonas sp.
	Acinetobacter sp.	S. hyicus	S. epidermidis	Acinetobacter	Acinetobacter sp.		Bacillus sp.
	Aeromonas sp.			Serratia sp.			
	S. aureus						

NG- there was no bacterial growth

Water Samples. Only water samples from one boar stud purification system (BS 6) were bacteria-free (Table 2). We observed growth of only one bacterial genus in 16.6% (1/6) of the samples, whereas 83.3% (5/6) of the remaining samples harbored a mixed microbial population. The most prevalent bacterial agents isolated from the reverse osmosis system were Alcaligenes faecalis (42.8%-3/7), *S. aureus* (42.8%-3/7), *Acinetobacter* sp. (42.8%-3/7) and *Yersinia* sp. (42.8%-3/7). Additional microbial agents such as *Aeromonas* sp., *Klebsiella* sp., *Pseudomonas* sp., *Serratia* sp. and *Escherichia coli*, as well as few yeast strains were isolated from some studs (Table 2). Water samples presented a microbiota predominantly Gram-negative (66.6%; 6/9).

Surface Swabs. Out of the samples collected from semen processing benches and equipment that come in direct contact with semen, 57.1% of the studs (5/7) presented two or more bacterial contaminants. *Staphylococcus* sp. was present in 85.7% (6/7) of the boar studs (Table 2).

Semen Samples. Bacterial growth of at least two different microorganisms was present in all raw semen and extended semen samples. The most prevalent strains in the raw semen were *Staphylococcus hyicus* (42.8%; 3/7), *Escherichia coli* (28.5%; 2/7), and *Alcaligenes faecalis* (28.5%; 2/7), whilst samples of extended semen

presented contamination with *Staphylococcus* sp. (36.3%; 4/11), and Escherichia coli (18.8%; 2/11) with predominance of Gram-negative microbiota (Table 2).

Total Bacterial Count. The average number of CFU mL^{-1} varied among boar studs. Mesophilic bacteria significantly ranged ($p<0.05$) from 1.66 (BS 6) to 3.78 (BS 4) Log CFU mL^{-1}. The number of coliform bacteria (Table 3) varied from 1.76 (BS 3) to 2.52 (BS 4) Log CFU mL^{-1}, however no significant differences were identified.

TABLE 3.

Table 3. Average number of CFU mL^{-1} found in boar studs.

Boar Stud	Log10 CFU mL^{-1}	
	Mesophiles	Coliforms
1	3.76 ± 0.55^{a}	2.52±1.49
2	3.34 ± 1.28^{ac}	2.22±0.88
3	3.16 ± 1.55^{ac}	1.76±1.00
4	3.83 ± 1.34^{a}	2.11±0.62
5	2.82 ± 1.06^{ac}	2.09±1.09
6	1.66 ± 0.66^{bc}	2.18±0.48
7	2.02 ± 1.01^{bc}	2.31±0.78

^{a,b,c} different superscripts in columns indicate statistical differences ($p<0.05$; Tukey-Kramer). Data are presented as mean ± SEM.

It was observed that there were different degrees of contamination among the boar studs and the source of contamination (Table 4). This is probably due to different management and hygiene procedures adopted in different boar studs.

Susceptibility Tests. All bacteria isolated from the BS were tested for susceptibility to antimicrobial agents. There was an increased antimicrobial susceptibility to gentamycin (87.5%), neomycin (87.5%), and ceftiofur (81.2%) in contrast to the majority of other antimicrobial agents tested. Bacterial isolates presented remarkably low sensibility to penicillin (25%) and lincomycin (12.5%). Therefore, all bacterial samples isolated from BS 3 and 7 were resistant to lincomycin, whereas percent microbial resistance to penicillin was 60, 75, 75, and 100% for BS 3, 5, 6, and 7, respectively.

TABLE 4.

Table 4. Average number of CFU mL^{-1} for mesophiles and coliforms in different boar studs (BS) according to source of contamination.

Sample	Culture	Log10 CFU mL^{-1}						
		BS 1	BS 2	BS 3	BS 4	BS 5	BS 6	BS 7
Water	Mesophiles	3.47	3.28	3.69	5.47	2.79	NG	3.04
	Coliforms	1.47	2.47	3.47	2.28	NG	NG	2.74
Surface	Mesophiles	3.47	3.89	2.97	3.73	2.63	1.00	2.41
	Coliforms	3.13	2.81	1.51	1.95	3.29	NG	1.57
Raw Semen	Mesophiles	4.61	2.30	2.53	3.47	3.39	1.86	1.53
	Coliforms	4.00	1.47	1.96	2.32	1.86	2.18	2.31
Extended Semen	Mesophiles	3.50	4.00	3.58	3.38	2.11	2.04	1.65
	Coliforms	1.47	1.47	1.15	1.76	1.00	NG	2.60

NG - there was no bacterial growth

DISCUSSION

Bacterial contamination is a common event during routine collection and processing of swine semen (4,9,20). Likewise, our results indicate that 86% of semen samples, raw semen, collected in different BS, contained one or more microorganism. According to Goldberg et al (26), the presence of more than 10^2 CFU mL^{-1} of aerobic mesophiles in the semen indicates a highly contaminated sample, which might affect the semen quality. In our study the average was 10^3 CFU mL^{-1}; suggesting a great potential effect on semen quality.

The water used in the processing of semen was the major source of contamination, since 85.7% (6/7) of BS had, at least, one or more microorganisms in water samples. The quality of water is critical and its contamination represents a significant risk factor, contributing to poor semen quality (7,14). Reicks (15) reported that, among 15 bacterial species isolated from semen, the water system was the primary source of the three most common genera, Alcaligenes, Pseudomonas, and Burkholderia. Payne et al (16) observed that the contamination of the water distillation system with *Achromobacter xylosoxidans*, a Pseudomonas-like bacterium led to endometritis and subsequent reproductive failure in sows and gilts. In this sense, Gary (17) performed a study, reporting that there was a decrease of 8% in sow pregnancy rate, as well as a reduction in 0.7 piglets born per litter, by using water with low quality. In our study, the BS that showed bacterial contamination of water, 66.6% (4/6) had one or more genus of the *Enterobacteriaceae* family. Therefore, the contamination may have occurred due to hygiene failures in the processes of purification, storage or handling of water. Studies have shown that nearly all large water purification systems can cause the formation of biofilm in the tubing. This biofilm can spread microorganisms within the system and contribute to an increase in particles and bacteria (23). Bresciani et al (24) also identified *Enterobacteriaceae* as the major boar semen contaminant. It is well documented that the presence of contaminants induces sperm agglutination (6) and reduces the viability and fertility of the semen (4).

Analyzing bacterial growth on surfaces (countertops, barrels, pipes), we observe that two BS (28.6%) had enterobacterial contamination. In virtually all BS *Staphylococcus* sp., was the predominant microflora. The presence of *S. aureus* might lead to a decrease in the number of spermatozoa, suppression of motility, changes in morphology, and fertilizing capacity. A considerable number of both spoilage and pathogenic microorganisms are able to participate in both adhesion and biofilm formation processes (19). Stainless steel, glass, rubber, and polypropylene surfaces can be contaminated either by spoilage or pathogenic microorganisms that, under certain conditions are deposited, adhered to, and interact with the surface, initiating cellular growth, and consequently leading to biofilm formation (19,20). Considering that the temperature of semen processing rooms in BS is controlled, the high prevalence of *Staphylococcus* sp., was biologically expected, since it is mesophilic agent, and might it has been one of the factors influencing the high prevalence on these surfaces. Corroborating to this hypothesis, in cultivating cells in tryptic soy broth at sub-optimal temperatures (20, 25 and 30°C), Rode et al (21) found the highest attachment capacity with *S. aureus* on polystyrene. De Souza et al (22) observed that *S. aureus* strains adhered in high numbers regardless the type of assayed surface and incubation temperature (7°C or 28°C). Therefore, our results suggest the need for better processes of inactivation or removal of contaminant agents in BS surfaces. Regarding biofilm organisms, the sanitization solution must penetrate the matrix of exopolymers and gain access to the microbial cells causing biofilm inactivation and removal (22).

The collection of a bacteria-free ejaculate is a significant challenge, since the microorganisms are inherent of the environment in which the ejaculate is collected, being present in dust particles in suspension, as well as on the normal microflora of the animal itself (5), representing a mixed microbiota. In this sense, our results pointed out that 57.14% of BS had ejaculated containing bacteria of the Enterobacteriaceae family. It is corroborated by a study of Úbeda et al (23), which also report the enterobacteria as the main ejaculate contaminants. These authors also observed that agents related to this family contaminated 40.68% of semen

samples assessed, and these are related to reduced motility and increased sperm pathologies. Moreover, these microorganisms might be involved in inflammatory processes of the endometrium of inseminated sows (5,24). While sperm motility, sperm morphology, and concentration of the SD produced are the parameters commonly used to assess semen quality, the microbiological evaluation is not a routine practice in boar semen evaluation, and may indicate other potential indicators of poor semen quality. Thus, considering poor semen quality as one of the primary causes of female reproductive failure in animals, including swine, the microbiological evaluation should be applied, especially because it consists of an easy and low-cost technique.

Considering the bacterial contamination found in water and raw semen, as expected, the analysis of results demonstrates that the highest contamination of mesophilic, in diluted SD, were found in BS who also had contamination in purified water supply and raw semen. Hygienic semen collection and proper processing techniques with stringent laboratory procedures are the first and primary lines of defense to successfully reduce contamination (18). Antibiotics were added to semen extenders as a preventive measure to reduce bacterial contamination (5,6). Some level of drug resistance has been observed among isolates from boar semen against antibiotics commonly used as preservative antimicrobials in commercial porcine semen extenders (3,8). Our results on evaluation of susceptibility to antimicrobial agents has shown a good efficacy (*in vitro*) of aminoglycosides (neomycin and gentamicin) on the main contaminants found in BS. It represents an important issue, since these antibiotics are the most commonly added to extenders of boar semen. However, susceptibility testes should be carried out more often, since our results differ significantly from other authors; in Italy, Bresciani et al (18) observed gentamicin resistance on isolate of *E. coli* (50%), *Serratia marcescens* (50%), *Proteus* sp. (50%), *Streptococcus* sp. (50%) and *Staphylococcus aureus* (100%).

On the other hand, our results showed an important resistance to penicillin (>75%) and lincomycin (>87.5%). It has been shown that when utilizing β-lactams in a mixed bacterial population of susceptible and resistant cells, resistant cells quickly (in as little as 2.5 h) degrade the β-lactam, eliminating whatever selection pressure the antimicrobial provided (25). Considering that mixed isolation was the major finding observed, we believe this mechanism of degradation might contributed to the high level of penicillin resistance observed in our results. Although the penicillin is an antibiotic recommended only for Gram positive bacteria, it was used because it is a constituent of most of commercial semen extenders, Since the origin of the contamination cannot be predicted. Lincomycin is an antibiotic with spectrum activity on Gram positive bacteria; thus, based on our bacterial isolation, the high degree of resistance was expected, since most of bacterial genera isolated corresponded to Gram negative.

Based on the results obtained, we concluded that 86% of semen samples, raw semen, collected in different BS, contained one or more genera of bacteria. Water was the major source of contamination. Additionally, our results on evaluation of susceptibility to antimicrobial agents has shown a good efficacy (in vitro) of aminoglycosides (neomycin and gentamicin) on the main contaminants found in BS. On the other hand, there was an important resistance to penicillin (>75%) and lincomycin (>87.5%). Therefore, the understanding of bacterial dynamics on contamination processes, as well as identification of major bacterial contaminants genera, and their source and profile of antimicrobial susceptibility is mandatory for a proper quality control and reduced risk of contamination of semen doses in boar studs.

Conflict of interest

None of the authors have any conflict of interest to declare.

REFERENCES

1. Bortolozzo FP, Menegat MB, Mellagi APG, Bernardi ML, Wentz I. New artificial insemination technologies for swine. Reprod Dom Anim. 2015; 50(Suppl. 2):80-84.

2. Riesenbeck A. Review on international trade with boar semen. Rep Domestic Anim. 2011; 46(Suppl. 2):1-3.

3. Morrell JM. Antimicrobials in boar semen extenders – A risk / Benefits analysis. J Antimicrob. 2016; 2(1):107-108.

4. Althouse GC. Sanitary Procedures for the Production of Extended Semen. Reprod Dom Anim. 2008; 43:374-378.

5. Althouse GC, Pierdon MS, Lu KG. Thermotemporal dynamics of contaminant bacteria and antimicrobials in extended porcine semen. Theriogenol. 2008; 70:1317-1323.

6. Bussalleu E, Yeste M, Sepúlveda L, Torner E, Pinart E, et al. Effects of different concentrations of enterotoxigenic and verotoxigenic E. coli on boar sperm quality. Anim Reprod Sci. 2011; 127:176-182.

7. Schultze M, Ammon C, Rudiger K, Jung M, Grobbel M. Analysis of hygienic critical points in boar semen production. Theriogenology. 2015; 83:430-437.

8. Schulze M, Grobbel M, Muller KM, Junkes C, Dathe M, Rudiger KR, Jung M. Challenges and limits using antimicrobial peptides in boar semen preservation. Reprod Dom Anim. 2015; 50(Suppl 2):5-10.

9. Grossfeld R, Peralta W, Weitze K, Waberski D. Antibiotic-free hypothermic storage of boar semen in Androstar +5 extender results in similar fertility results compared to semen stored at 17°C in extender with antibiotic content. Anim Reprod Sci. 2016; 169:125.

10. Schulze M, Ammon C, Rüdiger K, Kung M, Grobblel M. Analysis of hygienic critical control points in boar semen production. Theriogenol. 2015; 83(3):430-437.

11. Gaczarzewicz D, Udala J, Piasecka M, Blaszczyk B, Stankiewicz T. Bacterial contamination of boar semen and its relationship to sperm quality preserved in commercial extender containing gentamicin sulfate. Polish J Vet Sci. 2016; 19(3):451–459.

12. Markey B, Leonard F, Archambault M, Cullinane A, Maguire D. Clinical Veterinary Microbiology, 2 ed. Mosby: USA; 2013.

13. CLSI. Developtment of In Vitro Susceptibility Testing Criteria and Quality Control Parameters. 5th Ed. CLSI guideline 23. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

14. Althouse GC. Sanitary procedures for the production of extended semen. Reprod Dom Anim. 2008; 43:374-378.

15. Rodriguez AL, Soom AV, Arsenakis I, Maes D. Boar management and semen handling factors affect the quality of boar extended semen. Porcine Health Management. 2017; 3:15.

16. Payne BJ, Clark S, Maddox C, Ness A. Achromobacter xylosoxidans in extended semen causes reproductive failure in artificially inseminated sows and gilts. J Swine Health Prod. 2008; 16(6):316-322.

17. Gary E Ritchie. Water for Pharmaceutical Purposes USP 24/NF. Pharmacopeia. 2000; 30(5):1744. URL available in: http://www.pharmacopeia.cn/v29240/usp29nf24s0_c1231.html

18. Bresciani C, Cabassi CS, Morini G, Taddei S, Bigliardi E, Di Lanni F, Sabboni A, Parmigiani E. Boar semen bacterial contamination in Italy and antibiotic efficacy in a modified extender. Ital J Anim Sci. 2014; 13:3082.

19. Tremblay YDN, Hathroubi S, Jacques M. Les biofilms bactériens: leur importance en santé animale et en santé publique. Canadian J Vet Res. 2014; 78(2):110-116.

20. Fuster-Valls N, Hernández-Herrero M, Marín-de-Mateo M, Rodríguez-Jerez JJ. Effect of different environmental conditions on the bacteria survival on stainless steel surfaces. Food Cont. 2008; 19:308-314.

21. Rode TM, Langsrud S, Holck A, Moretro T. Different patterns of biofilm formation in Staphylococcus aureus under food-related stress conditions. Int J Food Microbiol. 2007; 116:372-383.

22. De Souza EL, Meira QG, Barbosa IM, Athayde AJAA, Conceição ML, Siqueira-Junior JP. Biofilm formation by Staphylococcus aureus from food contact surfaces in a meat-based broth and sensitivity to sanitizers. Braz J Microbiol. 2014; 45(1):67-75.

23. Úbeda JL, Ausejo R, Dahmani Y, Falceto MV, Usan A, Malo C, Perez-Martinez FC. Adverse effects of members of the Enterobacteriaceae family on boar sperm quality. Theriogenol. 2013; 80:565-570.

24. Mazurova J, Kukla R, Rozkot M, Lustykova A, Slehova E, Sleha R, Lipensky J, Opletal L. Use of natural substances for boar semen decontamination. Vet Med. 2015; 60:235–247.

25. Korpimäki T, Kurittu J, Karp M. Surprisingly fast disappearance of β-lactam selection pressure in cultivation as detected with novel biosensing approaches. J Microbiol Met. 2003; 53(1):37–42.

26. Goldberg AMG, Argenti LE, Faccin JE, Linck L, Santi M, Bernardi ML, Cardoso, MRI; Wentz, I; Bortolozzo, FP. Risk factors for bacterial contamination during boar semen collection. Res Vet Sci. 2013; 95:362–367.