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Physical, chemical and biological aspects of the urine of sows in a certificated swine reproduction farm in the city of Toledo, Paraná, Brazil

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Urinary tract infection in sows is among the main reproduction problems because of its influences on productivity of the herd, affecting mainly the general health of sows and has considerable increase in the replacement rate. Thus, it is considered as the most important endemic disease in the sow in the production phase. The aim of this study was to determine the physical, chemical and microbiological aspects of the urine of sows from a certified reproductive farm in the city of Toledo, Paraná, Brasil, and later isolate and identify the pathogens present and perform an antibiogram of the samples. Urine samples from 100 sows were evaluated, performing physical-chemical, microbiological and antibiogram examinations. Etiological agents isolated with greatest frequency were *Escherichia coli* (75%), *Salmonella* sp. (19%) and *Proteus vulgaris* (6%). All samples were negative for *Actinobaculum suis.* The most effective antibiotics in controlling urinary tract infection was ceftiofur (77%) and gentamicin (73%). However, those presenting greater resistance were lincomycin (100%). In the physical examination of the urine samples, a total of 59% were light yellow in color. Under chemical evaluation, there was absence (100% negative) of uribilinogen, glucose, ketone bodies and bilirubin, and the presence (positivity) of protein (3%), nitrite (83%) and blood (1%). In general, the mean density was 1.015. The pH did not present variation, and remained neutral in all samples.

Key words: Urinary tract infection, sows, productivity, antibiogram.

INTRODUCTION

Swine production in Brazil is facing continuous innovations in genetic, production, nutrition and disease control areas (Sesti, 1995). This increase in productivity, modernization and intensification of swine production has led to an increase in the incidence of multifactorial diseases, which is the case of urinary tract infection (Sobestiansky and Wendt, 1993).

The impact of swine production on the global economy,

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especially in Brazil, which has the 3rd largest herd of swine, and the State of Paraná as the 4th largest Brazilian producer of swine, has made it essential to have rigorous control of diseases affecting the economic yield and productivity of the heard, such as urinary infection (Dalla-Costa and Sobestiansky, 1999).

Urinary tract infections cause great economic losses due to puerperal diseases, post-weaning infertility, expenses with medications, reduction of weight gain in piglets and mortality rates (Brito et al., 2004). Urinary problems are responsible for 50% of sudden death of sows in production and are the main cause of mortality among adult animals (Perestrelo and Perestrelo, 1988).

Swine production in the west of Paraná is further multiplying income and employment in all sectors of the economy, leading to the expansion and modernization of the swine production commercialization and transformation sectors.

In the last decade, with the intensification and confinement in swine culture evidenced in many farms, productivity problems related to the high incidence of urinary infections prompt studies mainly regarding etiological agents (Sobestiansky et al., 1995).

Urinary tract infection is defined by the penetration and/or multiplication and colonization of pathogenic micro-organisms in the urinary tract, reaching all or part of the urinary system (Sobestiansky et al., 1995; Matos et al., 2005). Urinary infections in swine affect mainly sows, causing a pronounced reduction in productivity indexes of commercial farms, with an epidemiological prevalence of up to 30% (Alberton et al., 2000).

Urinary tract infection is a heard disease of multifactorial origin, and the number of sick sows in a herd is directly related with the set of risk factors present in the farm (Alberton and Werner, 1998).

According to Sobestiansky et al. (1999), clinical signs in sows with urinary infection can be observed individually (lack of appetite, progressive weight loss, difficulty to get up, purulent or bloody vulvar discharge, alteration in color and smell of urine and urine in streams), and in herd (high number of sows with lack of appetite, increase in the rates of returning to rutting, mortality, disposal and replacement of matrixes and reduction in productive indexes).

Diagnosis can be performed by observing clinical signs, physical, chemical and microbiological examinations, and prevention can be done by the elimination of risk factors present in the farm (Sobestiansky et al., 1999).

Due to its great importance, this study was performed with the objective of determining physical, chemical and microbiological aspects of the urine of sows in a certified reproducer farm in the city of Toledo, Paraná, Brasil.

MATERIALS AND METHODS

The experiment was conducted in the city of Toledo, Parana State, Brazil, in a farm certified by the Department of Agriculture and Supply (SEAB), for the production of sows and breeding pigs. Urine samples from 100 sows (housed in individual cages) were collected. All sows were pregnant with gestational ages between 50 and 90 days, aged 18-48 months. These urine samples were collected from sows belonging to following races: Landrace (25/100), Large White (44/100), Duroc (5/100) and Pietran (26/100).

The sows studied were randomly chosen, not necessarily for presenting or not, any clinical sign indicating urinary infection, as described by Sobestiansky et al. (1999). Since it is registered in the Ministry of Agriculture (MAPA), this farm adopts strict biosafety programs, having fences around the farm, bird and rodent traffic control, people entrance control system and sanitary empty space, animal quarantine system, quarterly and biannual examination of sows in the heard.

In this farm, the water supply system for the sows is provided in a continuous manner, that is, the sows receive water during the whole day, which originate from artesian well, stored in covered water tanks and treated with chlorine, without any type of analysis being performed on the water. The sanitation system of the facilities is done twice a day, at the beginning of the morning and at the end of the afternoon, both after feeding.

Urine collections were performed during spontaneous urination of sows, before the first feeding, discarding the first urine streams and colleting a minimum sample of 100 mL, starting from the second half of urination. These samples were collected in sterile flasks, open only at the time of collection (Vaz et al., 2007; Alberton et al., 2000).

After the collection, the urine samples were identified according to the breed and earing number of the sow, stored in isothermal boxes, with ice and a temperature of 5°C and immediately transported to the Preventive Veterinary Medicine and Public Health Laboratory at Universidade Paranaense, Campus Umuarama, in Paraná. The transport took about 90 min.

At first, the samples were submitted to physical evaluation, where it was possible to evaluate color, aspect and odor (Garcia-Navarro, 1996; Strasinger, 1998). According to Alberton et al. (2000), Pôrto et al. (2003) and Menin et al. (2008), coloring is obtained by macroscopic evaluation of the urine sample stored in transparent container, and can be classified into: colorless, light yellow and dark yellow. Chemical examination of urine samples was conducted using Uri-Color Check® reagent stripes to determine pH value, presence of nitrite, presence of blood, uribilinogen, glucose, ketonic bodies, bilirubin and density. After this, the samples were submitted to microbiological examinations where, first, there was the inoculation of 1 mL urine in BHI medium for enrichment, incubated for 24 h at 37°C. After enrichment, the sample was placed in MacConkey's Agar and Blood Agar (defibrinated sheep blood at 5%) plates, incubated for 24 h at 37°C. In order to identify *Actinobaculum suis,* all samples were sowed in blood agar and cultivated in anaerobic jars, added with anaerobiosis generators and incubated for 48 h at 37°C. After cultivation, the reading of plates was performed to observe bacterial growth, evaluating the macroscopic characteristics of the colonies (color, form, size) and microscopic characteristics of the colonies through GRAM coloring (morphologic, dying characteristics and biochemical test) (Quinn et al., 1994). After 24 h, the colony forming units (CFU) were counted, and samples with growth above 10^4 UFC/mL were considered as urinary tract infections (Jones, 1981).

Gram negative bacteria were submitted to biochemical tests for enterobacter differentiation (Gram-negative bacillus, positive catalase). Biochemical proofs were made using Newprov® enterobacter kit, where Gram-negative bacillus colonies isolated in the plates were inoculated in tubes containing biochemical media and incubated for 24 h. After this period, the tubes were read and the biochemical reactions that took place in the biochemical media

Table 1. Result of the resistance profile of urine examined through chemical tests, isolated in the urine of 100 sows in production phase, in a certified reproduction swine farm in the region of Toledo – PR. 2011.

Antibiotic	Sensitivity samples	Intermediary samples	Resistance samples	Total
Amoxicillin	42%	0 0%	58%	100%
Ceftiofur	77%	21 21%	2%	100%
Doxycycline	17%	25 25%	58%	100%
Streptomycin	21%	18 18%	61%	100%
Gentamicin	73%	6 6%	21%	100%
Lincomycin	0%	0 0%	100%	100%
Norfloxacin	46%	23 23%	31%	100%

were noted, according to the protocol recommended by the manufacturer. At last, each isolated was submitted to anti-microbial sensitive test (antibiogram), with the following antibiotics: lincomycin, amoxicillin, doxycyline, norfloxacin, penicillin G, ceftiofur, gentamycin and streptomycin, according to CLSI (2008), and the results obtained in millimeters were interpreted as resistant, sensitive and intermediary, according to the manufacturer indication.

Ethics committee

This experiment was submitted to the Ethics committee in Research Involving Animal Experimentation under protocol number 20101/2011.

Statistical analysis

Data obtained were analyzed in the statistical program SPSS, version SPSS 10.1 2001. Frequency determination of the studied variables was determined and for the correlation analysis among the variables, the Chi-square test was used, and variance analysis was done to check the influence of breed on urine pH and density, with significance level of 5%.

RESULTS

From the 100 urine samples analyzed, 59% presented light yellow, 34% colorless and 7% dark yellow coloring. In the chemical evaluation of the samples, there was absence (100% negative) of uribilinogen, glucose, ketonic bodies and bilirubin, and presence (positivity) of protein (3%), nitrite (83%) and blood (1%). Average pH found in the urine samples was 6.37 and the values obtained for density of the urine samples analyzed were placed into 6 groups (from 1000 to 1.020). Values found for density in urine samples were assigned to 6 groups, being 1.020 (31%), 1.015 (24%), 1.025 (21%), 1.010 (14%), 1.005 (7%), 1.000 (3%). It was found that 58 (58%) samples were positive for urinary tract infection. From the isolated bacteria, 75% were *Escherichia coli*, 19% *Salmonella* sp. and 6% *Proteus vulgaris*. All samples were negative for *Actinobaculum suis*.

The results found in the antibiogram were two antibiotics with the best performance, being ceftiofur (77%) and gentamicin (73%) with efficacy on bacterium and two antibiotics with the worst performance, namely lincomycin (100%) with inefficacy on the bacterium, as shown in Table 1.

DISCUSSION

Studies by Sobestiansky and Wendt (1993) state that the urine from sows with urinary tract infection tend to present dark yellow coloring, which did not happen in our study, where 59% samples (regardless of being with or without urinary tract infection) presented light yellow coloring. Our study corroborates with research performed by Alberton (1996), which found (62.5%) predominance of light yellow coloration in urine with infection, showing that this parameter can be influenced by a series of factors, and thus, cannot be used in isolation for estimating the presence of urinary infection in sows. In the Chi-square analysis, no significant correlation was found between the presence of urinary tract infection and urine coloring (P=0.173 and χ^2 =3.51).

Studies have shown a correlation between the presence of nitrite in urine and urinary infections in pigs (Garcia-Navarro, 1996). However, in the present paper, no significant correlation was found (P=0.390 and value of χ^2 = 1.25). This is contradictory to the result found by Garcia-Navarro (1996) and Sobestiansky et al. (1992), which found that urines containing this substance indirectly indicate a bacterial activity in the bowel, being a strong indication of the existence of cystitis, because the authors found 44 samples (44%) which were positive for nitrite and for cystitis, with significant presence of nitrite and cystitis. However, according to Carr and Done (1996), since not all bacteria are capable of reducing nitrate to nitrite, a negative result does not guarantee the absence of contamination in the urinary tract.

Regarding pH, the mean obtained among them were of a pH 6.37, which is considered normal, ranging from 5.5 to 6.5, according to Sobestiansky et al. (1995) and Alberton (1996). In this study, no correlation could be observed between the presence of cystitis and urine pH (Chi-square 1.043 and P=0.594). Carr and Walton (1992) found similar results when examining the urine of 52 sows. However, in the case of cystitis, it is expected to found alkali urine, since the bacterial flora located in the urinary tract transforms urea in ammonia, causing alkalization (Coles, 1989). In this paper, no correlation could be seen between urine density and cystitis (Chi-square 1.739 and P=0.187). In general, the mean density was 1.015, not differing between sows with or without infection. According to Sobestiansky et al. (1992), there is

a correlation between daily water ingestion and urine density in the first morning urination.

According to Alberton (1996), there is a direct relation between the restriction of water consumption and the occurrence of urinary infection, that is, the density of urine has direct relation with the amount of water ingested by the sow. Therefore, when the amount is sufficient, insufficient or is at a critical limit, the urine density is lower than 1.008, greater than 1.012 and between 1.008 and 1.012, respectively. Taking this relation into consideration, in can be concluded that from the sows studied in this paper, only 37.8% presented insufficient water ingestion. Jourquim et al. (1992) found urinary density of 1.015 for sows with daily water ingestion above 15 L/d, minimum volume recommended for pregnant pigs. Thus, values greater than 1.012 observed in this study can be considered within normality.

The best urine sample is the one collected at the first urination in the morning; since there is less ingestion of water during the night, the urine is more concentrated, presenting elements that translate the real situation of the urinary system (Veiga, 2006). Urination frequency directly influences the amount of bacteria in the urine and therefore, morning samples diagnosis has greater ease of urinary tract infections than those collected in the afternoon or early evening (Sobestiansky, 2007).

Chi-square analysis for the variables, number of births, age and breed of sow, regarding the presence of urinary infection have demonstrated no influence of these on the rate of infection Chi-square value and significance level for the variables studied were, respectively, χ^2 = 0.044, χ^2= 0.126 and χ^2 = 0.166 and P= 0.978, P=0.939 and P= 0.920. Other authors who have worked with urinary infections in sows, and reported deaths (Chagnon et al., 1991; Vearick et al., 2008), which did not occur in animals of this study. Probably such differences are related to the microorganisms involved. In the cited studies, the researchers report multibacterial infections and the animals of the research in question were analyzed only for *E. coli* infection.

In epidemiological surveys on the etiology of swine bacteria, it was verified that *E. coli* is the main microorganism found in urinary tract infections in 58% pigs in France, according to Madec and David (1983) and in 34% in Portugal (Perestrello and Perestrelo, 1988). In Brazil, the occurrence was 27% in Minas Gerais and 15% in Paraná (Reis et al., 1992). However, in our study, from the urine samples collected from 100 sows, 58% of the samples were positive for urinary tract infection.

From the bacteria isolated in this study, 75% were *E.coli.* This result corroborates with the study performed by Mazzutti (2010) in Curitiba, Paraná, where a greater isolation of *E. coli* was obtained, with frequency of 90.62% from 32 urine samples positive for urinary tract infection. Several other studies have also found *E. coli* as the most frequent bacterium in urinary tract infection cases in swine matrixes (Reis et al., 1992; Carr et al., 1995; Menin et al., 2008; Merlini et al., 2009).

According to Sobestiansky and Wendt (1993), the microbiota involved in urinary infection is characterized essentially for being fecal, and maybe this explains the high prevalence of isolation of *E. coli* in urinary infections. These opportunistic bacteria ascend to the bladder, taking advantage of the fact that the urinary tract of sows is naturally badly protected and there is a relatively small distance from the vulva to the urethra (Smith, 1883). According to Brito et al. (1999), the majority of *E. coli* samples from swine origin, isolated from urinary infections, present different plasmid profiles and multiple resistance to antimicrobial drugs. This antimicrobial resistance shows the need of new alternatives for the prevention and treatment of urinary infection. Mazzutti (2010) studied 44 urine samples and isolated (13.64%) *Proteus* sp. bacterium, a result which was higher than the one found in this study. However, Sansot et al. (1998), studying 408 sows found the presence of *Proteus* sp. in 6% urine samples, which corroborates with the same result found in our study. In this study, the authors have also isolated (19%) *Salmonella* sp. and did not found any reference to reports of isolation of this bacterium in urine from sows.

The result of identification of *A. suis* was negative for all samples surveyed, showing that in this study, there was no relation between the presence of *A. suis* and the occurrence of urinary tract infection. Study performed by Wendt and Vesper (1992), where a total of 943 sows from farms with problems with urinary infection were examined, obtained 55% relationship between cystitis and the occurrence of *A. suis.* According to Alberton et al. (2000), there might be competition between microbiota and fecal origin and *A. suis* and the positive interaction between the two bacterial flora starts to occur from the moment the first bacterium provokes initial lesion in the urinary mucosae, which allows the adhesion of *A. suis.* The results found in the antibiogram in the present study were: two antibiotics with better sensitivity to bacteria, namely, ceftiofur (77%) and gentamicin (73%), and two antibiotics with higher resistance to bacteria, that is, lincomycin (100%).

The *E. coli* samples tested revealed low levels of resistance to ceftiofur (2%) and gentamicin (21%). This sensitivity of the agent to these antimicrobials might be associated with the high cost of medication, which makes it be used with low frequency on the farm. However, the low antimicrobial sensitivity of *E. coli* to lincomicin (0%) and penicillin G (3%) in this study might have been related to the fact that the active principle of the medication had already been used for treating other infections in this farm, which might have brought an increase in the antimicrobial resistance to this antibiotic. Antimicrobial resistance of the *E. coli* bacterium observed

in the antibiogram in this study is an important factor to be considered, since this bacterium was the most frequently isolated one in this study (75%). Several other studies have also pointed *E. coli* as the most frequent bacterium in cystitis cases in sows (Sobestiansky et al., 1999).

This multi-resistance can be manifested by the presence of resistance genes F1, P type Fimbria, Aerobactin and Serum resistance, as observed by Brito et al. (2004), when studying virulence factors in uropathogenic *E. coli* samples, and Costa (2007) while studying pathotypical characteristics of *E.coli* isolates originating from pigs.

Conclusions

In physical examination, there was prevalence of light yellow color in 59% of the samples, and in the chemical examination, average pH in urine was 6.37, there was total absence (100% negative) of uribilinogen, glucose, ketonic bodies and bilirubin, and presence (positivity) of protein (3%), nitrite (83%) and blood (1%). The result of identification of *A. suis* was negative for all samples in this study, and the bacterium isolated with the highest frequency in the samples was *E. coli,* present in 75% samples. The authors found *Salmonella* sp. isolated in 19% of the urine samples from sows studied, which there was no previous report for. The antibiotic with highest sensitivity was ceftioflur and the ones presenting worst performance were lincomycin.

It could also be concluded that the physical and chemical tests can be performed on the farm, since they are safe and inexpensive examinations, and their performance can be used as screening method for sows for successive microbiological examination of urines.

Conflict of interests

The authors have not declared any conflict of interests.

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