Evaluation of the reagent test strips and microscopic examination of urine in the diagnosis of urinary tract infection in sows[1]

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ABSTRACT.- Mazutti K., Locatelli-Dittrich R., Lunardon I., Kuchiishi S.S., Lara A.C., Zotti E. & Alberton G.C. 2013. **Evaluation of the reagent test strips and microscopic examination of urine in the diagnosis of urinary tract infection in sows.** *Pesquisa Veterinária Brasileira 33(9):1103-1108.* Curso de Medicina Veterinária, Escola de Ciências Agrárias e Medicina Veterinária, Pontifícia Universidade Católica do Paraná, Campus Curitiba, Rua Imaculada Conceição 1155, Prado Velho, Curitiba, PR 80215-901, Brazil. E-mail: kelly.mazutti@pucpr.br

The diagnosis of the urinary tract infection (UTI) in sows is usually performed by using reagent test strips, since it is a fast and practical method, and capable of being done at the farm. The microscopic examination of the urine is rarely used at the farm since it is a more time consuming and difficult technique. However, there are no studies on the accuracy of those two techniques for the UTI diagnosis on this species. This study aims to assess the accuracy of the reagent test strip and the urine microscopic examination in the diagnosis of ITU in sows, comparing them with the bacteriological examination of urine. In order to select the sows for this study, a chemical reagent test strip was carried out previously and a total of 139 sows were selected, 66 sows of which showed positivity to nitrite in the reagent test strip and 73 without nitrituria. Then, the next day, a new sample collection for performing a complete urinalysis was carried out from those 139 sows, which included physical, chemical, microscopic and microbiological examination of these urine samples. The results revealed that the nitrite test of the reagent strip showed 100% of specificity and 93% of sensitivity. The specificity of the microscopic examination for bacteriuria was 82% and the sensitivity was 100%. The UTI diagnosis by using reagent strips and/or the urine sediment test is reliable if compared to the urine bacteriological examination, which makes possible the rapid diagnosis of UTI in sows at the farm.

INDEX TERMS: Urinary tract infection, cystitis, *Escherichia coli*, leukocyte esterase, nitrite, urinalysis, swine.

RESUMO.- [**Precisão da tira reagente e do exame microscópico da urina no diagnóstico de infecções do trato urinário em porcas.**] O diagnóstico de infecção do trato urinário (ITU) em porcas geralmente é feito com o auxílio de tiras reagentes, por ser um método rápido, prático e passível de ser realizado na própria granja. O exame microscópico da urina raramente é utilizado em granjas por ser uma técnica mais demorada e trabalhosa. No entanto, não existem estudos sobre a precisão destas duas técnicas no diagnóstico de ITU nesta espécie. O objetivo deste estudo foi avaliar a precisão da tira reagente e do exame microscópico da urina no diagnóstico de ITU em porcas, comparando-os com o exame bacteriológico da urina. Para selecionar as porcas que iriam compor o estudo foi realizado um exame químico prévio com tira reagente, do qual foram selecionadas 139 porcas, 66 positivas para nitrito na tira reagente e 73 negativas. No dia seguinte foi realizada uma nova coleta de urina destas 139 porcas para realização da urinálise completa, que incluiu os exames físico, quí-

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mico, microscópico e microbiológico destas amostras de urina. Os resultados demonstraram que a prova de nitrito da tira reagente apresentou 100% de especificidade e 93% de sensibilidade. A especificidade do exame microscópico para bacteriúria foi de 82% e a sensibilidade de 100%. O diagnóstico de ITU com o uso de tiras reagentes e/ou com exame microscópico da urina é confiável, quando comparado com o exame bacteriológico da urina, o que torna possível o diagnóstico rápido de ITU em porcas na granja.

TERMOS DE INDEXAÇÃO: Infecção do trato urinário, cistite, *Escherichia coli*, esterase leucocitária, nitrito, urinálise, suínos.

INTRODUCTION

Urinary tract infection (UTI) is the most important endemic disease affecting sows and also one of the main causes of reproductive failures, general health complications and reduction of the life expectancy of the herd (Girotto et al. 2000, Porto et al. 2004). The microorganisms most frequently found in these infections are *Escherichia coli*, *Proteus mirabilis, Staphylococcus* sp*., Streptococcus* sp*., Aeromonas hydrophila* and *Actinobaculum suis* (Meister 2006, Sobestiansky et al. 2007, Menin et al. 2008).

The clinical examination has a limited value in UTI diagnosis since in most of the cases the clinical signs are not evident (Fairbrother 2006), and it is necessary to perform a urinalysis to achieve a conclusive diagnostic. One of the routine practices used on farms is the collection of urine samples by spontaneous urination and the performance of the diagnosis by using reagent test strips. The preventive and/or healing procedures are carried out in accordance to the prevalence obtained through this method.

The use of the reagent strips method is widely used since it is fast, practical and can be performed at the farm. To supplement this method, a complete urinalysis, which includes the microscopic examination of the urine and the bacteriological test, may be also performed. However, the distance between the laboratories and the farms, the additional cost and the time demanded, are limiting factors for performing these tests.

Since the reagent strips used in Veterinary Medicine have been developed for diagnosing UTI in humans, they may, therefore, generate doubtful results when applied to swine. Furthermore, even on humans, there are various studies that show a wide variation in the sensitivity and specificity of the components of the strips used for diagnosing UTI (Kellogg et al. 1987, Bolann et al. 1989, Lachs et al. 1992, Holland et al. 1995, Sultana et al. 2001).

The aim of this study is to assess the accuracy of the reagent strip and of the urine microscopic examination as methods for diagnosing the urinary tract infections in sows, comparing obtained results to the bacteriological test.

MATERIALS AND METHODS

The experiment was carried out in two Piglet Production Units – PPU, one located in the State of Santa Catarina and the other in the State of Paraná, Brazil. The study was composed by 139 pregnant sows of commercial lineage, with different parity times, placed in individual cages with a channel drinker. All sows were pregnant, with gestational ages ranging between 50 and 70 days. The amount and the type of ration consumed by the animals during the time of the experiment followed the routine pattern already set by the farmers, in accordance with the length of pregnancy and in compliance with the NRC (1998) recommendations for pregnant sows. The animals were provided with plenty of water during the whole period of the experiment.

In order to select the sows for this study, a chemical reagent strip test was carried out previously and a total of 139 sows were selected, 66 sows of which showed positivity to nitrite in the reagent strip and 73 without nitrituria. Then, the next day, a new sample collection for performing a complete urinalysis was carried out from those 139 sows.

The urine samples were collected at dawn, before feeding and in sterile flasks. The collectors waited for the spontaneous urination of the sows and collected the middle urine, disregarding the first discharge. After each collection, the flasks were closed and placed behind the cages of the respective sows. After collecting the samples, the flasks were dried with tissue paper and numbered according to the sows earrings. The urine samples were placed into isothermal boxes with ice and taken to the laboratory on the farm for the immediate performance of the physical, chemical and microscopic tests.

The evaluation of the urine was performed in accordance with standard methods (Strasinger et al. 1998). The chemical test was carried out with reagent test strips (Uriquest®, Labtest Diagnóstica S.A., Brazil). The parameters evaluated were the following: nitrite, pH, urinary specific gravity and leukocytes. The urinary specific gravity was also obtained by refractometry and pH by pH meter. The microscopic examination of the urine (sedimentoscopy) was performed with a regular optical microscope in the 45 x objective. Leukocytes were quantified as a number per average of ten fields. The bacteria were classified in accordance with visual and subjective criteria and were recorded as absent (-), rare (R), discreet (+), moderate (++), pronounced or uncountable (+++).

The urine samples were placed in isothermal boxes with ice and were sent to the Animal Sanity Diagnosis Center – CEDISA, located in Concórdia/SC, for bacterial count and bacteriological isolation. The samples were seeded in 5% ovine blood Agar, Mac Conkey and in Tryptic Soy Agar (TSA) for colony count. Samples that showed a count equal or higher than 10^5 UFC/ml were considered positive for urinary tract infection (Fairbrother 2006). The bacteria were identified through Gram and biochemical tests (SIM, TSI, CIT, O/F, VM, Catalase). The bacteria identified as Gram negative were submitted to supplemental biochemical tests by using the Api 20 E commercial kit (BioMérieux®, Marcy l'Etoile, France).

The computations for determining the sensitivity and specificity of the reagent strip and of the microscopic examination of the urine were carried out in accordance with a described method (Menezes & Santos 1999). In the case of the reagent strip, the animals positive for nitrite in the reagent strip were considered positive. In the urine microscopic examination, samples with rare or absent bacteria were considered negative, while the samples with a discreet bacterial presence (+), moderate (++), pronounced or uncountable (+++) were considered positive. The results obtained from both techniques were compared to the urine bacteriological test, which is considered the "gold standard" diagnostic test for UTI.

During the statistical analysis, the data obtained with continuous numerical variables of normal distribution, were submitted to the *t* test and considering the statistical differences when the value of $P<0.05$. The statistical correlations were carried out by using the Pearson's test. The continuous numerical variables that did not follow the normal distribution were transformed into Log10 previously to the analysis. The agreement between

the bacterial count by seeding and by the bacteriological test was analyzed by Pearson's test, when using crosstabulation method (Statgraphics® 1982-2010), and by Kappa's test. The latter test was also used to establish the agreement between nitrite in the reagent test strip and the bacteriological test.

RESULTS

The UTI diagnosis with reagent test strip, considering the nitrite strip, showed 100% specificity, which means that all the positive samples for nitrite in the reagent strip (66) showed a bacterial count over 10^5 UFC/ml. The sensitivity of the reagent strip was 93% because five, from 73 of the negative samples for nitrite in the strip, showed a bacterial count of over 10^5 UFC/ml (Table 1). None of the samples was positive for leukocytes in the strip.

Urinary parameters of sows affected and non-affected by ITU can be seen in Table 2. There was no statistical difference in the urine pH, obtained by both the reagent test strip as obtained by pH meter, between positive and negative animals for ITU. There was a statistical correlation between the two techniques used (r=0.62, P<0.0001). The urine specific gravity was significantly higher in animals positive for UTI compared to the negative ones, in both techniques (Table 2), and there was also a correlation between urinary density obtained by the reagent test strip and the refractometer (r=0.86, P<0.0001).

The number of leukocytes per field was significantly higher in animals positive for UTI than in the negative ones, as well as bacterial count (Table 2). There was an agreement between the bacterial count obtained during the microscopic examination and the one obtained by seeding (r=-0.27, P=0.0013). The specificity of the microscopic examination for bacteriuria was 82% and sensitivity was 100% (Table 1).

According Landis & Koch (1977), the obtained values of Kappa's test revealed an almost perfect agreement between nitrite in the reagent test strip and the bacteriological test (K=0.928, P<0.001) and a substantial agreement between the bacterial count by seeding and by the bacteriological test (K=0.7, P<0.001) (Table 1).

The result of the urine culture of the 71 urine samples positive for UTI (Table 3) showed that the *Escherichia coli* bacteria was the most frequently isolated agent (81.69%).

Table 1. Results from Reagent test strip and Microscopic examination of urine, comparing to Bacteriological test results (gold standard)

	Reagent test strip	Microscopic examination of urine	Bacteriological test
Number of positive animals	66	84	71
Number of negative animals	73	55	68
True positives	66	72	
False positives	0	12	
True negatives	68	55	
False negatives	5	0	
Specificity	100%	82%	
Sensitivity	93%	100%	
Kappa's value*	0.928	0.7	

*According to the interpretation table of Kappa's value from Landis & Koch (1977), values of Kappa's test between 0.6 and 0.79 means a substantial agreement, and values between 0.8 and 1.00 means an almost perfect agreement (P<0.001).

DISCUSSION

The nitrite proof with reagent strip showed 100% specificity and 93% sensitivity for UTI diagnostic. The false-negative results may have two explanations: the first one is that not all the bacteria are capable of converting nitrate into nitrite; nevertheless, the Gram-negative, which are the main ones responsible for UTI, have this capacity (Morgan & McKenzie 1993, Strasinger 1998, Memişoğullari et al. 2010); the second explanation is that the reaction depends on the urinary stasis in the bladder for a minimum period of time of four hours (Almond & Stevens 1995); therefore, these sows may have urinated within an interval of time shorter than four hours.

There are several studies which evaluate the sensitivity and the specificity of the positive reaction for nitrite in the reagent strip for UTI diagnosis in human beings (Bollan et al. 1989, Tincello & Richmond 1998, Sultana et al. 2001, Devillé et al. 2004, Ali et al. 2007, Ducharme et al. 2007, Taneja et al. 2010). The values found range from 33 to 57% and 78 to 99%, respectively.

None of the samples showed leukocyturia in the reagent test strip, even when significant amounts of these were present in the urinary sediment (Table 2). The high number of leukocytes in urine is called pyuria and indicates the pre-

Table 2. Comparative results of urinary parameters of sows positive and negative for ITU

	pH reagent test strip	pH meter	Density reagent test strip	Density refractometer	Leukocyte/ field	Bacterial count (log10)
Positive animals	6.41±0.94	7.01±0.71	1012.1±8.60[a]	1012.2±7.23[a]	2.42±3.87[a]	7.38±0.75[a]
Negative animals	6.46±0.80	7.00±0.30	1006.9±6.17[b]	1007.2±6.00[b]	0.11±0.32[b]	2.79±1.29[b]
P value	0.749	0.965	<0.0001	<0.0001	<0.0001	<0.0001

[a,b] Different superscript letters in the same column indicate statistical significance (P<0,05).

Table 3. Results of the urine culture of 71 urine samples of sows positive for UTI

Isolated bacteria	Number of samples	Frequency %
Escherichia coli	58	81.69
Gram-negative Coccobacillus*	4	5.63
Escherichia coli / *Streptococcus* sp.	4	5.63
Escherichia coli / *Staphylococcus* sp.	1	1.41
Escherichia coli / *Proteus* sp.	1	1.41
Enterobacter sp.	1	1.41
Streptococcus sp.	1	1.41
Proteus sp.	1	1.41
Total	71	100

* Positive for negative Gram bacteria: excluding the possibility of being *Enterobacter* sp., *Klebsiella* sp., *Edwardsiella* sp., *Salmonella* sp. and *Escherichia coli*.

sence of infection or inflammation in the urogenital system. Among the most frequent causes are bacterial infections, such as cystitis, pyelonephritis, prostatitis and urethritis (Alberton & Locatelli-Dittrich 2010). The tests for leukocytes, or leukocyte esterase, are based on the hydrolysis of protein substrates esters with esterase activity. Human neutrophils produce up to 10 proteins with esterase activity. These proteins react with the substrate to produce alcohols and acids, which then react with other substances in order to produce a change in color that is proportional to the amount of esterase in the sample (Fuller et al. 2001). González & Silva (2006) say that the proof for leukocytes in the reagent strip is based on human leukocyte esterase and do not seem to be so sensitive in animals as in humans. Therefore, this study showed that the reagent strip was not a reliable parameter and the proof of the presence of leukocytes in the urine of sows must be obtained by analyzing the urinary sediment.

There was a correlation between the urinary specific gravity values obtained by reagent test strip and the refractometer (r=0.86, P<0.0001), demonstrating that the strip can be considered reliable for the assessment of urinary specific gravity. In both methodologies the values obtained were significantly higher in positive animals for UTI than in the negative ones. Urinary specific gravity is directly related to the amount of water intake by the sow. Thus, when the amount is sufficient, insufficient, or is in a critical limit, the urinary specific gravity is less than 1008, greater than 1012, and between 1008 and 1012, respectively (Sobestiansky et al. 1992). The water supply system was the same for all animals, with water *ad libitum*. A likely explanation for this observed difference would be that, probably, positive animals feel pain during urination caused by UTI and, thus, avoid urinating frequently, leading to a urinary stagnation and increasing consequently the urinary concentration. Also, because of the pain, these animals prefer to stay longer in bed, avoiding getting up to drink water.

There was also a correlation between the pH value obtained by the reagent test strip and by pH meter (r=0.62, P <0.0001). The average pH values were lower in the strip when compared to pH meter results (table 2). This may have two explanations: the first is that the pH meter might be more accurate in measuring pH than the reagent test strip, and the second is that the urine was evaluated immediately by reagent test strip, while in evaluation by the pH meter there was the transport time to the laboratory, which means that the urine may have a alkalization process at the elapsed time between the two evaluations. The pH values obtained by positive and negative animals for UTI, in both techniques, were within normal limits because, according Menin et al. (2008), pH values for sows urine between 5,5 to 7,5 are considered normal. It was expected that sows positive for ITU presented a more alkaline pH. According Coles (1989), in urinary infections is expected to find alkaline urine, due to the microorganisms located in the urinary tract. When they are endowed with the urease enzyme, they can turn the urea into ammonia, causing alkalinization. According Sobestiansky et al. (2007), a pH value of 8 or above, constitute an important sign of a predisposition to bacterial infections.

There was an agreement between the bacterial count carried out by sedimentoscopy and the bacterial count performed by seeding (r=-0.27, P=0.0013). This result shows that the microscopic examination of the urine is reliable for diagnosing UTI, since even the five sows with false negative diagnosis for UTI through the nitrite test were detected with bacteriuria in the sediment test. Therefore, even though the microscopic examination of the urine to detect the presence of leukocytes and bacteria is more time consuming and harder to perform than the reagent strip test (Downs 1999), the former may be performed safely at the farm, whereas the bacteriological examination, regarded as the reference method for diagnosing UTI (Zorc et al. 2005), needs to be performed in a laboratory, has a high cost and has the disadvantage of taking at least 48 hours for obtaining the results (Whiting et al. 2005).

The specificity and the sensitivity of the microscopic examination of the urine in this study were 82% and 100%, respectively. Hiraoka et al. (1995) set out to assess the usefulness of the microscopic examination of the urine for diagnosing UTI in human and obtained 91% sensitivity and 98% specificity for detecting bacteriuria. Memişoğullari et al. (2010) evaluated 250 human urine samples in order to compare the results obtained by the reagent strip with the microscopic examination of the urine and computed the performance characteristics of those tests. The sensitivity and the specificity of the microscopic examination of the urine were 91% and 68%, while in the reagent strip they were 80% and 60% respectively. The authors suggest that both urine analysis methods can be used for a quick diagnostic. Other authors obtained similar results (Vangone & Russo 1985, Vickers et al. 1991, Lohr et al. 1993, Al-Daghistani & Abdel-Dayem 2002). Taneja et al. (2010) evaluated the usefulness of the reagent strip (leukocyte esterase and nitrite) and of the microscopic examination of the urine for diagnosing UTI and concluded that these techniques must be used jointly for added safety in quickly UTI diagnosing.

Escherichia coli was the bacteria most frequently isolated in the urine culture of the samples positive for UTI (81.69%). Carr & Walton (1992) and Meister (2006) obtained similar results and found the *E. coli* bacteria as being the most frequent agent among the urine samples evaluated, with 90.38% and 70.45%, respectively. These findings corroborate various other studies that have also found *E. coli* as the most frequent bacteria in UTI cases in sows (Reis et al. 1992, Carr et al. 1995, Menin et al. 2008). Similarly, in human beings, *E. coli* is the most common etiological agent (Anderson et al. 2004, Mysorekar & Hultgren 2006, Rosen et. al 2008).

CONCLUSION

The UTI diagnosis with reagent strips and/or the microscopic examination of urine is reliable if compared with urine bacteriological examination, since the reagent test strip has shown 100% specificity and 93% sensitivity, and bacteriuria in the microscopic examination of the urine has shown 82% specificity and 100% sensitivity, which makes possible to diagnose rapidly UTI in sows on the farm.

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