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MICROBIOLOGY

Are stained and unstained methods of urine sediment from dogs in accordance with microbiological culture?

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ABSTRACT: Diagnosis of bacteriuria in veterinary medicine is commonly based on unstained urinary sediment evaluation. Nonetheless, amorphous particles can be confused with bacteria. This study aims to investigate whether the stained sediment increases the sensitivity and specificity of bacteriuria detection. One hundred urine samples were collected, with 60 obtained through voided specimens and 40 through catheterization, from dogs of various breeds, genders, and ages. Additionally, a 1 ml aliquot from each sample was subjected to quantitative bacteriological culture, serving as the gold standard test for comparison with the sediment analysis. Comparing to the bacteriology culture, the stained sediment of urine collected by catheterization exhibited a relative sensitivity of 60% and specificity of 70%, while to voided specimens showed a sensitivity of 93.3% and specificity of 57.8%. Unstained sediment presented a sensitivity of 100% and specificity of 10% to urine collected by catheterization, and 93.3% and 4.4% to voided samples, respectively. Both stained and unstained methods demonstrated negative predictive values > 60%, but the positive predictive value of unstained sediment ranged to 24.6% (voided samples) to 27% (catheterization) indicating a lower probability of true positives. Both techniques showed a low correlation coefficient (*kappa*) indicating that they cannot replace the gold standard method for confirming bacteriuria. However, Gram staining of urinary sediment improved the differentiation between amorphous substances and bacteriuria in canine urine samples, suggesting its potential applicability in laboratory routines.
Key words: urinalysis, stained urinary sediment, bacterial culture, bacteriuria, Gram.

A análise dos sedimentos urinários corado e não-corado de cães está de acordo com a cultura microbiológica?

RESUMO: O diagnóstico de bacteriúria em medicina veterinária é comumente baseado na avaliação do sedimento urinário não corado. No entanto, partículas amorfas podem ser confundidas com bactérias. Este estudo tem como objetivo investigar se o sedimento corado aumenta a sensibilidade e especificidade da detecção de bacteriúria. Foram coletadas 100 amostras de urina, sendo 60 obtidas por meio de micção natural e 40 por cateterismo, de cães de várias raças, sexos e idades. Adicionalmente, uma alíquota de 1 ml de cada amostra foi submetida à cultura bacteriológica quantitativa, servindo como teste padrão-ouro para comparação com a análise por US. Comparando com a cultura bacteriológica, o sedimento corado de urina coletada por cateterismo apresentou sensibilidade relativa de 60% e especificidade de 70%, enquanto que para amostras de micção natural apresentou sensibilidade de 93.3% e especificidade de 57.8%. O sedimento não corado apresentou sensibilidade de 100% e especificidade de 10% para urina coletada por cateterismo, e 93.3% e 4.4% para micção natural, respectivamente. Tanto a técnica de sedimento corado quanto não-corado demonstraram altos valores preditivos negativos (> 60%), mas o valor preditivo positivo do sedimento não-corado variou de 24,6% (micção) a 27% (cateterismo), indicando uma menor probabilidade de verdadeiros positivos. Ambas as técnicas apresentaram baixo coeficiente de correlação (*kappa*) indicando que não podem substituir o método padrão-ouro para confirmação de bacteriúria. No entanto, a coloração de Gram do sedimento urinário melhorou a diferenciação entre substâncias amorfas e bacteriúria em amostras de urina canina, sugerindo seu potencial de aplicabilidade em rotinas laboratoriais.
Palavras-chave: urinálise, sedimento urinário corado, cultura bacteriana, bacteriúria, Gram.

INTRODUCTION

Urinary tract infection (UTI) is a common disease in the clinical routine of small animals, occurring in approximately 14% of dogs that undergo clinical-veterinary evaluation. (BARTGES, 2004). Bacterial culture is necessary to confirm the diagnosis of UTI (THOMPSON et al., 2011), as urinalysis may erroneously lead to an unaltered exam.

Bacterial culture followed by susceptibility testing confirms infection, allows identification of resistant bacteria, and rules out non-infectious diseases that mimic UTI (WEESE et al., 2011). This approach avoids the indiscriminate use of antimicrobials, which would increase bacterial resistance to these drugs (GRANT et al., 2021).

However, the cost and time required for bacterial culture have been identified as possible

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obstacles to performing this test in clinical practice (DE BRIYNE et al., 2013). Thus, in some cases, the administration of antimicrobials is performed based on clinical signs and urinalysis findings compatible with UTI (MARQUES et al., 2016).

In the microscopic analysis of urinary sediment, amorphous particles similar to bacteria (pseudobacteria) are commonly reported as bacteria, regardless of the experience of the clinical pathologist who performs the examination (SWENSON et al., 2004). Knowing that the possibility of mistakenly identifying bacteria is a reality in routine and that the manufacture of stained slides of urinary sediment is a quick, inexpensive and relatively easy procedure (WAY et al., 2013), this study aims to verify the concordance between the analysis of the urine sediment stained or not, with the bacterial culture of urine of dogs for the confirmation of bacteriuria.

MATERIALS AND METHODS

Urine samples from female (n = 48) and male (n = 52) dogs, of different breeds, ages and collection methods, derived from routine clinical and surgical tests at the University Veterinary Hospital (HVU) at the Federal University of Santa Maria (UFSM) were processed for the detection of bacteriuria by analysis of the sediment stained or not by the Gram method. A 1ml aliquot of these samples was sent for bacteriological culture. Samples with less than 5 ml of urine or urine from animals treated with antibiotics were excluded from the experimental design.

Unstained sediment analysis

Five milliliters of urine were centrifuged at 1500 rpm for 5 minutes. After this process, the volume of 0.5 mL of supernatant was maintained, which was homogenized with the sediment. With the aid of a pipette, 10µL of this mixture was placed on a glass slide and superimposed by a cover slip for microscopy. Ten fields of the sediment were analyzed in an optical microscope (Zeiss®) with a 10X and 40X objective. The average number of bacteria was classified in a 400X objective, being classified as none, occasional (< 3 bacteria), few (3 to 10 bacteria), moderate (11 to 40 bacteria) and severe (> 40 bacteria) (SWENSON et al., 2004).

Gram-stained sediment analysis

The slides with the urinary sediment were subjected to dry fixation and then stained with a Gram-staining kit (Laborclin®) with the methodology according to the manufacturer's instructions. The stained slides were examined in an immersion objective (1000X) in 20 fields, being classified as none, occasional (1 to 4 bacteria), few (5 to 9 bacteria), moderate (10 to 20 bacteria) or severe (> 20 bacteria) (SWENSON et al., 2004).

Aerobic urine culture

A 0.1 ml aliquot of urine was seeded in blood and MacConkey agar medium using a sterile drigalski strap and incubated for 72 hours at 37 °C, aerobically. There was performed a serial dilution of the urine ranging from 10^{-1} a 10^{-5} and 0.1 ml of urine of each dilution was plated on the surface of each agar plate.

In collections by spontaneous urination, it was defined as significant bacteriuria from 100,000 CFU/ml. In urine collected by catheterization, samples with growth < 10,000 CFU/ml were considered evidence of contamination (Table 1).

Statistical analysis

The *kappa* coefficient was calculated to verify the concordance between the results of the culture with the analysis of the stained and the unstained sediment. In addition, sensitivity, specificity, positive and negative predictive value were also calculated.

RESULTS AND DISCUSSION

Urine samples from 102 dogs that were asymptomatic for inflammatory lower urinary tract were received during the period from May to July

Table 1 - Significance of quantitative urine cultures in dogs based on collection method (according BARTGES et al., 2004).

Method of collection	----------Significant----------	----------Suspicious----------	----------Contaminant----------
Catheterization	≥ 10000*	1000 - 10000	≤ 1000
Midstream voiding	≥ 100000	10000 - 90000	≤ 10000

*Colony-forming units per milliliter (CFU/ml). **For midstream voiding, the contamination level may be 10,000 CFU/ml or higher.

2022. Two samples were excluded from the study due to contamination in the bacteriological culture, remaining 25 positive and 75 negative samples in the microbiology culture. Fifteen samples were collected through voided specimens and ten through catheterization. Two dogs each provided two urine samples, and the remaining patients only one. Quantitative aerobic urine culture was positive in 10 samples collected by catheterization (10/40) and in 15 samples collected by voided specimens (15/60). In this last collection method, there were isolated 11 genera of bacteria.

Regarding the samples collected by catheterization (n = 40), bacteriuria was observed in 37 samples by analysis of unstained sediment, in 15 samples by stained sediment and in only 10 by bacterial culture (Tables 2 and 3). There were ten true positive results, three true negative results in unstained urine sediment, 27 false positive and none false negative results. Regarding the stained urinary sediment, six true positive results, 21 true negative results, 9 false positives and 4 false negatives were observed.

For samples collected by voided specimens (n = 60), bacteriuria was observed in 57 samples in the unstained sediment analysis, 33 in the stained sediment analysis and 15 in the bacterial culture (Table 4 and 5). There were 14 true positive, two true negative, one false negative and 43 false positive results for the unstained sediment analysis. There were 14 true positive, 26 true negative, 19 false positive and one false negative result for the stained sediment analysis.

Analysis of the stained sediment of urine collected by catheterization revealed a *kappa* coefficient of 0.265, which indicates a moderate concordance with the culture, while analysis of the unstained sediment evidenced a *kappa* coefficient of 0.053, which constitutes a minimal correlation (Table 2 and 3). When voided specimens were evaluated, the stained urine sediment showed a *kappa* coefficient of 0.365, which indicates a reasonable concordance with the culture, while analysis of the unstained sediment evidenced no correlation (*kappa* coefficient < 0) (Tables 4 and 5). Therefore, the Gram urinary sediment had a better concordance with the culture, the gold standard method for detecting bacteriuria, making the analysis of the stained sediment a more reliable test than the observation of the unstained sediment.

Despite the relative high rate of positive results, which increased sensitivity of unstained urinary sediment analysis (93.3%) and its low specificity in voided specimens (4.4%) this method is not suitable for use as a screening test for bacteriuria, requiring values of high specificity and sensitivity (GOULART & CHIARI, 2007). Based on this study, 30 (75%) of 40 samples collected by catheterization and 45 (75%) of 60 samples collected by voided specimens were considered negative by bacterial culture method. In unstained sediment obtained by voided method, there was no observation of bacteria in only 3 samples. However, this method detected 71.7% of false positive in voided samples, which could result in antimicrobial therapy unnecessary.

The analysis of the unstained sediment presented lower positive predictive values than the analysis of the stained sediment, resulting in more false positive results. Regarding the negative predictive values, both methods showed values > 60% indicating that both the stained and unstained sediment have a low chance of resulting in false negatives.

Table 2 - Obtained results in stained sediment and microbiology culture (gold-standard) of urine collected from dogs by catheterization method.

	Microbiology culture					
Stained sediment	Positive		Negative		Total	
	n	%	n	%	n	%
Positive	6	15.0	9	22.5	15	37.5
Negative	4	10.0	21	52.5	25	62.5
Total	10	25	30	75.0	40	100

Relative sensitivity: 60 %.
Relative specificity: 70 %.
Positive predictive value: 40%.
Negative predictive value: 84 %.
Kappa coefficient: 0.265.

Table 3 - Obtained results in unstained sediment and microbiology culture (gold-standard) of urine collected from dogs by catheterization method.

	Microbiology culture					
Unstained sediment	Positive		Negative		Total	
	n	%	n	%	n	%
Positive	10	25	27	67.5	37	92.5
Negative	0	0	3	7.5	3	7.5
Total	10	25	30	75	40	100

Relative sensitivity: 100 %.
Relative specificity: 10 %.
Positive predictive value: 27 %.
Negative predictive value: 100%.
Kappa coefficient: 0.053.

The present study had lower positive and negative predictive values compared to other studies (WAY et al., 2013; SWENSON et al., 2011). In the study carried out by WAY et al. (2013) the positive and negative predictive value were 100% and 93%, respectively. While in the study executed by SWENSON et al. (2004), there was the observation of positive and negative predictive value, respectively, of 94.5% and 98.7%. This difference may have been caused by differences in the population used in the studies, in the method of urine collection used and, in the threshold, used to consider bacteriuria significant by the culture method. Urine samples in the present study were collected by catheterization and voided specimens, which are methods more susceptible to contamination by bacteria from the lower urinary tract. Sample contamination can be confirmed by performing a quantitative bacteriological culture. Specimens that have counts below 10.000 are considered contaminated by the catheterization method and below 100.000 by the free catch method (Table 1) (BARTGES et al., 2004).

The high threshold for considering a sample with bacteriuria in samples collected by free catch and catheterization increased the number of false positive results in stained and unstained sediment. In addition to the low detection limit to consider a sample as presenting bacteriuria, the collection methods used in the present study contributed to the increase in the number of false positives, due to bacterial contamination, commonly present in lower urinary tract. Bacterial contamination of urine usually occurs when collecting a voided sample but it can also occur either during urethral catheterization or be associated with collection of urine from a contaminated surface (SMEE et al., 2013).

However, it is reliable to use the voided specimen's method, if veterinary reference intervals are used for counting CFUs/ml, if the samples are refrigerated and cultured within 4 h after collection.

Table 4 - Obtained results in stained sediment and microbiology culture (gold-standard) of urine collected from dogs by voided method.

	Microbiology culture					
Stained sediment	Positive		Negative		Total	
	n	%	n	%	n	%
Positive	14	23.3	19	31.7	33	55
Negative	1	1.7	26	43.3	27	45
Total	15	25	45	75	60	100

Relative sensitivity: 93.3 %.
Relative specificity: 57.8 %.
Positive predictive value: 42.4%.
Negative predictive value: 96.3 %.
Kappa coefficient: 0.365.

Table 5 - Obtained results in unstained sediment and microbiology culture (gold-standard) of urine collected from dogs by voided method.

	Microbiology culture					
Unstained sediment	Positive		Negative		Total	
	n	%	n	%	n	%
Positive	14	23.3	43	71.7	57	95
Negative	1	1.7	2	3.3	3	5
Total	15	25	45	75	60	100

Relative sensitivity: 93.3 %.
Relative specificity: 4.4 %.
Positive predictive value: 24.6%.
Negative predictive value: 66.7 %.
Kappa coefficient: -0.011.

The cleaning of external genital region with the objective of reducing the growth of bacteria, there is no great reduction of bacterial population. Thus, meticulous washing proved to be unnecessary (SØRENSEN et al., 2016).

Our data suggest that the collection method with the observation of false positive results, probably due to the previously established bacteriuria criteria for bacterial culture for the different methods of obtaining urine (SMEE et al., 2013). The false-positive results were due to the presence of small particles that resembled bacteria in size, shape and Brownian motion (Figure 1). These particles are usually small lipid molecules, cytoplasmic organelles, amorphous crystals or debris that do not grow in culture, but contribute to false-positive results due to the analysis of unstained urinary sediment (SWENSON et al., 2004). SWENSON et al. (2004) described the same effect when the sediment was stained with the Wright Giemsa stain. Gram staining has the advantage of being able to differentiate between Gram-positive and Gram-negative bacteria (WAY et al., 2013). SWENSON et al., (2004). The Gram staining method provides information about bacterial morphology that helps the clinician to decide the selection of the most appropriate antibiotic therapy (GOSWITZ et al., 1993). However, aerobic bacterial culture and susceptibility testing should be performed in all cases, to confirm the presence of bacteria and to identify the presence of resistant bacteria that may not respond to initial therapy (WEESE et al., 2011).

The stained sediment was not able to detect bacteriuria in 4 samples collected by catheterization and in one collected by voided specimens. This

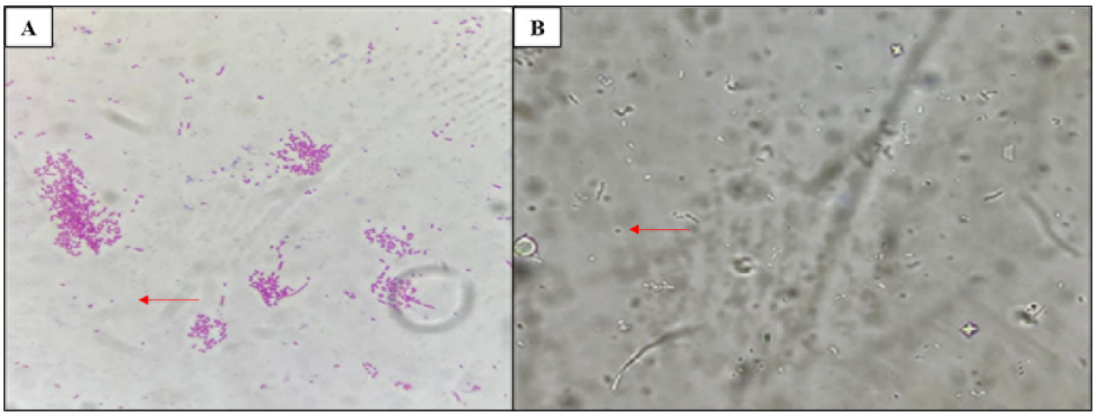

Figure 1 - (A) Stained urine sediment slides (arrow). Structure suggestive of the presence of bacteria. Objective: 40X, Gram. (B) Unstained urine sediment (arrow). Structure suggestive of the presence of bacteria. Objective: 40X.

occurrence cannot be attributed to a low count CFUs, since these were similar to the samples classified as having bacteriuria in the sediment analysis. Therefore, perhaps there could have been contamination of the culture medium or poor preparation of the sediment, as suggested by WAY et al. (2013).

The empirical use of antibiotics has been considered the main cause of the emergence of microorganisms resistant to multiple drugs (HALL et al., 2014). In Veterinary Medicine, the treatment of probable UTI based on the results of bacteriuria analysis of urinary sediment is the major cause of the indiscriminate use of antimicrobials (MARQUES et al., 2016).

Therefore, our preliminary results suggest that stained urinary sediment analysis should be routinely implemented to mitigate the false detection of bacteriuria in urinalysis. However, bacterial culture should be performed on all samples as it is the gold standard test for detecting bacteriuria.

CONCLUSION

The Gram staining technique of urinary sediment decreases the occurrence of false positive results in samples presenting bacteriuria. However, bacterial culture test should be performed to detect bacteriuria in urine samples collected by free catch and catheterization. Therefore, as it is an easy, fast and low-cost technique, it can be implemented in the laboratory routine, aiding in the trial diagnosis of bacterial infections of the urinary tract.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

All procedures carried out in this study were approved by the Ethical Committee for Animal Experimentation at the Universidade Federal de Santa Maria (CEUA-UFSM), under protocol 9964140622.

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