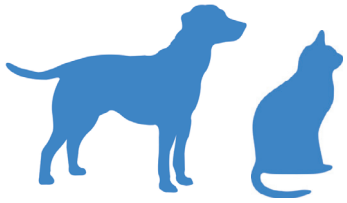
The role of urine sediment cytology in the evaluation of bacteriuria



Introduction and aim of the study - Urinary Tract Infections (UTIs) are a common disorder in the dog and cat and urinary culture is considered the "gold standard" for the final diagnosis. However, urine sediment cytology may give useful preliminary information. In this study we retrospectively evaluated the accuracy of cytology for the identification of bacteriuria and the agreement between cytology and bacteriology in identifying infections caused by cocci, rods and mixed bacteria.
Materials and methods - In this retrospective study we compared urine sediment bacteriology and cytology.
Results - A total of 148 urinary samples were included, 109 from dogs and 39 from cats. 69 of 148 (47%) were positive on microbiology (50 from dogs and 19 from cats). Sensitivity of cytology for bacteriuria was respectively 78.3% (total cases), 82% (dogs) and 68.4% (cats); specificity was 93.7% (total cases), 93.2% (dogs) and 95% (cats). Overall accuracy was 86.5% (total cases), 88% (dogs) e 82% (cats). The positive predictive value was 90.6% (total cases), 90.0% (dogs) and 92.2% (cats). The negative predictive value was 83.1% (total cases), 85.9% (dogs) and 76% (cats).
Discussion - In the study, urine sediment cytology was easily performed and showed a good overall accuracy. The agreement with urinary culture was good.

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INTRODUCTION

Urinary Tract Infections (UTIs) are an ordinary problem in the dog and cat and quantitative bacteriology cultures are the method of choice for diagnostic confirmation of bacteriuria.[1-7] However, a basic urine test with urine sediment evaluation may already exhibit microscopic findings suggestive of UTI: in fact, the presence of active sediment, with haematuria, leukocyturia and struvite crystalluria are often associated with UTI. In addition, urine sediment examination often allows the detection of bacteria. Previous studies have shown that the identification of bacteria in urine sediment assessed on a fresh preparation can be improved by using dry cytological smears and subsequent staining with routine haematology/cytology stains, such as Wright-Giemsa.[7-10] In these studies the diagnostic accuracy of bacterial detection after cytological preparation was shown to be higher than with direct fresh visualization.

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The retrospective study presented in this paper is based on an internal investigation carried out at the Città di Pavia Veterinary Hospital; aim of the study was to evaluate the agreement between urine sediment cytological findings versus urinary culture. In particular, it was decided to:
1) evaluate the sensitivity, specificity, positive and negative predictive values of the sediment cytological examination versus the culture examination in the samples of our case series and to compare such data with what reported in the literature;[7-10]
2) measure the diagnostic accuracy of cytology for the correct microscopic identification of cocci and rod-shaped bacteria, always using microbiology test results as the gold standard.

Urinary Tract Infections (UTIs) are an ordinary problem in the dog and cat and quantitative bacteriology cultures are the method of choice for the diagnostic confirmation of bacteriuria.

MATERIALS AND METHODS

The retrospective investigation was carried out utilizing the database of the Città di Pavia Veterinary Hospital; we first identified those urine samples which, after an initial standard urine test - including macroscopic evaluation, chemical-physical and urinary sediment examination -, were then also subjected to a subsequent culture test. For this purpose we reviewed the archived results of tests carried out in the period between November 2011 and March 2015.

A basic urine test with urine sediment evaluation may already present microscopic findings suggestive of UTI: in fact, the presence of active sediment, with haematuria, leukocyturia and struvite crystalluria are often associated with UTI.

Sediment tests were routinely performed after centrifugation for 5 minutes at 80 g. The supernatant was separated and used for the chemical-physical examination; an aliquot of sediment, equal to 10% of the original volume, was first re-suspended and then analysed fresh under the microscope at 10x and 40x magnification, as reported by Vap and Shropshire.[11] In the presence of active sediment, with >3 leukocytes per field at 40x magnification and/or bacteria, a cytological preparation was made, as follows: a small drop of sediment was gently smeared on a slide and then quickly dried with a source of hot air (hair dryer); the sample was stained with a Romanowsky-type technique (May-Grünwald-Giemsa or Diff-Quik).

To be included in the statistical analysis the samples had to fulfil the following inclusion criteria:
1) samples had to be taken by cystocentesis. Samples with active sediment, as described above, underwent a microbiological culture test. In the hospital in which the study was conducted the urine samples were routinely collected by clinicians in the morning, they were initially placed in a refrigerator and then brought back to room temperature for the routine analysis. When necessary, an aliquot was stored and sent within 3 p.m. to the microbiology lab, where the samples were seeded. Based on this approach, in no case more than 8-10 hours - often much less - passed between sample collection and seeding. Samples collected in the afternoon/evening or during the night were stored in the refrigerator until execution of the analysis, which was conducted by bringing the urine back to room temperature. Throughout this time the samples were kept within a sterile container and were not seeded on a transport medium;
2) the microscopic evaluation of the sediment, both fresh and cytological, was performed by a single operator (WB), who first described the presence or absence of bacteria and then, if present, classified them based on their morphology in cocci, rods or mixed. The assessment was carried out before the culture results and was therefore a blind evaluation.
The microbiological culture results were considered as the gold standard and were used as reference for the evaluation of sensitivity, specificity, total accuracy, positive and negative predictive value of the cytological test. The agreement between the two methods was evaluated with Cohen's Kappa coefficient.[12]

RESULTS

The study included 148 urine samples, of which: 109 dog samples, 57 males, 49 females and 3 dogs of unspecified sex; 39 cat samples, 20 males, 16 females and 3 cats of unspecified sex.
Of all the samples tested, 69 out of 148 (47%) were positive to bacteriological culture, 50 dogs and 19 cats, respectively. In dog patients with a positive urine culture, the most frequently identified bacteria were: *E. coli* 27/50 (54%), *Staphylococcus* spp. 9/50 (18%), *Streptococcus* spp. 4/50 (8%), *Pseudomonas* spp. 4/50 (8%) and *Proteus* spp. 3/50 (6%). In cats, the isolates were: *E. coli* 9/19 (47%), *Staphylococcus* spp. 5/19 (26%), *Streptococcus* spp. 3/19 (16%) and *Proteus* spp. 2/19 (11%).
Cytology samples were positive for bacteriuria in 59

cases out of 148 (40%), of which 45/109 (41%) dogs and 14/39 (36%) cats. Cytology identified rod-shaped bacteria in 42 total patients (Figure 1), 33 dogs and 9 cats; cocci-shaped bacteria in 11 total patients (Figure 2), 8 dogs and 3 cats, and mixed forms in 6 total patients (Figure 3), 4 dogs and 2 cats.

The agreement between cytology and bacteriology results is summarised in Table 1.

The coefficients of agreement between cytology and urine culture were: 0.70 (total cases), 0.71 (dogs) and 0.65 (cats). Table 2 shows the interpretation guidelines of the agreement values thus calculated.[12]

Table 1 - Summary of the comparison between cytological and bacteriological examination based on the agreement between the two methods

TOTAL RESULTS	
True Negatives - Complete Agreement	74
True Positives - Complete Agreement	48
False Negatives - Disagreement	15
False Positives - Disagreeement	5
True Positives - Partial Agreement	6
RESULTS IN DOGS	
True Negatives - Complete Agreement	55
True Positives - Complete Agreement	36
False Negatives - Disagreement	9
False Positives - Disagreeement	4
True Positives - Partial Agreement	5
RESULTS IN CATS	
True Negatives - Complete Agreement	19
True Positives - Complete Agreement	12
False Negatives - Disagreement	6
False Positives - Disagreeement	1
True Positives - Partial Agreement	1

Table 2 - Interpretation guidelines for Cohen's kappa coefficient[12]

Kappa	Agreement
< 0.01	none
0.01-0.20	minimal
0.21-0.40	weak
0.41-0.60	moderate
0.61-0.80	strong
0.81-1.00	excellent

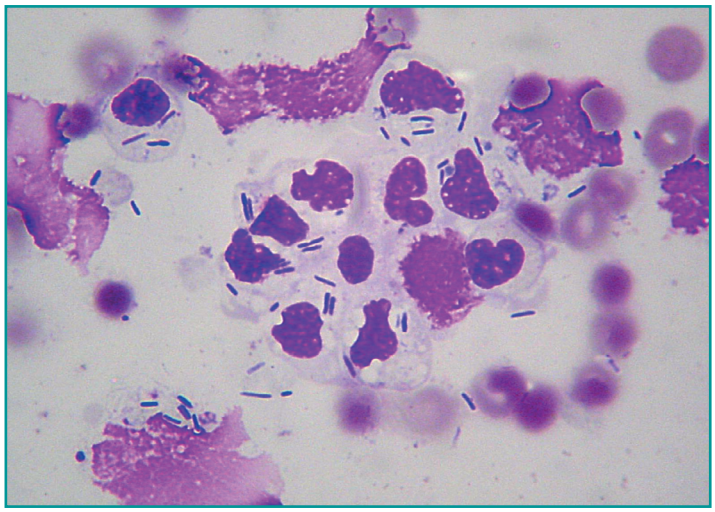

Figure 1 - Urine sediment of a dog: occasional erythrocytes, numerous degenerated (karyolytic) neutrophilic granulocytes and a single population of rod-like bacteria (May-Grünwald-Giemsa, 1000X).

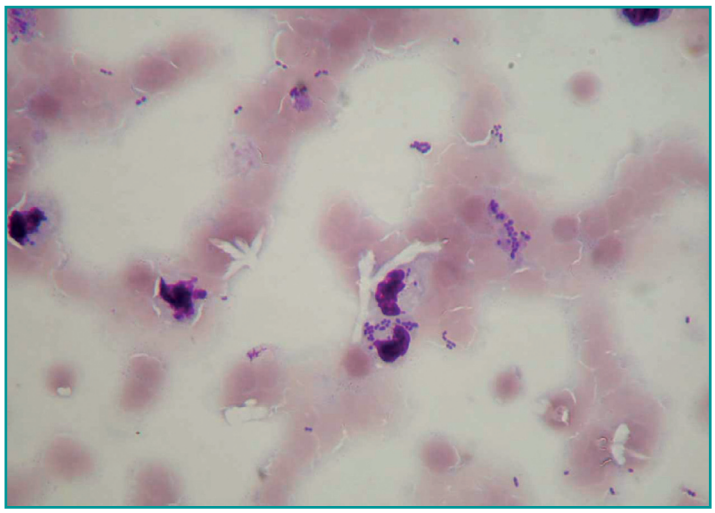

Figure 2 - Urine sediment of a cat: numerous erythrocytes, occasional degenerated neutrophilic granulocytes and a single population of cocci-like bacteria (May-Grünwald-Giemsa, 1000X).

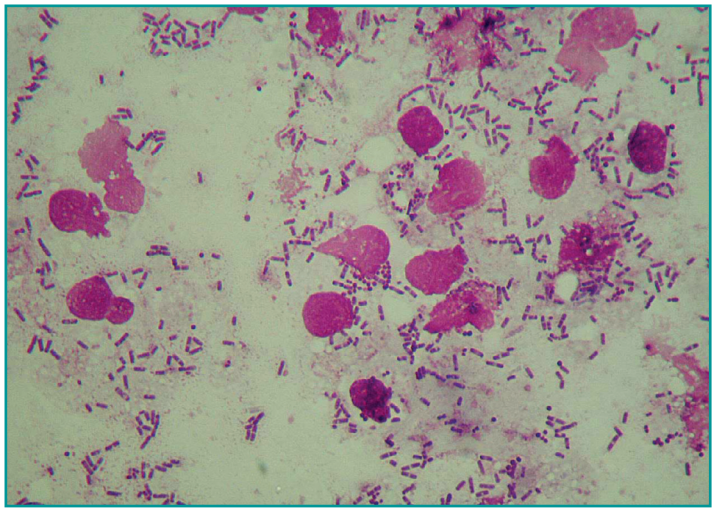

Figure 3 - Urine sediment of a dog: numerous degenerated (karyolytic) neutrophilic granulocytes and a mixed population of bacteria (May-Grünwald-Giemsa, 1000X).

Table 3 - Summary of the diagnostic accuracy of urinary sediment cytology, broken down by total cases, dogs and cats

	Total	Dogs	Cats
Sensitivity	78.3%	82%	68.4%
Specificity	93.7%	93.2%	95%
PPV	90.6%	90.0%	92.2%
NPV	83.1%	85.9%	76%
Accuracy	86.5%	88%	82%

Diagnostic accuracy

The sensitivity of cytology in detecting bacteriuria was 78.3% (total cases), 82% in dogs and 68.4% in cats, respectively, while specificity was 93.7% (total cases), 93.2% in dogs and 95% in cats. The overall diagnostic accuracy was 86.5% (total cases), 88% in dogs and 82% in cats. The positive predictive value was 90.6% (total cases), 90.0% in dogs and 92.2% in cats. The negative predictive value was instead 83.1% (total cases), 85.9% in dogs and 76% in cats (Table 3).

DISCUSSION

UTIs are a very frequent clinical problem in the dog and cat; while waiting for the results of the microbiological examination a standard urine examination can already provide useful clinical and therapeutic indications.[1-5,9] Antibiotic susceptibility tests take a few days, therefore either empirical, non-specific antibacterial therapies are initially used or antibiotics are not administered at all, until the arrival of the culture test report.

A rapid identification of bacteria allows clinicians to quickly establish an antibiotic treatment long before the urine culture results are available.

Sediment examination in fresh samples (as it is, or prepared with specific sediment dyes) is an extremely rapid and economical method for the identification of numerous clinically relevant alterations (e.g., presence of cells, rods, crystals, bacteria, etc.).[11,13] However, the identification of bacteria can be difficult and misleading, due to the presence of moving suspended particles (the so-called Brownian motion) or of dye precipitates in the case of stained preparations. In view of this, false positives can be extremely frequent[8,9] and can lead to the false identification of micro-organisms with consequent unnecessary or harmful treatments.

In the two studies by Swenson *et al.*,[8,9] the first on dogs and the second on cats, the subsequent preparation of cytological smears from sediment led to a significant reduction in the percentage of false positives (from 59.9% to 5.5% in dogs and from 40.7% to 1.3% in cats), with a diagnostic specificity that consequently increased from 76.4% to 99% in dogs and from 56.7% to 98.7% in cats. The same procedure also favoured the identification of true positives, with an increase of diagnostic sensitivity from 82.4% to 93.2% in dogs and from 75.9% to 82.8% in cats. In our study, on the total number of cases the sensitivity was 78.3% and the specificity 93.7%, values similar but lower than those found in both of the above mentioned studies by Swenson *et al.*[8,9] When comparing the results of our dog and cat samples, no relevant differences in specificity were found (93.2% in dogs vs 95% in cats); the sensitivity was instead markedly lower in our cat samples (68.4%) than in the dog samples (82%). As there were no marked differences in the proportions of bacilli or cocci infections between the two species, this discrepancy might be partly explained by the different sample size (109 dogs *vs* 39 cats). However, it should be noted that the highest number of false negatives was found in cocci infections, perhaps in view of them being more difficult to identify under the microscope compared to rods, even after cytological staining. In our study, if only pure infections are considered, out of 66 cases of individual isolates: 48 identified rod bacteria while 18 identified cocci. Of these, 6/48 (12.5%) and 7/18 (38.9%) were false negatives at cytology. These different proportions seem therefore to confirm that at cytology the identification of cocci is more difficult compared to rods. The different bacterial load, not evaluated in our study, may also have been a factor contributing to unsatisfactory results in some samples.

The correct identification of bacteria in the urine sediment can be improved by the preparation of dry cytological smears and subsequent cytological staining.

A separate mention should be made of cases in which mixed bacteria were isolated, that were only 3 in our case series (2 dogs and 1 cat). Within this scenario, from a diagnostic point of view cytology resulted highly inefficient, as it identified only one true positive case (33.3% sensitivity) and gave 5 false positive results. In 4 of these, cytology detected cocci and rods, but the former was not isolated in the culture test.

However, this last result requires additional investigation: microbiological examination was considered as the gold standard but it cannot be excluded that in the case of mixed infections only some of the bacterial species may in fact proliferate *in vitro,* while others may perhaps not be isolated under such conditions. For this reason, it cannot be excluded that the cytological findings may in fact have been correct.

The agreement between cytology and urine culture, our gold standard, was good. It should be noted that the calculation of the concordance was based on a very restrictive analysis of the concordances between the types of bacteria isolated; cases of partial concordance were counted as a discordance (e.g., mixed population in cytology vs. only rods in the urine culture).

The method of conservation of the samples may be a limitation of the study. All the samples were obtained by cystocentesis, but being the study retrospective it was not in fact possible to standardize the urine storing procedure. The time elapsed between collection and seeding of the sample varied from 1-2 hours to over 12 hours for samples collected in the evening or the night before. Moreover, in view of the nature of the study, the storage duration at refrigeration temperature and then at room temperature was not standardized. This may have favoured the discrepancy between cytology and culture examination results. At refrigeration temperature, bacteria may remain stable or die; *vice versa*, at room temperature, there may be an excessive proliferation of the bacteria present *in vivo*; but contaminating microorganisms that have accidentally ended up in the sample may also grow. Based on the data available in the literature, preservation at room temperature does not alter the results of the microbiological test, if conducted within a few hours of sampling; if the seeding is instead performed after 24 hours there can be up to 4% of false negative results and 50% of false positives.[14] Refrigeration of the sample may be an alternative, but a recent study has shown that compared to immediate seeding the refrigeration of urine samples reduces the sensitivity of the microbiological examination, thus leading to possible false negative results.[15] As previously mentioned this could be due to bacterial death or to growth inhibition *in vitro.* One possible remedy to this problem is the use of a seeding media suitable for the transport of the sample to the microbiology lab; with this approach the urine is immediately seeded onto the culture media and the bacteria can start to grow immediately.

The cytological examination of urine sediment is a simple and low-cost procedure which, if associated with the routine evaluation of fresh urine sediment, can provide additional valid clinical information.

In conclusion, we can state that urine sediment cytological examination is a simple and low-cost procedure which, if associated with the routine evaluation of fresh urine sediment, can provide additional valid clinical information. We have introduced this procedure in our daily clinical practice, whenever the initial sediment analysis shows the presence of cellular elements and/or microorganisms, in order to improve and refine the recognition of eventual bacteria. A rapid identification of bacteria allows clinicians to quickly establish an antibiotic treatment long before urine culture results are available. It should in fact be underlined that in the present study the microorganisms identified as cocci and rods were always gram-positive and gram-negative, respectively, to the culture test. This finding is of clinical importance, as it may allow a more targeted antibiotic therapy for these two different groups of bacteria, which often exhibit different sensitivities to antibiotic susceptibility tests.

KEY POINTS

- Urine sediment cytology showed a high diagnostic accuracy in the identification of bacteriuria.
- The agreement between sediment cytology and the bacteriological examination was good.
- The greatest discordance between urine sediment bacteriology and cytology was found with mixed infections.

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