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Evaluation of different sampling methods and criteria for diagnosing canine urinary tract infection by quantitative bacterial culture

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ABSTRACT

The use of voided urine specimens for bacteriological culture in dogs is discouraged because contamination from external genitalia could lead to misinterpretation of laboratory results. Quantitative culturing and defining significant bacteriuria could increase the usefulness of voided specimens. However, limited evidence exists for the cut-offs currently recommended. The aim of this study was to evaluate the accuracy of current veterinary cut-off values for significant bacteriuria in voided canine urine. A secondary aim was to investigate if accuracy improved when applying qualitative criteria used in humans. Paired urine specimens were collected by both cystocentesis and voiding, and quantitative bacteriological cultures were performed within the same day. Cystocentesis was used as the reference standard with a cut-off for significant bacteriuria of ≥1000 colony forming units (CFU)/mL. Voided specimens were compared to cystocentesis using: (1) the veterinary cut-off of ≥100,000 CFU/mL; and (2) various cut-offs depending on qualitative criteria (sex, clinical signs and complicating factors), adapted from human guidelines.

Ninety-four dogs with suspected urinary tract infection (UTI) were included for analysis. The veterinary cut-off yielded an accuracy of 94% with a sensitivity and specificity of 94% (95% confidence intervals [CI] 0.81, 0.99) and 94% (95% CI 0.86, 0.98), respectively. Applying the human guidelines did not improve overall accuracy (89%), and yielded a sensitivity and specificity of 97% (95% CI 0.86, 1.00) and 86% (95% CI 0.77, 0.92), respectively. The veterinary cut-off value of ≥100,000 CFU/mL for voided urine is appropriate for determining significant bacteriuria in the majority of dogs with suspected UTI if specimens are refrigerated and cultured on the day of collection.

Introduction

Urinary tract infection (UTI) is commonly diagnosed in dogs (Weese et al., 2011), and the diagnostic process poses several challenges. Clinical signs of UTI are not pathognomonic, and although urinalysis and microscopic examination increase the index of suspicion, quantitative bacteriological culture remains the reference standard for confirming bacteriuria (Bartges, 2004; Weese et al., 2011; Smee et al., 2013). Several pre-analytical and analytical factors could affect bacterial counts obtained by culture, e.g. storage and transportation of the urine specimen, laboratory procedures, and urine sampling method (Winkenwerder et al., 2004; Weese et al., 2011). This has led to the concept and definition of 'significant bacteriuria' to imply clinically relevant bacterial counts, applied in both veterinary and human medicine (Kass, 1957; Bush, 1977; Kivisto et al., 1977; Weese et al., 2011). In dogs, a cut-off value for significant bacteriuria in voided specimens of ≥100,000 colony forming units (CFU)/mL, independent of other characteristics, has been proposed on several occasions (Bush, 1978; Carter et al., 1978; Bartges, 2004; Weese et al., 2011). Nevertheless, the use of voided specimens for bacteriological culture in dogs is generally discouraged due to the risk of contamination, and according to the guidelines on the treatment of UTI proposed by the International Society for Companion Animal Infectious Diseases (ISCAID), voided specimens should be regarded as non-diagnostic (Weese et al., 2011). Cystocentesis, the recommended sampling method in dogs, is a simple but invasive procedure with a potential risk of complications. It requires trained staff and cooperative animals and might not be possible in all clinical situations.

The published evidence concerning the use of voided urine specimens compared to specimens obtained by cystocentesis in companion animals is relatively sparse. A few studies from the 1970s and 1980s examined small numbers of mainly healthy dogs (Finco and Kern, 1977; Carter et al., 1978; Comer and Ling, 1981; Thomsen et al., 1986), and their results were contradictory.

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In humans, voided specimens are widely used, and more complex quantitative cut-off values for significant bacteriuria have been defined in some guidelines, depending on age and gender, as well as the presence or absence of clinical signs or complicating conditions (Grabe et al., 2014).

The primary aim of this study was to investigate the accuracy of the current veterinary cut-off value of ≥100,000 CFU/mL in voided urine as an indicator of significant bacteriuria in dogs with suspected UTI. A secondary aim was to investigate whether the accuracy of urine culture analysis could be improved by adapting criteria for significant bacteriuria currently recommended in human medicine in Europe.

Materials and methods

Dogs

Dogs of any age, sex and breed presented to the University Hospital for Companion Animals, University of Copenhagen, with suspected UTI were prospectively enrolled. To enable easy application of human interpretive criteria, inclusion criteria were as follows: (1) clinical signs of lower urinary tract disease (dysuria, pollakiuria, stranguria, haematuria and/or malodorous urine); or (2) no clinical signs consistent with lower urinary tract disease, but conditions predisposing to or associated with UTI (history of recurrent/relapsing UTI, diabetes mellitus, hyperadrenocorticism, renal disease, urolithiasis, bladder tumours, micturition disorders, immunosuppression, polyuria, incontinence, or low urinary specific gravity). The latter group is hereafter referred to as 'dogs without clinical signs'. Specimens were excluded from the study if the dog had been treated with antimicrobials within 48 h prior to the consultation. This time limit was chosen based on elimination half-lives of the most commonly used antimicrobials in Denmark, as specified in the prescribing information provided by the manufacturer. Additionally, dogs were excluded if the owner declined to participate, or if only one urine specimen could be collected. Dogs were only allowed to participate in the study once.

Study design

The study was designed as a prospective observational study and was approved by the Ethical Administrative Committee at the Department of Veterinary Clinical and Animal Sciences before initiation (1 December, 2013; Approval number 2013-3).

Urine collection and handling

Paired urine specimens were collected from all dogs, first by antepubic cystocentesis (Scott et al., 1974) and then by voiding into a sterile container (Uripet, Rocket Medical) during a walk. External genitalia were inspected, visible dirt or hair was removed and the prepuce or vulva was flushed with sterile isotonic saline prior to collection of voided free catch specimens. Specimens were stored at 5 °C, and cultured within 24 h. Complete urinalysis was performed as part of the normal diagnostic workup for all cystocentesis specimens, and the sediment was stained with Hemacolor (Merck KGaA) for microscopic evaluation.

Culture and susceptibility testing

All urine specimens were analysed at the diagnostic laboratory Sund Vet Diagnostik, Department of Veterinary Disease Biology, University of Copenhagen. Specimens were cultured quantitatively on 5% calf blood agar plates by inoculating 1 μL and 10 μL of urine on each half of the plates, respectively. After overnight incubation at 37 °C, the number of colonies was counted on both halves of each plate, and the CFU per millilitre of urine was calculated as a weighted mean (Niemelä, 1983). If swarming by *Proteus* spp. made colony counting impossible, colony count was registered as ≥100,000 CFU/mL. All colony types were identified to the species level by matrix-assisted laser desorption/ionisation time of flight (MALDI-TOF) mass spectrometry (Vitek MS RUO, BioMérieux). Antimicrobial susceptibility was tested for all isolates obtained by cystocentesis and for a subset of isolates from voided urine using the broth microdilution method (Sensititre COMPAN1F, TREK Diagnostic System) according to the Clinical and Laboratory Standards Institute (CLSI, 2008).

Definition and classification of dogs with significant bacteriuria

Cystocentesis specimens were used as the reference standard, and a cut-off of ≥1000 CFU/mL was used to define significant bacteriuria. Voided specimens were evaluated for significant bacteriuria using: (1) the current veterinary cut-off of ≥100,000 CFU/mL; and (2) a set of criteria adapted from the human European Association of Urology (EAU) guidelines as shown in Table 1 (Weese et al., 2011; Grabe et al., 2014). In the voided specimens, contaminating growth was defined as growth of species that were not found in the corresponding cystocentesis specimen.

Table 1
Veterinary canine and adapted human medical criteria for interpretation of bacterial counts in voided urine specimens applied for different subgroups.

Veterinary ISCAID guidelines[a]		Human EAU guidelines[b]	
UTI group[c]	Criteria (CFU/mL)	UTI group	Criteria (CFU/mL)
		Symptomatic	
Uncomplicated	$\geq 10^5$	Uncomplicated	$\geq 10^3$
Complicated	$\geq 10^5$	Complicated[d]	$\geq 10^4$ (male) $\geq 10^5$ (female)
Subclinical	$\geq 10^5$	Asymptomatic Uncomplicated Complicated	$\geq 10^3$ (male) $\geq 10^5$ (female) $\geq 10^5$ of same species in two cultures minimum 24 h apart[d].

[a] Weese et al., 2011.
[b] Grabe et al., 2014.
[c] Defined as described in Materials and methods.
[d] In this study, urine was only sampled at one time point.
CFU/mL, colony forming units per millilitre; EAU, European Association of Urology; ISCAID, International Society for Companion Animal Infectious Diseases; UTI, urinary tract infection.

Dogs with significant bacteriuria on their cystocentesis specimen were classified as either: (1) uncomplicated bacterial cystitis cases when they presented with clinical signs of lower urinary tract disease but had no predisposing conditions; (2) complicated bacterial cystitis cases when they presented with clinical signs of lower urinary tract disease and had one or more predisposing condition(s); or (3) cases of subclinical bacteriuria when they presented without clinical signs of lower urinary tract disease (Weese et al., 2016).

Statistical analysis

For descriptive statistics, quantitative variables were presented as mean ± standard deviation (SD). Qualitative data were presented as total numbers and proportions. Sensitivity, specificity, positive and negative predictive values and accuracy of different criteria for diagnosing significant bacteriuria on voided urine specimens were calculated using cystocentesis as the reference standard. An alpha level of 0.05 was used for statistical significance, and calculations of one-sided 95% exact binomial confidence intervals were used. Statistical analyses were performed using SAS Enterprise Guide 9.4 software (SAS).

Results

Population

One hundred and two dogs were enrolled between 1 December 2013 and 17 August 2015. Eight of these dogs were excluded from the analysis, as they did not fulfil the inclusion criteria ($n = 5$), urine was not cultured within 24 h ($n = 1$) or because of prior participation ($n = 2$, second enrolment excluded). Of the 94 included dogs, 27 (29%) were intact females, 25 (27%) were spayed females, 29 (31%) were intact males, 12 (13%) were castrated males and sex was undetermined in one dog (1%). The study population represented 47 different breeds. The most commonly represented breeds were mixed-breed, Labrador retrievers, dachshunds and pugs. The majority of dogs (66%) had clinical signs of lower urinary tract disease. Demographic data and inclusion details are shown in Table 2.

Despite a time limit of 24 h, all urine specimens were cultured within 4 h. Quantitative results of paired cultures are shown in Table 3. Thirty-one dogs (33%) had significant bacteriuria on their cystocentesis specimens and were classified as uncomplicated bacterial cystitis cases (48%), complicated bacterial cystitis cases (29%) and cases of subclinical bacteriuria (23%), according to the criteria outlined in the Materials and methods and Fig. 1. Most cases of significant bacteriuria had bacterial counts at or above 100,000 CFU/mL, regardless of collection method, and only 2/31 dogs (6.4%) had counts between 1000 and 10,000 CFU/mL in the cystocentesis specimens. These two dogs also had counts between 1000 and 10,000 CFU/mL in their voided specimens.