Title

Prospective comparison of prostatic aspirate culture and cystocentesis urine culture for detection of bacterial infection in dogs with prostatic neoplasia

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Prospective comparison of prostatic aspirate culture and cystocentesis urine culture for detection of bacterial infection in dogs with prostatic neoplasia

Structured Summary

Objective: The purpose of this study was to determine whether prostatic aspirate culture is a superior method to detect infection compared to culture of urine collected by cystocentesis in dogs with prostatic neoplasia.

Methods: A prospective study was conducted and dogs with suspected or confirmed prostatic neoplasia were enrolled. Urinalysis was done and culture and antimicrobial susceptibility testing was performed on paired urine and prostatic aspirate samples collected at a single timepoint.

Results: Ten dogs with prostatic neoplasia were enrolled. All dogs had one or more clinical sign consistent with lower urinary tract disease. One dog (10%) had a positive urine culture, but negative prostatic aspirate culture, one dog (10%) had a positive prostatic aspirate culture, but negative urine culture, and one dog (10%) had both positive urine and prostatic aspirate cultures. Using prostatic aspirate culture as the reference standard, urine culture had a sensitivity for detecting infection of 87.5%; 95% CI [52.9-99.4] and specificity of 50%; 95% CI [2.6-97.4] in this population of dogs.

Clinical significance: Positive cultures were uncommon with both culture collection methods. Study results did not identify prostatic aspirate culture to be a more sensitive method of detecting prostatic infection than urine culture collected by cystocentesis in these dogs with prostatic neoplasia.

Introduction

Previous studies have indicated that urinary tract infections (UTI) or bacteriuria are common in dogs with urinary tract neoplasia (Budreckis *et al*. 2015, Wood *et al*. 2020). In a retrospective study of dogs undergoing treatment for transitional cell carcinoma, 55% of dogs had at least one positive urine culture over time (Budreckis *et al*. 2015). In that study, females were significantly more likely to have at least one positive culture than males (80% vs 29%). These results suggest that males are less predisposed to UTI or bacteriuria than females when diagnosed with urinary tract cancer. This aligns with results of previous research in the general canine population where female dogs are significantly more likely to have UTI or asymptomatic bacteriuria than male dogs, suggesting that anatomical differences influence bacteriuria risk (Wong *et al*. 2015, Byron 2019). When focusing on available data from male dogs with urinary tract neoplasia, it is interesting to note that a majority (71%) had prostatic involvement of their tumours and only 25% of male dogs with prostatic tumours had bacterial growth identified on urine culture (Budreckis *et al*. 2015). It is possible that prostatic tumours might not predispose affected dogs to bacterial infection or bacteriuria to the same degree as other lower urinary tract tumour locations. All culture samples in the previous retrospective study were urine, however, so it is also possible that urine culture results do not accurately reflect infections localized within the prostate.

Because of the structure and location of the prostate, prostatic infections can become walled off and may not shed bacteria into urine (Levy *et al.* 2014). This is known to occur in dogs with prostatitis or prostatic abscesses in which case sample collection directly from the prostate for culture is recommended (Levy *et al.* 2014, Parry 2006). A recent retrospective study of 82 dogs with prostatitis or prostatic abscessation found that results of urine and prostatic culture were discordant in 50% of cases and that, in 71% of those discordant cases, the urine culture was negative and the prostatic culture was positive (Lea *et al.* 2022). It is unknown whether walling off of infection occurs in dogs with prostatic neoplasia and studies have not been conducted to compare different samples for culture in dogs with urinary tract cancer.

The purpose of this study was to determine how results of prostatic aspirate sample cultures correlate with results of urine cultures collected by cystocentesis. Our hypothesis was that culture of prostatic aspirate samples would identify a greater number of prostatic infections compared to culture of urine collected by cystocentesis.

Materials and Methods

Inclusion Criteria

Male dogs with a presumptive or confirmed diagnosis of prostatic neoplasia presenting to the William R. Pritchard Veterinary Medical Teaching Hospital (VMTH) at the University of California, Davis were eligible for enrollment in this prospective study. Diagnoses were considered presumptive if dogs had clinical signs and ultrasonographic findings consistent with prostatic neoplasia, but prostatic sampling had not yet been attempted. Dogs with tumour involvement of the bladder, urethra, or with metastatic disease to other organs were included. Dogs had to be medically stable to be sedated at the judgement of the treating veterinarian. Previous non-steroidal anti-inflammatory drug (NSAID) therapy and/or chemotherapy were allowed, but dogs could not have received antimicrobial therapy for a minimum of 14 days prior to enrollment in the study. Dogs that had previously undergone urinary tract stenting, embolization, or radiation were excluded. Informed owner consent was obtained and the study was Institutional Animal Care and Use Committee (IACUC) approved (University of California, Davis IACUC #19420).

Data collected at the time of study enrollment included signalment, history of antimicrobial drug administration, tumour location based on contemporaneous imaging, diagnosis method, clinical signs at the time of sampling, and previous cancer treatment.

Sample Collection

Dogs were sedated and the ventral abdomen clipped of fur and swabbed with Zephiran (aqueous benzalkonium chloride) antiseptic solution (Spectrum Chemical, Gardena, CA). With ultrasound guidance, a cystocentesis was performed using a 3 ml syringe and 22-gauge needle. Additionally, two prostatic aspirates were performed with ultrasound guidance using a 3 ml syringe and 22-gauge needle. Dogs were monitored immediately after the procedure to assure safe recovery from sedation and sample collection.

Samples were immediately submitted to the central laboratory receiving area. Prostatic aspirate samples were submitted in the syringes with original needles and caps still attached. Urine samples had the needle removed and a sterile syringe cap placed on the syringe after collection. Urine samples were divided in the laboratory for urinalysis and urine culture.

Diagnostic Techniques

Urinalyses were run in the VMTH Laboratory using standard methodology. Cultures were performed at the VMTH Microbiology Laboratory. For urine cultures, 10 μL of urine were inoculated on to 5% sheep blood and MacConkey agars (Hardy Diagnostics, Santa Maria, CA) and incubated at 35°C in 5% CO_2. Colony number(s) were multiplied by 100 to determine the colony forming units/mL of urine tested. For prostatic cultures, aspirates were inoculated on to the same type of agar and incubated similarly. Because aspirated material from prostatic sampling was generally only in the hub of the needle submitted, the needle was removed and 1ml of brain-heart infusion broth (UC Davis Veterinary Medicine Biological Media Services, Davis, CA) was aspirated into the syringe with a fresh needle. The original needle was then replaced, and the aspirate/broth material was expressed on to the agar media and into 1 mL of tryptic soy enrichment broth (Hardy Diagnostics) and incubated as described above. Quantification of prostatic aspirate growth was reported in semi-quantitative terms (ie, very small, small, moderate, or large numbers of bacteria corresponding to growth in the first through fourth streaked quadrant of agar medium). All medium was incubated a total of 5 days before a no growth determination was made. Isolates were identified with conventional microbiological testing and/or matrix-assisted time of flight mass spectrometry (MALDI TOF MS).

Statistical Analysis

A two-stage design was used and a power analysis conducted to determine sample size for each stage (Ivanova 2016). The null hypothesis that the positive rate of urine culture in male dogs is 25% was tested against a one-sided alternative that prostate aspirate culture is superior with an estimated positive rate of 50%. These rate estimates were set based on previously published frequencies of positive urine cultures in male dogs with prostatic neoplasia and in all dogs with urinary tract neoplasia. Additionally, a large difference between testing methods was considered necessary to show a clinically significant improvement in infection detection to warrant the more invasive nature of prostatic aspirate culture compared to cystocentesis. In accordance with the power analysis, planned accrual in stage 1 was 9 dogs. If two or fewer positive prostatic cultures occurred in these nine patients, study closure was planned. If three or more positive prostatic cultures occurred, accrual of 15 additional dogs was planned in stage 2 for a total of 24 dogs. The null hypothesis is rejected if 10 or more positive prostatic cultures were observed in 24 dogs. This design yields a type I error rate of 0.05 and power of 0.8. Results are reported in terms of proportion of positive culture for each collection type and sensitivity and specificity were calculated for urine culture results using prostatic aspirate culture as the reference standard. Calculations were conducted using Graphpad Prism v 9.4.0.

Results

Signalment and Anamnesis

Ten dogs were enrolled in stage 1 of the study. The median age was 10.2 years (range, 6.8-14.7 years). All dogs were castrated males. Two beagles were enrolled and the remainder were various other pure breed dogs. Dogs had a median weight of 13.6 kg (range, 3.3-82 kg). Six dogs had neoplasia isolated to the prostate at the time of study participation, one also had lymph node involvement, one also had urethral involvement, one also had urethral and bladder involvement, and one also had lymph node, lung, kidney, and liver involvement. A diagnosis of carcinoma was confirmed via prostatic aspirate in 9 dogs. The remaining dog had ultrasonographic changes to the prostate consistent with prostatic neoplasia, but the cytologic sample contained only inflammation, necrosis, and hemorrhage.

All dogs had one or more clinical sign consistent with prostatic neoplasia at the time of enrollment. Reported clinical signs included pollakiuria (n=6), stranguria (n=5), hematuria (n=3), urinary incontinence (n=2), tenesmus (n=3), and change in stool shape (n=2). At the time of enrollment, seven dogs were receiving a non-steroidal anti-inflammatory drug (NSAID). One of those dogs had also previously received carboplatin chemotherapy. Three dogs had received no cancer specific treatment. No dogs had received antimicrobial therapy for at least 14 days prior to study enrollment in accordance with inclusion criteria.

Urinalyses

All urine samples were collected via cystocentesis as described in the study methods and all 10 dogs had urinalyses run. Protein was detected in the urine of eight of the 10 dogs. Three dogs had urine protein concentrations of 25 mg/dl, one dog had 75 mg/dl, and four dogs had 150 mg/dl. The urine protein to creatinine ratio was not measured in any dog. Pyuria (> 3 WBC/hpf) was detected in five of 10 dogs. Transitional epithelial cells were seen in all dogs to varying degrees. Bacteria were identified on urinalysis in one dog.

Urine and Prostatic Aspirate Cultures

All dogs had urine cultures conducted and 2/10 (20%) resulted in bacterial growth (Table 1). One urine culture grew *Staphylococcus pseudintermedius*. The other grew a Gram positive, non-sporeforming, catalase positive rod. The genus could not be further determined despite biochemical testing and use of MALDI TOF MS.

Ultrasound guided prostatic aspirate samples were successfully collected in all 10 dogs and there were no procedure-related complications. Sample volume was minimal in 9 dogs, but approximately 0.2 ml of fluid was collected from the prostate of one dog. Of the ten prostatic aspirate cultures, two (20%) resulted in bacterial growth (Table 1). One prostatic culture grew a Gram positive, non-sporeforming, catalase positive rod identical to the organism found on urine culture and described above. One prostatic aspirate yielded *Staphylococcus epidermidis* from the tryptic soy enrichment broth only. This finding was interpreted as most likely a contaminant because it was cultured only in enrichment broth and because this organism is plentiful on skin, but is rarely isolated from the prostate or urinary tract. This dog had a negative urine culture. The dog with a urine sample that grew *S.pseudintermedius* had a negative prostatic aspirate culture. This dog is also the only one in which bacteria were seen on urinalysis.

Statistical Results

For statistical analysis, the case in which *Staphylococcus epidermidis* was cultured from the prostatic aspirate sample was considered to be a positive culture. Using prostatic culture as the reference standard, urine culture had a sensitivity for detecting infection of 87.5% ; 95% CI [52.9-99.4] and specificity of 50%; 95% CI [2.6-97.4] in this population of dogs. Because only 2 positive prostatic cultures occurred out of 10 dogs enrolled in stage 1 of the study, the null hypothesis that prostatic aspirate culture is not more likely to identify prostatic infections than urine culture was confirmed and stage 2 was not conducted.

Discussion

The results of this study did not identify prostatic aspirate culture to be a more sensitive method of detecting prostatic infection when compared to urine culture collected via cystocentesis in these dogs with prostatic neoplasia. It was hypothesized that culture of prostatic aspirate material could help detect localized infections within the prostate and that a significant proportion of dogs would have an infection detected in the prostate sample, but not in urine collected via cystocentesis. There were no dogs in this study that had an infection identified by the prostatic aspirate culture, but not the urine culture. The rate of positive urine culture in this study (20%) is similar to the previous retrospective study that found only 25% of male dogs with prostatic neoplasia had positive urine culture (Budreckis *et al*. 2015). Two dogs (20%) had positive prostatic cultures, but one was clinically suspected to be a contaminant and the other dog's urine culture grew the same bacterial organism. In this group of dogs, prostatic aspirate culture did not appear to provide added diagnostic benefit.

The low rate of positive culture in prostatic aspirate samples in dogs with prostatic neoplasia suggests that undetected infections isolated to the prostate might not be common in this population. Normal prostate glands are known to be relatively resistant to infections due to mucosal defense barriers, high acidity in prostatic fluid, high urethral pressure and natural peristalsis, and mechanical flushing during urination (Parry 2006). These defense mechanisms may be impaired by neoplastic infiltration, but perhaps remain adequately intact to maintain some resistance to infection within the gland. In general, the male urinary tract appears to be more resistant to bacteriuria than the female urinary tract. A large study of urine cultures collected from dogs over a 51-month period at a single academic institution found that 74% of positive cultures were from female dogs (Wong *et al.* 2015). Although most of those dogs did not have urinary tract neoplasia, the data presented here supports a similarly low rate of positive urine cultures in male dogs with urinary tract neoplasia.

In this study, prostatic fine needle aspiration was a feasible and safe method of collecting samples for bacterial culture in dogs with prostatic neoplasia. Prostatic aspirate samples were successfully collected from all dogs in this study. Short-term complications of this procedure were not seen, though only a small number of dogs were included and long-term follow-up was not collected. It is possible that less common complications such as seeding of infection or tumour into surrounding tissues could occur in a larger population of dogs or with longer monitoring. Sedation was utilized to assure adequate immobilization and visualization of the prostate and to minimize risks associated with aspiration. Prostatic sampling for this study was also limited to two attempts to limit the possibility of procedure related complications. Prostatic sample handling was different than urine sample handling due to the small sample volume obtained from prostate aspirates. The lab utilized a small volume wash of enrichment broth to assure any sample left within the needle or needle hub were placed onto culture media. This method is used routinely in our laboratory for cultures of other small volume samples, but it is possible that study results could have been affected by this technique. Further research is necessary to determine whether alternative sample collection or handling methods could increase the sensitivity of detection of prostatic bacterial infections in dogs with prostatic neoplasia.

This study was powered to detect clinically meaningful results where prostatic aspirate culture is significantly superior to urine culture to warrant the increased cost, collection time, and risks associated with prostatic aspiration compared to cystocentesis. The positive urine culture rate of 20% was the same as the positive prostatic aspirate culture rate when the suspected contaminant is included and would have been higher if the contaminant were excluded. If culture of prostatic aspirate samples had been able to detect infection in 50% of dogs as estimated for power analysis, five dogs from the first cohort would have been expected to have positive prostatic aspirate cultures. This number would have warranted cohort expansion and, potentially, consideration of routine use of this collection method in dogs with prostatic neoplasia in clinical practice. Even when the suspected contaminant is included in the frequency of detected prostatic infections, cohort expansion would not have been triggered based on the planned two-stage study criteria. This power analysis method was designed to minimize enrollment of dogs if further enrollment was unlikely to alter results, but the small sample size could have resulted in an underestimation of the utility of prostatic aspirate culture in this population. Further, it is important to note that this study was not adequately powered to detect a small increase in infection frequency detected with prostatic aspirate culture and it is possible that a more minor benefit of the collection method could have been missed. Additional studies could be considered to confirm the results presented here.

Although a strength of this study is its prospective methodology, there are weaknesses that must be recognized. This study evaluated the utility of fine needle aspiration of the prostate to detect infection in dogs with neoplasia, but other techniques such as prostatic biopsy, massage and prostatic wash have been described to collect samples for culture. These techniques might be superior to prostatic aspirate culture for detecting occult infection in dogs with prostatic neoplasia and should be further investigated. Culture of urine collected by cystocentesis was utilized as the comparison group in this study because cystocentesis is the most common collection method utilized in our hospital and represented the majority of samples in the previous study by Budreckis *et al*. (2015). Though numbers of voided urine samples were relatively small in that study, culture of voided urine was more likely to be positive. This may be significant in dogs with prostatic neoplasia because urine collected from the bladder might not have sufficient exposure to prostatic tissue to result in a positive culture. Further research comparing various urine and prostatic sample collection techniques could be helpful to address unanswered questions about infection risk in dogs with prostatic neoplasia.

Another limitation of this study is in differentiating UTI from subclinical bacteriuria in this population of dogs. Bacteria may be found in urine of dogs without lower urinary signs and does not always indicate UTI. Guidelines published by the International Society for Companion Animal Infectious Diseases (ISCAID) recommend that a diagnosis of UTI be made by the presence of lower urinary tract signs in conjunction with laboratory evidence of infection through urinalysis and bacterial culture (Weese. *et al.* 2019). All of the dogs enrolled in this study had lower urinary signs, which is expected in dogs with prostatic neoplasia. It is difficult to determine within the confines of this study to what degree these signs were attributable to UTI or the neoplasia itself, but the low number of positive cultures suggests that neoplasia is a significant cause of clinical signs in these dogs. Further observation of antimicrobial treatments provided and changes in lower urinary signs over time might be helpful to assess how much UTI may contribute to clinical signs in dogs with concurrent prostatic neoplasia and bacteriuria.

In summary, the results of this study suggest that prostatic aspirate culture, while feasible, does not appear to be a more sensitive method for detection of infection than culture of urine collected by cystocentesis in dogs with prostatic neoplasia. Although this culture method might be considered in specific cases where there is clinical evidence of a walled-off prostatic infection, routine culture of prostatic aspirate samples is not likely to identify more infections than urine culture in most dogs with prostatic neoplasia.

Conflict of Interest: The authors declare no conflicts of interest.

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Table 1. Results of urine and prostatic aspirate cultures in 10 dogs with prostatic neoplasia. (* indicates suspected contaminant)

Case #	**Urine Culture**	**Prostate Culture**
1	No growth	No growth
2	No growth	No growth
3	No growth	No growth
4	*Staph. pseudintermedius*	No Growth
5	Gram positive rod	Gram positive rod
6	No growth	No growth
7	No growth	No growth
8	No growth	No growth
9	No growth	No growth
10	No growth	*Staph. epidermidis**