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Vaginal Microbiota of Spayed Dogs with or without Recurrent Urinary Tract Infections

R.G. Hutchins, S.L. Vaden, M.E. Jacob, T.L. Harris, K.D. Bowles, M.W. Wood, and C.S. Bailey

Background: Limited information is available regarding the vaginal microbiota of normal spayed dogs and spayed dogs with recurrent UTIs. Vaginal lactic acid-producing bacteria (LAB) have been associated with decreased frequency of recurrent urinary tract infection in women and may have a protective role within the urinary tract of female dogs.

Hypothesis/Objectives: Spayed dogs with historical recurrent UTI will have decreased prevalence of LAB and increased prevalence of uropathogenic bacterial populations in the vaginal microbiota when compared with the vaginal microbiota of healthy, spayed dogs.

Animals: Twenty-one client-owned adult spayed female dogs with historical recurrent UTI and 23 healthy, spayed female dogs without a history of recurrent UTI.

Methods: Dogs were placed into a recurrent UTI group or control group in this prospective study. Bacterial populations were isolated and characterized from vaginal swabs obtained from each dog.

Results: The most common bacterial isolates obtained from the vaginal tract of all dogs were *Escherichia coli* (11/44) and *S. pseudintermedius* (13/44). *E. coli* was isolated from the vaginal tract of 8 of 21 (38%) dogs in the rUTI group and 3 of 23 (13%) dogs in the control group ($P = .08$). LAB were isolated from 7 of the 44 dogs. Two of these 7 dogs were in the rUTI group and 5 of the 7 dogs were in the control group.

Conclusions and Clinical Importance: The vaginal microbiota of spayed female dogs with recurrent UTI was similar to the control population of normal, spayed female dogs.

Key words: Lactic acid-producing bacteria; Recurrent urinary tract infection.

Recurrent urinary tract infections (UTI) are a substantial source of morbidity in affected dogs and a major source of frustration and cost to owners. Although most UTIs in dogs occur as single episodes, recurrent UTIs affect approximately 3 of every 1,000 canine veterinary patients.[1–3] Often the development of recurrent infections can be linked to alterations in urine characteristics or abnormalities of urogenital anatomy, but in approximately 29% of the patient population an inciting cause is never identified, leading to repeated infections.[4,5]

Recently, an association between altered vaginal microbiota and development of recurrent UTI has been identified in women.[6,7] Women with recurrent UTIs are more likely to have vaginal microbiota predominated by uropathogenic bacterial populations, including *Escherichia coli*, *Klebsiella*, *Staphylococcus*, *Streptococcus*, and *Proteus* species, in conjunction with depletion of the normally predominant *Lactobacillus* species, especially at the time of UTI recurrence.[8–10] It is hypothesized that lactic acid-producing bacteria (LAB), including *Lactobacillus* and *Enterococcus* species, are essential in maintenance of urogenital health because these bacteria inhibit other microbial growth by a variety of mechanisms.[8]

Abbreviations:

CBA	Columbia blood agar
LAB	lactic acid-producing bacteria
MAC	MacConkey agar
MRS	de Man Rogosa Sharpe
UTI	urinary tract infection

Information regarding the vaginal microbiota of spayed dogs is limited and there are no published studies describing the vaginal microbiota of dogs prone to recurrent UTI. In 1 study that did quantify vaginal LAB in healthy and clinically ill dogs, LAB were detected in 41 of 42 samples.[11] These bacterial strains demonstrated inhibition of growth against common canine uropathogenic bacteria, suggesting that vaginal LAB also may have a protective role in the urinary tract of female dogs.[11]

The purpose of this study was to characterize the vaginal microbiota of spayed dogs with recurrent UTI and to compare the vaginal microbiota of these dogs with the vaginal microbiota of healthy, spayed dogs with no history of recurrent UTI. We hypothesized that spayed dogs with historical recurrent UTI would have a decreased prevalence of LAB and an increased prevalence of uropathogenic bacterial vaginal populations when compared to the vaginal microbiota of healthy, spayed dogs. Confirmation of this hypothesis would identify a new risk factor for recurrent UTI that may be targeted medically.

Materials and Methods

Case Selection Criteria

Twenty-three client-owned spayed female dogs >6 months of age admitted to the North Carolina State University Veterinary

From the Veterinary Specialty Hospital of the Carolinas, Raleigh, NC (Hutchins); the Department of Clinical Sciences (Vaden, Harris, Bowles, Wood, Bailey); and the Department of Population Health Pathobiology (Jacob), College of Veterinary Medicine, North Carolina State University, Raleigh, NC.

This work was performed at the College of Veterinary Medicine, North Carolina State University, Raleigh, NC.

Corresponding author: C.S. Bailey, Department of Clinical Sciences, North Carolina State University, 1600 William Moore Dr., Raleigh, NC 27607; e-mail: scott_bailey@ncsu.edu.

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Health Complex with a history of recurrent UTI were recruited for inclusion in this prospective study. Twenty-three spayed female dogs >6 months of age without a history of recurrent UTI were recruited from employees and students at the North Carolina State University Veterinary Health Complex for inclusion in the control group. Recurrent UTI was defined as >2 episodes of UTI in a 6-month period or >3 episodes of UTI in a 1-year period. Dogs were excluded from the study if they currently were receiving antibiotics, probiotic supplements, or cranberry supplements, or had received any of these within 2 weeks of enrollment. The study protocol was evaluated and approved by the North Carolina State University College of Veterinary Medicine Animal Care and Use Committee (IACUC), and owners of all dogs signed an informed consent form before enrollment of their pet in the study.

Recurrent UTI Patient Medical History Review

The medical record of each dog with recurrent UTI included in this study was retrospectively reviewed. Pertinent information regarding patient signalment, frequency of documented UTI, the causative organisms isolated, abnormal urogenital physical examination findings, and conditions identified that are recognized risk factors for recurrent UTIs were extracted and summarized. Urine culture results available from rUTI dogs within 1 week of obtaining vaginal samples were compared to vaginal flora. Urine samples for culture were not obtained from the control group.

Sample Collection

A vaginal sample was obtained from the cranial aspect of the vagina of each dog. Samples were collected with a sterile, double-guarded culturette[a] (Fig 1) to minimize contamination from other areas of the genitourinary tract. The outer guard of the vaginal swab was positioned beyond the urethral orifice and anterior to the vestibulovaginal junction in the vaginal vault. The inner guard and culturette were advanced through the sealed outer guard. The sterile swab was advanced beyond the inner guard as far cranially as possible with gentle pressure and rotated several times. Both the inner guard and swab were retrieved while the outer guard was held in place. The entire culturette was removed, with the sterile swab contained within the inner and outer guard, eliminating exposure to other areas of the genitourinary tract.[12]

Identification of Vaginal Bacterial Populations

The isolation of vaginal bacteria was conducted at the North Carolina State University Clinical Microbiology Laboratory. Vaginal swabs were plated onto Columbia blood agar (CBA) and MacConkey agar (MAC) plates[b] for the isolation of aerobic bacteria. Plates were incubated for a minimum of 48 hours at 35°C under 5% CO_2. A pure culture of each isolate was obtained and identified biochemically using commercially available panels,[c,d] for gram positive and negative organisms. Swabs also were inoculated onto 2 Rogosa agar plates.[b] These plates were incubated for a minimum of 48 hours at 35°C under both microaerophilic and anaerobic conditions for the isolation of LAB. Any morphologically distinct colony was isolated and gram stained; gram-positive organisms were saved as presumptive LAB. Finally, swabs were enriched in thioglycollate broth[b] and incubated at 35°C. If no colonies were obtained on the original CBA, MAC, or Rogosa plates after 48 hours, the enrichment was inoculated onto all 3 agar plates and again incubated for a minimum of 48 hours at 35°C. Plates were evaluated as described above. All isolates were frozen in glycerol at −80°C for further analysis. After completion of sample collection, all frozen presumptive LAB isolates (gram-positive rods obtained on Rogosa agar) were submitted for 16s rDNA sequencing at the National Veterinary Services Laboratory.[e]

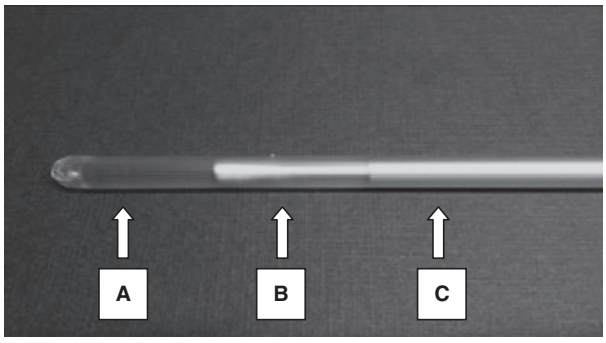

Fig 1. Picture of a canine sterile, double-guarded culturette used in this study to obtain vaginal samples from the cranial vaginal vault. **A**. Outer guard; **B**. sterile swab; **C**. sterile inner guard.

Statistical Analysis

A priori statistics were performed using the program G-Power 3.1.[13,14] For the purposes of this statistical analysis, a difference of 25% in the incidence of a bacterium was assumed to have clinical relevance. Using a previously published incidence of *Lactobacillus* spp. as a baseline (59%),[11] a priori statistics with $\alpha = 0.05$ and power = 0.95 indicated the need for 42 animals (21/group). The number of organisms isolated from vaginal samples was compared between groups using a Wilcoxon Rank Sum test because of a non-normal distribution pattern. The prevalence of specific organisms was compared between groups using Fisher's Exact test.

Results

Dogs

A total of 46 dogs were evaluated for entry in this study. Two dogs were excluded after enrollment because further review of the dogs' medical records identified an inadequate frequency of UTI in the year before being included in the study. Of the 44 remaining dogs included in the study, 21 dogs had a history of recurrent UTI (rUTI group). The remaining 23 dogs did not have a history of recurrent UTI (control group) and were included as a control population. Dogs in the rUTI group had a median age of 8 years (range, 1–14 years), whereas dogs in the control group had a median age of 5.5 years (range, 1–11.5 years). Breeds represented in the rUTI group included 3 mixed breeds, 2 Bichon Frise, 2 Golden Retrievers, and 1 each of American Staffordshire Terrier, Beagle, Boston Terrier, Cairn Terrier, Dachshund, Dalmation, English Bulldog, Greyhound, Mastiff, Labrador Retriever, Portuguese Water Dog, Rat Terrier, Rottweiler, and Standard Poodle. Breeds represented in the control group included 14 mixed breeds, 4 Labrador Retrievers, 2 German Shorthair Pointers, and 1 each of American Cocker Spaniel, American Staffordshire Terrier, and Catahoula Leopard Dog.

Recurrent UTI Patient Medical History Review

All dogs included in the rUTI group had ≥3 episodes of UTI during the 12-month period or ≥2 episodes of UTI during the 6-month period before enrollment in this study. Review of the medical records of dogs with recurrent UTI identified 25 occurrences of underlying disorders that could have contributed to recurrent UTI in 15 of 21 dogs (Table 1). Of the 25 occurrences, 9 different disorders were identified. Seven of 15 dogs had a single disorder identified, whereas 8 of 15 dogs had ≥2 disorders identified. Episioplasty had been performed in 4 of the 21 dogs in the rUTI group, resulting in normal vulvar configuration. All of these dogs, however, had persistent rUTI despite episioplasty. Urinary incontinence was reported in 3 of the 4 dogs and the remaining dog had no underlying disorder identified as a possible cause for recurrent UTI.

The median number of UTIs documented in the lifetime of the dogs in rUTI group was 3 (range, 2–15), with a median of 3 UTIs documented in the year before inclusion in this study (range, 2–8). The most common bacteria historically isolated from the urine of the dogs in the rUTI group was *E. coli*, which was identified in 15 of the 21 dogs. Other historical isolates from dogs in the rUTI group included *Enterococcus* (12/21), *Proteus* (7/21), *Staphylococcus* (4/21), *Streptococcus* (3/21), *Corynebacterium* (1/21), *Enterobacter* (1/21), *Klebsiella* (1/21), and *Pseudomonas* (1/21). The most common antimicrobial used for treatment of historical UTI was amoxicillin-clavulanic acid (15/21). Other antimicrobials historically used for treatment of UTI in the rUTI group include fluoroquinolones (11/21), cephalosporins (8/21), amoxicillin (6/21), trimethoprim sulfamethoxazole (3/21), and clindamycin (2/21). Urine culture results were available within 1 week of collection of vaginal swabs obtained for this study in 13 of 21 dogs in the rUTI group. Of the 13 samples, 7 (53%) had no growth. Urinary organisms isolated within 1 week of obtaining vaginal samples included *E. coli* (3/13), *Enterococcus faecium* (2/13), and *Enterococcus faecalis* (1/13).

Table 1. Distribution of disorders (n = 25) identified in 15 dogs with recurrent UTI that may have contributed to the development of recurrent UTI, divided by class.

Class of Disorder	Specific Condition	No. of Dogs Affected
Abnormal micturition (n = 8)	Incontinence, undetermined cause	8
Abnormal anatomy (n = 6)	Recessed vulva	5
	Persistent ligament	1
Alteration of urine composition (n = 5)	Chronic kidney disease	3
	Hypoadrenocorticism	2
Alteration of urothelium (n = 3)	Urolithiasis	2
	Transitional cell carcinoma	1
Impaired immunity (n = 3)	Chemotherapy	1
	Cyclosporine	2

Vaginal Bacterial Populations

Aerobic bacteria were isolated from 19 of 21 (90.5%; 95% CI, 0.68–0.93) dogs in the rUTI group and 18 of 23 (78.3%; 95% CI, 0.56–0.92) dogs in the control group ($P = .4$). Mixed bacterial populations were common in both groups, with a range of 0–4 (median, 2.0; 95% CI, 0.99–1.96) organisms isolated in dogs in the rUTI group and 0–3 organisms (median, 2.0; 95% CI, 1.3–2.5) isolated in dogs in the control group ($P = .28$). Two or more bacterial species were isolated from 11 of 21 dogs in the rUTI group and 11 of 23 dogs in the control group. The most common bacterial isolates obtained from the vaginal tract of all dogs were *E. coli* (11/44) and *S. pseudintermedius* (13/44; Table 2). *Escherichia coli* was isolated from the vaginal tract of 8 of 21 (38%; 95% CI, 0.19–0.61) dogs in the rUTI group and 3 of 23 (13%; 95% CI, 0.03–0.35) dogs in the control group. Of the 8 dogs in the rUTI group with *E. coli* isolated from the vaginal tract, 1 had pure growth of *E. coli* in historical urine cultures, whereas 4 dogs had various organisms isolated in historical urine cultures including *E. coli*, and the remaining 3 dogs did not have *E. coli* isolated in historical urine cultures. *Staphylococcus pseudintermedius* was isolated from the vaginal tract of 7 of 21 (33%; 95% CI, 0.15–0.57) dogs in the rUTI group and 6 of 23 (26%; 95% CI, 0.11–0.48) dogs in the control group ($P = .74$). Various *Enterococcus* species, including *E. faecalis* (7), *E. durans* (4), *E. avium* (3), and *E. gallinarum* (1) were obtained from the vaginal tract of all dogs. These *Enterococcus* species were isolated from the vaginal tract as single or multiple isolates from 8 of 21 dogs in the rUTI group and 4 of 23 dogs in the control group. Additional aerobic bacteria isolated with a low incidence included *Pasteurella canis, Pseudomonas aeruginosa, Proteus mirabilis, Sphingomonas paucimobilis, Streptococcus* spp., *Corynebacterium* spp., *Bacillus* spp., and *Enterobacter* spp.

Five of 13 dogs in the rUTI group for which urine culture results were available had isolates of either *E. coli* or *Enterococcus* spp. in both urine and vaginal

Table 2. Types of bacteria isolated from the vaginal tract of dogs with recurrent UTI and normal dogs.

	rUTI (n = 21)	Control (n = 23)
LAB *(Enterococcus canintestini)*	2	5
Staphylococcus pseudintermedius	7	6
Group G *Streptococcus*	3	6
Enterococcus spp.	11	4
E. coli	8	3
Pseudomonas aeruginosa	2	2
Pasteurella canis	0	1
Proteus mirabilis	4	1
Sphingomonas paucimobilis	0	1
Bacillus spp.	0	2
Corynebactierium spp.	2	1
Enterobacter spp.	2	2
Total organisms	40	35
Average organisms/animal	1.9	1.5

samples. Of these, 3 had identical cultures, 1 each for *E. coli, Enterococcus faecium*, and *Enterococcus faecalis*, whereas 2 dogs had pure growth of *E. coli* in urine and a mixed flora, including *E. coli,* in the vagina. Of the 7 dogs from the rUTI group with *E. coli* cultured from the vaginal sample for which concurrent urine culture was available, 3 had no growth on urine culture and 1 had *Enterococcus faecium* on urine culture (positive predictive value within the rUTI group, 0.43).

Lactic acid-producing bacteria were isolated on Rogosa agar from 7 of the 44 dogs. Two of these 7 dogs were in the rUTI group (2/21, 9.5%; 95% CI, 0.02–0.32) and 5 of the 7 dogs were in the control group (5/23, 21.7%; 95% CI, 0.08–0.44). There was no significant difference in the frequency of vaginal LAB populations between these 2 groups of dogs ($P = .42$). Lactic acid-producing bacteria isolated from the vaginal tract of these 7 dogs by LAB selective enrichment were identified as *Enterococcus canintestini* by sequencing.

Discussion

This study is the first to describe the vaginal microbiota of healthy spayed dogs and spayed dogs with rUTI. The results of this study suggest that there is no significant difference in the vaginal microbiota of healthy spayed dogs and those with recurrent UTI. *Escherichia coli* and *S. pseudintermedius* were the most prevalent organisms obtained from the vaginal tract of dogs in this study, whereas *Enterococcus canintestini* was the most common LAB isolated. The prevalence of common uropathogens, such as *E. coli* and *S. pseudintermedius*, was similar between the rUTI and control groups, but *E. coli* tended to be more common in patients with rUTI. Additionally, there was no significant difference between the 2 groups in the frequency of LAB isolated from the vaginal tract. LAB was an uncommon isolate in the vaginal vault of dogs in this study.

The most commonly isolated LAB from the vaginal microbiota of the dogs in this study was *Enterococcus canintestini*, whereas *Lactobacillus* species were not isolated. In health, *Lactobacillus* species are the predominant LAB of the vaginal tract of women.[8,15] However, this study demonstrates that this may not be true in dogs. *Lactobacillus* and certain *Enterococcus* species are both gram positive bacteria that are characterized as LAB. Both of these genera of bacteria have potential antimicrobial properties, such as production of lactic acid resulting in an acidic environment, bacteriocin, and hydrogen peroxide, which have been shown to regulate other urogenital microbiota.[16,17] Therefore, the presence of *Enterococcus canintestini* in the vagina of dogs may exert selective pressure on other bacteria as does *Lactobacillus* in the vagina of women.

In contrast to a previous study evaluating the vaginal microbiota in intact bitches, this study found a low incidence of LAB in both groups and a complete absence of *Lactobacillus* species. In a previous study, LAB were identified in 41 of 42 ill and healthy female dogs.[11] In our study, only 7 of 44 spayed female dogs had vaginal colonization by LAB. One possible explanation of this difference is that an unguarded swab was used to obtain cultures in the previous study, allowing for sampling from the vestibule, vulva, and vagina. The use of a guarded swab in our study limited the cultures to only the vagina. The contrasting results may indicate variable bacterial colonization of LAB even within the vestibule, vulva, and vagina of dogs. Alternatively, the aforementioned study was completed in a different geographical region and the contrasting results may be a result of geographical variation in normal canine vaginal microbiota.

In the present study, Rogosa agar supported the growth of *Enterococcus canintestini*, thereby resulting in initial overestimation of presumptive *Lactobacillus* species within the vaginal microbiota of these dogs. However, all isolates on Rogosa agar in this study were identified as *Enterococcus canintestini*. Because *Lactobacillus* and *Enterococcus* species are both gram positive, LAB, and both may grow on Rogosa agar, they may not be easily distinguished by conventional bacteriological analysis.[18] Therefore, the use of more advanced techniques, such as sequencing, is recommended in future studies investigating the prevalence of these bacterial species in the vaginal tract of dogs.

In health, LAB, specifically lactobacilli, are the predominant colonizers of the vaginal tract of women.[8,15] However, women with recurrent UTI often have alterations in vaginal flora, namely increased colonization by uropathogens and depletion of lactobacilli.[9,19] Unlike the vaginal microbiota of healthy women and women with recurrent UTI, the presence of common uropathogens, such as *E. coli* and *Staphylococcus* species, was not significantly different between groups of dogs in the present study. These findings are similar to those of previous studies of intact bitches, which describe the vaginal microbiota of healthy intact female dogs as variable, with normal microbiota characterized by light to moderate growth of a mixed population of bacteria.[20] The heterogeneity of the vaginal microbiota of healthy female dogs may account for the lack of statistical difference between the vaginal bacterial populations isolated in the 2 groups. However, it is also possible that a significant difference in the prevalence of specific uropathogens such as *E. coli* would be identified in a larger study population. Moreover, the present study recorded only the presence or absence of a bacterial strain, and not quantitative absolute colony counts. Thus, it is possible quantification of absolute colony counts may have detected significant differences between the 2 groups. Although research in women suggests that vaginal colonization of uropathogens is correlated with urogenital disease, this has not been demonstrated in the dog to date. Within the rUTI group of the present study, vaginal culture of *E. coli* was not strongly correlated with urine culture results. Additional diagnostic tests, such as typing by high-performance liquid chromatography or identification of virulence factors would provide additional insight into the relative importance of *E. coli* in vaginal microbiota.

Prior antibiotic treatment in the dogs with rUTI potentially could impact resistance profiles of the organisms isolated from both the urine and vaginal samples of the dogs included in this study. Ideally, exclusion of dogs receiving antibiotics up to 2 weeks before inclusion in this study limited the impact of recent antimicrobial treatment on the vaginal microbial populations identified in these dogs. However, antimicrobial resistance remains a major emerging problem in both animals and people, and the pressure to develop resistance is exacerbated by repeated antibiotic use.[21] This suggests an urgent need for the development of effective therapies that do not rely on repeated antibiotic administration.

In conclusion, recurrent UTI in dogs is a well-recognized condition that is often difficult and expensive to manage. Although the vaginal microbiota is recognized to play a key role in the prevention of recurrent UTI in women, no previous studies have investigated this in dogs. As in women at risk for UTI, this study found a low incidence of LAB in spayed dogs, but no significant difference was identified between spayed dogs with rUTI and healthy spayed dogs. In contrast to other studies in both women and intact dogs, *Enterococcus canintestini* was the most common LAB isolated from the vagina in this study. Further investigation of the role of this organism in the urogenital tract, as well as the potential presence of LAB in the vestibule and vagina, is warranted in dogs. Moreover, additional study of uropathogens in the vaginal tract of dogs with recurrent UTI, including quantitative absolute colony counts, prevalence of virulence factors associated with urogenital disease, and resistance to commonly used antibiotics, also is warranted in a larger study population.

Footnotes

[a] 14" Canine Guarded Culture Swab, Reproduction Resources, Walworth, WI
[b] Remel; Thermo Fisher Scientific, Lenexa, KS
[c] Vitek 2; Biomeriux, Durham, NC
[d] Streptex; Remel, Lenexa, KS
[e] National Veterinary Services Laboratory, Ames, IA

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