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Evaluation of the Live Biotherapeutic Product, Asymptomatic Bacteriuria *Escherichia coli* 2-12, in Healthy Dogs and Dogs with Clinical Recurrent UTI

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Background: Antimicrobial resistance is an emerging problem.

Hypothesis/Objective: To investigate the safety and efficacy of a live biotherapeutic product, ASB *E. coli* 2-12 for UTI treatment.

Animals: Six healthy research dogs; nine client-owned dogs with recurrent UTI.

Methods: Prospective noncontrolled clinical trial. For safety data, research dogs were sedated, a urinary catheter was inserted into the bladder; 10^{10} CFU/mL of ASB *E. coli* 2-12 was instilled. Urine was cultured on days 1, 3, and 8 post-instillation and dogs were observed for lower urinary tract signs (LUTS). For client-owned dogs, ASB *E. coli* 2-12 was instilled similarly and urine cultures analyzed on days 1, 7, and 14 days postinstillation.

Results: No LUTS were noted in any of the 6 research dogs after ASB *E. coli* 2-12 infusion. Pulse field gel electrophoresis (PFGE) studies confirmed the bacterial strains isolated matched that ASB *E. coli* 2-12 strain. Four of the nine client-owned dogs had complete or nearly complete clinical cures by day 14. Of these four dogs, 3 also had microbiologic cures at day 14; one of these dogs had subclinical bacteriuria (in addition to ASB *E. coli* 2-12). Three of these four dogs had ASB *E. coli* 2-12 isolated from their urine at day 14. With the exception of mild, temporary, self-limiting, hyporexia in two dogs on the day of biotherapeutic administration, there were no major adverse effects.

Conclusions and Clinical Importance: These results suggest ASB *E. coli* 2-12 is safe and should be investigated in a larger controlled study evaluating clinical UTI in dogs.

Key words: Antimicrobial resistance; Cystitis; Dog; Urinary tract.

Uncomplicated bacterial urinary tract infections (UTI), also known as sporadic bacterial cystitis, are common and occur in approximately 14% of dogs evaluated for medical care.[1] Simple, uncomplicated UTI in small animals generally occur in otherwise healthy animals with no evidence of underlying disease and resolve with proper antimicrobial treatment.[2] In human beings, bacterial UTI is the second most common infectious disease.[3] Recurrent UTI also occurs in dogs and human beings and occasionally are associated with an underlying comorbidity.[4] At a referral hospital, 0.3% of the canine population was diagnosed with recurrent infections, that is, reinfections, persistent, or relapsing infections over a 26-year period.[5] Antimicrobials are prescribed for clinical UTI and antimicrobial resistance has been an emerging problem in both dogs and humans,[6–8] and other treatment modalities are being investigated.

Recently, researchers have investigated administration of asymptomatic bacteriuria (ASB) 83972 into atonic bladders of human patients with chronic bacteriuria secondary to spinal cord injuries as an alternative treatment. Instillation of ASB *E. coli* 83972 improved the patients' quality of life,[9] and none of the patients in this clinical trial developed signs of bacteremia or sepsis. Moreover, in patients where long-term bladder colonization by the bacteria was achieved, no clinical signs of UTI developed. No adverse impact of ASB *E. coli* 83792 on renal function was reported.[10] The mechanisms by which ASB *E. coli* provides protection for recurrent UTI are not fully understood but might be due to immunomodulation or bacterial interference,

Abbreviations:

ASB	asymptomatic bacteriuria
CBC	complete blood count
CC	clinical cure
MC	microbiologic cure
PFGE	pulse field gel electrophoresis
UPEC	uropathogenic *Escherichia coli*
UTI	urinary tract infection

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whereby the ASB strains colonize the bladder and prevent colonization with uropathogenic *E. coli* (UPEC) strains that cause inflammation and result in lower urinary tract signs.

Asymptomatic bacteriuria *E. coli* can significantly reduce bacteriuria in murine UTI models of acute cystitis.[11] Various ASB *E. coli* strains also have antiinfective and visceral analgesic activity when evaluated in these mouse models using UPEC strain NU14 to induce clinical cystitis.[11] Moreover, the strain ASB *E. coli* 2-12 provided the greatest analgesic activity against cystitis pain and was superior to ciprofloxacin when evaluating tactile allodynia of the pelvic region in mice by mechanical stimulation. The superior analgesic properties of ASB *E. coli* 2-12 suggest this strain might be a viable option for management of isolated acute and perhaps recurrent UTI in human beings and dogs. Whereas safety trials evaluating the effects of intravesicular administration of ASB *E. coli* 83972 were performed in two different canine pilot studies,[12,13] neither the safety nor efficacy of ASB *E. coli* 2-12 administration has been evaluated in dogs or humans with acute or recurrent UTI.

The purpose of this study was twofold: (1) to evaluate the safety of intravesicular administration of ASB *E. coli* 2-12 as well as the duration of bladder colonization in healthy Beagle dogs and (2) to evaluate the microbiologic and clinical effect of intravesicular administration of ASB *E. coli* 2-12 to dogs with naturally occurring recurrent clinical UTI.

Material and Methods

ASB E. coli 2-12 Preparation

ASB *E. coli* 2-12 is a clinical isolate of *E. coli* obtained from the urine of a patient with asymptomatic bacteriuria.[11,14] ASB *E. coli* 2-12 was cultured through serial passages at 37°C in Luria broth supplemented with 50 mg/mL streptomycin and 100 mg/mL ampicillin under the following conditions: shaking at 225 rpm for 16-18 hours, subsequently diluted 1:1,000 into fresh broth, cultured under static conditions for 24 hours, and followed by an additional dilution into fresh medium and culture for another 24 hours under static conditions. Bacterial cultures were centrifuged at 6000 rpm for 20 minutes at 4°C. Pelleted bacteria were washed with ice-cold Dulbecco's PBS and centrifuged again as above. Bacteria were quantified at $OD_{420\ nm}$ and then recovered by centrifugation as above. The bacterial cell pellet was resuspended in sterile, ice-cold inulin preservation solution (4.5% inulin (Alfa Aesar) and 1.0% glycerol in ddH2O) to achieve a final concentration of $2 \times 10\hat{}10$ CFU/mL, and 1 mL single-dose aliquots were transferred to sterile cryovials. Lyophilization was carried out at room temp for 18-24 hrs, and vials were then stored at −80°C. After reconstitution in sterile PBS, preserved and stored ASB *E. coli* 2-12 aliquots retained approximately 69% of viability.

Safety Studies

In this prospective, non controlled clinical safety trial, six female intact 3-year old purpose-bred Beagle dogs were utilized for the first part of the study. All dogs were deemed healthy based on a normal physical examination and the absence of abnormalities on a CBC, serum biochemical panel, and urinalysis. All dogs had a negative baseline aerobic bacterial urine culture before entering the study and were housed at the University of California-Davis Animal Care Facilities.

On day 0, dogs received 10 μg/kg dexmedetomidine and 0.3 mg/kg of butorphanol IM to provide chemical restraint. Once appropriate sedation was achieved, the vulvar area was clipped to remove excess hair and cleaned with chlorhexidine solution. A 6 Fr catheter[a] was inserted into the urinary bladder by aseptic technique. All urine was removed from the bladder. A total of 10^{10} colony-forming units (CFU) of ASB *E. coli* 2-12 (reconstituted in 10 mL of sterile saline from lyophilized bacteria) was instilled into the bladder. This bacterial strain is highly sensitive to the most commonly used antimicrobials but is characterized by resistance to ampicillin and ticarcillin. The catheters were left in place for approximately 1 hour, while the dogs recovered from sedation. Dogs were monitored daily for changes in appetite and lower urinary tract signs (LUTS) including stranguria, hematuria, and pollakiuria.

Urine specimens (5 mL) were collected by antepubic cystocentesis on days 1 and 3, and by urinary catheterization on day 8. After collecting urine on day 8, instillation of bacteria was repeated as described above and urine collected by cystocentesis on days 9, 11, and 16. All urine specimens were submitted to the University of California-Davis Veterinary Medical Teaching Hospital (VMTH) microbiology laboratory for routine urinalysis and aerobic bacterial urine culture by inoculation of 10 μL of urine on to 5% defibrinated sheep blood and MacConkey agars incubated at 35°C in room air with added 5% CO_2. Susceptibility testing by the Clinical Laboratory Standards Institute, methodology was performed.[15] If protein was detected on urinalysis, the urine protein/creatinine ratio was determined. A final urine culture was also performed on day 30. Bacterial strain typing was performed with pulsed field gel electrophoresis (PFGE) as previously described.[16] This portion of the study was approved by the Institutional Animal Care and Use Committee at the University of California-Davis (protocol #17782). All animals were adopted after study completion.

Clinical Efficacy Study

This was also a nonplacebo controlled, prospective clinical pilot based on Simon stage 2 design. At least 9 patients with the same histology or molecular target need to be treated with the investigational drug to test the null hypothesis of insufficient efficacy; therefore, this was considered a phase II pilot study.[17] Assuming the likelihood of spontaneous regression of UTI is less than 5% and expecting at least a 25% response rate for the ASB *E. coli* 2-12 treated dogs to be clinically useful, with a $P = 0.05$ (type I [a] error; false positive) and a power of 0.8 (type II [b] error; false negative), then 9 dogs would be needed in this study.

Dogs with a history of three or more recurrent clinical UTIs over a one year time period where no underlying predisposing factors were documented, except those limited to voiding disorders, were eligible for enrollment. Dogs were enrolled at two study sites, the University of California-Davis VMTH in Davis, California, USA and the Koret School of Veterinary Medicine, Hebrew University of Jerusalem, Israel. Exclusion criteria included dogs with underlying comorbidities that could predispose to UTI including cystic neoplasia, cystic and/or renal calculi, moderate or severe polypoid cystitis, ectopic ureters, and other urogenital congenital abnormalities, as well as systemic disorders such as systemic neoplasia, diabetes mellitus, and hyperadrenocorticism. Dogs were also excluded if antimicrobials had been administered within the 3 days before enrollment, or if glucocorticoids had been administered within 2 weeks before enrollment. Only standard ectoparasite and endoparasite preventative medications were allowed to be administered during the study period. If dogs had

received the long-duration cephalosporin cefovecin,[b] they were also deemed ineligible, due to the variable half-life of that antibiotic and duration of its effect.

All dogs had a complete blood count, serum biochemical panel, urinalysis, and abdominal ultrasound performed before ASB *E. coli* 2-12 instillation to ensure there was no evidence of any of the exclusion criteria. If pyuria and bacteriuria were noted on the urinalysis, the urine was submitted for culture, and the dog was sedated with 2.5-10 μg/kg of dexmedetomidine (sufficient for urinary catheterization), and the ASB *E. coli* 2-12 was infused as described for the healthy dogs.

The sedation was not reversed and the catheter was removed approximately 30 minutes after the ASB *E.coli* 2-12 was instilled. Only dogs with positive urine cultures were finally enrolled. The dogs were evaluated again at their respective institutions on days 1, 7, and 14 post-ASB *E. coli* 2-12 infusion, and a urine specimen was collected by ultrasound guided cystocentesis for urinalysis and aerobic bacterial urine culture.

Any *E. coli* isolates from these clinical dogs were stored at −80°C from each dog on each visit, and PFGE was performed, according to a protocol developed by the Centers for Disease Control and Prevention (CDC) for *Salmonella*, *Escherichia coli* O157:H7, non-*Escherichia coli* O157:H7, and *Shigella* using *Salmonella enterica* serotype Braenderup H9812 as the control strain.[18]

Owners were also asked to complete a daily questionnaire regarding their dog's lower urinary tract voiding habits during the two-week study period. Owners were asked to rank on a 0-3 scale the presence and frequency of pollakiuria, hematuria, stranguria, licking of the penis/vulvar area, and the presence or absence of urinary incontinence (see Appendix S1). This portion of the study was approved by Institutional Animal Care and Use Committee at the University of California-Davis (protocol #18658) and the Koret School of Veterinary Medicine, The Hebrew University, Jerusalem, Israel (protocol # KSVM_VTH/04-2015).

Results

Safety Studies

All baseline CBC, biochemical panel, and urinalysis findings in the purpose-bred Beagle dogs were within reference ranges. No complications were encountered during instillation of the ASB *E. coli* 2-12. Lower urinary tract signs were not detected in any of the dogs during the study period.

Urine culture and relevant urinary sediment results are shown in Tables S1 and S2. ASB *E. coli* 2-12 was present in the urine of 4 of 6 dogs 1-day post-instillation, 1 of 6 dogs 3 days postinstillation, and none of the dogs 8 days postinstillation, as determined by urine culture and PFGE. One dog (dog 5) had a low magnitude (3×10^2 CFU/mL) growth of *Streptococcus* colonies, 8 days postinstillation, but had a negative urine culture on day 9. No dogs had pyuria on urine sediment examination.

One day after the second instillation of ASB *E. coli* 2-12 (day 9), one dog had minimal growth (1×10^5 CFU/mL) of ASB *E. coli* 2-12 and all subsequent cultures were negative. Pyuria was again not detectable in any of the dogs. On day 30, culture of the urine from one dog yielded 3×10^2 CFU/mL of a nonenteric bacterial species as well as *Mycoplasma* spp. No LUTS were noted in that dog, and a follow-up culture was not obtained. The UPC was consistently ≤0.2 (within reference range), when proteinuria was detected on dipstick examination from all urine specimens.

The *E. coli* strains isolated had the same susceptibility profile and were consistently resistant to ampicillin and ticarcillin. Furthermore, PFGE analysis confirmed that the PFGE type of all *E. coli* strains isolated matched that of the ASB *E. coli* 2-12 strain (Fig 1).

Clinical Efficacy of ASB 2-12

Nine dogs were recruited for this aim of the study; five from the University of California-Davis, and four from The Koret School of Veterinary Medicine. Four dogs were mixed breeds and one of each of the following: Golden retriever, Anatolian shepherd, German shorthaired pointer, German shepherd, and a dachshund. Median weight was 21 kg (range, 7.6-29.6 kg). Seven were female (6 spayed and 1 intact), and 2 were castrated males; the median age was 8 years (range, 0.5-13 years). One dog had clinical signs of pollakiuria only, and four dogs had pollakiuria, hematuria, and stranguria. These dogs had no other comorbidities. One dog, dog 3, had its right thoracic limb amputated 3 years before enrollment. This dog had stranguria, pollakiuria, and hematuria. Dog 4 had a T3-L3 myelopathy and was evaluated for pronounced urinary incontinence and mild hematuria that responded to antimicrobial treatment associated with its UTI. Dog 7 had a right pelvic limb amputated 6 years before

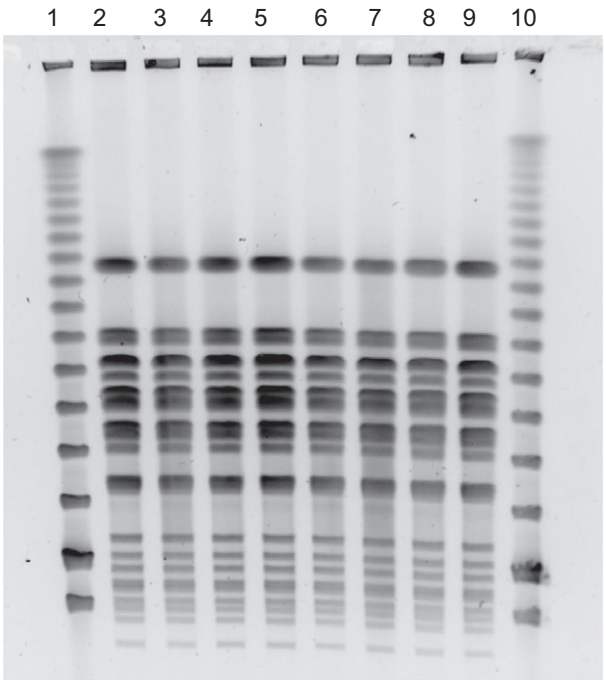

Fig 1. Pulse field gel electrophoresis profiles from urine *E.coli* isolated from research dogs. Lane 2 is ASB *E coli* 2-12 (lane 2) control from the lyophilized sample. Lanes 3-9 represent the seven *E. coli* isolates obtained from the research dogs after bladder instillation. The *E. coli* isolates were genetically identical. Low range PFGE biomarkers are represented in lanes 1 and 10.

enrollment due to an osteosarcoma. This dog had mild osteoarthritis and weakness in his left pelvic limb and was evaluated for stranguria and pollakiuria. Dog 8 was a dachshund that had intervertebral disk disease, required a cart for mobility, and assistance with micturition by manual bladder expression. This dog developed hematuria and pronounced urinary incontinence that were associated with UTI and these signs were reported to have resolved with proper antimicrobial treatment. With the exception of mild normocytic normochromic nonnegative anemia (HCT 36%, reference range, 40-55%) in one dog (dog 9), none of the other eight dogs had abnormalities on CBC and serum chemistry before enrollment.

Urinalysis and Microbiologic Outcomes

All 9 dogs screened had a positive urine culture and were formally enrolled in the study (Table S3). Three dogs (dogs 7, 8, and 9) showed persistently positive culture results, and in the remaining 6 dogs, the infecting pathogen was not isolated by day 14. Of the latter 6 dogs, 3 (dogs 1, 2, and 3) also had MC at day 14 and a fourth dog (dog 4) had MC at days 1 and 7 followed by subclinical reinfection (in addition to ASB *E. coli* 2-12) at day 14, and antimicrobials were not clinically indicated. Three of the four dogs with MC had ASB *E. coli* 2-12 isolated from their urine on day 14 documented by PFGE. Two dogs (dogs 5 and 6) had MC documented with only ASB *E. coli* 2-12 growth on day 1, but by day 7, clinical signs and reinfections with new strains of bacteria were documented. In the remaining dog (dog 9), ASB *E. coli* 2-12 was never isolated.

Of the three dogs considered microbiologic failures, ASB *E. coli* 2-12 was isolated from one dog on days 1 and 7, but both the original pathogen and clinical signs persisted. In one dog, emphysematous cystitis was documented 24-hours post-ASB *E. coli* 2-12 bladder instillation, and this dog was deemed a clinical failure. All clinical signs and evidence of emphysematous cystitis resolved after initiation of antimicrobial treatment. One dog showed no colonization with ASB *E. coli* 2-12 and no eradication of the initial infecting pathogen.

Long-term follow-up urine cultures were only available in dogs 5 and 6. ASB *E. coli* 2-12 (as determined by PFGE analysis) was isolated 60 days after bacterial instillation and these two dogs remained free of LUTS for more than 6 months.

Clinical Outcomes

Four dogs had complete or nearly complete clinical cures by day 14 based on voiding diary scores (Fig 2). Three of these dogs also had microbiologic cures. Of the dogs that had clinical improvements, the median total LUTS score on day 0 was 8 (range, 5-11). On days 1, 7, and 14 after ASB *E. coli* 2-12 instillation, median scores were 8 (range, 4-11), 4 (range, 0-12), and 1 (range, 0-2), respectively (Fig 2). Although ASB *E. coli* 2-12 was never isolated from dog 2, a clinical cure was documented for 11 months.

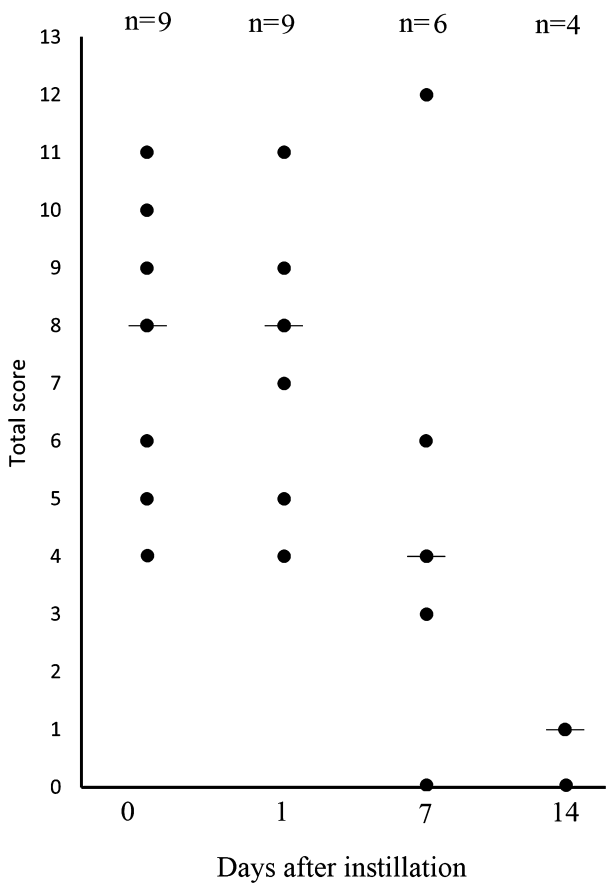

Fig 2. Scatter plot of voiding diary scores of the dogs that had clinical improvements noted. Dash line indicates the median.

Adverse Effects

The only clinical adverse effects reported post-instillation of ASB *E. coli* 2-12 were in two dogs in which the owners described a mild, self-limiting decrease in appetite at the day of the procedure, which resolved in both dogs within 24 hours. Ultrasonographic evidence of emphysematous cystitis was also noted in one dog.

Discussion

We have documented a clinically applicable, easy, and safe method to administer the live biotherapeutic product (LBP) ASB *E. coli* 2-12 to dogs. By evaluating the ASB *E. coli* 2-12 strain in healthy dogs first, we were able to develop a protocol that translated well into the clinical setting (procedure time <20 minutes). The isolated *Streptococcus* spp. from the urine of one research dog just before the second instillation of ASB *E. coli* 2-12 might have been a contaminant from urine collection by catheterization on day 8. ASB *E. coli* 2-12 was isolated from the dogs after inoculation; however, bacterial colonization was short-lived. These findings are similar to a previously published study where ASB *E. coli* was cleared from the urine of healthy mice within days after instillation.[11] Similar findings were reported in dogs treated with ASB *E. coli* 83972.[13,19]

However, that canine study evaluated a multidose regimen[13] requiring an indwelling urinary catheter throughout the study period, which allowed multiple administrations of the ASB *E. coli* 83972 strain. This might have been one reason a single dog had colonization of the bacteria for 28 days. We chose to design a protocol that would allow easier translation to the clinical setting and would not place dogs at high risk for iatrogenic bacterial infection due to prolonged urinary catheterization, so indwelling catheters were not utilized.

After a single instillation of ASB *E. coli* 2-12 into the bladders of 9 dogs with clinical recurrent UTI, five dogs had a MC of the initial bacterial infection on day 14, although two had documented reinfections with a new bacterial strain. Clinical cures were documented in four cases on day 14. PFGE confirmed at least transient colonization with ASB *E. coli* 2-12 in 6 dogs (range, 1-60 days). We also noted a lack of recurrent infections in three dogs, which is intriguing. These results were achieved in both the presence (dogs 2 and 3) and absence (dog 1) of ASB *E. coli* 2-12. The combination of these acute microbiologic and clinical effects coupled with some durable prophylactic benefits suggests that ASB *E. coli* 2-12 has potential antimicrobial and analgesic activity which was noted in the murine studies.[11]

Of several ASB strains that have been investigated for treatment of recurrent UTIs, ASB *E. coli* 2-12 has shown the greatest analgesic activity in mouse models when compared to *E. coli* 83972, which is why we selected this strain for these studies.[11] Intravesicular or intravaginal administration of ASB *E. coli* 2-12 in a NU14 *E. coli* - induced cystitis mouse model led to a rapid and significant decrease in UTI-associated allodynia. Furthermore, ASB *E. coli* 2-12 exhibited superior analgesic activity compared to ciprofloxacin for UTI induced by non-UPEC bacteria (eg., *Proteus mirabilis* and *Klebsiella pneumoniae*) in mouse studies. It is also possible that ASB *E. coli* 2-12 might exert an immunostimulatory, adjuvant-like activity that promotes an effective host immune response sufficient to clear uropathogens after ASB clearance, as has been recently shown for BCG-mediated clearance of *E. coli* in human patients.[20] Thus, we speculate that the clinical efficacy of ASB *E. coli* 2-12 in canine UTI results from a combination of immunomodulation that promotes microbiologic relief and analgesic activity that provides symptomatic relief.

No major adverse events were reported. However, one dog had ultrasonographic evidence of emphysematous cystitis. It is unknown if the original bacteria or the instillation of ASB *E. coli* 2-12 were the cause of this observation and the dog's signs resolved quickly with antimicrobial administration. Two dogs experienced hyporexia that resolved within 24 hours after the infusion of the ASB *E. coli* 2-12, which might have been the result of residual effects of the sedatives, as seen commonly in veterinary practice after other procedures involving sedation. In some of the dogs with naturally occurring bacterial cystitis, the duration of ASB *E. coli* 2-12 colonization was longer when compared to the healthy female Beagles. It is possible that dogs with recurrent lower UTI have compromised local defense mechanisms that are less effective at clearing bacterial infections, which enables more prolonged colonization by the ASB *E. coli* 2-12 strain compared to healthy dogs.[21]

While some dogs were considered clinical failures, one should consider that dogs in this study had complicated UTI. Although we excluded cases with immunosuppressive disorders and overt nidus for infection that was documented, we enrolled dogs with micturition abnormalities, as long as the historic clinical signs associated with the UTI responded to antimicrobial treatment. For example, three dogs had incomplete micturition due to orthopedic or neurologic problems. Two of these dogs relapsed with clinical signs early in the course of treatment; one developed emphysematous cystitis and the other had intervertebral disk disease. While dog nine also had a T3-L3 myelopathy, this dog's clinical signs waxed and waned during the initial 2 weeks after the instillation of the ASB *E. coli* 2-12, but further antimicrobial treatment was not clinically indicated for this dog again until approximately 6 months later. Cocolonization of ASB *E. coli* 2-12 with the other strains of *E. coli* in the bladder was noted.

Alternative approaches for prevention and treatment of recurrent UTI that have been investigated in human beings as well as animal UTI models include the use of cranberry extract,[22,23] cranberry juice,[24] probiotics,[25,26] live biotherapeutic products (LPBs),[10,11] vaccines,[27] and various other alternative treatments.[28] Live biotherapeutic products appear promising for treatment of recurrent UTI for several reasons. Intravesicular administration of *E. coli* ASB 83972 in human beings with recurrent UTI can reduce symptoms of UTI and protect some patients from recurrent UTI after serial catheterizations[29] which might reduce the need to antimicrobial treatment.

This study had limitations. First, the study was short term and we only carefully followed dogs during the initial 14 days post-ASB *E. coli* 2-12 instillation. However, long-term information on a few of these dogs was available and provided on an individual case basis. Second, this was a pilot study including only 9 dogs. A larger number of dogs with a control group are needed to establish clinical efficacy of this treatment modality, as some apparent responses might have resulted from immune clearance or waxing and waning of clinical signs over time. Bacterial cystitis can resolve spontaneously in some patients; therefore, we must consider that some dogs might have responded with or without the ASB *E. coli* 2-12 instillation. Prospective-controlled clinical trials are warranted. Finally, we only investigated one dosing regimen. Studies evaluating *E. coli* 83972 for clinical recurrent UTI in humans with spinal cord injury were inoculated postantimicrobial treatment 3 times daily for 3 days. Preliminary findings suggested the colonization decreased UTI in these treated patients.[9] Therefore, evaluating multiple instillations of ASB *E. coli* 2-12 in clinical dogs could be evaluated to see if improved clinical outcomes are noted.

Conclusion

In summary, our study demonstrated that a single intravesicular administration of ASB *E. coli* 2-12 was not associated with major adverse effects in a limited number of healthy dogs. Data from the pilot study in dogs with clinical recurrent UTI were promising. Judicious case selection and tailoring the dosing intervals of ASB *E. coli* 2-12 should be explored in future studies. Additional placebo-controlled clinical trials are warranted based on the results of this study to more clearly determine the clinical potential of this novel LBP.

Footnotes

[a] Convenia, Zoetis Parsippany, NJ
[b] DLC-6P, Life Tech. Stafford TX

Acknowledgments

Conflict of Interest Declaration: Jane Sykes serves as Associate Editor of the Journal of Veterinary Internal Medicine. She was not involved in the review of this manuscript.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Urinalysis and aerobic urine culture result from six healthy Beagle dogs after first instillation of ASB *E. coli* 2-12 into the urinary bladder. CFU/mL = colony-forming units/milliliter; WBC/HPF = white blood cells per high power field; RBC/HPF = red blood cells per high power field; NG = no growth.

Table S2. Urinalysis and aerobic bacterial urine culture result from six healthy Beagle dogs after a second instillation of ASB *E. coli* 2-12 into the urinary bladder 8 days after the first treatment. CFU/mL = colony-forming units/milliliter; WBC/HPF = white blood cells per high power field; RBC/HPF = red blood cells per high power field; NG = no growth.

Table S3. Urine culture outcomes in 9 dogs with naturally occurring recurrent clinical bacterial cystitis. **E. coli* isolate is ASB *E. coli* 2-12 as identified by pulse field gel electrophoresis. MC = Microbiologic Cure. CC = Clinical Cure, MF = Microbiologic Failure, CF = Clinical Failure. NG = No growth. NA = Not applicable.

Appendix S1. Please circle the number that corresponds best to the answer regarding your pet's urination habits.