Title

Evaluation of the efficacy of a live Escherichia coli biotherapeutic product (asymptomatic bacteriuria E. coli 212).

Journal

Journal of Veterinary Internal Medicine, 38(5)

Authors

Segev, Gilad

Chen, Hilla

Dear, Jonathan

et al.

Publication Date

2024

DOI

10.1111/jvim.17167

Peer reviewed

Received: 21 January 2024 | Accepted: 31 July 2024

DOI: 10.1111/jvim.17167

STANDARD ARTICLE

Journal of Veterinary Internal Medicine Open Access

ACVIM American College of Veterinary Internal Medicine

Evaluation of the efficacy of a live *Escherichia coli* biotherapeutic product (asymptomatic bacteriuria *E. coli* 212)

Gilad Segev[1] | Hilla Chen[1] | Jonathan D. Dear[2] | Beatriz Martínez López[2] | Jully Pires[3] | David J. Klumpp[4] | Anthony J. Schaeffer[4] | Jodi L. Westropp[2]

[1]Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel

[2]Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, Davis, California, USA

[3]Veterinary Center for Clinical Trials, University of California, Davis, Davis, California, USA

[4]Department of Urology, Feinberg School of Medicine, Northwestern University, Evanston, Illinois, USA

Correspondence
Jodi L. Westropp, Department of Veterinary Medicine and Epidemiology, School of Veterinary Medicine, University of California Davis, 2108 Tupper Hall, Davis, CA 95616, USA.
Email: jlwestropp@ucdavis.edu

Funding information
Morris Animal Foundation, Grant/Award Number: D20CA-044

Abstract

Background: Recurrent bacterial cystitis, often referred to as recurrent urinary tract infection (UTI), can be difficult to manage and alternative treatments are needed.

Hypothesis/Objective: Intravesicular administration of asymptomatic bacteriuria (ASB) *E. coli* 212 will not be inferior to antimicrobial treatment for the management of recurrent UTI in dogs.

Animals: Thirty-four dogs with >1 UTI in the 12 months before presentation.

Methods: All dogs were deemed normal otherwise based on absence of abnormalities on physical examination, CBC, serum biochemical panel, and abdominal ultrasonography. Dogs were randomized to 1 of 2 treatment groups: Group 1 antimicrobials for 7 days or group 2 intravesicular administration of ASB *E. coli* 212. Owners were provided a voiding questionnaire regarding their dogs' clinical signs, which was completed daily for 14 days to assess clinical cure. Dogs were examined on days 7 and 14 to assess clinical cure, and urine specimens were submitted for urinalysis and bacterial culture.

Results: Clinical cure rates for ASB *E. coli* 212–treated dogs were not inferior to 7 days of antimicrobial treatment with a 12% margin of difference to determine non-inferiority. No significant difference was found between the treatment groups on days 7 and 14 in the proportion of dogs achieving ≥50% or ≥75% reduction in their clinical score compared with baseline.

Conclusions and Clinical Importance: These data suggest that intravesicular administration of ASB *E. coli* 212 is not inferior to antimicrobials for the treatment of recurrent UTI in dogs. This biotherapeutic agent could help alleviate the need for antimicrobials for some dogs with recurrent UTI, improving antimicrobial stewardship.

KEYWORDS
antimicrobial resistance, cystitis, dog, urinary tract

Abbreviations: ASB, asymptomatic bacteriuria; LUTS, lower urinary tract signs; RIA, rapid immunoassay; SDMA, symmetric dimethylarginine; UC, urine culture; UPEC, uropathogenic *Escherichia coli*; UTI, urinary tract infection.

1 | INTRODUCTION

Bacterial cystitis is a common cause of morbidity in dogs and often is treated using antimicrobials. The most common clinical signs of lower urinary tract infection (UTI) in dogs include stranguria, hematuria, and pollakiuria, although malodorous urine,[1] peri-genital licking[1,2] and urinary incontinence,[2,3] also have been noted in dogs with positive urine cultures. Sporadic cystitis (hereafter called UTI) generally occurs in otherwise healthy dogs with no evidence of underlying disease and resolves with antimicrobial treatment.[4] Recurrent UTI also occurs in dogs and occasionally is associated with underlying comorbidities.[5] Antimicrobials are prescribed for UTI, but antimicrobial resistance has been an emerging problem in both dogs and humans,[6-8] and thus other treatment modalities are being investigated.

Asymptomatic bacteriuria (ASB) *Escherichia coli* strains have been isolated from human patients with persistent subclinical bacteriuria. In murine models using uropathogenic *E. coli* (UPEC) strain NU14 to induce acute clinical cystitis, ASB *E. coli* strains have been shown not only to significantly decrease bacteriuria but to also have anti-infective and visceral analgesic activity.[9] Asymptomatic bacteriuria *E. coli* 83972 strain has been reported to improve the quality of life in humans with UTI[10] without major adverse events. Moreover, when long-term bladder colonization by the bacteria was achieved, no symptoms of UTI developed, with no adverse impact of ASB *E. coli* 83792 on renal function.[11] The mechanisms by which ASB *E. coli* provides protection for recurrent UTI are not fully understood but might result from immunomodulation or bacterial interference, whereby an ASB strain colonizes the bladder and prevents subsequent colonization with UPEC strains that cause inflammation and result in lower urinary tract signs (LUTS).

In a pilot study evaluating the instillation of 10^{10} colony-forming units of ASB *E. coli* 212 reconstituted in 10 mL of saline from lyophilized bacteria into the urinary bladder of healthy research dogs, long-term bacterial colonization was not achieved, but no dogs experienced any adverse events. When this biotherapeutic was instilled into the bladders of 9 client-owned dogs with recurrent UTI, 4 of these 9 dogs had complete or nearly complete clinical cures by day 14. Of these 4 dogs, 3 had microbiological cures of their original pathogen on day 14 and 1 had subclinical bacteriuria (in addition to ASB *E. coli* 212) isolated from its urine. Three of these 4 dogs had ASB *E. coli* 212 isolated from their urine on day 14. With the exception of mild, temporary, self-limiting, hyporexia in 2 dogs on the day of biotherapeutic administration, no major adverse effects were observed.[12]

Because of these preliminary data, our study was designed to evaluate ASB *E. coli* 212 in a randomized prospective non-inferiority trial to evaluate the efficacy of this biotherapeutic compared to antimicrobial administration for 7 days. We hypothesized that ASB *E. coli* 212 would not be inferior to standard antimicrobial treatment for dogs with recurrent UTI within a 12% margin of difference to determine noninferiority.

2 | MATERIALS AND METHODS

In this prospective, randomized multicenter clinical trial, dogs 3 months of age with LUTS including pollakiuria, stranguria, hematuria, persistent genital licking, and >1 UTI associated with these signs in the past 12 months before presentation based on a positive urine culture (UC) of urine collected by cystocentesis were eligible for enrollment. Furthermore, dogs with urinary incontinence associated with a storage disorder such as urethral sphincter mechanism incompetence as their sole clinical sign also were eligible as long as the urinary incontinence previously was associated with bacteriuria, and the urinary incontinence completely resolved with prior antimicrobial administration.

Dogs were excluded if underlying comorbidities such as urinary calculi, bladder neoplasia, emphysematous cystitis, hooded vulva with concurrent perivulvar pyoderma or dermatitis, pyelonephritis, prostatitis, acute kidney injury, or chronic kidney disease were identified by laboratory tests, abdominal ultrasonography or a combination of these. Dogs with *Corynebacterium* spp. UTI were not eligible for enrollment because of the risk of encrusting cystitis.[13] Dogs diagnosed with polypoid cystitis or proliferative urethritis also were excluded. Dogs with a recent history of cefovecin administration were excluded because of unpredictable drug elimination kinetics.[14] Upon enrollment, CBC, serum biochemistry panel, and serum symmetric dimethylarginine (SDMA) concentration were performed. All dogs had urine collected by cystocentesis for urinalysis, including urine sediment, and evaluated by their respective clinical laboratories. Some dogs also were screened for bacteriuria using a rapid immunoassay (Rapidbac Vet, SilverLake Medical) test. If a positive result was confirmed or evidence of bacteriuria and pyuria were noted on urinalysis, dogs were randomized to receive either the antimicrobial treatment (group 1) or ASB *E. coli* 212 (group 2). Only dogs with positive urine cultures continued in the trial. Clients were provided a questionnaire regarding their dog's lower urinary tract clinical signs, which was completed daily for 14 days (Supporting Information S1). Recorded clinical signs included stranguria, pollakiuria, hematuria, dysuria, urinary incontinence, preputial or perivulvar licking, and the presence or absence of an odor to the urine. Aggregate scores could range from 0 to 17.

2.1 | Dogs treated with antimicrobials (Group 1)

Dogs were treated with the empirical antimicrobial, amoxicillin with clavulanic acid (14-19 mg/kg PO q12h), pending urine susceptibility results. Once the susceptibility results were available, the antimicrobial was changed if the bacteria were resistant to amoxicillin-clavulanic acid. Antimicrobials were administered for 7 days (or 7 days from when an antimicrobial was changed based on susceptibility test results). Urinalysis and aerobic bacterial UC were repeated on days 7 and 14. If clinical signs developed or persisted during antimicrobial treatment, the dog was deemed a clinical failure, removed from the study and treated with another antimicrobial based on susceptibility.

2.2 | Study dogs (Group 2)

Dogs were treated with a sedative, approximately 2-5 μg/kg dexmedetomidine and 0.3 mg/kg of butorphanol IV, to provide chemical

restraint. Once appropriate sedation was achieved, the vulvar or preputial area was clipped to remove excess hair and cleaned with chlorhexidine solution. An appropriately sized catheter was inserted into the urinary bladder using aseptic technique. All urine was removed from the bladder. A total of 10^{10} colony-forming units of ASB *E. coli* 212 from lyophilized bacteria (reconstituted in 10 mL of sterile saline) were instilled into the bladder. The catheter was removed, and the dogs were allowed to recover and discharged to their owners when the investigator determined they were stable. If at least a 50% reduction in their clinical score was not achieved by day 3, a second ASB *E. coli* 212 infusion was offered to the client to be administered using the same protocol. If the clinical score persisted 36 hours after the second infusion, the dog was deemed a clinical failure and treated with antimicrobials for 7 days based upon UC susceptibility testing. Urinalysis and UC were repeated on day 7 and day 14 for all dogs, and additionally on day 3 for dogs that received a second infusion. In both groups, the UC results and bacterial strain were recorded. Asymptomatic bacteriuria *E. coli* 212 is highly susceptible to commonly used antimicrobials but is characteristically resistant to ampicillin and ticarcillin.[12] This susceptibility pattern was used to distinguish *E. coli* strains on follow-up UC.

The study was approved by Institutional Animal Care and Use Committee at the University of California, Davis (protocol #22771), and the Koret School of Veterinary Medicine, The Hebrew University, Jerusalem, Israel (protocol # MD-2016230-3).

3 | STATISTICAL ANALYSES

Statistical analysis to show noninferiority between 2 treatments was conducted using a multilevel regression analysis to evaluate the magnitude of the difference of the means (effect size) between the 2 treatments (antimicrobials and ASB *E. coli* 212) on the UTI clinical scores using both *P* values and confidence intervals.[15] The multilevel modeling approach was the most appropriate analysis to account for the repeated measures design (ie, dog identification is a random effect). We defined noninferiority as a margin of 2 points in the clinical score. This margin (12%) was set in advance based on clinical and statistical reasons. Proportions were compared between the 2 groups using the Fisher's exact test. Analyses were conducted in R (v. 4.3.1.) using packages lmer[16] and confinterpret.[17]

A sample size calculation using the function epi.ssninfcin in the epiR package[18] was performed. We assumed a mean clinical score of 3 in the treatment group, a mean clinical score of 3 in the control group, an expected population SD of the clinical score of 2, and a clinically meaningful difference (margin/limit) in the clinical score of 2. We assumed 80% power and a 5% level of significance. A minimum of 26 dogs needed to be enrolled in the trial, 13 in group 1 and 13 in group 2.

4 | RESULTS

Thirty-four dogs with positive UC were enrolled in the clinical trial. All dogs that had pyuria and bacteriuria, a positive radioimmunoassay test or combination of the 2 also had growth on the UC. Seventeen dogs were randomized to receive antimicrobial treatment (group 1) and 17 were treated using intravesicular ASB *E. coli* 212 (group 2). One dog in group 1 was lost to follow-up, and only 16 were included in the data analyses. One dog in group 2 died on day 4 because of presumed neoplasia in the pleural cavity, which was not thought to be related to the lower urinary tract disease or treatment, leaving 16 dogs in the statistical analyses from this group.

The median age of dogs in group 1 was 5.5 years (range, 0.5-12 years) and the median weight was 21.5 kg (range, 7.6-39.8 kg). There were 11 spayed female, 4 intact female dogs, and 1 castrated male dog in this group. The median age of dogs in group 2 was 6.5 years (range, 0.25-13 years) and the median weight was 14.6 kg (range, 5.5-63.7 kg) and included 14 spayed female dogs and 2 intact female dogs. A number of different breeds were represented, including Cavalier King Charles spaniels (2 in group 1 and 3 in group 2) and 1 each of various other breeds in both groups. Two dogs in group 1 had ectopic ureters that were corrected using laser ablation before enrollment, 1 of these dogs also had a urethral occluder placed before enrollment. One dog enrolled in group 2 also had a urethral occluder placed for refractory urethral sphincter mechanism incompetence 2 years before study enrollment.

Serum creatinine concentration was within the respective laboratories' reference ranges for all dogs in both groups on day 0. No other clinically relevant abnormalities were noted on the CBC or serum biochemical panel from any dogs. The SDMA concentrations were available for only 11/16 dogs in group 1 (because of insufficient available serum), of which 2 had SDMA concentrations of 15 and 18 µg/dL, respectively. In 1 dog, SDMA concentrations decreased from 18 µg/dL to 15 µg/dL by day 14. All SDMA concentrations for dogs in group 2 were within the reference range. No major adverse events were noted in any dogs, and none exhibited clinical signs suggestive for pyelonephritis or appeared systemically ill during the trial period.

Thirteen of 16 dogs in group 1 were treated with amoxicillin-clavulanic acid for the entire 7-day duration. Based on urine culture susceptibility results, 2 of these 16 dogs initially were treated with ofloxacin (11.5 mg/kg q24h and 12.5 mg/kg q24h, respectively), but treatment was changed to cephalexin (33 mg/kg q8h) for 1 dog. One dog in group 1 initially was treated with nitrofurantoin (4 mg/kg q8h) but was changed to amoxicillin-clavulanic acid after the bacterial susceptibility results obtained from day 7 returned. Six dogs in group 2 were given a second treatment of ASB *E. coli* 212 on day 3.

Bacterial isolates that were cultured on day 0 are presented in Figure 1. The most common isolates from both groups were *E. coli*. All dogs in group 1 had a single isolate cultured from their urine specimen. Fourteen dogs in group 2 had only 1 isolate cultured from their urine, but 2 dogs in group 2 had growth of 3 bacterial isolates.

4.1 | Clinical cure

The clinical scores of dogs in groups 1 and 2 on days 0 and 7 are presented in Figure 2. The median clinical scores at baseline for groups

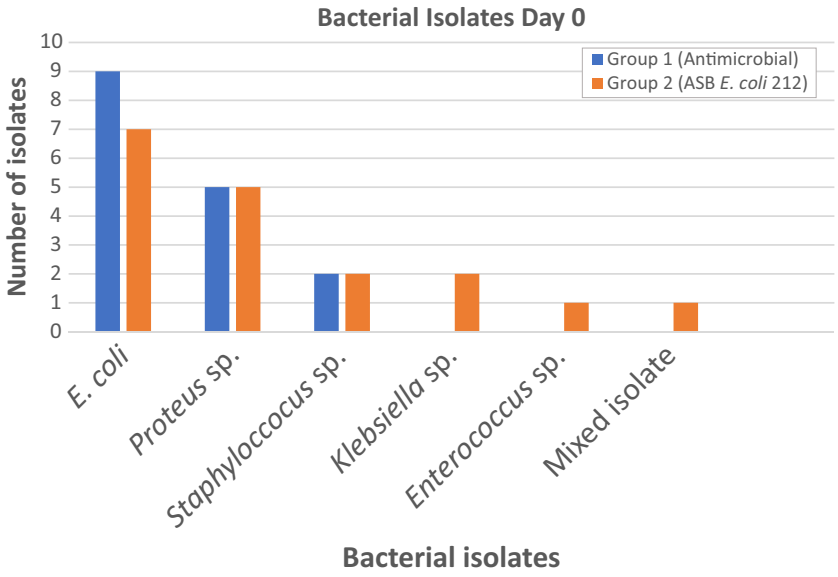

FIGURE 1 Bacterial isolates cultured from urine specimens from all dogs on day 0.

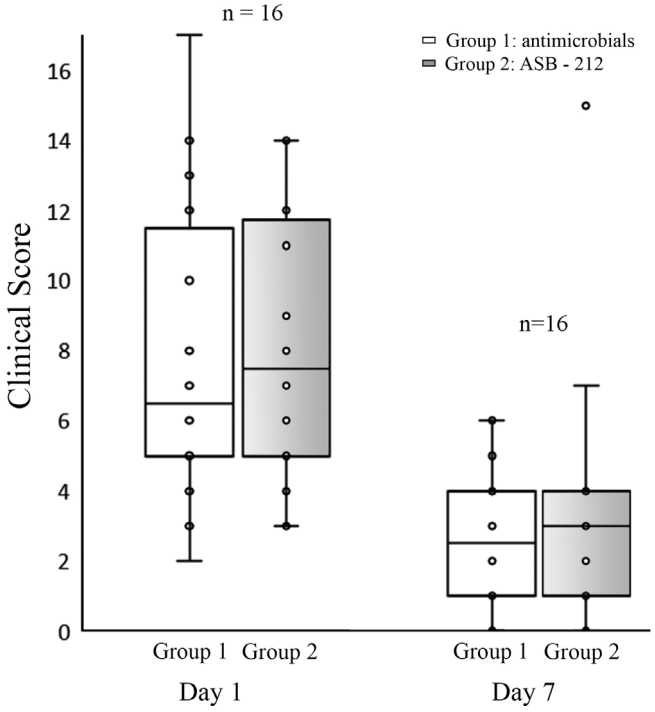

FIGURE 2 The clinical scores (median and range) of dogs in groups 1 and 2 on days 0 and 7.

1 and 2 were 6.5 (range, 2-17) and 7.5 (range, 3-16), respectively. Three dogs in group 2 were deemed clinical failures after day 7 and were treated with antimicrobials. No dogs from group 1 were removed from the study. The percent reduction in clinical scores on days 7 and 14 is provided in Table 1. No significant difference was found in the proportion of dogs achieving ≥50% or ≥75% reduction in their clinical scores compared with their baseline between the treatment groups, both on days 7 and 14. Dogs in group 2 that were deemed clinical failures and received antimicrobials were included in these datasets as failures (0% reduction in clinical sign score). Although scores for 1 dog in group 2 were not available on day 14, the client notified us that the dog was clinically well, but the owner failed to complete the daily survey. This dog's data is not included in the day 14 analyses in Table 1.

TABLE 1 Number and percentage of dogs in each group that had either a 50% or ≥75% reduction in their clinical score compared to baseline.

	≥50% ↓ Clinical signs	≥75% ↓ Clinical signs
Day 7		
Group 1	13/16 (81%)	8/16 (50%)
Group 2	13/16 (81%)	8/16 (50%)
P value	1.0	1.0
Day 14		
Group 1	9/16 (56%)	6/16 (38%)
Group 2	11/15 (73%)[a]	7/15 (47%)[a]
P value	.53	.76

[a]Dogs in group 2 that withdrew early because of persistent lower urinary tract signs and were deemed clinical failures are included in these dataset as failures (0% reduction in clinical signs).

The results of our multilevel regression analysis used in our non-inferiority trial are presented in Table 2 and Figure 3. The confidence interval for the difference between ASB *E. coli* 212 and antimicrobial treatment was located to the left of the noninferiority margin and, because lower clinical scores were considered to be the beneficial outcome, we concluded that ASB *E. coli* 212 was noninferior to antimicrobial treatment. Similarly, the *P* value obtained for ASB *E. coli* 212 treatment was not significant, which indicated that the intravesicular administration of ASB *E. coli* 212 was not inferior to antimicrobial treatment for the management of recurrent UTI.

TABLE 2 Results of the multilevel regression analysis to evaluate the magnitude of the difference between group 1 (antimicrobial treatment) and group 2 (ASB *E. coli* 212) on UTI clinical scores.

	Score		
Predictors	**Estimates**	**CI**	***P***
Intercept	3.78	2.58-4.98	<.001
Treatment (ASB212)	−0.23	−1.91 to 1.45	.79
Random effects			
σ^2	5.98		
$\tau_{00\ ID}$	5.56		
ICC	0.48		
N_{ID}	33		

Note: The *P* value obtained for ASB *E. coli* 212 treatment was not significant, which indicated that the intravesicular administration of ASB *E. coli* 212 was not inferior to antimicrobial therapy for the management of recurrent UTI. Observations = 436. Marginal R^2/Conditional R^2 = 0.001/0.482.

Abbreviations: ASB, asymptomatic bacteriuria; ICC, intraclass correlation coefficient; N_{ID}, number of clusters (ie, dogs in this case); $t_{00\ ID}$, residual variance; UTI, urinary tract infection; σ^2, random effect variance.

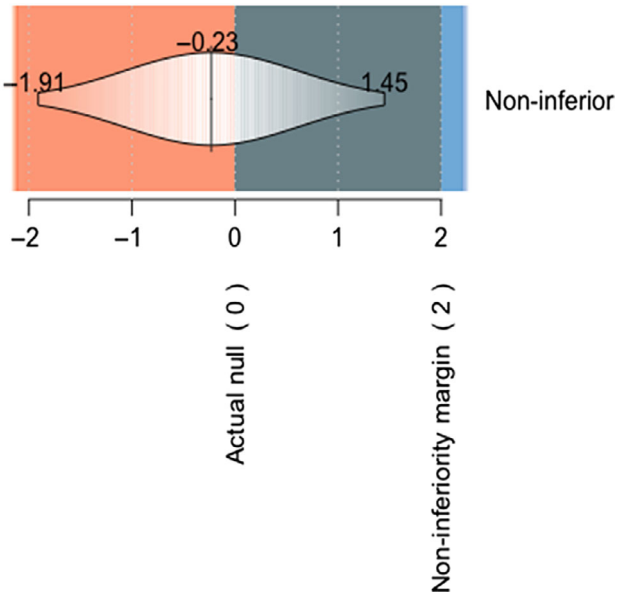

FIGURE 3 Boundaries of noninferiority for the 2-sided 95% confidence interval of the difference between group 1 (antimicrobial treatment) and group 2 (ASB *E. coli* 212) treatment and groups. The confidence interval for the difference between ASB *E. coli* 212 and antimicrobial treatment was located on the left of the noninferiority margin, therefore ASB *E. coli* 212 was noninferior to the antimicrobial treatment. Graph generated using the R package "confinterpret." ASB, asymptomatic bacteriuria.

4.2 | Microbiological cure

The bacterial isolates cultured from each individual dog's urine on days 0, 3 (if applicable), 7, and 14 can be found in Table S2. Seven of 16 dogs in group 1 had a negative UC on day 14; however, only 3/16 dogs in group 2 had only ASB *E. coli* 212 isolated on day 14. Fifteen dogs in group 1 had a negative UC on day 7. Fifteen of 16 dogs had a reduction in their clinical score on day 7 compared with baseline. One dog had a positive UC and no change in clinical signs on day 7. Nine of these 16 dogs had positive UCs on day 14. Although 14 dogs in group 1 still had reductions in their clinical score on day 14 compared to baseline, 8 of these dogs had an increase in their score compared with day 7. One dog in group 1 had a 100% reduction in clinical signs on day 14 compared with baseline.

All dogs in group 2 had positive UC on days 7 and 14. Three of these dogs were clinical failures and none of these 3 had ASB *E. coli* 212 isolated from their urine. Seven of the 16 dogs in group 2 had growth of ASB *E. coli* 212 in their urine on day 7 or day 14 or both. Only 1 of these dogs had an increase in its clinical sign score on day 14. Of the 12 dogs in group 2 that remained in the study on day 14, 4 dogs had increased clinical sign scores compared with day 7. The owner of 1 dog reported that the dog was well, but failed to return and did not complete the voiding questionnaire. Three dogs in group 2 had a 100% reduction in their clinical scores on day 14 compared with baseline.

4.3 | Longer term follow-up

We were able to obtain longer term information regarding clinical cures for 9 dogs in group 1 and 9 dogs in group 2. Six of the 9 dogs in group 1 had clinical relapses within 21 days of completing the study; 1 dog remained free of LUTS until day 65. One of these 9 was euthanized 10 months after the trial because of intracranial disease, the medical record contained no mention of LUTS. Another dog was diagnosed with systemic mast cell tumor 5 months after the trial ended and was euthanized 8 months after the trial; there was no mention of LUTS in the medical record during this time period.

Of the 9 dogs in group 2 that completed the 14-day trial for which longer term follow-up was available, 1 dog had mild urinary incontinence and was lost to follow-up at 1 month, 6 dogs had no LUTS until 14 days, 1 month (2 dogs), 2 months, 3 months, and 8 months after the trial; 1 of these 6 dogs subsequently was diagnosed with polypoid cystitis. Another dog remained free of LUTS at 6 months after ASB *E. coli* 212 instillation, whereas information on another indicated no LUTS for 1 year. At that time, doxycycline was prescribed for a cough by the referring veterinarian and LUTS returned shortly thereafter. Another dog was clinically well for 13 months but presented with signs compatible with pyelonephritis at that time. This dog had a urethral occluder placed 2 years prior and it was determined that the occluder was causing partial urethral obstruction. *Escherichia coli* was isolated from the urine specimen, but the antimicrobial susceptibility results were not suggestive for ASB *E. coli* 212, in contrast to results that were documented a few months prior.

5 | DISCUSSION

The biotherapeutic, ASB *E. coli* 212, was not inferior to antimicrobial treatment when evaluating clinical cure for dogs with recurrent UTI in

this 2-week clinical trial. Furthermore, longer term outcomes demonstrated promising results in some dogs, whereby clinical cure was documented for as long as 13 months. No dogs experienced any major adverse events. For most female dogs, the entire bladder instillation procedure took approximately 20 minutes to perform. Unlike previous studies that evaluated another asymptomatic strain of *E. coli* in healthy dogs (*E. coli* 83 972),[19,20] our protocol did not require indwelling urinary catheters, and dogs were discharged to their owners soon after the sedative was reversed. These findings support the positive results from our previously published study in which this biotherapeutic was used in 9 dogs with ≥3 UTI within a 12-month period.[12] In that study, 4 of the 9 client-owned dogs had complete or nearly complete clinical cure by day 14. Long-term follow-up urine cultures only were available in 2 of those dogs, and ASB *E. coli* 212 was isolated 60 days after bacterial instillation. These 2 dogs remained free of LUTS for > 6 months. The ASB *E. coli* 212 isolates from that study, which were confirmed by pulse field gel electrophoresis, always were resistant to ampicillin and ticarcillin, allowing us to extrapolate this information for our current study.

We believe we have used the best methodological approach, accounting for both clinical and statistical considerations, to demonstrate noninferiority of ASB *E. coli* 212 compared with antimicrobial treatment for UTI in dogs. The use of a multilevel regression model allowed us to account for the repeated measures design and analyze the data clustered in groups (ie, multiple observations of the same dog over time). The extent of noninferiority is usually better observed using the confidence interval, and for that reason, some researchers prefer the confidence interval approach over the use of *P* values and hypothesis testing.[21] Therefore, we provided both confidence intervals and *P* values to allow a better interpretation of the findings as recommended previously.[22] The selection of the noninferiority margin is also a critical but challenging decision because there is no gold standard criterion for appropriate margins.[23] Our decision was based both on clinical relevance and previous noninferiority trials evaluating UTI in dogs,[4,24] which utilized a 20% margin of difference between the novel treatment and standard of care as well as statistical properties. We assumed that a 2-point margin in the clinical score was reasonable (ie, the new treatment is at least 88% as effective as the standard treatment), and, therefore, we assumed that <12% difference between the new and the standard treatment was clinically unimportant. If we selected 1.5 points of margin (ie, the new treatment is at least 90% as effective as the standard treatment) to be more conservative, we were still able to demonstrate noninferiority. This finding reinforces our noninferiority results when comparing ASB *E. coli* 212 treatment versus antimicrobial treatment for recurrent UTI.

To increase enrollment for our trial, we amended the standard definition of recurrent UTI[25] to include dogs that had >1 UTI in the 12 months before presentation. Unlike our pilot study, dogs that were randomly allocated to group 2 could receive a second ASB *E. coli* 212 administration if their clinical scores were not reduced by at least 50% by day 3. Six of the 16 dogs in this group, in which clinical signs did not sufficiently improve by day 3, had a second intravesicular administration. Whereas 2 of these dogs were considered clinical failures, 4 clinically improved after the second treatment, suggesting the additional infusion might have helped alleviate their clinical signs. Therefore, additional instillation of the ASB strain or higher concentration of ASB *E. coli* 212 might improve outcomes and should be considered if this treatment becomes commercially available, especially when considering the lack of associated adverse events.

In 9 dogs from group 2, ASB. *E coli* 212 was never isolated from their urine specimen at any time point, but 8 of these dogs had improved clinical scores on days 7 and 14. The combination of these short-term microbiologic and clinical results coupled with some durable prophylactic benefits suggests that ASB *E. coli* 212 has potential antimicrobial and analgesic activity, which was noted in murine studies.[9] Of several ASB strains that have been investigated for the treatment of recurrent UTI, ASB *E. coli* 212 has shown the highest analgesic activity in mouse models when compared to *E. coli* 83972, which is why we selected this strain for our studies.[9] Intravesicular or intravaginal administration of ASB *E. coli* 212 in a NU14 *E. coli*-induced cystitis mouse model led to a rapid and significant decrease in UTI-associated allodynia. This effect was greater than that observed with PO ciprofloxacin treatment. Furthermore, ASB *E. coli* 212 exhibited superior analgesic activity compared to ciprofloxacin for UTI induced by non-UPEC bacteria (eg, *Proteus mirabilis* and *Klebsiella pneumoniae*) in mouse studies. These data suggest ASB *E. coli* 212 could have provided analgesia for some of the dogs with clinical recurrent UTI in our study that remained free of clinical signs despite persistent bacteriuria.

Emergence of antibiotic resistance is a global problem and is considered to be an important health concern. Thus, efforts should be made to practice antimicrobial stewardship, thereby decreasing the risk of antimicrobial resistance. UTIs are a common reason for prescribing antibiotics. When UTI is recurrent, the dog should undergo diagnostic evaluation to identify and manage conditions that could predispose to UTI, but an underlying cause is not always identified or, if identified, cannot be eliminated (eg, neoplasia), thus recurrence is expected and therapeutic options eventually decrease. In these cases, veterinarians might elect to use highly or critically important antimicrobials, which might be selected for progressive resistance. The administration of intravesicular ASB *E. coli* 212 has been shown in a previous pilot study and in our current study to be an easy-to-administer alternative, which is not inferior to the use of antimicrobials and is associated with minimal adverse effects. As a result, intravesicular administrations of ASB *E. coli* 212 were well accepted by the dogs' owners, because it replaced the need for daily medication and resulted in noninferior clinical results compared with the conventional antibiotic treatment.

Our study had several limitations, including that it was only intended to evaluate dogs over 14 days. We selected this time frame based on previous studies and our clinical experience in dogs with recurrent UTI. Based on the number of positive UC from dogs in group 1 and increasing LUTS, 14 days appeared to be an appropriate time frame to evaluate for recurrence of bacteriuria and clinical signs. Uncomplicated UTI can be self-limiting in humans[26] and it is possible that neither treatment provided a beneficial effect. However, information from the medical records suggested that longer intervals free of clinical signs were noted in dogs after treatment with ASB *E. coli*

212 compared to before the treatment with this biotherapeutic. Because our study took several years to enroll the necessary number of dogs, we were able to follow a similar number of dogs in each group over a longer time period to evaluate their clinical response. These data provided more support that antimicrobial treatment might be able to be reserved for selected cases of UTI. However, these findings should be interpreted with caution because we did not have longer term follow-up on all dogs, and therefore, we were not certain if they continued to develop recurrent clinical UTI. Cystourethroscopy was not required before study enrollment, but it is uncommon for dogs that have normal genitourinary abdominal ultrasound findings to have clinically relevant urodendoscopy findings.[27] However, 7 dogs in group 1 and 8 dogs in group 2 did have uroendoscopy performed. Except for the 2 dogs with ectopic ureters, no underlying comorbidities to account for the recurrent UTI were noted in the other 13 dogs.

Although we did not perform molecular diagnostics to confirm the ASB *E. coli* 212 after instillation, based on our previous studies, this biotherapeutic has an identifiable antimicrobial susceptibility pattern. Furthermore, if this treatment becomes available for clinical use, it will provide general practitioners a method to easily identify the strain. Finally, the concentration of bacteria in each instillation remained constant. Some of the dogs improved only after a second ASB *E. coli* 212 administration. Thus, it is possible there might be a dose-dependent response to ASB *E. coli* 212 and increased administered concentrations of bacteria could have led to a higher proportion of the dogs achieving clinical cure, microbiological cure, or both.

6 | CONCLUSION

In summary, we demonstrated that up to 2 intravesicular administrations of ASB *E. coli* 2-12 were not inferior to antimicrobial administration for the treatment of recurrent UTI in dogs. Neither group had any serious adverse effects in our study. This biotherapeutic appears to be a reasonable treatment for dogs with recurrent clinical UTI. Future studies should evaluate the ideal dose and frequency of ASB *E. coli* 212 concentrations.

ACKNOWLEDGMENT

Funding was provided by a grant from the Morris Animal Foundation, D20CA-044.

CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Approved by IACUC of the University of California, Davis (protocol #22771) and the Koret School of Veterinary Medicine, The Hebrew University, Jerusalem, Israel (protocol # MD-2016230-3).

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

ORCID

Gilad Segev https://orcid.org/0000-0003-4714-3159
Hilla Chen https://orcid.org/0000-0001-5549-9484
Jonathan D. Dear https://orcid.org/0000-0002-7166-1442
Jodi L. Westropp https://orcid.org/0000-0003-1287-3979

REFERENCES

1. Sorensen TM, Holmslykke M, Nordlund M, et al. Pre-test probability of urinary tract infection in dogs with clinical signs of lower urinary tract disease. *Vet J*. 2019;247:65-70.
2. Llido M, Vachon C, Dickinson M, Beauchamp G, Dunn M. Transurethral cystoscopy in dogs with recurrent urinary tract infections: retrospective study (2011-2018). *J Vet Intern Med*. 2020;34:790-796.
3. Grant DC, Nappier MT, Corrigan VK. Diagnostic accuracy of a point-of-care test using voided urine samples for detection of bacteriuria in dogs with signs of lower urinary tract disease. *J Vet Intern Med*. 2021;35:993-996.
4. Westropp JL, Sykes JE, Irom S, et al. Evaluation of the efficacy and safety of high dose short duration enrofloxacin treatment regimen for uncomplicated urinary tract infections in dogs. *J Vet Intern Med*. 2012;26:506-512.
5. Wong C, Epstein SE, Westropp JL. Antimicrobial susceptibility patterns in urinary tract infections in dogs (2010-2013). *J Vet Intern Med*. 2015;29:1045-1052.
6. Chang SK, Lo DY, Wei HW, et al. Antimicrobial resistance of Escherichia coli isolates from canine urinary tract infections. *J Vet Med Sci*. 2015;77:59-65.
7. Weese JS. Antimicrobial resistance: time for action. *Vet Rec*. 2011;169:122-123.
8. Weber G, Riesenberg K, Schlaeffer F, Peled N, Borer A, Yagupsky P. Changing trends in frequency and antimicrobial resistance of urinary pathogens in outpatient clinics and a hospital in southern Israel, 1991-1995. *Eur J Clin Microbiol Infect Dis*. 1997;16:834-838.
9. Rudick CN, Taylor AK, Yaggie RE, Schaeffer AJ, Klumpp DJ. Asymptomatic bacteriuria Escherichia coli are live biotherapeutics for UTI. *PLoS One*. 2014;9:e109321.
10. Hull R, Rudy D, Donovan W, et al. Urinary tract infection prophylaxis using Escherichia coli 83972 in spinal cord injured patients. *J Urol*. 2000;163:872-877.
11. Darouiche RO, Thornby JI, Cerra-Stewart C, et al. Bacterial interference for prevention of urinary tract infection: a prospective, randomized, placebo-controlled, double-blind pilot trial. *Clin Infect Dis*. 2005;41:1531-1534.
12. Segev G, Sykes JE, Klumpp DJ, et al. Evaluation of the live biotherapeutic product, asymptomatic bacteriuria Escherichia coli 2-12, in healthy dogs and dogs with clinical recurrent UTI. *J Vet Intern Med*. 2018;32:267-273.
13. Bailiff NL, Westropp JL, Jang SS, Ling GV. Corynebacterium urealyticum urinary tract infection in dogs and cats: 7 cases (1996-2003). *J Am Vet Med Assoc*. 2005;226:1676-1680.
14. Stegemann MR, Sherington J, Blanchflower S. Pharmacokinetics and pharmacodynamics of cefovecin in dogs. *J Vet Pharmacol Ther*. 2006;29:501-511.
15. Schumi J, Wittes JT. Through the looking glass: understanding non-inferiority. *Trials*. 2011;12:106.
16. Bates D, Maechler M, Bolker B, et al. Fitting linteral mixed-effects models urine IMe4. *J Stat Softw*. 2015;67:1-48.
17. Vine J. Descriptive interpretations of confidence intervals. R package version 1.0.0. 2017. https://CRAN.R-project.org/package=confinterpret

18. Stevenson M, Evan S, Cord H, et al. Tools for the analysis of epidemiological data. R package version 2.0.70. 2024. https://CRAN.R-project.org/package=epiR
19. Thompson MF, Totsika M, Schembri MA, Mills PC, Seton EJ, Trott DJ. Experimental colonization of the canine urinary tract with the asymptomatic bacteriuria Escherichia coli strain 83972. *Vet Microbiol*. 2011;147:205-208.
20. Thompson MF, Schembri MA, Mills PC, Trott DJ. A modified three-dose protocol for colonization of the canine urinary tract with the asymptomatic bacteriuria Escherichia coli strain 83972. *Vet Microbiol*. 2012;158:446-450.
21. Durrleman S, Simon R. Planning and monitoring of equivalence studies. *Biometrics*. 1990;46:329-336.
22. Greene CJ, Morland LA, Durkalski VL, Frueh BC. Noninferiority and equivalence designs: issues and implications for mental health research. *J Trauma Stress*. 2008;21:433-439.
23. Greene WL, Concato J, Feinstein AR. Claims of equivalence in medical research: are they supported by the evidence? *Ann Intern Med*. 2000;132:715-722.
24. Clare S, Hartmann FA, Jooss M, et al. Short- and long-term cure rates of short-duration trimethoprim-sulfamethoxazole treatment in female dogs with uncomplicated bacterial cystitis. *J Vet Intern Med*. 2014;28:818-826.
25. Weese JS, Blondeau J, Boothe D, et al. International Society for Companion Animal Infectious Diseases (ISCAID) guidelines for the diagnosis and management of bacterial urinary tract infections in dogs and cats. *Vet J*. 2019;247:8-25.
26. Ferry SA, Holm SE, Stenlund H, Lundholm R, Monsen TJ. Clinical and bacteriological outcome of different doses and duration of pivmecillinam compared with placebo therapy of uncomplicated lower urinary tract infection in women: the LUTIW project. *Scand J Prim Health Care*. 2007;25:49-57.
27. Hsieh ES, Palm C, Segev G, Johnson EG, Leung K, Westropp JL. Diagnostic yield of uroendoscopy compared to ultrasonography for evaluating lower urinary tract disorders in dogs. *J Vet Intern Med*. 2022;36:1700-1707.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Segev G, Chen H, Dear JD, et al. Evaluation of the efficacy of a live *Escherichia coli* biotherapeutic product (asymptomatic bacteriuria *E. coli* 212). *J Vet Intern Med*. 2024;38(5):2548-2555. doi:10.1111/jvim.17167