The use of *Escherichia coli* strain Nissle 1917 shows promise for improving gastrointestinal and urinary health in dogs

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**OBJECTIVES**
To investigate the probiotic *Escherichia coli* Nissle 1917 (EcN) in canine idiopathic diarrhea and urinary tract infections.

**ANIMALS/SAMPLES**
The utility of EcN was explored in a 3-phase study from March 2017 to June 2020. Eighty-nine dogs with idiopathic diarrhea were included in phase 1, 3 healthy dogs were included in phase 2, and uropathogenic *E coli* (UPEC) isolates from 38 dogs with urinary tract infections were included in phase 3.

**PROCEDURES**
In phase 1, dogs with diarrhea were prospectively enrolled in a randomized study to receive EcN (10⁸ EcN bacteria/mL; < 10 kg received 5 mL/dose, 10 to 25 kg received 10 mL/dose, or > 25 kg received 15 mL/dose) or placebo for 3 days, followed by a 15-day observation phase. In phase 2, healthy dogs received EcN as described in phase 1, with feces analyzed for *E coli* populations and microbiome composition at days 0, 3, and 7. In phase 3, EcN efficacy was tested by in vitro plate assay against UPEC isolates.

**RESULTS**
Median duration of abnormal stool consistency, time to response, and duration of diarrhea were shorter for dogs that received EcN (5.0, 3.0, and 2.0 days, respectively) versus the placebo (7.0, 5.0, and 4.0 days, respectively) (P = .21, P = .05, and P = .039, respectively). EcN induced shifts in *E coli* diversity in healthy dogs while having minimal impact on overall microbiome structure. Furthermore, 68% of the canine UPEC isolates were susceptible to EcN in vitro.

**CLINICAL RELEVANCE**
EcN improved the treatment of idiopathic diarrhea, colonized the gastrointestinal tract during the trial, and displayed in vitro competition with UPEC.

**Introduction**
Probiotics are a commonly used therapy in human and veterinary medicine.1–2 *Escherichia coli* Nissle 1917 (EcN) is classified as a nonpathogenic probiotic based on phenotypic, biochemical, and genomic criteria.1–6 EcN has been used in human and veterinary medicine for over 100 years due to antimicrobial
properties, stimulation of host defensive mechanisms, restorative effects on epithelial surfaces, and modulation of mucosal immune responses.\textsuperscript{6–9}

EcN is approved for use in Canada and Europe and in human subjects treats diarrhea and alleviates symptoms of irritable bowel diseases, ulcerative colitis, and Crohn’s disease.\textsuperscript{6,10–18} To date, there has been limited evaluation of EcN in veterinary species; however, positive outcomes were observed in calves and pigs.\textsuperscript{19,20} Gastroenteritis and colitis are 2 of the most common problems in domesticated dogs that may benefit from EcN therapy.

EcN also displays antimicrobial activity against human pediatric and feline uropathogenic \textit{E coli} (UPEC) isolates in vitro.\textsuperscript{21,22} Urinary tract infections (UTIs) are one of the most common infections in dogs. UPEC initially resides as a commensal within the intestine and upon introduction into the urinary tract causes the majority of diagnosed UTIs.\textsuperscript{23,24} The use of EcN to eradicate UPEC from the gastrointestinal reservoir would represent a novel potential preventative strategy for UTIs.

Probiotic therapy for gastrointestinal and urinary disease continues to be a rapidly growing research focus. Alternative therapies for these disorders, such as antimicrobial use, are associated with marked impacts on the microbiome and microbial resistance patterns.\textsuperscript{25–27} Based on its use in human gastrointestinal disease and preliminary research in human UPEC isolates, EcN may have the potential to be a useful probiotic in common canine diseases including diarrhea and UTIs.

\textbf{Materials and Methods}

A 3-phase study was conducted to evaluate the ability of EcN to treat idiopathic diarrhea, the effect of EcN on the microbiome, and an in vitro analysis of EcN’s effectiveness against canine UPEC. Phase 1 was carried out in Germany in accordance with German legislation relating to animal welfare. Phase 1 was additionally carried out in compliance with International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use and Good Clinical Practice (GCP) guidelines.\textsuperscript{28} Phases 2 and 3 were approved by the Institution Animal Care and Use Committee at The Ohio State University (2019A00000008 and 2017A00000093). Before enrollment, informed owner consent was acquired for all animals and specimens used.

\textbf{EcN and placebo formulation and dosing (phase 1 and 2)}

The placebo suspension formulation (purified water with NaCl, KCl, MgSO\textsubscript{4}, CaCl\textsubscript{2}, and MgCl\textsubscript{2}) was supplemented with 10\textsuperscript{8} viable EcN bacteria/mL (EcN) under the trade name Ponsocol for use in animals (Pharma-Zentrale GmgH). Formulations were provided in identical 15-mL plastic ampules and stored at 4°C until use with temperature monitoring. The probiotic was provided to owners for at-home administration along with guidelines from the manufacturer on proper storage. Empirical dosage was determined according to body weight: < 10 kg received 5 mL/dose, 10 to 25 kg received 10 mL/dose, or > 25 kg received 15 mL/dose based on manufacturer recommendations. The placebo was matched in body-weight dosing, appearance, and packaging. The pharmaceutical form of the placebo, as well as the shape and color of ampules, corresponded to the verum, except the active substances were not contained. Animals were provided formulations orally by syringe every 12 hours for 3 days, followed by a 15-day observation phase (Supplemental Figure S1). The first dose of either EcN or placebo was administered after the initial examination. All further doses were given in the morning and the evening after feeding the standardized diet (Gastrointestinal, Royal Canin).

\textbf{Phase 1: efficacy of EcN suspension on reducing the duration of idiopathic diarrhea in dogs}

Dogs presenting with at least a 24-hour history of diarrhea were considered eligible for enrollment in a single-blind, placebo-controlled, parallel-group comparison 18-day clinical trial investigating the efficacy of EcN. Dogs were diagnosed with idiopathic diarrhea based on clinical history with the only noted problem being diarrhea, physical examination that was not indicative of systemic disease, and diagnostics including at least a complete blood count, biochemical profile, and negative zinc sulfate fecal floatation without clinically significant abnormalities. Additional diagnostics were performed at the discretion of the attending clinician based on individual case presentation. Dogs were not eligible for enrollment if they were unable to be fed the standardized easily digestible diet for the duration of the study. Diet change was immediate without a transition period. Dogs were not eligible if they had received EcN in the previous 3 months; had evidence of concomitant disease (eg, cardiac, hepatic, renal); had obstructive or mechanical gastrointestinal dysfunction; were receiving antimicrobials, probiotics, immunosuppressives, or antidiarrheal treatments within 3 months of enrollment; underwent a diet change within 2 weeks before enrollment; or were already on the study diet at the time of enrollment. Dogs were considered to have acute diarrhea if present for less than 14 days, chronic diarrhea if present for greater than or equal to 14 days, or acute on chronic if experiencing an acute relapse of previous controlled chronic diarrhea.

Once enrolled, dogs were randomized to receive either EcN or a placebo (Figure 1). Dogs were evaluated at study inclusion on day 4 and at the conclusion of the study on day 18. Dogs were monitored using an owner diary for efficacy and safety until completion of the study (Supplemental Appendix S1). Owner diaries were completed on a twice-daily basis until study day 4 and then continued on a daily basis until completion of the study. Completion of dairy entries was verified by investigators on study days 4 and 18. All owners completing
Dogs Enrolled
n=89

EcN Group
n=48

Placebo Group
n=41

Dogs excluded due to drop-outs, lost to follow-up, or protocol deviations.

EcN Group
n=38

Placebo Group
n=33

Figure 1—CONSORT diagram for phase 1. A total of 89 dogs were included in phase 1 of the study. There were 48 dogs randomized to the *Escherichia coli* Nissle 1917 (EcN) group and 41 dogs randomized to the placebo group. There were 10 (20.8%) dogs excluded from the EcN group and 8 (19.5%) dogs excluded from the placebo group due to study drop out, lost-to-follow-up, or major protocol deviations. In the 60% of 10 dogs in the EcN group, the study was prematurely terminated due to lack of efficacy compared to 7 (87.5%) of 8 dogs in the placebo group (Fisher’s exact test, *P* = .31). A total of 38 dogs completed in the EcN group and a total of 33 dogs completed in the placebo group.

The study were fully compliant. The primary efficacy endpoint was the sum of days with abnormal stool consistency (normal stools, mild, moderate, or severe diarrhea). Secondary efficacy variables were time to response, dichotomous overall outcome classified as “responder” or “nonresponder,” change in stool consistency, owner assessment of health (very good, good, moderate, or poor), owner assessment of tolerability (very good, good, moderate, or poor), and owner assessment of efficacy (very good, good, moderate, or poor). Outcome was defined as responder if stool consistency was normal for at least 3 consecutive days before the end of the study. The 3 days of normal stool consistently could include the final 3 days of the study. If clinical signs returned after normalization, the dog was not classified as a responder. Safety and tolerability were assessed by monitoring adverse events, laboratory parameters, as well as owner-assessed tolerability and general state of health. The severity criteria and causality criteria of adverse events were based on WHO Collaborating Centre for International Drug Monitoring guidelines.

**Evaluation of short-term colonization of EcN in dogs with acute diarrhea by PCR assay (phase 1)**—To investigate whether EcN was able to pass through and colonize the gastrointestinal tract, fecal samples were obtained from dogs in phase 1. Using an online random number generator, 6 animals were selected randomly from the EcN treatment group and 4 were selected randomly from the placebo group for further analysis. Feces were collected on study days 1, 2, 3, 4, 6, 10, and 18 and stored at −80°C until analysis. DNA was extracted from the fecal samples using a commercial kit (QiAamp Power Fecal DNA; Qiagen). A multiplex PCR assay with 3 primer sets designed to amplify 3 genetic loci of EcN was used. PCR amplification of EcN-specific genes was accomplished using the primer pairs Muta 5/6, Muta 7/8, and Muta 9/10 as previously described. This multiplex PCR approach detects the native plasmids pMUT1 and pMUT2, which are stable and thus well suited for the detection of the EcN strain. The relative abundance of EcN is reported based on the PCR band signaling strength from weak (+) to strong (++++) on each day analyzed.

**Phase 2: pilot study on the impact of EcN on healthy dog microbiome and quantitative analysis of strain level diversity of E coli in healthy dogs following ingestion of EcN**

Three healthy dogs based on clinical history, physical examination, CBC, biochemical profile, and urinalysis prior were enrolled in the phase 2 pilot study. All dogs had no reported gastrointestinal signs, had not received probiotic/antimicrobials, or had diet changes in the 6 months before the study. Each dog received EcN (10⁸ viable EcN bacteria/mL) orally at the same dosing regimen used in phase 1. The validated competition-based plate assay and the PCR assay described above were used to assign an individual strain as EcN or non-EcN. Only *E coli* isolates that were positive for competition and contained the EcN-specific genes by PCR assay were identified as EcN, all other isolates were deemed to be non-EcN strains of *E coli*. Assays were completed in triplicate on 3 independent occasions.

Twenty individual colonies were isolated from the feces on lactose MacConkey agar to select for *E coli*. These could be either random *E coli* strains or EcN as they are morphologically indistinguishable. Each strain was first screened for the ability to be outcompeted by EcN as a first indicator that the isolate may be EcN by evaluation of the inhibition of microbial growth of a laboratory-adapted strain, MG1655, using a previously validated plate assay. In brief, each individual fecal isolate and the MG1655 strain were grown separately in Luria broth centrifuged at 8 X g and 37 °C for 24 hours. The MG1655 strain was spread on a Luria broth agar plate. The antagonist, the fecal isolate, was then stab inoculated into the background. Following inoculation, the plate was incubated aerobically at 37 °C and examined at 24 hours. A positive result was defined as an area of clearing (growth inhibition) around the stab of the fecal isolate. A negative result was defined as the absence of a zone of clearing. As a secondary measure, each *E coli* isolate was also analyzed using the EcN-specific multiplex PCR analysis described above.
Freshly voided fecal samples were collected from the 3 healthy dogs in the pilot study (phase 2) at baseline and on study days 3 and 7. Feces were flash frozen and stored at -80 until DNA extraction. DNA was extracted from the feces using standard protocols and a commercially available kit (QIAamp PowerFecal DNA kit; Qiagen) according to manufacturer instructions.

The microbiome impact of EcN was assessed using 16S ribosomal RNA (16S rRNA) sequencing analysis. The hypervariable regions V3 to V5 of the 16S rRNA genes were amplified from purified genomic DNA using primers 341F and 926R. PCR amplification was performed according to the protocol developed by the Human Microbiome Project.\(^{39}\) Nextera indexes were added to the purified amplicons based on the Illumina MiSeq specifications (Illumina). 16S rRNA amplicon libraries were purified, quantified using quantitative PCR, and pooled for sequencing on Illumina MiSeq platform (Illumina). Paired-end reads of 300 bp were obtained for each sample.

A data-cleaning process was applied to all sequence data before analysis. Briefly, low-quality bases with a Phred quality value lower than 20 were trimmed off the read ends. The read pairs were removed if any of the 2 reads were trimmed to shorter than 200 bp or had ≥ 3% uncertain bases. The 16S rRNA sequences with paired reads were analyzed using an open-source bioinformatics pipeline, Quantitative Insights Into Microbial Ecology (QIIME).\(^{31}\) The paired-end reads were joined by overlap, and the assembled sequences were clustered with 97% similarity threshold. Taxa with an average ≥ 1% relative abundance in each sample are shown. The microbial community evenness and richness were measured by alpha diversity, and the similarity between individual microbial communities was measured by beta diversity. Alpha diversity (Shannon index) and beta diversity (weighted and unweighted UniFrac) were calculated using QIIME.

Phase 3: susceptibility of UPEC isolates to EcN-mediated competition

In phase 3, EcN was tested against canine UPEC isolates from dogs that had been diagnosed with a clinical UPEC UTI. All dogs with positive E coli urine cultures were eligible for inclusion in this study. Once a dog was identified with positive urine culture for E coli, the owner was contacted and signed consent was obtained to include residual culture samples in the study. Clinical case data from each dog was collected and at minimum included a complete clinical history, physical examination, urinalysis, urine culture, and susceptibility results. Competition of UPEC growth by EcN was assessed using a previously validated and described plate assay.\(^{22}\) The area of clearing or overgrowth was measured at maximal diameter. Assays were completed in triplicate on each plate and the experiment was repeated 3 times for each canine UPEC strain. The maximal diameter of inhibition or overgrowth were averaged among the total number of tests.

Statistical analyses

All data were tested for normality with the Shapiro-Wilk test. Normally distributed data are reported as mean and standard deviation, and nonparametric data are reported as median and range. In phase 1, Fisher’s exact test, chi-squared test, 2-tailed unpaired t-test, and Mann-Whitney test were used to test for differences between placebo and experimental groups. Kaplan-Meier analysis was performed for the median time to response in dogs in phase 1. In phases 2 and 3, descriptive statistics are reported for all results. Nonparametric multivariate analysis (ANOSIM) was conducted in Mothur to test whether the microbial community similarity within groups is statistically different from the similarity between groups. Alpha diversity between groups was compared using a 2-tailed, unpaired t-test. Statistical significance was set at \(P < .05\).

Results

Phase 1

A total of 89 dogs were screened for enrollment into the study (EcN group, \(n = 48\); placebo group, \(n = 41\)). There were no differences in age (EcN group, 5.2 years [0.4 to 15 years]; placebo group, 4.3 years [0.3 to 12 years]), body weight (EcN group, 22.0 kg [3.5 to 50 kg]; placebo group, 23.3 kg [2.4 to 74 kg]), or sex (EcN group, 50% male, 50% female; placebo group, 48.8% male, 51.2% female) between groups (\(P\) values all > .05). EcN and placebo groups did not differ significantly in regards to clinical data pertaining to type of diarrhea (all \(P\) values > .05) (Table 1). Two dogs (experimental group, \(n = 2\)) were removed from the study due to concurrent disease. Three dogs were lost to follow-up (experimental group, \(n = 2\); placebo group, \(n = 1\)). There were 13 dogs (experimental group, \(n = 6\); placebo group, \(n = 7\)) who prematurely terminated study participation due to lack of efficacy. The dogs terminating due to lack of efficacy occurred during both the initial 3-day treatment phase and afterward during the observation phase in both groups. The number of dogs terminating prematurely did not differ between groups (\(P = .31\)). There were 71 dogs (experimental group \(n = 38\); placebo group, \(n = 33\)) who completed the entire study period (Figure 1). Efficacy results are reported for this study population.

The median [mean] number of days with abnormal stool consistency was significantly decreased in subjects receiving EcN (5.0 [6.1] days, 1 to 18) vs the placebo (7.0 [8.5] days, 1 to 18) (\(P = .021\)). The time to response was also significantly reduced in the EcN-treated subjects (3.0 [5.6] days, 2 to 16) compared to placebo (5.0 [8.2] days, 1 to 16) (\(P = .050\)) (Figure 2). In addition, the duration of the diarrhea was significantly reduced in the EcN-treated subjects (2.0 [4.9] days, 1 to 18) compared to placebo (4.0 [7.9] days, 1 to 18) (\(P = .039\)). The overall response rate was higher in the EcN group (89.5%) compared to the placebo group (75.8%), but these results were not statistically different (\(P = .11\)).}

Stool consistency at baseline, stool consistency at the final visit on day
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18, and change in stool consistency between base-
line and final visit did not differ significantly between
the EcN and placebo groups ($P = .22$, $P = .35$, and
$P = .31$, respectively).

A subgroup analysis was performed for the pri-
mary outcome variable based on the type of diar-
rhea at presentation. The median [mean] number
of days with abnormal stool consistency in the
acute diarrhea dogs was not significantly decreased
in the subjects that received EcN (5.0 [5.8] days,

Table 1—Descriptive variables characterizing the
diarrhea as well as the owner’s perception of the
Escherichia coli Nissle 1917 (EcN)/placebo efficacy,
tolerability, and overall health assessment for patients
enrolled in the phase 1 diarrhea response trial.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group</th>
<th>EcN</th>
<th>Placebo</th>
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<tr>
<td>Reproductive status</td>
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<tr>
<td>Acute</td>
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<td>17</td>
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<tr>
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<td>2</td>
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<tr>
<td>&lt; 3/day</td>
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Categorized data for the entire population are presented as reported at study inclusion. Data on overall health assessment at time of study inclusion and change in health assessment over the course of study (day 1 compared to day 18) are also reported. Categorized data for the entire population completing the trial are presented.
1 to 16) versus the placebo (6.5 [7.4] days, 1 to 18) \((P = .141)\). The median [mean] number of days with abnormal stool consistency in the chronic diarrhea dogs was significantly decreased in the subjects that received EcN (5.0 [5.7] days, 1 to 15) vs the placebo (8.0 [9.7] days, 1 to 18) \((p = .019)\). There were insufficient numbers to analyze the acute on chronic diarrhea dogs separately.

There was no significant difference in the global assessment of the efficacy of the trial medication, tolerability, global assessment of health, or change in global health assessment throughout the study as scored by the owners \((P \text{ values } > .05)\) (Table 1). Adverse events (AEs) were not significantly different between the EcN (12.5%) and placebo groups (4.5%) \((P = .28)\). AEs included progressive diarrhea \((n = 3)\), vomiting \((2)\), liver enzyme activity \((1)\), kidney tumor \((1)\), and hematochezia \((1)\). These AEs include dogs who were removed due to identification of concurrent disease. All but 1 AE were causally listed as not related according to WHO guidelines. The remaining AE, 1 dog with self-limiting vomiting, was classified as possibly caused by the EcN probiotic. The majority (94.6%) of study participants rated their dog's tolerability of EcN to be good or very good.

The presence of EcN was determined by PCR analysis of the feces on each day indicated after initiation of the study (Supplemental Table S1). EcN was not detected in the feces of the dogs on the first day of treatment, except for a weak signal that was observed in the feces of dog 37. On the second day, EcN was already detected in the feces of 4 out of 5 dogs that received EcN (subjects 31, 33, 37, and 39). One subject (number 35) did not provide a fecal sample on day 2. Although variable in intensity, EcN was detected in the feces of all the dogs that received EcN. All of the feces in the placebo treatment group were negative at all times evaluated. EcN is readily shed in the feces of diseased dogs that receive EcN for a minimum of 15 days after the conclusion of treatment.

Phase 2

Twenty individual colonies of \(E\) \textit{coli} were isolated from the shed feces at baseline and 7 days following ingestion of EcN. None of the colonies were determined to be EcN before ingestion (Figure 3). However, on day 7, 75% to 90% of the isolates were determined to be EcN, suggesting that EcN becomes the predominant \(E\) \textit{coli} strain. The alpha diversity, measurement of richness and evenness of the microbial community, was not significantly different before, during, and after EcN ingestion in the 3 dogs analyzed (Figure 3). The beta diversity, measurement of the similarity between samples, was not significantly different among the samples collected from the same dogs before, during, and after EcN ingestion, suggesting that EcN does not alter the overall microbiome structure. The beta diversity significantly differed among individual dogs (ANISOM; weighted,
The phase 3 study involved 38 UPEC isolates from 38 individual dogs with UTI included in the study. Thirty-four of these dogs were females (29 spayed, 5 sexually intact) and 4 dogs were males (2 neutered, 2 sexually intact). The median age at diagnosis was 9 years (range, 0.29 to 16 years). Dogs were reported as mixed breeds (n = 10), Labrador Retrievers (6), West Highland White Terrier (3), Shetland Sheepdogs (2), Cavalier King Charles Spaniel (n = 2), Golden Retriever (2), Toy Poodle (2), Miniature Dachshund (2), and Yorkshire Terrier (2) and 1 of each of the following: English Bulldog, Pug, Havanese, Brussels Griffon, English Springer Spaniel, and Miniature Pinscher.

Clinical signs varied among these dogs. Twenty-eight dogs were reported to show primarily lower urinary tract signs (pollakiuria, stranguria, dysuria, frequent voiding, and frequency). Ten dogs exhibited primarily lower urinary tract signs (pollakiuria, stranguria, dysuria, frequent voiding, and frequency). Eight dogs had a history of previous UTI previously confirmed by urine culture and sensitivity testing. Eight dogs had a history of previous UTI previously confirmed by urine culture and sensitivity documented in the medical record. Two dogs were suspected of having pyelonephritis at the time of diagnosis of the UTI. Presumptive pyelonephritis was suspected based on a positive urine culture in combination with systemic signs of illness (fever, polyuria, and polydipsia) and azotemia. Comorbidities were present in many of the dogs including diabetes mellitus (n = 10), neoplasia (2), chronic enteropathy (2), atopic dermatitis (2), neurogenic bladder (2), urinary incontinence (2), and hyperadrenocorticism (1). The results of the urinalyses are summarized in Table 2. All urine samples were collected by cystocentesis using standard protocol. Active sediments were a frequent finding characterized by changes in color, turbidity, hematuria, inflammatory cells, and presence of bacteria. Canine UPEC susceptibility and sensitivity testing was performed for 16 clinically relevant antimicrobials and the results are presented in Table 2.

With the use of the plating assay, 68% (26) of the isolates in this study were susceptible to EcN competition. There was no effect noted in 12 strains (32%) in this population. The average effect zone was 5.1 mm (SD = 1.61 mm). The 2 isolates resistant to 2 separate antimicrobial classes were susceptible to EcN (n = 2). The 3 isolates resistant to 4 separate antimicrobial classes were also susceptible to EcN (n = 3). The isolate resistant to 5 antimicrobial classes was not susceptible in vitro to EcN. Thus, EcN appears to be effective against the majority of dog UPEC strains including strains that exhibit significant resistance to clinically relevant antimicrobials.

### Table 2—Descriptive variables characterizing the urinalyses associated with urinary tract infection (UTI) patients enrolled in the phase 2 uropathogenic Escherichia coli (UPEC) susceptibility trial.

<table>
<thead>
<tr>
<th>Property</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5 5.5 6 6.5 7 7.5 8 8.5 9</td>
</tr>
<tr>
<td>No. of dogs</td>
<td>6 0 8 5 3 1 10 2 3</td>
</tr>
<tr>
<td>Color</td>
<td>Yellow Light yellow Red</td>
</tr>
<tr>
<td>No. of dogs</td>
<td>31 6 1</td>
</tr>
<tr>
<td>Turbidity</td>
<td>Clear Cloudy Hazy</td>
</tr>
<tr>
<td>No. of dogs</td>
<td>18 9 11</td>
</tr>
<tr>
<td>Squamous cells</td>
<td>None Rare 0-1/hpf 1-2/hpf 2-5/hpf 5-10/hpf 10-15/hpf</td>
</tr>
<tr>
<td>No. of dogs</td>
<td>18 4 11 4 0 1 0</td>
</tr>
<tr>
<td>Transitional cells</td>
<td>None Rare 0-1/hpf 1-2/hpf 2-5/hpf 5-10/hpf 10-15/hpf</td>
</tr>
<tr>
<td>No. of dogs</td>
<td>22 7 4 4 1 0 0</td>
</tr>
<tr>
<td>RBC</td>
<td>None Rare 0-1/hpf 1-2/hpf 2-5/hpf 5-10/hpf 10-15/hpf</td>
</tr>
<tr>
<td>No. of dogs</td>
<td>3 7 4 6 3 7 8</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>None Rare 0-1/hpf 1-2/hpf 2-5/hpf 5-10/hpf 10-15/hpf</td>
</tr>
<tr>
<td>No. of dogs</td>
<td>2 2 4 0 2 10 18</td>
</tr>
<tr>
<td>Protein</td>
<td>None Negative Trace 1+ 2+ 3+</td>
</tr>
<tr>
<td>No. of dogs</td>
<td>5 8 10 10 5</td>
</tr>
<tr>
<td>Glucose</td>
<td>None Negative Trace 1+ 2+ 3+</td>
</tr>
<tr>
<td>No. of dogs</td>
<td>28 0 3 0 7</td>
</tr>
<tr>
<td>Bacteria</td>
<td>None Rare Present Many TNTC</td>
</tr>
<tr>
<td>No. of dogs</td>
<td>7 5 9 4 13</td>
</tr>
</tbody>
</table>

All urine samples were from dogs where urine cultures yielded at minimum 105/mL colony counts for E. coli. Categorized data for the entire population are presented as reported at time of UTI diagnosis. 

hpf = Hours postfertilization. RBC = Red blood cell. TNTC = Too numerous to count.
Discussion

This study indicates a potentially clinically important role of the EcN probiotic in small animal veterinary medicine. In all study phases, there were positive effects of the probiotic observed. In phase 1, diarrhea was resolved sooner, resulting in a longer period of time diarrhea free in the EcN group than in the placebo group. In phase 2, EcN remained within the shed feces of healthy dogs without significant changes to the microbiome structure. In phase 3, EcN demonstrated similar effectiveness against canine UPEC strains as has been demonstrated previously for human and feline isolates in vitro. Importantly, this study utilized 2 highly prevalent and often frustrating clinical scenarios for owners and veterinarians alike, diarrhea and UTIs that are commonly treated empirically with antimicrobials. The need for novel, probiotic approaches like EcN to manage infections grows rapidly as published data continue to increasingly describe the detrimental effects of antimicrobial use on the microbiome and microbial resistance patterns.25,26

In this study, dogs presenting with idiopathic diarrhea of different durations and enrolled in phase 1 demonstrated reduced duration of clinical signs and improved response times following administration of a short course of EcN as compared with placebo. Previously, multiple different probiotics have been used in the treatment of both acute and chronic enteropathies in dogs.1,2 These studies provide some evidence for the use of probiotics in a preventative manner for acute enteropathy, infectious enteropathies, and treatment for nonspecific acute diarrhea. Studies in chronic enteropathies of different etiologies have often been confounded by the concurrent use of traditional therapies making interpretation of results challenging. To our knowledge, there are 2 studies that have shown an effect of probiotics in chronic diarrhea management. The first study used a single strain Lactobacillus acidophilus probiotic, and it resulted in improved defecation frequency, fecal consistency, and fecal dry matter. The second study used a multistrain probiotic in combination with a high fiber, easily digestible, hypoallergenic diet. This study demonstrated an improvement in clinical signs; however, the exact role of the diet versus the probiotic was unable to be discerned in the absence of control groups. In both of the studies demonstrating an effect, a Lactobacillus acidophilus strain was all or part of the probiotic product. However, neither study investigated an Escherichia coli probiotic like EcN, which is documented to have very different effects on the gastrointestinal tract and on host responses. Furthermore, direct comparisons to the previous literature are challenging given not only the differences in probiotics but also study design, disease processes, probiotic dosing, and concurrent therapies. However, the results of phase 1 of this study expand the existing literature demonstrating the benefit of probiotics in idiopathic diarrhea.

The dogs enrolled in phase 1 comprised a population presenting with a mix of acute, acute-on-chronic, and chronic forms of diarrhea and often lacked a definitive cause of the clinical signs. This is a limitation of the study and its ability to be directly applied to specific clinical scenarios. However, a positive effect of EcN in this varied population was noted, and further study should be pursued looking at subsets of dogs with specifically defined gastrointestinal disorders and clinical signs. In human studies of chronic gastrointestinal disease, EcN has shown efficacy in ameliorating gastrointestinal symptomology in multiple diseases. However, it is possible that EcN is more or less effective in certain situations than others for dogs, and these data would help guide future clinical recommendations. The probiotic should also be studied in a preventative setting as well where it may be able to minimize the occurrence of severity of diarrhea similar to other probiotics by either microbial
shifts or induction of favorable immune responses by the host providing a protective effect.

Direct PCR from prepared fecal samples showed the successful passage of EcN through the gastrointestinal tract. The data in this study demonstrate EcN sheds for up to 2 weeks following ingestion, suggesting at least transient colonization of the gastrointestinal tract and providing a potential additional opportunity for long-term effects even after probiotic cessation. This is in contrast to many other commercially available Lactobacillus and Bifidobacterium-based probiotics that do not typically engraft into the microbiome.66,67 The potential for engraftment and resulting long-lasting effects following cessation of administration are a potential major benefit to the EcN probiotic.

Phases 1 and 2 of the study also demonstrated that EcN product administered in live dogs was well tolerated. There was no difference in AE rate between the EcN and placebo groups and the majority (94.6%) of study participants rated their dog’s tolerability of EcN to be “good” or “very good.” The only AE determined by preset criteria to be potentially related to EcN administration resolved within 24 hours and did not require removal from the study or cessation of EcN administration. Future safety studies should be designed to examine EcN effects on all aspects of host health and safety including the systemic and local effects. Additional studies beyond our preliminary information on the impact of EcN on the gastrointestinal microbiome are also needed. From these studies, it is unclear whether the changes in abundance of EcN correlates with overall changes in the shedding of E coli or due to variations in the shedding of EcN. Nonetheless, the phenotypic, genomic, and biochemical data published currently indicate a high inherent level of safety with this probiotic.3–5

Coriobacteriaceae were decreased after EcN treatment and is consistent with the previous study by Mokszycki et al46 that showed that Actinobacteria, specifically those of the family Coriobacteriaceae, were found only in the absence of colonized EcN. This suggests that EcN may compete against Coriobacteriaceae in the gut microbiome. It is possible that other microbiome shifts occurred at a lower taxonomic level than our analysis. Full comparison of individual taxa within our subjects was not completed given the low number of healthy dogs used in the phase 2 pilot study. Comparison of individual taxa across dogs was not performed as the microbiomes were already considered different based on beta-diversity analysis.

The aim of the third phase of this study was to assess the in vitro bactericidal efficacy of EcN against E coli cultured from urine samples of dogs presenting with UTI. UPEC colonizes the gastrointestinal tract and enters the urinary tract through direct contamination of fecal material into the bladder to cause UTI.23,24 The empiric use of antimicrobials was once thought to do no harm and that treatment is better than neglecting an infection. However, recent studies underscore the importance of antimicrobial stewardship and emphasize the ineffectiveness of antimicrobials in the eradication of UPEC from the primary reservoir in the gastrointestinal microbiome. The results of this phase demonstrate the canine UPEC strains share many similarities with human and feline UPEC strains in susceptibility to the EcN probiotic when tested in vitro, including a 68% effectiveness rate against tested strains.21,22 Antimicrobial stewardship and patient management could be greatly advanced through the incorporation of EcN to reduce UTI through competition and eradication of UPEC from the canine gastrointestinal reservoir.

Previous in vitro and in vivo studies50,51 have demonstrated successful EcN colonization in the bowel and that displacement of pathological intestinal bacteria is mediated by competition. Thirty-two percent of isolates were unaffected by the presence of EcN. It is unknown whether this was due to the presence of immunity factors within the UPEC isolates, similar growth rate characteristics between organisms, or a separate response by the specific UPEC strains that were unaffected. Interestingly, we observed that EcN was effective against antimicrobial-resistant UPEC isolates, suggesting that EcN could be used to reduce the burden of antimicrobial-resistant strains of E coli within the gastrointestinal tract. Additionally, host mucosal response to the probiotic may play a role in the efficacy of the probiotic and should be examined. Further research in this area could expand our understanding of canine UPEC biology.

The use of probiotics for UTIs is an ongoing area of research in human medicine where in vitro success with the EcN probiotic has been documented against pediatric uropathogens.21 However, there have been much less frequent attempts in veterinary medicine to examine the ability of probiotics to provide a health benefit in the setting of UTI. In 2 studies52,53 of healthy dogs and dogs with recurrent UTI, the effect of lactic-acid producing bacteria probiotics was examined with mixed results. The basis of this probiotic approach is that the production of lactic acid changes the vaginal pH making it challenging for uropathogenic infections. Alternatively, EcN would provide a novel probiotic approach to UTI by directly competing with and excluding uropathogens from the gastrointestinal reservoir.

If the results of this study can be translated to an in vivo model, it will represent a paradigm shift in the way that canine UTI patients could be managed in clinical practice. As such, EcN represents a promising novel therapeutic strategy for preventing UTI patients in veterinary medicine. In dogs, UTIs afflict animals of all ages as well as those with urogenital maldevelopment, micturition disorders, or systemic disease.22 Furthermore, increasing attention in both human and veterinary medicine has focused on the prevention of subclinical bacteriuria, uncomplicated UTIs, and recurrent UTIs, which previously relied on antimicrobial prophylaxis, a strategy originally recommended based solely upon expert opinion.23 This in turn has led to the frequency of multiple antimicrobial-resistant UPEC to increase significantly over the last 20 years and an urgent need for nonantimicrobial approaches.24 In addition, the transmission of
E coli between humans and their pets is known to occur, and sharing of E coli is more common if one member of the family (animal or human) has a history of UTI, suggesting that antimicrobial-resistant strains could be spread within the household. The in vitro efficacy of EcN against antimicrobial-resistant dog strains of UPEC suggests that the use of EcN will reduce sharing of antimicrobial-resistant strains within households and positively contribute to the One-Health Initiative.

There are also limitations to this study that are important to address. As mentioned previously, phase 1 was completed on dogs with nonspecific idiopathic diarrhea. This may have affected the results and further study is needed on specific disease syndromes and subclassifications of dogs affected by diarrhea to better guide its use. Additionally, this study was short in duration. All dogs responding had a sustained response, but the short duration of the observation period may have precluded our ability to detect relapses in these dogs in a longer term setting. Further studies should examine the long-term impact, ability to control clinical signs, and effect of the probiotic in dogs with diarrhea. Phase 2 was a pilot study, and while the results were promising of a minimized microbiome effect, much larger studies are needed to confirm the results of the phase 2 pilot study. Further study should also expand beyond the impact on the microbiome and consider both the host mucosal response as well as metabolic impact. Finally, phase 3 is an in vitro study and for clinical efficacy a pivotal study translating these studies into an in vivo model is required.

Conclusion

This report provides evidence for the use of EcN in the treatment of diarrhea, short-term colonization of the gastrointestinal tract following administration, preliminary evidence for effectiveness against UPEC, and initial safety data in a population of dogs. Further studies are required to better assess the efficacy of EcN in specific gastrointestinal disorders, as a preventative for gastrointestinal signs, and in vivo against canine uropathogens and to determine the utility of EcN as a UTI prevention tool in dogs.

Acknowledgments

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The results of this study were presented in part, as an oral abstract, at the American College of Veterinary Internal Medicine Symposium, Phoenix, AZ, June 2019.

References


**Supplementary Materials**

Supplementary materials are posted online at the journal website: avmajournals.avma.org.