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Escherichia coli-associated granulomatous colitis in dogs treated according to antimicrobial susceptibility profiling

Alison C. Manchester | **Belgin Dogan** | **Yongli Guo** | **Kenneth W. Simpson**

Cornell University College of Veterinary Medicine, Ithaca, New York

Correspondence
Alison C. Manchester, 230 Translational Medicine Institute, 2350 Gillette Dr, Fort Collins, CO, 80523.
Email: alison.manchester@gmail.com

Kenneth W. Simpson, Cornell University, College of Veterinary Medicine, VMC2011, 602 Tower Road, Ithaca, NY, 14853.
Email: kws5@cornell.edu

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Abstract

Background: Eradication of intramucosal *Escherichia coli* correlates with remission of periodic acid-Schiff-positive *E coli*-associated granulomatous colitis (GC). Treatment failures attributed to multidrug resistant (MDR) bacteria necessitate alternative approaches.

Hypothesis/objectives: Determine clinical outcome of *E coli*-associated GC in dogs treated based on antimicrobial susceptibility profiling and characterize *E coli* phylogeny and resistance mechanisms.

Animals: Twenty Boxers and 4 French Bulldogs with *E coli*-associated GC.

Methods: Culture, antimicrobial susceptibility profiling, and molecular characterization of *E coli* were performed and response to treatment was evaluated.

Results: Initial biopsy sample culture yielded fluoroquinolone-sensitive (FQ-S) *E coli* from 9/24 dogs and fluoroquinolone-resistant (FQ-R) *E coli* from 15/24. All but 1 FQ-R *E coli* were MDR with susceptibility to macrophage-penetrating antimicrobials restricted to carbapenems in 13/15 dogs. Of 22/24 treated based on susceptibility profiling, 8/9 FQ-S dogs had complete initial clinical response (CR) during fluoroquinolone (FQ) treatment, whereas 9/13 FQ-R dogs had complete or partial response (PR) during meropenem or doxycycline treatment. In 5/9 FQ-S and 12/13 FQ-R dogs with follow-up ≥3 months, CR was sustained in 5/5 FQ-S (median, 25 months; range, 4-46) whereas 6/12 FQ-R had long-term CR (median, 59 months; range 15-102), 4/12 PR (median, 19 months; range, 5-65), and 2/12 had no response (NR). Four dogs with long-term follow-up died within 4 years of diagnosis, including 2 euthanized for refractory colitis. *Escherichia coli* were genetically diverse. Fluoroquinolone resistance was associated with mutations in *gyrA* and *parC*, with plasmid-mediated resistance less common.

Conclusions and Clinical Importance: Antimicrobial treatment guided by susceptibility profiling was associated with positive long-term outcomes in >80% of cases.

Abbreviations: PAS, periodic acid-Schiff; GC, granulomatous colitis; AIEC, adherent-invasive *E coli*; CD, Crohn's disease; FQ, fluoroquinolone; FQ-S, fluoroquinolone-sensitive; FQ-R, fluoroquinolone-resistant; FISH, fluorescence in situ hybridization; MIC, minimum inhibitory concentrations; MDR, multidrug resistant; CR, complete clinical response; PR, partial clinical response; NR, no response; RAPD, random amplified polymorphic DNA; PMQR, plasmid-mediated quinolone resistance; QRDR, quinolone-resistance determining region.

Kenneth W. Simpson contributed equally to this manuscript as the corresponding author.

Fluoroquinolone-resistance was widespread and not clonal. Further study is required to optimize treatment for dogs with MDR *E coli*-associated GC.

KEYWORDS
antibiotic, boxer, canine, diarrhea, fluoroquinolone, resistance

1 | INTRODUCTION

Mucosally invasive *Escherichia coli* are causally associated with the etiopathogenesis of periodic acid-Schiff (PAS)-positive granulomatous colitis (GC) of Boxer dogs, French Bulldogs, and breeds in the mastiff cluster.[1-5] *Escherichia coli* typically are observed in clusters within macrophages, and genomic and functional analyses indicate they are most similar to extraintestinal pathogenic *E coli*, including adherent-invasive *E coli* (AIEC) associated with Crohn's disease (CD).[1,6-8] Periodic acid-Schiff-positive *E coli*-associated GC is familial in Boxers and French Bulldogs, with genetic susceptibility linked to a region encoding the CD48/SLAM family of genes on chromosome 38.[5] Genes in this region have been implicated in IBD in humans, and the selective sensing and killing of *E coli* by murine macrophages,[9-11] suggesting that susceptibility of Boxers and French Bulldogs to this form of GC is related to ineffective killing of intracellular *E coli*.

Clinical and histopathological remission of *E coli*-associated GC hinges upon eradication of intramucosal *E coli*.[1,3] Uniformly positive clinical responses initially were observed in dogs treated using fluoroquinolone (FQ) antimicrobials.[1,12,13] The positive clinical outcomes with FQs and chloramphenicol[2,3] but not various other antimicrobials[12-14] suggest that a drug's ability to penetrate macrophages and kill intracellular *E coli* is important.[15] However, resistance to FQs emerges rapidly and correlates with failure to respond fully to prescribed treatments and, in many cases, euthanasia.[3,14,16]

The purpose of our study was to determine if antimicrobial susceptibility profiling of colonic *E coli* improves the clinical outcome of dogs with *E coli*-associated GC. Isolates were tested against 12 antimicrobials, including 5 known to penetrate macrophages. The initial responses to treatment and long-term clinical responses were determined for dogs with fluoroquinolone-sensitive (FQ-S) and -resistant (FQ-R) *E coli*. Additionally, we evaluated the relationship of *E coli* genotype and phylogroup to antimicrobial resistance (AMR) and the molecular basis of FQ-R.

2 | MATERIALS AND METHODS

2.1 | Patients and identification of mucosal *E coli*

Inclusion criteria for our study were histologic evidence of GC with PAS-positive macrophages, confirmation of intramucosal *E coli* based on fluorescence in situ hybridization (FISH), and *E coli* cultured from intestinal biopsy specimens with antimicrobial susceptibility information. Case records of 86 boxers and French Bulldogs with suspected GC evaluated by *E coli* FISH[1] at the Simpson Laboratory of Cornell University's Veterinary Clinical Science Department between January 2010 and February 2014 were reviewed for inclusion.

2.2 | Isolation and antimicrobial susceptibility testing of mucosal *E coli*

Escherichia coli was isolated from intestinal biopsy samples as previously described[1] at Cornell University's Animal Health Diagnostic Center and stored in glycerol broth at −70°C. The minimum inhibitory concentrations (MICs) of antimicrobials against *E coli* were determined by microdilution and interpreted using Clinical Laboratory Standards Institute (CLSI) criteria.[17,18] The MIC was recorded as the lowest concentration of the antimicrobial inhibiting visible bacterial growth. Isolates were classified as susceptible, intermediate, or resistant based on breakpoints accepted at the time the assay was run. The panel of antimicrobials included amikacin, cefazolin, cefovecin, chloramphenicol, clindamycin, doxycycline, enrofloxacin, erythromycin, gentamicin, imipenem, oxacillin, and trimethoprim-sulfamethoxazole. In 2 dogs, culture was performed elsewhere using comparable laboratory standards and a panel including cefovecin, chloramphenicol, enrofloxacin, and trimethoprim-sulfamethoxazole or enrofloxacin, gentamicin, and imipenem.

Escherichia coli resistant to ≥1 antimicrobial in ≥3 mechanistic categories were considered multidrug resistant (MDR).[19]

Selective culture for *Campylobacter* and *Salmonella* was performed on biopsy samples from 21 and 22 of 24 dogs, respectively.

2.3 | Clinical outcome

Response to treatment was determined by contacting the referring veterinarian. Each dog's response was evaluated during the first 4 weeks of antibiotic treatment (initial response) and at last follow-up ≥3 months after initial biopsy (long-term response). A dog was classified as having a complete clinical response (CR) if they were clinically normal during and after treatment. A dog with improvement in clinical condition but ongoing signs (eg, hematochezia, unacceptable stool quality, increased frequency of defecation) was classified as having a partial response (PR). A dog with no improvement or progressive clinical signs was considered to have no response (NR) to treatment.

Colonoscopic mucosal biopsy was repeated during or after treatment or both in a subset of dogs. *Escherichia coli* culture and

susceptibility testing, *E coli* FISH, or both, were performed as described previously.

2.4 | Molecular characterization of *E coli*

Escherichia coli was identified using the computer-controlled Sensititre System (TREK Diagnostic Systems, Cleveland, Ohio) and confirmed by species-specific PCR. Colonies with different morphologies on eosin methylene blue agar plates were selected for further characterization. Isolates were genotyped using random amplified polymorphic DNA (RAPD)-PCR with informative primer 1283, followed by additional primer 1290 to distinguish isolates with similar banding patterns[20] as well as assigned to a phylogenetic group (A, B1, B2, D) using triplex PCR.[21] The serotype of 7 strains isolated over 13 months from 1 dog (#10) was identified by Pennsylvania State University's *E coli* Reference Center.[22]

2.5 | Fluoroquinolone resistance

The presence of mutations and genes involved in FQ-R was determined by PCR.[23-29] Genes encoding plasmid-mediated quinolone resistance (PMQR) included the enzymatic modification gene *aac* (6′)-*Ib-cr* (encodes variant aminoglycoside acetyltransferase, conferring resistance to tobramycin, amikacin, kanamycin), efflux pump gene *qepA*, and target protection PMQR genes: *qnrA*, *qnrB*, *qnrC*, and *qnrS*. Additionally, *E coli* were screened for mutations in the quinolone-resistance determining region (QRDR), impacting DNA gyrase (*gyrA*), and topoisomerase IV (*parC*, *parE*) genes. Three FQ-S *E coli* isolates were included as negative controls.

2.6 | Statistical analysis

Fisher's exact test was used to determine if prebiopsy FQ administration was associated with the likelihood of a dog harboring MDR *E coli* and to compare proportions of isolates that were FQ-R and MDR vs FQ-S and MDR. A chi-squared test was used to determine if phylogroup (A, B1/B2, D) was related to FQ-R. For the purposes of statistical analysis, intermediate isolates were coded as resistant. Differences were considered significant if the calculated *P*-value was <.05.

3 | RESULTS

3.1 | Patient population

Twenty-four of 86 dogs with histologic evidence of PAS-positive GC (20 Boxer dogs; 12 male, 8 female; 4 French Bulldogs; 2 male, 2 female) met inclusion criteria for the study. Sixty-two dogs were excluded because of absence of invasive *E coli* on FISH (n = 38), positive FISH but no concurrent bacterial culture and susceptibility testing (n = 21), or inadequate medical records (n = 3).

The 24 eligible dogs had a median age of 1.2 years (range, 1-4 years) and clinical signs typical of chronic colitis including abnormal fecal consistency with hematochezia, increased frequency of defecation, and tenesmus (Supplemental Table 1). The median duration of clinical signs before initial biopsy was 10.5 months (range, 2-39 months).

Eight dogs had no history of FQ treatment before definitive diagnosis of *E coli*-associated GC. Sixteen dogs were treated with ≥1 FQ (14/16 enrofloxacin, 2/16 marbofloxacin, 1/16 levofloxacin, 1/16 ciprofloxacin) before definitive diagnosis of GC. Details of enrofloxacin treatment were available for 9/14; median dose and duration was 6.5 mg/kg (range, 5-10) PO q24h for 8 weeks (range, 3-20), respectively. Detailed medication history was available for 21/24 dogs. Seventeen of 21 dogs were treated with ≥1 non-FQ PO antimicrobial (median, 2; range, 1-5), most commonly metronidazole (13/21 dogs, Supplemental Table 1). Other treatments utilized before definitive diagnosis included anthelmintics (n = 11), diet change (n = 10), prednisone (n = 6), supplemental fiber (n = 4), and probiotics (n = 4). One dog each was treated with diphenhydramine, For-Bid (Alpar Laboratories, La Grange, Illinois), polyethylene glycol, Pro-Pectalin (Vétoquinol, Fort Worth, Texas), aminopentamide, diphenoxylate, sulfasalazine, and cobalamin.

Before colonoscopy, responses ranged from no improvement to complete resolution of clinical signs with subsequent relapse during or after antimicrobial treatment. In 24/24 dogs, empirical treatment before definitive diagnosis of *E coli*-associated GC was unsuccessful at inducing lasting clinical remission, prompting colonic mucosal biopsy with FISH analysis and culture with antimicrobial susceptibility testing of mucosal *E coli*. Six of 24 dogs had >1 colonic biopsy procedure with samples submitted for *E coli* culture, *E coli* FISH, or both.

3.2 | Isolation and antimicrobial susceptibility testing of mucosal *E coli*

Forty-two representative *E coli* isolates were cultured from initial colonic mucosal biopsy samples from 24 GC dogs (median, 2 isolates per dog; range, 1-6). All isolates were sensitive to imipenem and amikacin and resistant to clindamycin, erythromycin, and oxacillin. In 9/24 dogs, all isolates were FQ-S, whereas in 15/24 dogs, ≥1 isolate was FQ-R (Table 1, Supplemental Table 2). Overall, 20/42 isolates from initial biopsy samples were FQ-S whereas 22/42 were FQ-R.

Fluoroquinolone administration before colonic mucosal bacterial culture was strongly associated with the presence of MDR *E coli* ($P < .001$; 20/21 MDR vs 2/20 non-MDR isolates), and MDR status was strongly associated with FQ-R ($P < .001$; 20/21 FQ-R vs 2/20 FQ-S isolates).

Culture for *Campylobacter* was negative in 21/21 dogs. Two of 22 dogs (#12, 19) had positive growth of *Salmonella* (few group C1, few group D1).

TABLE 1 Antimicrobial susceptibility profiles of *E. coli* from initial biopsies from 15 *E. coli*-associated GC dogs with at least one FQ-R isolate. Date refers to month and year in which *E. coli* was isolated. All isolates were resistant to clindamycin, erythromycin and oxacillin. At the time of testing, canine-specific interpretive breakpoints did not exist for chloramphenicol, imipenem or trimethoprim sulfamethoxazole. Thus, interpretations were extrapolated from human *Enterobacteriaceae* isolate data.

Dog #	Isolate	AMK	CFZ	CFV	CHL	DOX	ENR	GEN	IPM	SMZ
11	DT1	S	S	S	R	I	R	S	S	R
	DT10	S	R	R	S	S	S	S	S	R
12	DBF1	S	S	S	S	S	R	S	S	S
	DBF2	S	R	R	I	R	R	I	S	R
13	-	S	S	S	R	R	R	R	S	R
	-	S	S	S	S	R	S	S	S	R
14	DSP	S	S	S	R	R	R	R	S	R
15	DRX	S	R	R	R	I	R	R	S	R
16	CUD	S	S	S	R	R	R	R	S	R
	CUD2	S	R	R	R	R	R	R	S	R
17	DMX1	S	S	S	R	R	R	S	S	R
	DMX2	S	R	R	S	S	R	S	S	R
18	CUD49	S	S	S	R	R	R	R	S	R
19	CUD68	S	R	R	R	R	R	S	S	R
	CUD69	S	S	S	S	R	S	S	S	S
20	CUD66	S	S	S	S	S	R	R	S	S
21	CUD72	S	S	S	R	S	R	R	S	R
22	CUD97	S	S	S	R	R	R	S	S	R
	CUD98	S	S	S	R	R	R	S	S	R
23	CUD106	S	R	R	R	R	R	S	S	R
24	CUD120	S	S	S	R	R	R	R	S	R
	CUD121	S	S	S	S	S	R	R	S	S
	CUD122	S	S	S	R	R	R	R	S	R
25	-	NI	NI	NI	NI	NI	R	S	S	NI

Abbreviations: AMK, amikacin; CFV, cefovecin; CFZ, cefazolin; CHL, chloramphenicol; DOX, doxycycline; ENR, enrofloxacin; FQ, fluoroquinolone; GEN, gentamicin; I, intermediate; IPM, imipenem; R, resistant; S, sensitive; SMZ, trimethoprim sulfamethoxazole.

3.3 | Follow-up isolation and antimicrobial susceptibility testing of mucosal *E coli*

Colonic mucosal biopsy was repeated in 6 dogs: 4 with refractory colitis (#10, 12, 16, 22) and 2 with positive responses to treatment (#4, 13) to confirm eradication of invasive *E coli*. Five dogs had a single repeat procedure whereas dog #10 had 3 repeat procedures.

Escherichia coli FISH, performed in 5 cases, was positive in 3 dogs with refractory colitis (#10, 16, 22) and negative in 2 clinically healthy (CR) dogs (#4, 13). Biopsy samples from 4 dogs (#4, 10, 12, 13) were cultured for *E coli* (10 isolates; median, 1.5; range, 1-3 per dog). Antibiograms showed static or increased AMR (Supplemental Table 3). All isolates remained susceptible to imipenem. Multidrug resistance was identified in 0/2 FQ-S vs 8/8 FQ-R isolates (P = .02). Cultures of biopsy samples from both CR dogs (#4, 13) yielded MDR *E coli*.

4 | TREATMENT AND CLINICAL RESPONSE

We recommended that veterinarians use antimicrobial susceptibility testing (Table 1; Supplemental Table 2) to guide initial treatment. Initial and long-term treatment responses are summarized in Figure 1.

4.1 | Dogs with fluoroquinolone-sensitive *E coli*

On the basis of previous studies,[1,3,4,12,13] we recommended treating dogs with FQ-S *E coli* with enrofloxacin at 7.5 mg/kg PO q24h for 6 to 8 weeks. In practice, 8/9 dogs received enrofloxacin and 1/9 marbofloxacin. Adjunct empirical treatment was employed in 4 dogs (#5, 6, 7, 10) according to the preference of the primary veterinarian (Supplemental Table 4).

Eight of 9 dogs with FQ-S *E coli* had a CR during antibiotic treatment. Follow-up ≥3 months after initial biopsy was available for 5/8 dogs treated with a FQ initially with long-term CR in 5/5 and median duration of remission 25 months (range, 4-46).

One dog (#10) did not improve after 3 weeks of enrofloxacin (6.5 mg/kg PO q24h) and a second colonoscopy with biopsy for bacterial culture was performed (4 weeks after the initial procedure). Multidrug resistant *E coli* was isolated (FISH not repeated at this time), and subsequent treatments are described below.

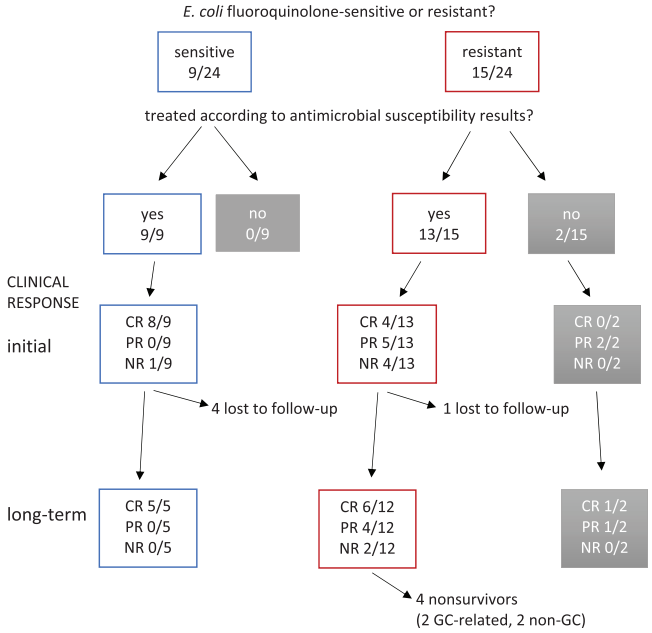

FIGURE 1 Flow-chart depicting colonic mucosal *Escherichia coli* fluoroquinolone-sensitivity or resistance at initial biopsy, treatment according to antimicrobial susceptibility profiling or not, as well as clinical response initially (during first 4 weeks of treatment) and long-term (at last follow-up at or beyond 3 months postbiopsy) of 24 dogs with histologically proven, FISH-positive *E coli*-associated GC. CR, complete clinical response; GC, granulomatous colitis; NR, no response; PR, partial clinical response

4.2 | Dogs with fluoroquinolone-resistant *E coli*

Treatment guided by susceptibility results:

In 13/15 dogs with FQ-R *E coli* at initial diagnosis, susceptibility testing indicated imipenem/meropenem as the only macrophage-penetrating antimicrobial to which isolates were susceptible (Table 1). We recommended treatment with meropenem 10 mg/kg SC q12h for at least 6 weeks. Eleven of 13 dogs were treated accordingly (Supplemental Table 4).

In 2/15 dogs with FQ-R *E coli* at initial diagnosis (#20, 21), *E coli* were susceptible to doxycycline (Table 1). For these dogs, we recommended treatment with doxycycline, 10 mg/kg PO q24h for at least 6 weeks.

4.3 | Initial responses

During the first 4 weeks of meropenem or doxycycline administration, clinical signs improved in 9/13 (69%) FQ-R dogs: CR in 4 (#11, 13, 19, 21) and PR in 5 (#12, 14, 22, 23, 25). Four of 13 dogs (31%; #15, 16, 17, 20) had NR during the first 4 weeks of definitive treatment. One dog (#20) subsequently was lost to follow-up (Figure 1).

4.4 | Long-term responses

Follow-up ≥3 months after initial biopsy was available for 12/13 dogs with FQ-R *E coli* treated according to susceptibility results. Long-term CR (median, 59 months; range, 15-102) was documented in 6/12 dogs (#11, 13, 19, 21, 23, 25) after meropenem or doxycycline (Figure 1).

Long-term PR (median, 19 months; range, 5-65) was documented in 4/12 dogs (#14, 15, 16, 22). Two of the long-term PR dogs initially had NR, and 2/4 had initial PR. All 4 dogs were managed long-term using symptomatic colitis treatments including sulfasalazine, fiber and diet. Dog #10, that did not respond to enrofloxacin and then had MDR *E coli* cultured, subsequently was treated using meropenem (10 mg/kg SC q12h). This treatment resulted in clinical improvement but was discontinued after 3 weeks because of its high cost and replaced with chloramphenicol and ciprofloxacin. Clinical signs recurred, and meropenem was restarted. Ultimately, this dog was treated with 3 separate courses of meropenem, other antimicrobials, acupuncture, colloidal silver, and Chinese herbs. At last reevaluation 46 months postdiagnosis, the dog had no clinical signs of GC.

Four FQ-R dogs (#12, 14, 17, 22) died or were euthanized within 4 years of initial diagnosis. Two dogs (1 PR, #14; 1 NR, #17) were euthanized because of refractory colitis. Dog 14 had recurrent severe hematochezia soon after completing 7 weeks of meropenem. No improvement was seen with numerous antibiotics, nutraceuticals, and probiotics. After transient improvement in clinical signs after fecal transplantation, the dog once again deteriorated and was euthanized (27 months postdiagnosis; age, 3.8 years). Dog 17 showed no improvement with meropenem treatment and was euthanized within 4 months of diagnosis because of unrelenting colitis (age, 2.8 years).

In the other 2 dogs, death or euthanasia was not directly linked to GC. Dog 22 improved during meropenem treatment but declined clinically 1 month after cessation of treatment. Repeat FISH identified persistent intramucosal *E coli* (concurrent culture not performed). Subsequently, the dog continued to decline and died suddenly at home 3 weeks later (5 months postdiagnosis; age, 1.8 years). Necropsy was not performed. Dog 12 had recurrence of clinical signs during the first month of meropenem after transient improvement. Another biopsy sample was obtained and MDR *E coli* was isolated (FISH not repeated). Large bowel clinical signs persisted. The dog was euthanized because of aggressive behavior 16 months after initial diagnosis (age, 2.3 years).

At the time of last follow-up, 4 other dogs (# 11, 15, 21, 25) had been euthanized for unrelated causes (8.5, 5.4, 6, 3.8 years, respectively, postdiagnosis). Colitis resolved during initial antimicrobial treatment without recurrence in 3/4.

Both of the dogs with positive *Salmonella* cultures also had MDR *E coli* and were treated using meropenem. One dog (#12) initially had a PR but ultimately was a nonsurvivor whereas the other (#19) had an initial and long-term CR.

In summary, 17/22 dogs (77%) treated based on antimicrobial susceptibility testing with or without empirical supportive treatment showed clinical improvement during the first 4 weeks. Considering the 17 dogs with ≥3 months follow-up, 14 had positive long-term clinical outcomes (82%). These included 100% (5/5) of dogs with FQ-S *E coli* and 75% (9/12) of dogs with FQ-R *E coli*. Median durations of positive responses (PR + CR) in dogs treated based on susceptibility testing were: 25 months for FQ-S *E coli* and 46 months for FQ-R *E*

coli. Two of 17 (12%) dogs were euthanized because of refractory colitis.

Treatment not guided by susceptibility results:

In 2/15 dogs with FQ-R *E coli* at initial biopsy, treatment with meropenem was cost-prohibitive, and combination protocols were developed by the primary veterinarian. Dog 24 was treated with enrofloxacin (isolates resistant) and metronidazole. The severity of diarrhea decreased, but at last follow-up 3 months postdiagnosis, the dog continued to have 5 to 8 bowel movements per day (long-term PR). Dog 18 was treated with 10 months of cephalexin (isolates susceptible; 500 mg PO q12h) and tylosin and initially was a PR. Eight months after cessation of antibiotics, the dog was doing well on a prescription diet and was classified as having a CR. To summarize findings in the 2/24 dogs not treated according to antimicrobial susceptibility testing, some improvement (PR) was documented in both initially. Although 1 dog was considered a long-term PR, it was lost to follow-up 3 months postdiagnosis, precluding full assessment of the efficacy of its antimicrobial regimen. The other dog was classified as a long-term CR after prolonged cephalosporin treatment, to which its *E coli* was susceptible, but the drug had not been suggested given lack of macrophage penetration.[15]

5 | ESCHERICHIA COLI MOLECULAR CHARACTERIZATION

5.1 | Molecular basis of fluoroquinolone-resistance

All 26 FQ-R *E coli* isolates had mutations in *gyrA* (24, double; 2, single) and 24/26 had mutations in *parC* (Supplemental Table 5). Mutations in *parE* were present in 11/26 FQ-R isolates. Genes indicative of plasmid-mediated resistance were identified in 7/26 isolates from 5/15 dogs. No dogs had *E coli* carrying *qnrA*, *qnrB*, or *qnrC* genes. The lone FQ-R isolate that was not MDR (DBF1) had a unique pattern of substitutions in the QRDR. No mutations were identified in the 3 FQ-S used as controls (Supplemental Table 5).

5.2 | Genomic and phylogenetic characteristics of *E coli*

Phylogroup was determined for 43 isolates, including 35 isolates from initial biopsy samples (20 dogs) and 8 from follow-up samples (3 dogs). Isolates were distributed across the *E coli* phylogenetic space: 37% (16/43) belonged to phylogroup A, 34.8% (18/43) belonged to phylogroup B1/B2, and 20.9% (9/43) belonged to phylogroup D.

The RAPD PCR genotyping of 33 mucosal *E coli* isolates using initial biopsy samples from 20 dogs (28 isolates) and follow-up biopsy samples from 3 dogs (5 isolates) indicated substantial diversity within and between dogs (Figure 2). Overall, 20 different genotypes were found among the 33 isolates. Identical RAPD patterns using primers 1283 and 1290 were identified in isolates from 4 pairs of dogs (#10 + 12, 11 + 19, 17 + 20, 18 + 24). Despite having identical RAPD banding patterns, *E coli* strains from dogs 11 (DT1) and 19 (CUD68), as well as dogs 17 (DMX2) and 20 (CUD66) had different antibiograms and different substitutions in the QRDR (Supplemental Table 5). Isolates from dogs 18 (CUD49) and 24 (CUD120, CUD122) had the same mutations in resistance genes but differed in susceptibility to ticarcillin and cephalothin. This finding led us to conclude that these dogs were colonized by unique strains of *E coli*.

The RAPD genotype was determined for *E coli* cultured from dog #10 on 4 separate occasions. We identified 3 phylogroup D serotype O102:H+ strains (DCAT11, DCAT21, DCK1) with identical RAPD on 3 separate occasions over the course of 11 months (Figure 3).[22] All strains had consistent mutations in the QRDR indicating persistent colonization.

An identical strain (DBF2) based on RAPD genotyping, phylogroup, and AMR genes was isolated from dog 12 at initial biopsy, suggesting 2/24 dogs were colonized by a clonal *E coli* strain.

6 | DISCUSSION

Our objective was to determine the clinical outcome of dogs with *E coli*-associated GC treated based on antimicrobial susceptibility profiling. We also examined the relationship of individual *E coli* strains with antimicrobial resistance.

Antimicrobial susceptibility profiling determined that 62.5% of GC dogs in our study were colonized by FQ-R *E coli* at initial diagnosis, substantially higher than the 0% to 43% reported previously.[3,4,14] Fluoroquinolone resistance was closely associated with MDR in our *E coli* isolates. These findings parallel the growing prevalence of FQ-R, and the association of FQ-R with MDR, described in *E coli* from canine and human patients over the past 2 decades.[30-33] Fluoroquinolone administration before bacterial culture correlated with isolation of FQ-R/MDR *E coli*, in agreement with previous findings in dogs with GC[3,14,16,34] and the population as a whole receiving veterinary care.[35,36] In our study, 13/15 dogs with FQ-R *E coli* harbored strains only susceptible to imipenem and amikacin. The macrophage-penetrating ability of carbapenems[37] as well as the toxicity of aminoglycosides made meropenem the treatment of choice. Carbapenems are a critically important class of drugs according to the World Health Organization,[38] and our recommendation to administer meropenem was driven by the high likelihood of euthanasia for dogs with *E coli*-associated GC that fail to respond to antimicrobials.[2,3,14,39,40]

We found that antimicrobial treatment guided by susceptibility profiling and macrophage-penetrating ability resulted in positive long-term outcomes (median, 28 months; range, 4-102) in 14/17 dogs having at least 3 months of follow-up. This included 9/12 dogs with FQ-R *E coli* at initial biopsy. There are few previous reports of positive clinical responses in GC dogs with FQ-R *E coli*,[16,34] and dogs often are euthanized because of unrelenting disease.[3,14] Only 2 of these 12 dogs were euthanized because of GC. Clinical remission was achieved with

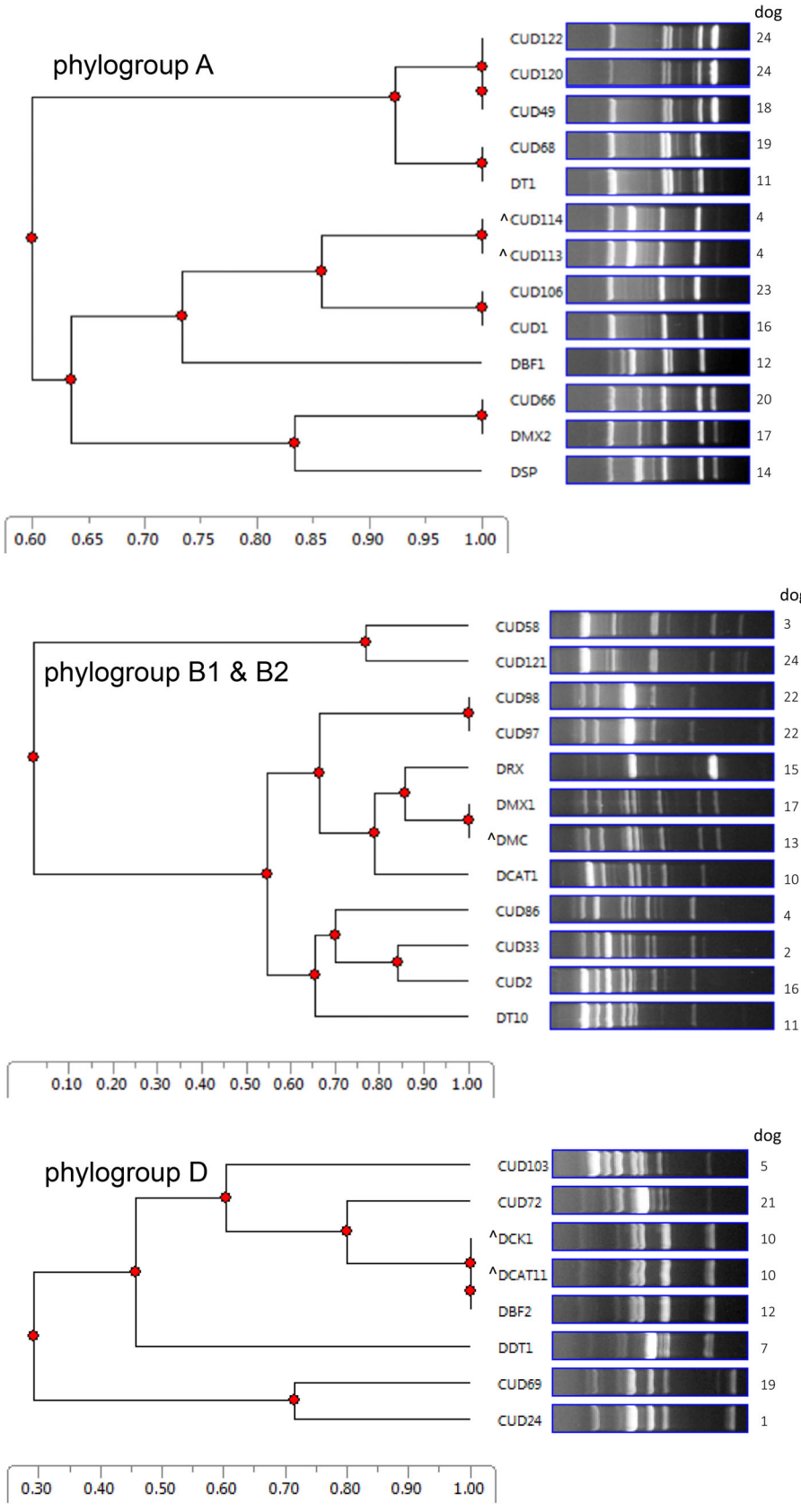

FIGURE 2 Random amplified polymorphic DNA (RAPD)-PCR genotype (primer 1283) and phylogeny (A, B1, B2, D) among 33 granulomatous colitis (GC) *Escherichia coli* isolates from colonic biopsies of 21 dogs with *E coli*-associated GC. The identity of each isolate and dog are shown on the left and right of the RAPD profile. Five isolates were obtained from follow-up colonic biopsies, indicated by^; all other isolates were obtained from initial colonic biopsies. Dendrograms were constructed using TotalLab CLIQS software

meropenem (n = 8) and doxycycline (n = 1), 2 therapeutic options not previously evaluated for GC dogs with FQ-R/MDR *E coli*. The presence or absence of *E coli* in posttreatment samples correlated with presence or absence of colitis signs, respectively, supporting the relationship between eradication of invasive *E coli* and clinical remission observed previously.[3]

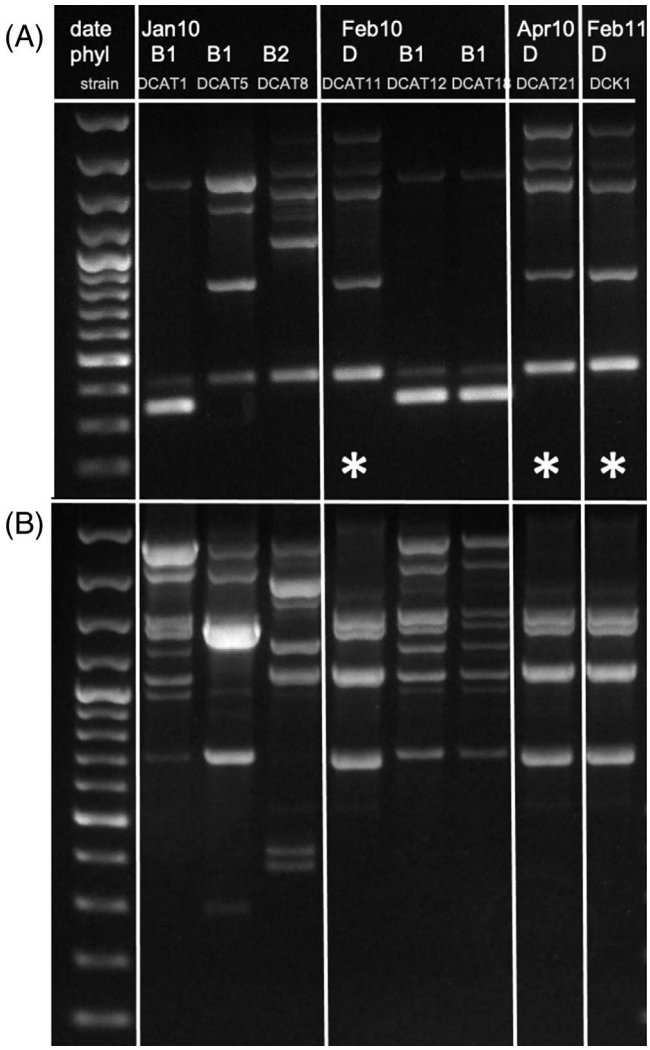

FIGURE 3 In dog #10, *Escherichia coli* isolated at multiple time points was subjected to random amplified polymorphic DNA (RAPD)-PCR analysis with informative primer sets 1290 (A) and 1283 (B). Three *E coli* isolates from biopsies from different time belonged to phylogroup D *E coli* strain had identical banding patterns with both RAPD primer sets (as indicated by *). These strains were serotyped as O102:H+. Left-most lane contains DNA ladder. Phyl, phylogroup

The clinical response to antimicrobial treatment guided by the aforementioned criteria was not uniform. Although 8/9 dogs with FQ-S *E coli* had initial CR with FQ, only 4/13 dogs with FQ-R *E coli* had initial CR, with 5/13 achieving PR. Four of 13 dogs had no improvement initially and 2 dogs were euthanized because of refractory GC within 2.5 years of diagnosis. Failure to eradicate the *E coli* isolated at diagnosis or reinfection with a different strain could explain the inconsistent outcomes. Serial biopsy and FISH analysis of 3 dogs (#10, 16, 22) not responsive to initial treatment identified persistent intramucosal *E coli*. Molecular characterization of *E coli* from 1 of these dogs (#10) indicated presence of a clonal MDR strain (Figure 3) still present despite 8 weeks of meropenem treatment. Further analysis identified this strain as serotype O102:H+, a serotype that was identified in feces from 10/35 healthy dogs in Japan,[41] but that also was overrepresented in 6/21 carbapenem-resistant samples from human patients at a Chinese hospital.[42]

The reason for suboptimal outcomes in dogs with meropenem-susceptible *E coli* is unclear. It is possible that the causative isolate was actually resistant. In our study, dogs were colonized by a median of 2 different *E coli* strains (range, 1-6). We may have undersampled the diversity of colonic *E coli*, allowing carbapenem-resistance to go undetected. Carbapenem-resistant *E coli* from dogs with naturally occurring infections were reported in 1.3% of 2390 isolates submitted to a private veterinary diagnostic laboratory between 2008 and 2013.[32]

Boxer dogs are predisposed to various chronic granulomatous diseases such as protothecosis,[43] cutaneous tuberculosis,[44] and urinary bladder malakoplakia (Simpson KW unpublished data), supporting the notion of immunodeficiency in these dogs. Genome-wide analyses in Boxers and French Bulldogs have identified a susceptibility locus related to sensing and killing of *E coli*.[5] Glucocorticoids were administered before or concurrent with antimicrobial treatment in 7 dogs with complete medication history. The glucocorticoids could have compromised antimicrobial efficacy. However, the proportions of dogs with known prednisone administration before or during susceptibility-guided antibiotic treatment were similar in the initial PR/NR (3/8) and CR (4/12) dogs.

Of the 5 dogs that showed no improvement (NR) during the first 4 weeks of susceptibility-guided antimicrobial treatment, 3 ultimately had a PR or CR. These responses are both encouraging and difficult to explain, but could be related to patient age. To our knowledge, all dogs that survived until 4 years of age went on to have positive outcomes. Age is an important factor in local and systemic inflammatory reactions in murine *Clostridioides difficile* enteritis[45] but, in humans, increasing age generally is thought to increase risk of gastrointestinal infection and inflammation.[46] Previous work has documented an age-related decline in lamina propria macrophages in the canine intestine,[47] which could have implications for persistence of intramucosal *E coli*. Further work is needed to better understand the reasons for refractory disease and potential age-related susceptibility in dogs with *E coli*-associated GC.

We found no instances of disease recrudescence in dogs that had complete resolution of clinical signs during the first 4 weeks of susceptibility-guided antibiotic administration and treated for 6 to 8 weeks. This finding is in contrast to previous reports of relapses after cessation of antimicrobial treatment in GC dogs.[16,48] Factors contributing to an individual GC dog's ability to achieve lasting remission remain unclear. We suspect that the presence of an intact mucosal barrier prevents *E coli* from accessing susceptible macrophages in the colonic lamina propria. This hypothesis is supported by the isolation of FQ-R/MDR *E coli* from follow-up biopsy samples of 2 dogs (#4, 13) in clinical remission that were FISH negative and had resolving colonic inflammation. We speculate that longer duration of antimicrobial treatment prevents recolonization while the intestinal barrier heals, but it was beyond the scope of our study to investigate the impact of antimicrobial treatment duration on clinical outcome.

We observed positive long-term outcomes in 2/15 dogs with FQ-R *E coli* not treated according to susceptibility testing. This response included dog #18 that eventually achieved CR with a prolonged course of cephalexin and tylosin. Dog #18's *E coli* strains were susceptible to cephalosporins in vitro, but neither of these drugs are expected to have intracellular efficacy.[15] These findings resemble the positive outcomes in 2 French Bulldogs with FQ-R *E coli*-associated GC treated with PO amoxicillin-clavulanic acid[16] with or without cefovecin.[34] Drug synergism could occur. In vitro evidence suggests that the combination of ciprofloxacin, tetracycline, and trimethoprim kills intramacrophage *E coli* more efficiently than does ciprofloxacin alone.[15] Despite suggestive histologic, FISH, and culture results, it is also possible that the FQ-R isolate was not responsible for the clinical signs. Regardless, in vitro antimicrobial susceptibility testing may underestimate antimicrobial efficacy, as suggested previously.[14] We caution against empirical administration of antibiotic combinations in this era of growing AMR, with experimental treatments reserved for cases that fail to respond to rational treatment. Moreover, because many causes of chronic colitis are much more prevalent than *E coli*-associated GC, definitive diagnosis is strongly recommended before prescribing prolonged courses of broad-spectrum, potentially cartilage-damaging antimicrobials.

Antimicrobials are the cornerstone of management for dogs with *E coli*-associated GC. Still, minimizing pro-inflammatory cross-talk between the luminal environment and colonic epithelium and promoting mucosal barrier healing through dietary and pharmacological interventions might also help affected dogs.[49] Aminosalicylates (sulfasalazine, 5-acetylsalicylic acid, 5-ASA) have been used for decades to induce and maintain remission in people with ulcerative colitis.[50] The clinical benefit of aminosalicylates had been attributed to modulation of colonic mucosal inflammatory responses.[51] More recently, a study documented 5-ASA's ability to inhibit the growth, adherence, and invasion of IBD-associated *E coli*. These findings were in addition to inhibition of inflammatory responses of cultured colonic epithelial cells.[52] Only 2/24 of dogs in our study received sulfasalazine (1, CR; 1, NR) along with antimicrobials. Further study is necessary to determine if aminosalicylates could benefit dogs with *E coli*-associated GC. Adjunctive treatment could be considered in refractory cases. Development of keratoconjunctivitis sicca however could preclude long-term administration.[53]

Severe enteric infections associated with *E coli* have been linked to specific pathotypes and strains (eg, EHEC O157).[54] To determine if specific *E coli* are associated with GC and FQ-R/MDR, we genotyped disease-associated strains. We found substantial genetic diversity within and between dogs, with no evidence of clonal FQ-R/MDR *E coli* as the reason for the higher prevalence of FQ-R in our isolates as compared with previous studies. The overall genetic diversity and lack of clustering by phylogroup are consistent with previous studies in dogs with GC and humans with AIEC and CD.[7,14,55-58] Considerable overlap exists in virulence genes between *E coli* from GC and healthy dogs, lending credence to this form of GC being host- rather than pathogen-driven.[59]

We also explored the molecular mechanisms underlying FQ-R. Mutations in *gyrA* +/− *parC* were identified in 100% of FQ-R isolates, whereas plasmid-mediated resistance was identified in 27% (7/26). The preponderance of mutational over plasmid-mediated resistance mimics findings in *E coli* from dogs receiving veterinary care, as well as humans, in the United States and Europe.[23,31,60-62] Similar to previous studies involving FQ-R *E coli* isolated from urinary, fecal, and respiratory samples from dogs, *gyrA parC* mutants prevailed.[60,61,63] Combined mutations in *parC* (S80I) and *gyrA* (S83L, D87N) may endow *E coli* with improved fitness over susceptible and single mutant strains[64] and could explain the repeated isolation of FQ-R *E coli* from dogs biopsied sequentially without repeated FQ exposure.

Five of the 16 dogs with QRDR mutations had concurrent plasmid-mediated FQ-R, conferring *E coli* with high level FQ-R.[22] Plasmid-mediated FQ-R may be linked to MDR in a subset of GC dogs, facilitating rapid transmission of AMR to bacteria including, but not limited to, *E coli*.[22] *Escherichia coli* from 4/5 dogs with PMQR genes were resistant to third-generation cephalosporins, paralleling previous findings in FQ-R *E coli* from humans, dogs, and food-producing animals.[22,60,63,65] Given the close proximity of dogs and humans in many households, and the increased likelihood of fecal contamination during episodes of diarrhea, the potential for transmission of MDR *E coli* must be considered.[30,66] Judicious use of antimicrobials, including but not limited to FQ, is necessary because PMQR can be acquired even in the absence of FQ exposure.[31]

Limitations of our study include the retrospective, nonstandardized approach to monitoring clinical response to treatment. We were not able to control treatments before initial biopsy or adjunctive treatments prescribed by referring veterinarians. Although we intended to sample all *E coli* colonies with different morphologies, we may have undersampled diversity. Clinicians should encourage their microbiology laboratories to screen multiple colony phenotypes to increase the chance of capturing representative bacteria. The susceptibility results reported here reflect the existing breakpoints at the time utilized at Cornell University's Animal Health Diagnostic Center, which are applicable for this laboratory alone; canine-specific breakpoints for *Enterobacteriaceae* did not exist for chloramphenicol, imipenem, and trimethoprim sulfamethoxazole. In the absence of definitive information on antimicrobial concentrations required to eliminate *E coli* in canine macrophages, we relied on breakpoints derived from isolates causing systemic infections in humans.[67,68] Doing so could account for the lack of clinical improvement in dogs treated based on susceptibility testing.

7 | CONCLUSIONS

Antimicrobial treatment informed by susceptibility testing, with or without empirical symptomatic treatment, was associated with positive long-term clinical outcomes in >80% of cases. All patients achieving CR in the first 4 weeks of such treatment had durable positive responses. Resistance to FQ and other classes of antimicrobials was widespread but not clonal. Given the association between empirical

FQ treatment and isolation of MDR *E coli*, definitive diagnosis of *E coli*-associated GC is strongly recommended before initiating FQ treatment. Further study is required regarding the acquisition and maintenance of MDR *E coli* in dogs with *E coli*-associated GC dogs to optimize clinical outcomes.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Neither azithromycin, ciprofloxacin, levofloxacin, meropenem, metronidazole, nor tylosin is FDA approved for any use in dogs. Cephalexin and doxycycline are approved only for canine use, but not for treatment of bacterial enteritis.

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

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

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

ORCID

Alison C. Manchester https://orcid.org/0000-0003-3507-0589

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SUPPORTING INFORMATION

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

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