

Prevalence of Diarrhea and Enteropathogens in Racing Sled Dogs

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Background: Diarrhea is highly prevalent in racing sled dogs, although the underlying causes are poorly understood.

Hypothesis: *Clostridium perfringens* enterotoxin (CPE) and *Clostridium difficile* Toxin A and B are associated with diarrhea in racing sled dogs.

Animals: One hundred and thirty-five sled dogs.

Methods: Freshly voided feces were obtained from 55 dogs before racing and from 80 dogs after 400 miles of racing. Samples were visually scored for diarrhea, mucus, blood, and melena. CPE and *C. difficile* Toxin A and B were detected by ELISA. Samples were cultured for *C. perfringens*, *C. difficile*, *Campylobacter*, *Salmonella*, and *Escherichia coli* 0157; *Giardia* and *Cryptosporidium* spp. were detected via immunofluorescence.

Results: Diarrhea occurred in 36% of dogs during racing, and hematochezia, fecal mucus or melena, or all 3 occurred in 57.5% of dogs. *Salmonella* was isolated from 78.2% of dogs before racing, and from 71.3% of dogs during racing. *C. perfringens* and *C. difficile* were isolated from 100 and 58.2% of dogs before racing, and from 95 and 36.3% of dogs during racing. Dogs were more likely to test positive for CPE during than before racing (18.8 versus 5.5%, $P = .021$); however, no enteropathogens or their respective toxins were significantly associated with hematochezia or diarrhea.

Conclusions and Clinical Importance: Sled dogs participating in long distance racing have a high prevalence of diarrhea and hematochezia that is not associated with common enteropathogens. It is possible that diarrhea and hematochezia represent the effect of prolonged exercise on the gastrointestinal tract.

Key words: Anaerobic bacteria; Bacterial microbiology; Diarrhea and vomiting; Gastroenterology; *Salmonella* bacterial species; Sports medicine.

Diarrhea affects a substantial proportion of sled dogs during long distance racing. The prevalence of diarrhea was reported to be 7.5% in a study of morbidity in long distance racing sled dogs; however, anecdotally the problem occurs with much greater frequency, and diarrhea represents a leading cause of morbidity in racing sled dogs (S. Nelson, Jr, Iditarod Trail Committee, personal communication).¹ Although diarrhea rarely results in illness severe enough to mandate withdrawal of the dog from competition, the authors believe that teams with one or more diarrheic dogs are likely to suffer some degree of performance loss. The additional time and attention that a musher must expend attending to a diarrheic dog is frequently the primary reason that affected dogs are removed from racing.

Despite the frequency of diarrhea in racing sled dogs, studies of the possible etiology of this problem are limited.^{2,3} Racing sled dogs have a very high prevalence of *Salmonella* infection; however, the prevalence of *Salmonella* infection has not been shown to differ between

Abbreviations:

CPE	<i>Clostridium perfringens</i> enterotoxin
GDH	glutamate dehydrogenase

diarrheic and nondiarrheic sled dogs, and is likely attributable to consumption of a raw diet with subsequent passive shedding of the organism.^{2,4} Additionally, a recent study that documented substantial increases in serum titers to canine parvovirus in sled dogs competing in the 2006 Iditarod Trail Race did not identify clinical disease associated with this phenomenon.⁵ However, these findings underscore the substantial risk of exposure to enteropathogens that racing sled dogs encounter as a result of competition.

Multiple studies suggest that the bacterial enteropathogens *Clostridium perfringens* and *Clostridium difficile* are significantly associated with the occurrence of diarrhea in companion dogs.⁶⁻⁹ Concern that *Clostridium* spp. might also be a significant cause of diarrhea in racing sled dogs has resulted in some mushers reporting the adapted use of a *Clostridium* vaccine in their dogs (S. Nelson, Jr, Iditarod Trail Committee, personal communication).

The objectives of the current study were to determine the prevalence of diarrhea in purpose-trained sled dogs participating in a long-distance race, and to determine if *C. perfringens* and *C. difficile* and their respective toxins were associated with diarrhea in actively racing sled dogs. We hypothesized that the presence of *C. perfringens*, *C. difficile*, or both and their respective toxins would be substantially higher in diarrheic versus nondiarrheic sled dogs during racing. A 3rd objective of the study was to determine if diarrhea associated with *C. perfringens* or *C. difficile* was exacerbated by coinfection with other putative enteropathogens, including *Campylobacter*, *Salmonella*, *Giardia*, and *Cryptosporidium* spp.

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Materials and Methods

This study was approved by the Institutional Animal Care and Use Committee of Oregon State University. All dogs enrolled in the study underwent physical examination, a complete blood count, serum biochemistry panel, and electrocardiogram to fulfill mandatory requirements for participation in the 2008 Iditarod Trail Race, which was approximately 1000 miles in length (S. Nelson, Jr, Iditarod Trail Committee, personal communication).^a Additionally, all participating dogs were mandated by race rules to be treated for *Echinococcus* infestation within 10 days preceding the race, and to be vaccinated against canine distemper virus, canine parvovirus, and canine adenovirus within the year preceding the race.^a Ninety-six teams started the 2008 Iditarod Trail Race, comprising 12–16 dogs per team.

Naturally voided fresh fecal samples were immediately collected into a sterile plastic container from a convenience sample of 55 sled dogs within 4 hours before the start of the race. No attempts were made to specifically collect samples from diarrheic or nondiarrheic dogs or from specific teams. As dog teams arrived at the race site and were unloaded from the trucks they were monitored by the investigators for approximately 20 minutes per team. Fecal samples were collected from all dogs that defecated during observation of a team to ensure that only freshly voided samples were obtained. Musherers were consulted regarding the presence of diarrhea in their team and any medications that were being administered currently or within the 10 days preceding the race. Fecal consistency was subjectively evaluated at the time of collection and each fecal sample was scored based on a modification of a fecal scoring system for dogs^b using a 1–4 scale where a score of Grade 1 was given for liquid diarrhea; Grade 2 for soft nonformed feces; Grade 3 for soft-formed feces; and Grade 4 for firm, formed fecal specimens. A score of grade 1 or 2 was considered consistent with diarrhea. Feces were also visually examined for the presence of fresh blood, mucus, or melena.

During the race, freshly voided fecal samples were obtained from a convenience sample of 80 sled dogs at the McGrath checkpoint, approximately 400 miles into the race. As before, mushers were consulted regarding the presence of diarrhea in their team before and during the race, and the use of any medications before and during the race.

Detection of *C. perfringens* enterotoxin (CPE) and *C. difficile* Toxins A and B were performed on fecal samples within 6 hours of collection with commercially available ELISA test kits according to the manufacturers' instructions.^{c,d} All fecal samples were kept at room temperature (approximately 21°C) before ELISA testing. Samples were stabilized with the manufacturer provided chemical buffer to preserve them during the brief delay before analysis. The ELISA reaction was subjectively graded by visual inspection relative to internal positive and negative control wells. In addition, detection of *C. difficile* common antigen (glutamate dehydrogenase [GDH]) was performed on fecal samples within 6 hours of collection, with a commercially available assay.^e

Remaining portions of each fecal sample were placed into anaerobic culture medium^f and transported to the University of California, Davis, Microbiology Laboratory for isolation of *C. perfringens*, *C. difficile*, *Escherichia coli* 0157, *Campylobacter*, and *Salmonella* within 72 hours of collection. Portions of each fecal sample were also placed into dilute formalin (5%) and were transported to the University of California, Davis, Parasitology Laboratory where they were analyzed for the presence of *Giardia* cysts and *Cryptosporidium* oocysts with a commercially available immunofluorescence assay according to manufacturer's instructions.^g

For isolation of *C. perfringens*, 0.5 g of feces was inoculated onto McClung's egg yolk agar to test for lecithinase production. All plates were incubated anaerobically at 37°C for 24–48 hours. In addition,

a reverse-CAMP test was performed on blood agar to observe the characteristic arrowhead-shaped hemolytic pattern consistent with *C. perfringens*. Identification of *C. perfringens* was based on a positive reverse-CAMP test, lecithinase production, and a Gram-stain demonstrating large, nonspore forming, boxcar-shaped Gram-positive bacilli.

For isolation of *C. difficile*, fecal specimens were inoculated onto prereduced cycloserine-cefoxitin-fructose broth (CCFB) and incubated anaerobically at 37°C for 24–48 hours. Positive CCFB-feces mixture was subcultured onto prereduced selective medium containing cycloserine-cefoxitin-fructose agar (CCFA). Plates were incubated anaerobically at 37°C for 72 hours. Plates were examined for the presence of nonswarming yellow colonies exhibiting a ground glass appearance. Yellow colonies were subcultured to prereduced *Brucella* agar (BA) and incubated anaerobically for 24 hours at 37°C. Colonies were Gram stained and identification of *C. difficile* was made on the basis of lack of aerotolerance, colony morphology, fluorescence, odor, and detection of L-proline-aminopeptidase on both CCFA and BA.

For isolation of *E. coli* 0157, fecal specimens obtained from 44 dogs during racing were plated to sorbitol MacConkey agar plates^h and incubated aerobically at 37°C for 24 hours. All plates were screened for sorbitol-negative colonies typical of *E. coli*.ⁱ

For isolation of *Campylobacter* spp., fresh feces were plated with a sterile swab onto a selective *Campylobacter* agar containing cefoperazone, vancomycin, and amphotericin B, and streaked for isolation. Plates were incubated at 42°C by use of a microaerophilic gas generating system. Plates were examined for growth at 48–72 hours. Suspect colonies were Gram stained and subcultured to 5% SBA. Tests for catalase, oxidase, indoxyl acetate, nitrate, hippurate hydrolysis, and susceptibility to 30 µg disks of cephalothin and nalidixic acid were performed.

For isolation of *Salmonella* spp. fecal specimens were plated with a sterile swab onto MacConkey agar and streaked for isolation. One gram of feces was inoculated into 4% selenite broth for *Salmonellae* enrichment. Incubated selenite broth was subcultured to xylose-lysine-tergitol 4 (XLT4) agar. All cultures were incubated without CO₂ for 24–48 hours at 37°C, except selenite broth, which was incubated for 24 hours. Lactose-negative colonies from MacConkey and H₂S-positive colonies from XLT4 were subcultured to biochemical media according to identification schema used by the diagnostic microbiology laboratory at the University of California, Davis. *Salmonella* isolates (4 per positive culture) were serotyped at the National Veterinary Service Laboratory, Ames, IA.

Associations in the data were analyzed by logistic regression with robust error estimation to account for clustering by sled dog team; results are presented as prevalence odds ratios (POR) and 95% confidence intervals (95% CI). Prevalence estimates are presented with 95% CI derived from variance estimates that account for team clustering. *P* values < .05 were considered significant.

Results

Before racing, freshly voided fecal samples were successfully obtained from 55 dogs among 12 different teams (mean, 5 samples per team; range, 2–11 samples per team). Fresh blood was observed in the feces of 1 dog, and mucus in the feces of another dog on the same team (only 2 samples were collected from this team). Fecal scores were recorded for 49 dogs (Table 1). Diarrhea was documented in 6/49 dogs (12.2%, 95% CI = 0–26.0%, fecal score of 2). No dogs had a fecal score of 1. Thirty-four dogs from which samples were obtained were currently receiving pharmaceuticals, psyllium, or both. Eleven dogs on 2 teams were receiving probiotics, and 11

Table 1. Fecal scores recorded in sled dogs before and during the 2008 Iditarod trail race.

Fecal Score	1	2	3	4
Before racing (<i>n</i> = 49)	0	6 (12.2%)	13 (26.5%)	30 (61.2%)
During racing (<i>n</i> = 80)	7 (8.8%)	22 (27.5%)	35 (43.7%)	16 (20%)

Racing was significantly associated with diarrhea (fecal score of ≤ 2) ($P = .021$).

dogs on 1 team were receiving psyllium. Famotidine was administered to 12 dogs among 4 teams for the purpose of gastric ulcer prevention. No treatments were administered to 15 dogs on 4 teams. Treatment status was unknown for 1 team of 6 dogs. No dogs were reported to receive antibiotics before the race.

Fecal samples collected during racing were obtained from 80 dogs, representing 18 teams (average, 4.4 samples per team; range, 1–13 samples per team). Sampled teams were spread throughout the 1st 60 teams arriving in the checkpoint, with the arrival times of the sampled teams ranging from 2 days and 10 hours to 3 days and 1 hour after the race start. Diarrhea (fecal score ≤ 2) was documented in 29/80 dogs (36.3%, 95% CI = 24.2–48.3%) during the race, and 7 of these dogs (8.8%, 95% CI = 0.86–16.6%) had liquid diarrhea (Table 1). Racing (versus before racing) was significantly associated with the presence of diarrhea (POR = 4.1, 95% CI = 1.2–13.4, $P = .021$). Treatment status was successfully recorded for 66/80 (82.5%) dogs examined during racing. The treatment status for 1 team of 13 dogs and 1 additional dog was unknown. Three dogs on 2 teams did not receive any treatments. Famotidine was administered to 51 dogs from 12 teams during the race. Administration of psyllium was reported for 34 dogs on 7 teams and administration of probiotics was reported for 27 dogs on 5 teams. One dog on 1 team was treated with metronidazole for diarrhea, and 2 dogs on another team were treated with metronidazole and cephalixin (1 dog each) for diarrhea, but treatment for both dogs was altered to kanamycin in the 24-hour period before fecal samples were obtained. Racing dogs were significantly more likely to receive famotidine (POR = 10.5, 95% CI = 1.5–71.6, $P = .017$) than dogs before racing; however, there was no association between treatment with famotidine and the presence of diarrhea in dogs during racing

($P = .15$). During racing, frank blood, mucus, or melena were observed in fecal samples from 48/80 dogs (57.5%, 95% CI = 40.3–74.7%). Frank blood was observed in 30/80 samples (37.5%, 95% CI = 17.4–57.6%); 13/80 samples (16.3%, 95% CI = 4.6–27.9%) contained mucus (of these, 9/80 samples [11.3%, 95% CI = 0.68–21.8%] contained mucus without blood and 4/80 samples [5.0%, 95% CI = 0–10.8%] contained mucus and blood); and 7/80 samples (8.8%, 95% CI = 0.46–17.0%) were very dark or tarry. Twelve of the 18 teams (67%) had at least 1 dog with frank blood in the feces or melena. A single team of 13 dogs had no abnormalities of fecal character apart from 3 dogs with mucus in the feces, and an additional 3 teams did not have dogs with blood or melena; however, samples were only obtained from 1 or 2 dogs in each of these teams. Five of the 7 dogs with melena were currently receiving famotidine. Racing was significantly associated with the presence of frank fecal blood (POR = 32.4, 95% CI = 3.5–296.2, $P = .002$) and mucus (POR = 10.5, 95% CI = 1.1–95.8, $P = .037$). During racing, diarrhea was significantly associated with the presence of mucus in the feces (POR = 8.4, 95% CI = 2.2–31.7, $P = .002$). Diarrhea was not significantly associated with the presence of blood in the feces.

Culture results for *C. perfringens* and *C. difficile*, and ELISA results for CPE, *C. difficile* Toxins A and B, and GDH

C. perfringens was isolated from the majority of samples obtained from dogs before and during the race (Table 2). *C. difficile* was isolated from 32/55 dogs (58.2%) and GDH was detected in 43/53 dogs (81.1%) before the race. In contrast, *C. difficile* was isolated from 29/80 dogs (36.3%) and GDH was detected in 66/78 dogs (84.6%) during the race. The isolation of *C. perfringens* and *C. difficile* from the feces of dogs before and during racing was not significantly associated with the presence of diarrhea or hematochezia, and during racing, dogs with a positive culture for *C. difficile* tended to be less likely to have diarrhea than dogs with a negative culture for *C. difficile* (POR = 0.32, 95% CI = 0.097–1.04, $P = .057$). Racing dogs were no more likely to have a positive ELISA result for the common antigen of *C. difficile* (GDH) than dogs before racing ($P = .65$) and a positive GDH result was not associated with the presence of diarrhea or blood in the feces in either group. There was no association between treatment with famotidine and the

Table 2. Culture and ELISA results for *Clostridium perfringens* and *Clostridium difficile* in sled dogs before and during the 2008 Iditarod trail race.

	<i>C. perfringens</i> Culture	CPE ELISA	<i>C. difficile</i> Culture	GDH ELISA ^a	<i>C. difficile</i> Toxin A/B ELISA
Before racing (<i>n</i> = 55)	55 (100%)	3 (5.5%)	32 (58.2%)	43 (81.1%)	0
During racing (<i>n</i> = 80)	76 (95%)	15 (18.8%)	29 (36.3%)	66 (84.6%)	0

^aGDH ELISA: before racing, *n* = 53; during racing, *n* = 78.

CPE, *Clostridium perfringens* enterotoxin; GDH, glutamate dehydrogenase.

culture of *C. difficile* from the feces or a positive ELISA result for GDH.

The percentage of dogs with a positive result for CPE was significantly (POR = 4.0, 95% CI = 1.2–12.9, $P = .021$) higher during racing than before racing (Table 2). However, CPE was not significantly associated with diarrhea in dogs before racing or during racing. None of the 3 dogs with a positive test for CPE before racing had diarrhea. During racing, 4/15 dogs (26.7%, 95% CI = 0–65.4%) with a positive test for CPE had diarrhea whereas 25/65 dogs (38.5%, 95% CI = 27.3–49.6%) with a negative test for CPE had diarrhea. Additionally, dogs that tested positive for CPE during racing were significantly less likely to have blood present in their feces (POR = 0.20, 95% CI = 0.054–0.76, $P = .018$). Twenty-eight of 65 dogs (43.1%, 95% CI = 23.0–63.2%) had blood in their stools, despite a negative test for CPE, whereas 2/15 dogs (13.3%, 95% CI = 0–36.3%) had blood in their stools with a positive CPE test. No dogs tested had a positive result for *C. difficile* toxins (TcdA and TcdB) before or during racing (Table 2).

Culture results for *E. coli*, *Campylobacter*, and *Salmonella* and immunofluorescence results for *Giardia* and *Cryptosporidium*

There was a significant difference in isolation proportions of *Campylobacter* (POR = 0.45, 95% CI = 0.20–1.00, $P = .049$) but not of *Salmonella* ($P = .59$) from dogs during racing versus dogs before racing (Table 3). *E. coli* was isolated from all dogs before and during racing; however, *E. coli* 0157 was not identified in any samples tested. Additionally, there was no significant association between diarrhea and a positive culture for *Campylobacter* or *Salmonella* in dogs before racing or during racing ($P = .31$ and $.059$, respectively). Additionally, dogs were no more likely to have a positive immunofluorescence result for *Giardia* or *Cryptosporidium* during racing as compared with before racing ($P = .72$ and $.71$, respectively, Table 3), and *Cryptosporidium* was not associated with diarrhea in dogs before racing or during racing ($P = 1.0$ and $.78$, respectively). However, *Giardia* was significantly associated with fecal score during racing ($P < .001$). All 5 dogs positive for *Giardia* during racing had a fecal score of 2, whereas the 5 dogs positive for *Giardia* before racing had fecal scores of 3 (2 dogs) and 4 (3 dogs). The presence of blood in the feces was not significantly associated with the presence of *Campylobacter*, *Salmonella*, *Giardia*, or *Cryptosporidium* ($P \geq .14$).

Table 3. Culture results for *Campylobacter* and *Salmonella* and immunofluorescence results for *Giardia* and *Cryptosporidium* spp.

	<i>Campylobacter</i>	<i>Salmonella</i>	<i>Giardia</i>	<i>Cryptosporidium</i>
Before racing	6/39 (15.4%)	43/55 (78.2%)	5/53 (9.4%)	1/53 (1.9%)
During racing	6/80 (7.5%)	57/80 (71.3%)	5/67 (7.5%)	2/67 (3.0%)

There was no association between having 2 or more pathogens versus none or 1 pathogen and the presence of diarrhea.

Results of *Salmonella* serotyping

Fifteen serotypes of *Salmonella* representing 5 serogroups (B, C1, C2, D, and E) were isolated from samples obtained before racing (Table 4). Multiple (≥ 2) serotypes of *Salmonella* were identified in 27.9% (95% CI = 0.94–54.9%; 12 of 43) samples containing *Salmonella* obtained before the race. In fecal samples obtained during the race, 9 serotypes were identified from the same 5 serogroups isolated in samples obtained before the race. Multiple (≥ 2) serotypes were identified in 40.4% (95% CI = 28.6–52.1%) of fecal samples containing *Salmonella* during the race.

Discussion

This study critically assesses both the prevalence of diarrhea and the association between diarrhea and multiple enteropathogenic bacteria and parasites in a relatively large cohort of racing sled dogs before and during a long distance race. The prevalence of diarrhea during racing was 36%, with nearly 9% of dogs having liquid diarrhea. This is considerably higher than previously reported, but is compatible with reliable anecdotal accounts (S. Nelson, Jr, Iditarod Trail Committee, personal communication). The significance of the problem is further underscored by the fact that the majority of mushers reported using one or more treatments for the prevention or control of diarrhea in their team during racing.

Despite the suspicion among some members of the mushing community of *C. perfringens* being a cause of diarrhea in racing sled dogs, the current study did not identify any association between the isolation of *C. per-*

Table 4. *Salmonella* serotypes isolated in fecal samples obtained from sled dogs before and during the 2008 Iditarod trail race.

<i>Salmonella</i> Serotype	Number of Samples Positive before Racing	Number of Samples Positive during Racing
<i>S. Infantis</i>	17	1
<i>S. Newport</i>	14	24
<i>S. Typhimurium</i> (copenhagen)	6	5
<i>S. Typhimurium</i>	5	13
<i>S. Dublin</i>	4	4
<i>S. Kentucky</i>	3	0
<i>S. Mbandaka</i>	2	6
<i>S. Hadar</i>	1	0
<i>S. Reading</i>	1	4
<i>S. Heidelberg</i>	1	0
<i>S. Montevideo</i>	1	0
<i>S. Muenster</i>	1	14
<i>S. Uganda</i>	1	7
<i>S. Orion</i>	1	0
<i>S. Anatum</i>	1	0
<i>S. Agona</i>	0	7

fringens or *C. difficile* or the detection of their respective toxins, and the presence of diarrhea in racing sled dogs. *C. perfringens* was isolated from 97% of dogs in the current study, consistent with its nature as a commensal organism of the canine intestinal tract.^{6,7} However, although the percentage of both CPE positive dogs and dogs with diarrhea increased with racing, there was no statistically significant association between these 2 phenomena. Furthermore, we did not identify toxins of *C. difficile* in any dogs before or during racing, although the apparent rate of intestinal carriage of *C. difficile* indicated by the results of fecal culture and the GDH assay appears to be substantially higher than reported in previous studies of companion dogs.^{6,7,10,11} Common antigen (GDH) was detected in more dogs compared with isolation of *C. difficile*, and it is plausible that the delay in performing fecal cultures on selective medium could have resulted in a lower diagnostic yield. Testing for common antigen via ELISA is an extremely valuable diagnostic test to help rule out infection with *C. difficile*, as the lack of detection of GDH and TcdA in diarrheic people and dogs has been shown to have a high negative predictive value, with a specificity of 98% reported.^{11,12} Nevertheless, it is important to recognize that tests with a high negative predictive value can have a poor sensitivity, and therefore be unable to adequately diagnose disease. It would have been optimal to perform PCR testing on *C. difficile* isolates to determine whether the strains were toxigenic in an effort to better characterize the association between *C. difficile* and diarrhea; however, this was not performed in the current study. A previous study in 143 dogs did identify a good correlation between the in vitro tissue culture cytotoxin-B assay (CTA) and detection of toxigenic isolates confirmed via PCR testing.¹¹ However, due to the relatively expensive and time consuming nature of CTA, more effort should be focused on PCR testing of isolates in an effort to help characterize *C. difficile* infection in dogs. The reason for the higher prevalence of *C. difficile* in dogs in the current study is not known; however, antibiotics and gastric acid suppressants are commonly administered to sled dogs during racing and both represent risk factors for *C. difficile* infection in humans.¹³ We did not demonstrate such a relationship in the current study.

It is important to recognize the limitations of the immunoassays used for the detection of *C. difficile* toxins (TcdA and TcdB) and CPE. The immunoassays currently utilized by all veterinary reference laboratories were developed for use in people, and have not been validated to date for use in animals. This concern is underscored by a recent study documenting the low sensitivity of 5 human-based ELISAs for TcdA and TcdB, in which sensitivities for the assays tested on 143 canine fecal specimens ranged from 7 to 33%.¹¹ In addition, the human-based ELISA used for detection of CPE in this study has been associated with positive results in 5–13% of clinically healthy dogs.^{7,10} Lastly, the limitations of single fecal cultures for the diagnosis of *Salmonella* are well documented, and it is plausible that the prevalence of *Salmonella* might have been even higher if more than 1 fecal culture was performed on each dog. The intermit-

tent shedding of *Salmonella* has been well documented in dogs.¹⁴ It is also plausible that the delay in processing of fecal specimens at the microbiology laboratory might have compromised our culture results, despite the immediate placement of all fecal specimens in appropriate transport medium. Nevertheless, the high prevalence of *Salmonella* observed in both diarrheic and nondiarrheic dogs in this study parallels the findings of previous studies and highlights the potential for zoonotic transmission of this organism.^{2,15}

The other enteropathogens studied in the current project also did not appear to be associated with the presence of diarrhea during racing. Although a positive immunofluorescence test for *Giardia* was significantly associated with the presence of diarrhea during racing, the prevalence of *Giardia* was relatively low and was not different in dogs before and during racing. This suggests that *Giardia* infection was not a significant factor in the majority of sled dogs with diarrhea in the current study, in accord with previous findings.³ Hence, the high prevalence of diarrhea in racing dogs is not attributable to currently identifiable enteropathogens, at least as a single etiology.

In the current study, a strong association was observed between racing and the presence of blood, mucus, or both in the feces, with 46.5% of dogs studied during racing displaying gross evidence of gastrointestinal bleeding (hematochezia or melena). However, it is possible that the prevalence of gastrointestinal bleeding was even higher because the raw meat contained in the diet the dogs consumed precluded the use of more sensitive methods of testing for occult fecal blood. Gastrointestinal bleeding has been suspected to occur frequently in racing sled dogs, based on documented declines in hematocrit and serum albumin and increases in serum urea nitrogen during racing.^{16,17} Additionally racing sled dogs have a prevalence of up to 61% of potentially hemorrhagic gastric lesions, including gastritis and gastric ulcerations.^{18,19} However, the high prevalence of hematochezia, in addition to diarrhea, indicates that colonic dysfunction is also an important phenomenon in sled dogs during racing. The current study did not identify any of the enteropathogens studied to be associated with the presence of hematochezia or melena. Gastrointestinal problems, including gastrointestinal hemorrhage and diarrhea, also occur with a high incidence in human athletes during long distance running.^{20,21} Hematochezia has been reported in up to 16% of runners and occult fecal blood in up to 85% of ultramarathon athletes.^{22,23} Similar to sled dogs, gastric lesions occur in human runners, and the stomach is a common source of gastrointestinal hemorrhage associated with exercise.^{24,25} However, a positive correlation has been demonstrated between the occurrence of fecal blood and lower gastrointestinal clinical signs in long distance runners, and the colon is also reported to be an important site of inflammation and hemorrhage associated with running activity.^{25–27}

Proposed etiologies for gastrointestinal dysfunction associated with running include gastrointestinal ischemia, the actions of GI hormones and neuropeptides

released during exercise, endotoxemia, mechanical stimulation or trauma to the bowel, and exercise induced alterations of colonic motility.^{26,28,29} Exercise, even at moderate intensity, can significantly reduce splanchnic blood flow, which may have consequences for mucosal integrity and intestinal contractility.^{30,31} A recent study demonstrated mucosal destruction and altered intestinal contractility in a murine model of repetitive treadmill running.³² Although aerobic training helps sustain mesenteric blood flow and exerts a protective effect against oxidative injury to the intestine, even highly trained athletes commonly suffer from gastrointestinal problems during competition, including severe ischemic colitis.^{26,33–36} Human intestinal tissue has a very rapid repair mechanism in response to ischemic injury, which may explain the usually transient nature of exercise associated gastrointestinal injury and may complicate definitive identification of ischemic injury in affected athletes.^{26,37,38} It is possible that impaired splanchnic blood flow may also contribute to gastrointestinal dysfunction in racing sled dogs. A previous study of exercising sled dogs did not identify a reduction in mesenteric blood flow; however, the dogs were run relatively modest distances of 30 miles or less.³⁹ The effect on mesenteric blood flow of the more rigorous and much longer duration exercise that dogs encounter in an event like the Iditarod is not known; however, dogs racing the Iditarod develop a high incidence of gastric lesions concurrently with increased intestinal permeability.¹⁹

A study of meat samples fed during the 2003 Iditarod Trail race identified *Salmonella* organisms in 12.7% of samples tested, including a nalidixic acid-resistant Newport MDR-AmpC strain.¹ In the current study, susceptibility testing was not performed on isolated serotypes from fecal samples to determine antibiotic resistance patterns; however, serotype Newport was the most frequently isolated strain. Serotype Typhimurium, frequently linked to human salmonellosis, was also isolated from dogs before and during racing.⁴⁰ Although there is no evidence that *Salmonella* infection is associated with pathologic consequences in racing sled dogs, the high prevalence of *Salmonella* carriage in racing sled dogs represents a significant public health concern, particularly if antibiotic resistant strains are involved.

Iditarod mushers utilize a range of medications and methods to combat diarrhea in their dogs, some of which are potentially deleterious to the animal, such as the use of untested vaccines. The use of most agents is largely empirical, with minimal controlled clinical research support, though psyllium may provide some beneficial effects.⁴¹ However, the widespread use of antibiotics, especially given that a bacterial cause of diarrhea has not been established, is to be questioned, particularly because antibiotic treatment may have an adverse impact on the gastrointestinal flora of diarrheic dogs.⁴²

In conclusion, the current study confirmed a high prevalence of diarrhea and hematochezia in sled dogs during long distance racing. However, neither phenomenon was associated with any of the bacterial or protozoal enteropathogens studied. Further research is warranted to determine the cause of gastrointestinal dysfunction in

racing sled dogs and to determine if the syndrome is analogous to that occurring in human running athletes.

Footnotes

- ^a <http://www.iditarod.com/mushers/iditarodrules.html>
^b Purina Fecal Scoring System for Dogs and Cats, Nestle-Purina Pet Food Co, St Louis, MO
^c *C. perfringens* enterotoxin test (T5006), Techlab Inc, Blacksburg, VA
^d Tox A/B Quik Chek (T5029), Techlab Inc
^e C. diff Quik Chek (T5033), Techlab Inc
^f Parapak C & S single vial (cat # 900612), Fisher Scientific, Pittsburgh, PA
^g Para-Tect *Cryptosporidium* /*Giardia* direct fluorescence assay (MCC-C/G-DFA), Medical Chemical Corp, Torrance, CA
^h Remel, Lenexa, KS
ⁱ Lee JA, Hinchcliff K, Munro DS, et al. Nalidixic acid-resistant MDR-AMP C *Salmonella enterica* serotype Newport isolated from a canine raw meat diet. Proceedings. Seventh Congress of the International Sled Dog Veterinary Medical Association. Anchorage, AL, 2004:94 (abstract)
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