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ORIGINAL RESEARCH

Coliform and Pathologic Bacteria in Sierra Nevada National Forest Wilderness Area Lakes and Streams

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Objective.—To analyze backcountry-area water quality in US Department of Agriculture Forest Service–designated wilderness areas for the presence of coliform and potentially pathogenic bacteria.

Methods.—Thirty-one backcountry lakes and streams were selected that would stratify the risk based on use by backpackers, pack animals, commercial grazing animals, or natural unaffected wilderness areas. Sites included Desolation Wilderness (10 sites), Carson-Iceberg Wilderness (4 sites), Emigrant Wilderness (3 sites), Hoover Wilderness (6 sites), John Muir Wilderness (3 sites), and Golden Trout Wilderness (5 sites). Water was collected in sterile tubes and quantification was performed through Millipore bacterial samplers. On return to the laboratory, bacteria were harvested from the samplers and subjected to qualitative analysis that identified species according to standard laboratory methods.

Results.—Coliform bacteria were detected in 14 of 31 sites (45%). Eight sites had high levels of coliforms. All 8 of these sites correlated with heavy human use or commercial grazing. Coliforms were identified as *Escherichia coli*. In addition, 1 sample contained *Yersinia entercolitica*. All samples contained expected amounts of normal aquatic bacteria, including *Pseudomonas* spp, *Rahnella aquatilis*, *Serratia* spp, and other nonpathogenic species of *Yersinia* in concentrations of 600 to 10 000 colony-forming units per 100 mL.

Conclusions.—In this study, coliform bacteria were found at nearly half of the sampling sites. High coliform levels correlated with high-impact human use or cattle grazing.

Key words: wilderness bacteria, wilderness water, coliforms, *Escherichia coli*, Sierra Nevada

Introduction

Wilderness areas within US Department of Agriculture National Forest Service lands provide a unique and important resource for the United States. In concept, a balance of animals, plants, microbiologic life, and ecologic systems is preserved in their natural state and protected into the distant future. Watersheds are important within wilderness areas and essential to the ecological balance.[1] However, the charge of the Forest Service is termed *multiuse land management.*[2] Therefore, even specific wilderness areas within Forest Service districts may be used for commercial cattle, sheep grazing, or commercial pack-animal trips. Such activities may have an impact on the water quality in these wilderness areas. Despite multiuse land management, remote wilderness lakes and streams have, for the most part, remained oligotrophic and free from both chemical and bacterial pollution.[1] Wilderness water quality is important to summer backpackers, day hikers, fishermen, and other recreational users, as well as downstream municipal water districts. Many wilderness areas are used by children who may acquire pathogens by inadvertently swallowing water while swimming or playing in it.[3,4] This problem is potentially significant because many wilderness trailheads are a short distance from lakes or streams.

Until the 1980s, little was written on potentially pathogenic organisms in Sierra Nevada wilderness lakes and streams. However, after that time, a number of studies raised the issue of *Giardia lambdia* as a microbial pollutant of alpine lakes and streams.[5–8] Although *G lambdia* has been the subject of attention in relationship to waterborne disease, we believe that bacteria pose an equal or greater risk of causing waterborne disease in humans. Pathogenic bacteria in wilderness water may

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originate from a number of sources, including cattle and sheep grazing, pack animals, and humans visiting wilderness areas.[9–11] Animal manure may be swept into watersheds by summer storms or annual snowmelt. To further characterize the bacteriology of watersheds in Forest Service wilderness areas, we conducted a study to search and identify coliforms, pathogenic bacteria, and nonpathogenic bacteria.

Methods

FIELD-SITE SELECTION

A total of 31 sites were selected in advance, which statistically would differentiate among environmental risk for different types of bacterial risk among wilderness areas of the Sierra Nevada range in California. Wilderness areas included Desolation Wilderness near Lake Tahoe, Carson-Iceberg Wilderness between California Highways 4 and 108, Emigrant Wilderness to the northwest of Yosemite National Park, Hoover Wilderness to the east of Yosemite, John Muir Wilderness north of Kings Canyon National Park, and Golden Trout Wilderness south of Sequoia National Park. Risk classifications included 1) high use by backpackers, 2) high use of pack animals, 3) tracts where cattle or sheep grazed, and 4) sites not contaminated by humans or domesticated animals. Trailhead quotas and personal observations were used to determine human impact. Cattle or sheep grazing was determined by actual observation of animals or evidence of extensive manure and effects on landscapes, as well as cattle tract allotment data.

FIELD-WATER COLLECTION

Water samples were collected from June through September 2003. Water was collected in sterile test tubes and Millipore total coliform-count samplers (Millipore Corporation, Bedford, MA). Samples were collected in duplicate, were cooled following standardized procedures, and were transported to the University of California, Davis.[12] Sample devices measured bacteria for 1 mL of sample. This was multiplied by 100 as per standardized procedure of reporting colony-forming units (CFU) per 100 mL in the water literature. Water temperature was measured at each site with a stream thermometer (Cortland Line Company Inc, Cortland, NY).

WATER ANALYSIS

The quantitative analyses for both coliform counts and total bacterial counts were obtained from incubated Millipore counting-plate paddles. Bacterial colonies were harvested from counting plates and transport tubes for qualitative analysis that would provide specific identification of bacterial organisms. These colonies were initially plated onto sheep blood agar and MacConkey agar. Further screening was done by subplating again onto sheep blood agar and also Sorbitol-MacConkey agar, *Yersinia* selective agar (Remel, Lenexa, KS), triple sugar iron agar tubes, and lysine iron agar tubes. An analysis was done to confirm the presence of coliform bacteria and to identify other pathogenic bacteria according to standardized automated microbiology laboratory procedures. Coliforms were also subjected to further analysis to determine the presence of *Escherichia coli* O157 by using latex agglutination techniques.

Results

A total of 31 different sites in the wilderness areas were sampled. Five sites were sampled in both early and late season for a total of 37 samples (Table). Water temperatures ranged from a low of 3°C, at Cottonwood Lake (elevation 11 100 feet) in July in John Muir Wilderness to 21°C in August at Hidden Lake in Desolation Wilderness.

No coliform bacteria were found in 17 sites. In analyzing these sites for risk factors, we determined that no cattle or sheep grazing occurred above or near these sites. However, coliform-free sites were found both in areas with absent or minimal human usage and in areas with moderate human impact.

Coliform bacteria were detected in 14 samples at 14 different locations. Low levels of coliforms (50–100 CFU/100 mL) were found at 5 locations, which included areas both lightly and moderately affected by human activity. Higher levels of coliforms (≥150 CFU/100 mL) were found at 8 sites: 1) Ralston Lake in Desolation Wilderness, 2) Disaster Creek in Carson-Iceberg Wilderness, 3) Powell Lake in Emigrant Wilderness, 4) Molybdenite Creek in Hoover Wilderness, 5) Burt Canyon in Hoover Wilderness, 6) Buckeye Creek in the proposed addition to Hoover Wilderness at Big Meadow, 7) Buckeye Creek site 2 in the proposed addition to Hoover Wilderness, and 8) Horseshoe Meadow in Golden Trout Wilderness. Cattle, stock, or sheep grazing occurred in the last 5 of these areas, whereas heavy use by backpackers or day hikers occurred in the first 3.

NONCOLIFORM BACTERIA

All collected specimens grew naturally occurring aquatic bacteria. Counts ranged from 600 to 10 000 CFU/100 mL. These bacteria were identified and included *Rahnella aquatilis*, *Yersinia frederiksenii*, *Aeromonas hydro-*

Wilderness-area water analysis for pathogenic bacteria, summer 2003

Wilderness area	*Location**	*Elevation (feet)*	*Water temperature (°C)*	*Coliform bacteria*	*Other bacteria†*
Desolation	Genevieve Lake	7400	20	None	1100
Desolation	Crag Lake	7460	20	None	2500
Desolation	Hidden Lake	7500	21	None	5600
Desolation	Stony Ridge Creek	7800	19	None	2350
Desolation	Phipps Creek	7600	18	100	5500
Desolation	Generals Creek	7500	20	None	8500
Desolation	Meeks Creek	7000	20	None	8900
Desolation	Tamarack Lake	7820	19	None	1350
Desolation	Ralston Lake	7780	20	200	1650
Desolation	Ropi Lake	7544	19	None	4100
Carson-Iceberg	Arnot Creek, early season	6500	10	100	3600
Carson-Iceberg	Arnot Creek, late season	6500	14	0	6300
Carson-Iceberg	Disaster Creek, early season	6700	9	0	1000
Carson-Iceberg	Disaster Creek, late season	6700	‡	200	7200
Carson-Iceberg	Clark Fork, early season	6700	9	None	800
Carson-Iceberg	Clark Fork, late season	6700	‡	None	4400
Carson-Iceberg	Rock Lake	7315	20	None	3800
Emigrant	Blue Canyon, early season	9100	11	None	600
Emigrant	Blue Canyon, late season	9100	14	None	2000
Emigrant	Powell Lake, early season	8800	‡	0	3400
Emigrant	Powell Lake, late season	8800	‡	200	6500
Emigrant	Stanislaus River above Kennedy Meadows, early season	6600	9	None	1000
Emigrant	Stanislaus River above Kennedy Meadows, late season	6600	‡	None	4800
Hoover	Molybdenite Creek	9100	10	300	2500
Hoover	Little Walker River (Burt Canyon)	8750	10	200	1450
Hoover	Buckeye Creek at the ‘‘Forks’’	8400	8	50	1650
Hoover	Side Creek into Buckeye Creek	8100	8	100	2900
Proposed Hoover	Buckeye Creek at Big Meadow	7550	11	200	3100
Proposed Hoover	Buckeye Creek above Big Meadow	7700	9	550	5800
Muir	Taboose Creek	7200	9	150	4000
Muir	Cottonwood Lake no. 1	11 100	3	None	10 000
Muir	Cottonwood Creek	9600	5	None	10 000
Golden Trout	Horseshoe Meadow	9900	9	500	2000
Golden Trout	Mulky Meadows (side creek)	9300	17	None	4000
Golden Trout	SF Kern at Tunnel	8900	7	None	4000
Golden Trout	Golden Trout Creek at Tunnel	8900	7	None	5500
Golden Trout	Little Whitney Meadow	8400	9	None	6000

*Early = May/June; late = August/September.
†Colony-forming units per 100 mL.
‡Temperature not taken.

phila, *Pseudomonas* spp, *Serratia fonticola*, and *Serratia plymuthica*. We found only 1 site that grew a colony of an established human pathogen. *Yersinia entercolitica* was found at a site at Disaster Creek in Carson-Iceberg Wilderness.

Altitude and water temperature were analyzed regarding total bacterial counts and total coliform counts. No correlation could be made between altitude or water temperature regarding the presence or absence of coliforms or the colony counts of naturally occurring aquatic bacteria.

Discussion

Seventeen of 31 sites in the wilderness lakes and streams were free of *E coli* and other coliforms. Some of these sites included areas below moderate human use, such as Clark Fork, Rock Lake, the upper Stanislaus River, and Cottonwood Lake. The absence of coliforms at these sites may have been a result of a lack of contamination of the water, the timing of sampling, or the limited survival of coliforms under certain conditions. For example, direct sunlight decreases the surviving *E coli*.[13] Furthermore, underground water flow in some areas may provide a filtering effect. The absence of coliforms in moderately used backpacker areas was also found in a study of Sierra Nevada national parks.[14]

Coliforms were found at 14 locations in the current study. The presence of coliforms at these sites could also be a result of contamination by human visitors, pack animals, or cattle or sheep grazing, or it could be a permanent condition of the natural ecosystem. Coliform bacteria have been used as indicators of fecal pollution or contamination of waterways in the United States.[15] The coliform group of bacteria consists of several genera belonging to the family Enterobacteriaceae.[15] These are gram-negative, non–spore-forming, rod-shaped bacteria that ferment lactose when incubated at 35°C. The most common genera and species associated with human or animal fecal contamination include *E coli*, *Klebsiella*, or *Enterobacter*. Of the 14 sites at which coliforms were found, 8 were in areas of moderate human use or cattle or sheep grazing. In fact, all sites sampled below alpine meadows used for grazing contained coliforms.

E coli and other coliform bacteria can survive in aquatic environments for many weeks depending on the nutrient availability, pH level, and water temperature. In nutrient-rich eutrophied tropical waters, *E coli* not only survives, but it also can replicate. The number of years that *E coli* can survive in cooler aquatic environments has been subject to discussion.[16,17] In a previous study, we found significant concentrations of *E coli* below cattle-grazed meadows in Golden Trout Wilderness 9 months after the last cattle-grazing activity.[18] We believe grazing cattle and sheep pose a risk to the quality of wilderness water. Range cattle are noted to carry the *E coli* strain O157:H7 at a rate of 1%, potentially placing humans who drink untreated water below established cow pastures at risk for a very serious pathogenic disease.[12] In addition, other non-O157 *E coli* are capable of inducing serious disease in humans.[19] The most serious among the diseases caused by *E coli* O157:H7 is the hemolytic uremic syndrome. Children are especially vulnerable; hemolytic uremic syndrome develops in an estimated 10% of infected children under 10 years of age.[20] Furthermore, a non-O157:H7 strain of *E coli* (O121:H19) was documented as the causative agent in 11 cases of hemolytic uremic syndrome associated with a contaminated lake in Connecticut.[19]

Studies of wilderness water have suggested a correlation between total bacterial counts and use by backpackers.[21] Although late-season total counts were higher in watersheds used by backpackers, we did not perform early and late samplings at sites where no visible impact by humans occurred. Noncoliform pathogenic bacteria were detected at only 1 site, where *Y entercolitca* was found. Other studies of wilderness water have found *Campylobacteria*, *Salmonella*, and *Yersinia* spp.[9,18,21] High water run-off from abundant snowfall as well as wilderness-management practices may have contributed to our lack of finding significant numbers of pathogenic bacteria other than the single *Yersinia* colony.

STUDY LIMITATIONS

This study focused only on the identity and prevalence of bacteria. Other waterborne organisms capable of causing human disease, including the protozoa *G lambdia*, were not analyzed. Water samples were a single point in time and would not identify fluctuations in bacteria that could occur during the summer season. To fully study water pathogens in the Sierra Nevada range, a multiyear project that samples each site regularly throughout each summer should be undertaken. In addition, such a study should also include an analysis for protozoal organisms. Finally, molecular typing to differentiate human vs animal *E coli* would provide knowledge on the precise source of contamination.

Conclusion

Many wilderness lakes and streams studied contained low to moderate levels of coliforms. The highest levels of coliforms occurred in watersheds heavily used by humans, livestock, or cattle grazing. The low levels of coliform bacteria found in a very few streams and lakes may be part of a natural ecological environment or may be secondary to occasional contamination from humans, pack animals, or natural wild animals. Noncoliform and nonpathogenic aquatic bacteria were present in counts consistent with other studies on environmental bacteria.

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