

ORIGINAL RESEARCH

Coliform Bacteria in Sierra Nevada Wilderness Lakes and Streams: What Is the Impact of Backpackers, Pack Animals, and Cattle?

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Objective.—The presence of coliform bacteria indicates a watershed risk for harboring microbes capable of causing human disease. We hypothesized that water from watersheds that have different human- or animal-use patterns would have differing risks for the presence of coliform bacteria.

Methods.—Water was collected in wilderness areas of the Sierra Nevada range in California. A total of 60 sites from lakes or streams were selected to statistically differentiate the risk categories: 1) high use by backpackers, 2) high use by pack animals, 3) cattle- and sheep-grazing tracts, and 4) natural areas rarely visited by humans or domestic animals. Water was collected in sterile test tubes and Millipore coliform samplers during the summer of 2004. Water was analyzed at the university microbiology lab, where bacteria were harvested and then subjected to analysis by standardized techniques. Confirmation was performed with a Phoenix 100 bacteria analyzer. Statistical analysis to compare site categories was performed with Fisher exact test.

Results.—Only 1 of 15 backpacker sites yielded coliforms. In contrast, 12 of 15 sites with heavy pack-animal traffic yielded coliforms. All 15 sites below the cattle-grazing areas grew coliforms. Differences between backpacker and cattle or pack-animal areas were significant ($P \leq .05$). Only 1 of the 15 wild sites rarely visited by humans grew coliforms. All coliforms were identified as *Escherichia coli*. All samples grew normal aquatic bacteria of the genera *Pseudomonas*, *Ralstonia*, and *Serratia* and nonpathogenic strains of *Yersinia*. No correlation could be made with temperature or elevation. Sites below cattle-grazing tracts and pack-animal usage areas tended to have more total bacteria.

Conclusions.—Alpine wilderness water below cattle-grazing tracts or areas used by pack animals are at risk for containing coliform organisms. Areas exclusively used by backpackers were nearly free of coliforms.

Key words: water, Yosemite National Park, Kings Canyon National Park, Sierra Nevada, *Escherichia coli*

Introduction

The Sierra Nevada range snowpack serves as an important water source for California; its watershed provides nearly 50% of the state's freshwater supply.¹ It is important that this watershed be protected from microbial, chemical, and toxic pollution for users both downstream and upstream.

Within the Sierra Nevada range, over 3 000 000 acres of land have been designated as official wilderness by

the National Park Service or United States Department of Agriculture (USDA) Forest Service and protected from development, logging roads, and motor vehicles.^{2,3} Some wilderness areas have quotas to limit overnight camping by backpackers and use by pack animals. Most of these protected areas are in high alpine regions between 2000 and 4200 m in elevation. These high alpine lakes and streams are an especially important watershed for California because of presumed purity of water and a large quantity of precipitation in the form of snow. The water is important for not only the distant water users but also the local water users such as backpackers, campers, fishermen, and the USDA Forest Service and

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National Park Service. However, this land is potentially subject to pollution by day hikers, backpackers, horses and pack animals, and also commercial cattle and sheep grazing. Pollution may occur from potential harmful substances that include microbial organisms or toxic substances.⁴ Microbial organisms that may cause illness in humans include pathogenic bacteria such as coliforms and protozoa such as *Giardia* or *Cryptosporidium*.⁵ Chemicals or toxins may be imported or synthesized by microbes, zooplankton, or phytoplankton from precursors imported by humans. Debate has ensued on the impact of backpackers, cattle grazing, or livestock such as mules and horses polluting the watersheds in wilderness areas. We completed 2 studies in a previous year that surveyed remote Sierra Nevada lakes and streams.^{6,7} However, these studies did not provide the statistical power to show significant differences for risk factors. This current study was designed to provide a direct comparison of risk factors.

Coliform bacteria have been established as indicators of fecal pollution or contamination of waterways in the United States.^{8,9} Coliforms may originate from a single source or a combination of sources: 1) backpackers, 2) pack animals, 3) grazing animals (cows, sheep), and 4) wild animals. Coliform pollution of wilderness areas by humans occurs through inadequate burial and disposal of fecal material. In addition, bathing or swimming in alpine lakes may also result in microbial pollution.⁹ Pack animals may pollute by deposition of manure either directly into lakes and streams or indirectly onto trails or meadows, from which it may be washed into waterways by summer storms and annual snowmelt. The USDA Forest Service "leases" tracts in wilderness areas for cattle grazing.² As a result, a high density of cattle manure may be found in certain alpine watersheds, either in meadows or as a result of direct deposit into streams or lakes. Finally, coliform or other bacteria may originate from natural, wild animal zoonotic reservoirs.

We hypothesized that wilderness freshwater from watersheds that have different human- or animal-use patterns would have differing risks for the presence of coliform bacteria. Therefore, the purpose of the study was to analyze wilderness freshwater samples for coliforms and compare results from watersheds that have different use patterns among the following groups: 1) backpackers, 2) horses and mules (pack animals), 3) cattle grazing, and 4) isolated areas affected only by natural wild animals.

Methods

FIELD SITE COLLECTION

Sixty sites were prospectively selected to differentiate among environmental risks for different types of bacte-

rial contamination in wilderness areas of Kings Canyon National Park, Sequoia National Park, and Yosemite National Park as well as the following USDA Forest Service wilderness areas: Mokelumne, Carson-Iceberg, Emigrant, Hoover Wilderness, Adams, John Muir, and Golden Trout. The Hall Natural Research Area, adjacent to the eastern boundary of Yosemite National Park and the southern boundary of Hoover Wilderness, was also included. No overnight camping or motor vehicles are allowed in the Hall area, and the remote areas have minimal visits by humans.

Risk classifications included 1) high use by backpackers, 2) high use of pack animals, 3) cattle-grazing tracts, and 4) natural sites (wild ecologies) not likely contaminated by humans or domesticated animals. Sites were risk stratified with the assistance of the National Park Service and USDA Forest Service on the basis of user nights by backpackers, pack animals, and cattle allotments in grazing tracts. Cattle grazing is not permitted in national parks, so all samples in cattle-grazing tracts were taken from within USDA Forest Service wilderness areas.

FIELD WATER COLLECTION

Water samples were collected from May through September in 2004. Water was collected in sterile test tubes and Millipore total coliform count samplers (Millipore Corporation, Bedford, MA). All samples were collected in duplicate, cooled according to standardized procedures, and transported to the University of California, Davis.¹⁰ Sample devices measured bacteria for 1 mL of sample. This was multiplied by 100 as per standardized procedure of reporting colony-forming units per 100 mL in the water literature. Water temperature was measured at each site with a stream thermometer (Cortland Line Company Inc, Cortland, NY).

BACTERIAL ANALYSIS OF WATER SAMPLES

Details of analysis for bacteria have been described elsewhere.^{6,7} The analysis for coliform counts and total bacterial counts required incubating Millipore counting plate paddles at 35°C for 24 hours. Bacterial colonies were counted and then harvested for further analysis. Colonies were initially plated onto sheep blood and MacConkey agars (Remel Inc, Lenexa, KS). Lactose fermenting colonies from MacConkey plates were presumed to be coliform bacteria and were subject to further testing. Further screening and initial identification was performed by subplating onto C.I.N. (*Yersinia*) agar, Sorbitol-MacConkey agar, L.I.A., and T.S.I. tubes. Precise identification of bacteria genera and species analysis

Table 1. Sites with heavy backpacking*

Wilderness area	Place	Elevation (m)	Temperature (°C)	<i>Escherichia coli</i> CFU/100 mL	Other bacteria CFU/100 mL
Yosemite	Yosemite Creek	2278	11.1	None	200
Yosemite	Budd Creek	2701	7.8	None	600
Yosemite	Townsley Lake	3154	13.3	None	5200
Emigrant	Wire Lakes	2694	19.4	None	3800
Emigrant	Blue Lake	3048	17.8	None	1100
Mokelumne	Round Top Lake	2834	17.2	None	800
Kings Canyon	East Lake	2493	13.9	None	6400
Kings Canyon	North Fork Woods Creek	2621	11.1	None	1900
Kings Canyon	South Fork Kings River (Upper Basin)	3078	12.2	None	4400
John Muir	Chicken Foot Lake (Little Lakes Valley)	3288	11.6	200	2900
John Muir	Ruwau Lake	3366	12.2	None	4100
Golden Trout	Chicken Spring Lake	3429	15.6	None	4600
Sequoia	Upper Rattlesnake Creek	3169	14.4	None	1100
Sequoia	Kern River	2031	16.7	None	3800
Desolation	Meeks Creek	2133	17.8	None	8900

*CFU indicates colony-forming units.

were performed by standardized automated laboratory procedures. In addition, analysis was also performed with a Phoenix 100 bacteria autoanalyzer. Strains were grown on Colombia agar with 5% sheep red blood cells for 16 to 24 hours at 37°C, replated, and grown again for 16 to 24 hours at 37°C just before testing. A suspension of 0.5 McFarland (accepted range, 0.5–0.6) was prepared in the identification (ID) broth (Becton Dickinson, Erembodegem, Belgium) and poured within 30 minutes into the panel, which was then loaded into the instrument within 30 minutes. Four quality-control strains (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Klebsiella pneumoniae* ATCC 700603, and *Pseudomonas aeruginosa* ATCC 27853) were loaded with each study batch, which always met quality-control criteria. The Phoenix instrument gives an ID result when a species or group of species is identified with more than 90% confidence. The confidence value is a measure of the likelihood that the issued ID is the only correct ID. The average time required to reach an ID result ranged from 3 to 12 hours. The autoanalyzer provided a computer printout identifying the bacteria. *E. coli* colonies were also subjected to analysis to determine the presence of *E. coli* O157 by using latex agglutination methodology.

Statistical significance among groups was calculated with Fisher exact test by STATA 8 Software (STATA Corporation, College Station, TX).

Results

The results are summarized in Tables 1 through 4. Significant differences were found among sample groups. All 15 samples that were taken below areas in which cattle grazed or had recently grazed were positive for coliform growth. From areas frequented by pack animals, 12 of 15 samples had coliforms. In contrast, coliforms were found in only 1 of 15 areas of heavy backpacking. Only 1 of 15 sites rarely visited by humans or pack animals contained coliforms. Backpacker and natural-site groups had significantly fewer sites with coliforms when compared with the cattle-grazing group ($P \geq .01$). Likewise, the pack-animal group had significantly more sites with coliforms when compared with the backpacker and natural areas ($P \geq .05$). No statistical differences were found in numbers of coliform bacteria according to water temperature or elevation.

Noncoliform aquatic bacteria were also identified from the samples. The most common bacteria found included *Achromabacter* species, *Pasteurella haemolytica*, *Rahnella aquatilis*, *Ralstonia paucula*, *Serratia odorifera*, *Serratia plymthica*, *Yersinia intermedia*, *Yersinia kristensenii*, *Yersinia frederiksenii*, *Pseudomonas putida*, and *Pseudomonas fluorescens*. No correlation could be made between site use and types of noncoliform bacteria or total bacteria counts, except for the Hall Natural Research Area, where the total bacteria range was the lowest of any group of samples. Total bacteria in the Hall

Table 2. Sites with stock (horses and pack animals)*

Wilderness area	Place	Elevation (m)	Temperature (°C)	<i>Escherichia coli</i> CFU/100 mL	Other bacteria CFU/100 mL
Hoover	W. Walker River	2262	11.1	250	3100
Emigrant	Horse/Cow Meadow Stream	2686	10.0	200	3000
Emigrant	Grouse Lake inlet stream	2179	5.0	550	2500
Emigrant	Piute Creek—Groundhog Meadows	2286	7.8	300	2000
Emigrant	Spring Meadow Creek	2590	23.3	900	10 000
Kings Canyon	Arrow Lake	3154	17.2	350	2100
Kings Canyon	Kings River—Paradise Valley	1981	14.4	500	1500
Yosemite	Fletcher Lake	3095	15.0	None	5800
John Muir	Long Lake (Bishop Pass Trail)	3277	12.2	150	5000
John Muir	Rock Creek at Wilderness Boundary	3154	11.1	300	8200
Yosemite	Tuolumne River (Lyell Canyon)	2804	16.1	200	3000
Kings Canyon	Dollar Lake	3115	17.2	None	1800
Kings Canyon	Rae Lake (middle)	3211	16.7	None	3100
Golden Trout	Horseshoe meadow	3017	10.0	300	1500
John Muir	Cottonwood lakes	3383	8.9	200	10 000

*CFU indicates colony-forming units.

Natural Research Area ranged from 200 to 500 per 100 mL. Temperature or elevation was not a factor, as other sites with similar temperature and elevation had higher baseline levels of aquatic bacteria. The marked absence of human impact distinguished this area.

Discussion

In this study, areas frequented by cattle or pack animals had the greatest degree of fecal contamination into the wilderness watershed. We are not surprised at the finding of coliforms below cattle-grazing areas. In most of these areas, moderate amounts of cattle manure were observed during field collections. We identified all coliforms in our study as *E coli*. In some respects, finding coliforms below grazing areas serves as a positive control for the study. One might expect coliforms in watersheds with high densities of cattle.¹¹ However, we are surprised at the finding of coliforms in areas frequented by pack animals. National parks and the USDA Forest Service have strict requirements on management of livestock in wilderness areas. It is not possible to exclude a human contribution to this finding, as high-volume pack-animal areas are also used by humans. In previous years we have examined Sierra Nevada water for coliform bacteria.^{6,7} However, those studies were from water taken primarily from watersheds polluted by both pack animals and humans, and we were unable to fully determine associated risks for coliform pollution. This current study identified and included sampled sites used exclusively by backpackers and not pack animals. In addition, this current

study added sites that were unused by humans, pack animals, or cattle. The absence of coliforms in most of those areas used exclusively by humans and the absence of pack animals would suggest that pack animals are most likely the source of coliform pollution. Pack animals produce high volumes of manure, which is deposited directly onto the surface of trails, soil, or meadows.^{12,13} Manure deposited on the ground may be swept into streams during summer rains or spring snow runoff. During the field operations of the study, pack animals were observed on several occasions to be defecating directly into bodies of freshwater. Fecal contamination as indicated by the finding of coliforms would place the watershed at risk for harboring microbes capable of causing human disease. Some of these infections are a potential threat to humans. This includes certain pathogenic strains of *E coli*, *Salmonella*, *Campylobacter*, and *Aeromonas* and protozoa such as *Giardia*, all of which have animal reservoirs. The organism *Yersinia enterocolitica* has been previously cultured in high alpine areas of the Sierra Nevada range and may have a natural reservoir in small mammals and birds.¹⁴ Pack animals entering the High Sierra have been subject to analysis, and *Giardia* samples were found in their manure.¹⁵

E coli and other pathogenic bacteria can survive in aquatic environments for long periods depending on the nutrient availability, pH, and water temperature. The number of years that *E coli* can survive in aquatic environments has been debated.¹⁶ A study of Lake Michigan shore water showed that *E coli* may sustain itself indefinitely in appropriate environmental situations.¹⁷

Table 3. Cattle-grazing sites*

Wilderness area	Place	Elevation (m)	Temperature (°C)	<i>Escherichia coli</i> CFU/100 mL	Other bacteria CFU/100 mL
Carson	Upper Clark Fork River	2072	11.2	250	10 000
Carson	Lower Clark Fork River	2316	8.9	300	2600
Carson	Disaster Creek—north fork	2366	10	350	1300
Carson	Disaster Creek—east fork	2438	10.6	200	5700
Carson	Arnot Creek	2000	11.1	100	4600
Carson	Woods Gulch	1976	11.7	100	5200
Hoover	Buckeye Creek (Big Meadows)	2274	12.8	500	3800
Hoover	Buckeye Creek side creek	2377	8.9	450	4700
Hoover	Molydunite Creek	2773	11.1	400	3400
Hoover	South Fork Walker River (Burt Canyon)	2719	11.1	250	2800
Golden Trout	Mulkey Meadows	2840	15.6	100	3500
Golden Trout	Little Whitney Meadow	2560	16.7	100	3500
Emigrant	Borland Lake	2264	8.9	250	8400
Adams	East Fork Chiquito Creek	2212	14.5	100	5200
Adams	Cold Creek	2503	14	150	4600

*CFU indicates colony-forming units.

Open-range cattle are noted to carry *E coli* strain O157:H7 at a rate of 1%, placing humans who drink untreated water below established cow pastures at risk for a very serious disease.¹³ Studies on this strain have also shown it to survive in cold water.¹⁸ In addition, many non-O157 *E coli* are capable of inducing serious disease in humans.¹⁰ Although it is possible to genetically differentiate human from animal and ecologic *E coli*, these tech-

niques are very expensive and available only in limited laboratories in the United States.

Finally, we wish to comment on the noncoliform bacteria found in the study. Aquatic bacteria are part of a normal ecosystem of lakes and streams.¹⁹ Indeed, if bacteria were absent, the normal food chain from frogs to fish, as well as the ecological balance, would be in jeopardy. The most common bacteria we found was *R aqua-*

Table 4. Low-impact sites: rare visits by humans*

Wilderness area	Place	Elevation (m)	Temperature (°C)	<i>Escherichia coli</i> CFU/100 mL	Other bacteria CFU/100 mL
Hall area	Green Treble Lake—lower	3115	10	None	300
Hall area	Green Treble Lake—upper	3116	10	None	400
Hall area	Maul Lake	3117	10.6	None	200
Hall area	Spuller Lake	3132	11.1	None	500
Kings Canyon	Avalanche Creek	1554	8.9	None	5000
Yosemite	Middle Dana Fork Creek	3016	12.8	None	1200
Yosemite	Parker Pass Creek	2971	13.9	None	1500
Yosemite	Granite Lake	3167	14.5	None	1200
Kings Canyon	Cunningham Creek	2621	14.0	None	2300
Sequoia	Upper Buck Creek	2209	16.7	None	3400
John Muir	Little Cottonwood Creek	2996	14.5	None	1900
Kings Canyon	North Guard Creek	2895	14.0	None	2600
Sequoia	Side Spring Creek Franklin Pass Trail	3078	5	None	1200
Sequoia	Laurel Creek	2063	13.9	None	4700
Yosemite	Miguel Creek—upper north fork	1503	12.8	100	1800

*CFU indicates colony-forming units.

tilis. Several nonpathogenic species of *Yersinia* were also cultured. Many bird species can be carriers of non-pathogenic species of *Yersinia* and *Y enterocolitica*.²⁰ Previous studies of wilderness water suggest a correlation between total bacterial counts and usage by backpackers.^{6,7} Freshwater from remote alpine areas has been shown to be a source of *Campylobacter*, *Salmonella*, and *Y enterocolitica*, although these were not found in the current study.^{21,22,23}

Conclusion

The risk for finding coliform bacteria in alpine wilderness water was determined by the use of the adjacent watershed. Water in areas used extensively by pack animals or for cattle grazing was routinely contaminated, whereas water in those areas used exclusively by backpackers or rarely visited by humans was rarely contaminated.

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