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ORIGINAL RESEARCH

An Analysis of Wilderness Water in Kings Canyon, Sequoia, and Yosemite National Parks for Coliform and Pathologic Bacteria

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Objective.—To determine the prevalence of coliform and potentially pathogenic bacteria in remote backcountry alpine lakes and streams of national parks in the Sierra Nevada mountains.

Methods.—Water was sampled at 55 predetermined lakes and streams that would stratify the risk, based on sites used by backpackers, sites used by pack animals, and uncontaminated wild areas. Sites were distributed among Kings Canyon (15), Sequoia (17), and Yosemite (23). Water was collected using Millipore bacterial samplers, which provided specific counts of coliform and other bacteria in each water sample and also served as a transport media from the wilderness to the laboratory. On return to the laboratory, bacteria were harvested from the samplers and subjected to specific identification and qualitative analysis using standard microbiology techniques for the analysis of water.

Results.—Coliform bacteria were detected in 22 of the 55 sites. All of these sites were below areas used by backpackers or pack animals. Thirty-three sites were free of coliforms. These sites included both those used lightly by backpackers and those with no human or domestic animal use. All samples contained expected amounts of normal aquatic bacteria including *Pseudomonas, Rahnella aquatilis, Serratia* spp, and nonpathogenic species of *Yersinia*.

Conclusions.—Most sampling sites in these national parks are free of coliform or pathogenic organisms. Low levels of coliform bacteria are found in some bodies of water where the watershed has been affected by human or pack animal travel.

Key words: Yosemite, Sequoia National Park, Kings Canyon National Park, coliforms, wilderness water

Introduction

The quality of water in wilderness streams and lakes in Kings Canyon, Sequoia, and Yosemite national parks is important to multiple users. Backcountry national park water is used by summer backpackers, day hikers, fishermen, and other recreational users. Precipitation that collects as snow during the winter storm season provides continuous water for streams into late summer from snow runoff.[1,2]

Currently, an emphasis has been placed on *Giardia* as the major harmful water microbial contaminant in wilderness areas.[3–5] Although certain mammals such as beavers have been thought to be natural reservoirs of the infection, we believe the seriousness of exposure in the wilderness to *Giardia* has been overemphasized. The average concentration of less than 10 cysts/1000 L reported in studies of Sierra Nevada wilderness water poses minimal risk to humans.[6,7] In one study of Sierra Nevada backpackers who developed diarrhea, none had *Giardia*.[6] Although portable water filters may remove *Giardia* and other protozoal organisms, they easily become clogged with sediment and may be less useful on extended trips. In addition, some water filters used by backpackers may be effective at filtering out *Giardia* but not bacteria.[8]

We believe that bacteria, not protozoa such as *Giardia*, pose a greater risk of causing waterborne disease in humans. This has also been suggested by others.[9–11] Pathogenic bacteria may originate from ''imported'' sources, such as pack animals and humans visiting wil-

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derness areas, or may be present from natural environmental sources. Manure may be swept into streams and rivers by summer storms as well as annual snowmelt. Areas of high human use may result in the contamination of waterways with pathogenic bacteria. Finally, other bacteria may originate from natural wild animal zoonotic reservoirs. Some of these zoonotic infections are a potential threat to humans. These include certain strains of *Escherichia coli*, *Salmonella*, *Campylobacter*, and *Aeromonas*. The organism *Yersenia enterocolitica*, which has previously been cultured in high alpine areas of the Sierra, may have a natural reservoir in small mammals.[12,13] *Leptospirosis*, *Listeria*, and certain species of *Vibrio* and *Aeromonas* are found in some animals and aquatic environments and potentially may be found in Sierra water.

Methods

FIELD SITE COLLECTION

A total of 55 predetermined sites were selected that statistically differentiated among environmental risk for different types of bacterial risk in Kings Canyon, Sequoia, and Yosemite national parks. Risk classifications included 1) sites with high use by backpackers; 2) sites with high use by pack animals; and 3) natural sites (wild ecologies) not contaminated by humans or domesticated animals. Sites were selected in Kings Canyon (15 sites), Sequoia (17 sites), and Yosemite (23 sites). Sites were risk stratified with the assistance of the National Park Service.

FIELD WATER COLLECTION

Water samples were collected from June through September 2003. We defined early season as June and July and late season as August and September. Water was collected in 1) sterile test tubes, and 2) total coliform count samplers (Millipore Corporation, Bedford, MA). Samples were collected in duplicate and were then cooled following standardized procedures and transported to UC Davis (Sacramento, CA).[14] Sample devices measured bacteria for 1 mL of sample. This was multiplied by 100 per the standardized procedure of reporting colony-forming units (CFU) per 100 mL in the water literature. Water temperature was measured at each site by a stream thermometer (Cortland Line Company Inc, Cortland, NY).

BACTERIAL ANALYSIS OF WATER SAMPLES

The quantitative analysis for coliform counts and total bacterial counts was obtained after incubating Millipore counting plate paddles at 35°C for 24 hours. Bacterial colonies were then harvested from counting plates and transport tubes for qualitative analysis. Colonies were initially plated onto sheep blood and MacConkey agars. Further screening and initial identification were performed by subplating onto CIN (*Yersinia*) agar, sorbitol-MacConkey agar, LIA tubes, and TSI tubes. Specific identification of bacteria genera and species analysis were performed to confirm the presence of coliform bacteria and to identify other pathogenic bacteria using standardized automated laboratory procedures. Further analysis was performed using 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 Phoenix 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 (*E coli* ATCC 25922, *Klebsiella pneumonia* ATCC 13883, *K 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. A computer printout identifying the bacteria was provided by the autoanalyzer. *E coli* colonies were also subjected to analysis using latex agglutination methodology to determine the presence of *E coli* O157.

Results

A total of 55 different sites were sampled in the national parks. Twelve of these sites were sampled both early and late season. The results from Kings Canyon National Park are displayed in Table 1, the results from Sequoia National Park are displayed in Table 2, and the results from Yosemite National Park are displayed in Table 3. Water temperatures ranged from a low of 4°C at several early-season streams to 17°C during August at Dollar Lake in Kings Canyon.

COLIFORM BACTERIA

No coliform bacteria were found in 33 of the locations. Some of these locations also included watersheds used by livestock and backpackers—for example, Bubbs

Table 1. Kings Canyon National Park wilderness water analysis for pathogenic bacteria, summer 2003

Stream/Lake	*Exact location*	*Elevation*	*Coliform bacteria**		*Other bacteria**	
			Early†	*Late†*	*Early†*	*Late†*
Rae Lake	Lower Outlet	10 535	⋯	None	⋯	7500
Dollar Lake	Outlet	10 220	⋯	100	⋯	4000
South Fork Woods Creek	At confluence with North Fork	8600	None	100	1650	4300
North Fork Woods Creek	At confluence with South Fork	8600	None	None	1700	2500
South Fork Kings River	Above confluence of Woods Creek	6696	None	None	11 500	500
South Fork Kings River	Lower Paradise Valley	6500	None	100	3000	1800
Bull Frog Lake	Outlet	10 610	⋯	None	⋯	4000
Bubbs Creek	Vidette Meadow	9500	⋯	None	⋯	5000
East Creek	At confluence of Bubbs Creek	8180	⋯	100	⋯	8000
Bubbs Creek	Junction Meadow	8100	⋯	None	⋯	4750
Bubbs Creek	At confluence of Kings	5150	None	None	200	900
Copper Creek	100 yd above trail	5100	100	100	1200	2300
Granite Creek	100 yd above stock trail	5000	None	Dry	3000	Dry
Roaring River	Pool at waterfall base	7200	⋯	None	⋯	850
Lewis Creek	100 yd above road	4000	100	100	1400	3900

*Colony-forming units per 100 mL.
†Early = May/June; late = August/September.

Table 2. Sequoia National Park wilderness water analysis for pathogenic bacteria, summer 2003

Stream/Lake	*Exact location*	*Elevation*	*Coliform bacteria**		*Other bacteria**	
			Early†	*Late†*	*Early†*	*Late†*
Upper Rattlesnake Creek	Treeline Meadow	10 460	⋯	None	⋯	1100
Lower Rattlesnake Creek	Above trail crossing Kern Valley	6563	⋯	None	⋯	5000
Kern River	Above confluence of Big Arroyo	6666	⋯	None	⋯	4000
Big Arroyo	100 yd above Kern Trail	6696	⋯	None	⋯	4800
Laurel Creek	100 yd above Kern Trail	6450	⋯	None	⋯	5100
Soda Springs	Near Kern RS	6405	⋯	300	⋯	None
Coyote Creek	100 yd above Kern Trail	6477	200	100	4000	7500
Kern River	At park boundary bridge	6300	None	100	1800	8000
Lone Pine Creek	Above Hamilton Lake Trail	7300	100	⋯	2900	⋯
Bear Paw Meadow	Backpackers' water faucet	7600	2000	⋯	6500	⋯
Buck Creek	100 yd above trail	7200	100	⋯	750	⋯
9 Mile Creek	At trail crossing	7550	None	⋯	1200	⋯
Franklin Creek	Below dam	9934	⋯	100	⋯	2000
Franklin Creek	Mineral King Trail crossing	8377	⋯	None	⋯	10 000
Side spring	Franklin Lake Trail	9737	⋯	None	⋯	350
Crystal Creek	100 yd above trail	7963	⋯	200	⋯	2000
South Fork Kaweah	Lady Bug Trail	4700	⋯	None	⋯	3900

*Colony-forming units per 100 mL.
†Early = May/June; late = August/September.

Table 3. Yosemite National Park wilderness water analysis for pathogenic bacteria, summer 2003

Steam/Lake	*Exact location*	*Elevation*	*Coliform bacteria**		*Other bacteria**	
			Early†	*Late†*	*Early†*	*Late†*
Flecher Lake	Outlet	10 220	None		500	⋯
Vogelsang Lake	Outlet	10 341	None	⋯	10 000	⋯
Bernice Lake	Outlet	10 217	None	⋯	500	⋯
Booth Lake	East Shore	9850	100	⋯	2700	⋯
Emeric Lake	East Shore	9400	None	⋯	2800	⋯
Babcock Lake	East Shore	8983	None	⋯	600	⋯
Washburn Lake	Outlet	7600	None	⋯	1200	⋯
Merced Lake	North Shore	7200	1000	⋯	5500	⋯
Rafferty Creek	100 yd above JMT crossing	8790	None	⋯	300	⋯
Dana Fork	At Parker Pass Trail	9500	None	⋯	600	⋯
Tuolumne River	Tuolumne Meadows	8550	100	None	2200	6500
Tuolumne River	JMT upper bridge (Glen Aulin)	8330	⋯	None	⋯	11 000
Tuolumne River	200 yd below Glen Aulin bridge	7800	⋯	None	⋯	5700
Tuolumne River	At Cathedral Creek confluence	5600	⋯	500	⋯	4400
Tuolumne River	Just above Pate Valley	4832	⋯	None	⋯	3900
Return Creek	At confluence of Tuolumne	6200	⋯	200	⋯	4000
Rogers Creek	At confluence of Tuolumne	5350	⋯	None	⋯	7000
Piute Creek	Pate Valley	4365	⋯	None	⋯	5800
Yosemite Creek	¼ mi. above Highway 120	7474	100	None	700	10 500
Snow Creek	¼ mi. above Highway 120	8430	None	1000	350	12 000
Kibby Creek	Trail Crossing above Lake	4700	⋯	250	⋯	8000
Chain of Lakes	Outlet	8900	None	⋯	2000	⋯
South Fork Merced River	1 mi. west of Chain of Lakes	8100	None	⋯	800	⋯

*Colony-forming units per 100 mL.
†Early = May/June; late = August/September.

Creek (Kings Canyon) at the confluence of the Kings River and Big Arroyo River (Sequoia) and portions of the Tuolumne River above Hetch Hetchy (Yosemite).

Coliform bacteria were detected at 22 of the 55 sites. These were all identified as *E coli* species. At 13 locations, low levels of coliforms were found (50–100 CFU/100 mL). Backpacker use above these locations occurred. These locations included 1) Kern River at the park boundary; 2) Lone Pine Creek at the High Sierra Trail; 3) Buck Creek at the High Sierra Trail; 4) Franklin Creek below the dam; 5) Dollar Lake at the outlet; 6) South Fork Woods Creek above the confluence of North Fork; 7) South Fork Kings River at Lower Paradise Valley; 8) East Creek at the Bubbs Creek confluence; 9) Copper Creek; 10) Lewis Creek; 11) Booth Lake; 12) Upper Yosemite Creek; and 13) Tuolumne River below Tuolumne Meadows.

At 9 locations, higher levels of coliforms were found:

1. Soda Springs near the Kern River Ranger Station in southern Sequoia National Park, which has high visitation by humans, had 300 CFU/100 mL.
2. Crystal Creek, near Mineral King, is also affected by humans and had 200 CFU/100 mL.
3. Coyote Creek near the Kern River Ranger Station had coliforms identified both early and late season. During spring runoff in May 2003, we found 200 CFU/100 mL. This may be because of animal contamination or residual contamination from the prior season. The midsummer analysis at Coyote Creek showed 100 CFU/100 mL.
4. The water faucet at the Bear Paw Meadow campground yielded 2000 CFU/100 mL, the highest found during this study.
5. Merced Lake (1000 CFU/100 mL)
6. Tuolumne River at the confluence of Cathedral Creek (500 CFU/100 mL)
7. Return Creek near the confluence of the Tuolumne (200 CFU/100 mL)
8. Snow Creek below May Lake (1000 CFU/100 mL)

Table 4. Aquatic bacteria cultured

Kings Canyon
Achromabacter species
Pasteurella haemolytica
Rahnella aquatilis
Serratia odorifera
Serratia plymthica
Yersinia intermedia
Yersinia kristensenii
Sequoia
Pseudomonas putida
Pseudomonas species undetermined
R. aquatilis
S. plymythica
Yersinia frederiksenii
Yosemite
P. haemolytica
Pseudomonas fluorescens
P. putida
R. aquatilis
Ralstonia paucula
Serratia fonticola
Y. frederiksenii
Y. intermedia
Yersinia odorifera
Yersinia ruckeri

9. Kibby Creek in Yosemite (250 CFU/100 mL)

OTHER BACTERIA

Normal aquatic bacteria were cultured at all sample sites. Locations with high bacterial counts (>5000 CFU/100 mL) included the outlet of Lower Rae Lake, Bubbs Creek below Vidette Meadow, East Creek at the confluence of Bubbs Creek, Lower Rattlesnake Creek, Coyote Creek, Kern River at the park boundary, and Franklin Creek at the lower trail crossing. These bacteria included 1) *Rahnella aquatilis*, 2) nonpathogenic *Yersinia* spp, and 3) *Pseudomonas* spp (see Table 4). *R aquatilis* was the most frequently discovered bacteria, found at 50% of the sampling sites equally spread among the 3 national parks, followed by various *Pseudomonas* spp found at 30% of the sites.

Twelve sampling sites were studied both early and late season. Total bacterial counts were higher during late season at all but 1 site. At the other sites, total bacteria at least doubled and, in one instance, increased fourfold. The mean temperature at these sites increased between early- and late-season sampling times from 9°C to 12°C. We did not detect other pathogenic bacteria in this study.

Discussion

Most backcountry lakes and streams in Kings Canyon, Sequoia, and Yosemite national parks do not contain *E coli* or other coliforms. The very low levels of coliforms found at 13 of 22 positive locations could either be part of the natural environment or ecosystem or occur as a result of contamination by human visitors or pack animals. *E coli* and other coliforms can be found in the fecal material of many animals and birds.[15] Therefore, some of the *E coli* identified may be solely the result of the natural animal and bird populations. The higher levels found at 9 locations were in watersheds clearly affected by humans and pack animals.

Coliform bacteria have been used as indicators of fecal pollution or contamination of waterways in the United States. The coliform group of bacteria consists of several genera belonging to the family Enterobacteriaceae.[16] These bacteria are gram-negative, nonspore-forming, rod-shaped bacteria that ferment lactose when incubated at 35°C. The most common species associated with human or animal fecal contamination include *E coli*, *Klebsiella*, and *Enterobacter*. All coliforms in this study were *E coli*.

It is generally accepted that *E coli* and other coliform bacteria can survive in aquatic environments for at least several weeks, depending on the nutriment availability, pH, and water temperature. The number of years that *E coli* can survive in aquatic environments has been debated.[17] A recent study of the beaches of Lake Michigan suggests that *E coli* sustains itself indefinitely in appropriate environmental situations.[18] Indeed, we have found significant concentrations of *E coli* below cattle-grazed meadows in the Golden Trout Wilderness 9 months after the last cattle-grazing activity.[14] Although less relevant in national park environments, range cattle are noted to carry *E coli* strain O157:H7 at a rate of 1%, potentially placing persons who drink untreated water below established cow pastures at risk for a very serious pathogenic disease.[19] Studies of this strain have also shown it to survive in cold water.[20] Potentially, runoff from Golden Trout Creek is relevant to Sequoia and Kings Canyon national parks. In addition, many non-O157 *E coli* strains are capable of inducing serious disease in humans.[21]

It is difficult to explain the higher coliform counts found at 4 locations. Significant human and pack animal use occurs in the vicinity of Soda Springs and Crystal Creek. Activity also occurs in the Coyote Creek watershed, but we do not know to what extent. Ongoing studies need to be conducted to determine if the contamination is from wild animals, pack animals, or human sources. Although it is possible to differentiate human

from animal/ecologic *E coli* genetically, these techniques are very expensive and are available only in limited laboratories in the United States. The one finding of high levels of coliforms at the Bear Paw Meadow Campground should be considered a single-point sample only and would require confirmation with multiple samples taken during a summer season. However, this wilderness camping area is one of the most heavily used areas in Sequoia National Park and receives heavy pack animal traffic. A spring feeding the camp area water system is in close geographic proximity.

TOTAL BACTERIAL COUNTS

Aquatic bacteria are part of a normal ecosystem of lakes and streams. Indeed, if bacteria were absent, the normal food chain, from frogs to fish, as well as the ecologic balance would be in jeopardy. The most common bacterium found was *R aquatilis*. Several nonpathogenic species of *Yersinia* were also cultured. Some bird species are carriers of *Yersinia*.[22] A previous study of wilderness water suggested a correlation between total bacterial counts and use by backpackers.[14] Although during late season, total bacterial counts were higher in watersheds used by backpackers, we did not take enough samples at the same sites both early and late season to draw conclusions. Most remote alpine Sierra Nevada lakes have very limited essential nutriments, elements, and organic compounds and are considered oligothrophic in scientific terms. This limits algae growth and may create an environment that supports only limited preservation of bacteria. Eutrophication (nutriment loading) of heavily used lakes is of concern, because it may lead to the formation of algae blooms and upset the natural ecologic balance. This nutriment loading may result from pack animal manure, phosphate-containing soap used by bathing humans, and clothes washing, among other activities. The increased bacteria observed could be secondary to nutrient loading. To study this observation further, data on phosphates, nitrates, and phytoplankton must be obtained.

We did not detect noncoliform pathogenic bacteria in this study. However, other studies of wilderness water have found *Campylobacteria*, *Salmonella*, and *Y entercolitica*.[9,14,23] High water runoff from abundant snowfall as well as wilderness management practices may have contributed to our not finding these bacteria in Kings Canyon, Sequoia, and Yosemite national parks.

Conclusion

The wilderness lakes and streams studied in Kings Canyon, Sequoia, and Yosemite national parks contain expected levels of normal aquatic bacteria. Most sampling sites are free of coliform bacteria. The low levels of coliform bacteria found in some streams and lakes may be part of a natural ecologic environment, or they may be secondary to contamination from humans, pack animals, or natural wild animals. Further studies are necessary to answer this question.

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