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-
Bacterial counts was obtained after incubating Millipore
coated filters. The quantitative analysis for coliform counts and total
bacterial counts 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).

**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, Se-
quoa, 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
cultures) 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 Sep-
tember 2003. We defined early season as June and July
and late season as August and September. Water was
scoated 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 transpor-
ted to UC Davis (Sacramento, CA). Sample devices
measured bacteria for 1 mL of sample. This was multi-
plied 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 per-
formed 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 bac-
teria and to identify other pathogenic bacteria using stan-
dardized automated laboratory procedures. Further anal-
ysis was performed using a Phoenix 100 bacteria au-
toanalyzer. Strains were grown on Columbia 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 (ac-
teped 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 qual-
ity control strains (E coli ATCC 25922, Klebsiella pneu-
monia 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 col-
onies were also subjected to analysis using latex agglu-
tination 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

<table>
<thead>
<tr>
<th>Stream/Lake</th>
<th>Exact location</th>
<th>Elevation</th>
<th>Coliform bacteria*</th>
<th>Other bacteria*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rae Lake</td>
<td>Lower Outlet</td>
<td>10 535</td>
<td>...</td>
<td>None</td>
</tr>
<tr>
<td>Dollar Lake</td>
<td>Outlet</td>
<td>10 220</td>
<td>...</td>
<td>100</td>
</tr>
<tr>
<td>South Fork Woods Creek</td>
<td>At confluence with North Fork</td>
<td>8600</td>
<td>None</td>
<td>100</td>
</tr>
<tr>
<td>North Fork Woods Creek</td>
<td>At confluence with South Fork</td>
<td>8600</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>South Fork Kings River</td>
<td>Above confluence of Woods Creek</td>
<td>6696</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>South Fork Kings River</td>
<td>Lower Paradise Valley</td>
<td>6500</td>
<td>None</td>
<td>100</td>
</tr>
<tr>
<td>Bull Frog Lake</td>
<td>Outlet</td>
<td>10 610</td>
<td>...</td>
<td>None</td>
</tr>
<tr>
<td>Bubbs Creek</td>
<td>Vidette Meadow</td>
<td>9500</td>
<td>...</td>
<td>None</td>
</tr>
<tr>
<td>East Creek</td>
<td>At confluence of Bubbs Creek</td>
<td>8180</td>
<td>...</td>
<td>100</td>
</tr>
<tr>
<td>Bubbs Creek</td>
<td>Junction Meadow</td>
<td>8100</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Bubbs Creek</td>
<td>At confluence of Kings</td>
<td>5150</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Copper Creek</td>
<td>100 yd above trail</td>
<td>5100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Granite Creek</td>
<td>100 yd above stock trail</td>
<td>5000</td>
<td>None</td>
<td>Dry</td>
</tr>
<tr>
<td>Roaring River</td>
<td>Pool at waterfall base</td>
<td>7200</td>
<td>...</td>
<td>None</td>
</tr>
<tr>
<td>Lewis Creek</td>
<td>100 yd above road</td>
<td>4000</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

*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

<table>
<thead>
<tr>
<th>Stream/Lake</th>
<th>Exact location</th>
<th>Elevation</th>
<th>Coliform bacteria*</th>
<th>Other bacteria*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper Rattlesnake Creek</td>
<td>Treeline Meadow</td>
<td>10 460</td>
<td>...</td>
<td>None</td>
</tr>
<tr>
<td>Lower Rattlesnake Creek</td>
<td>Above trail crossing Kern Valley</td>
<td>6563</td>
<td>...</td>
<td>None</td>
</tr>
<tr>
<td>Kern River</td>
<td>Above confluence of Big Arroyo</td>
<td>6666</td>
<td>...</td>
<td>None</td>
</tr>
<tr>
<td>Big Arroyo</td>
<td>100 yd above Kern Trail</td>
<td>6696</td>
<td>...</td>
<td>None</td>
</tr>
<tr>
<td>Laurel Creek</td>
<td>100 yd above Kern Trail</td>
<td>6450</td>
<td>...</td>
<td>None</td>
</tr>
<tr>
<td>Soda Springs</td>
<td>Near Kern RS</td>
<td>6405</td>
<td>300</td>
<td>...</td>
</tr>
<tr>
<td>Coyote Creek</td>
<td>100 yd above Kern Trail</td>
<td>6477</td>
<td>200</td>
<td>100</td>
</tr>
<tr>
<td>Kern River</td>
<td>At park boundary bridge</td>
<td>6300</td>
<td>None</td>
<td>100</td>
</tr>
<tr>
<td>Lone Pine Creek</td>
<td>Above Hamilton Lake Trail</td>
<td>7300</td>
<td>100</td>
<td>...</td>
</tr>
<tr>
<td>Bear Paw Meadow</td>
<td>Backpackers' water faucet</td>
<td>7600</td>
<td>2000</td>
<td>...</td>
</tr>
<tr>
<td>Buck Creek</td>
<td>100 yd above trail</td>
<td>7200</td>
<td>100</td>
<td>...</td>
</tr>
<tr>
<td>9 Mile Creek</td>
<td>At trail crossing</td>
<td>7550</td>
<td>None</td>
<td>...</td>
</tr>
<tr>
<td>Franklin Creek</td>
<td>Below dam</td>
<td>9934</td>
<td>...</td>
<td>100</td>
</tr>
<tr>
<td>Franklin Creek</td>
<td>Mineral King Trail crossing</td>
<td>8377</td>
<td>...</td>
<td>None</td>
</tr>
<tr>
<td>Side spring</td>
<td>Franklin Lake Trail</td>
<td>9737</td>
<td>...</td>
<td>None</td>
</tr>
<tr>
<td>Crystal Creek</td>
<td>100 yd above trail</td>
<td>7963</td>
<td>...</td>
<td>200</td>
</tr>
<tr>
<td>South Fork Kaweah</td>
<td>Lady Bug Trail</td>
<td>4700</td>
<td>...</td>
<td>None</td>
</tr>
</tbody>
</table>

*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

<table>
<thead>
<tr>
<th>Stream/Lake</th>
<th>Exact location</th>
<th>Elevation</th>
<th>Coliform bacteria*</th>
<th>Other bacteria*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Early†</td>
<td>Late†</td>
<td>Early†</td>
</tr>
<tr>
<td>Flecher Lake</td>
<td>Outlet</td>
<td>10,220</td>
<td>None</td>
<td>500</td>
</tr>
<tr>
<td>Vogelsang Lake</td>
<td>Outlet</td>
<td>10,341</td>
<td>None ...</td>
<td>10,000</td>
</tr>
<tr>
<td>Bernice Lake</td>
<td>Outlet</td>
<td>10,217</td>
<td>None ...</td>
<td>500</td>
</tr>
<tr>
<td>Booth Lake</td>
<td>East Shore</td>
<td>9,850</td>
<td>100 ...</td>
<td>2,700</td>
</tr>
<tr>
<td>Emeric Lake</td>
<td>East Shore</td>
<td>9,400</td>
<td>None ...</td>
<td>2,800</td>
</tr>
<tr>
<td>Babcock Lake</td>
<td>East Shore</td>
<td>8,983</td>
<td>None ...</td>
<td>600</td>
</tr>
<tr>
<td>Washburn Lake</td>
<td>Outlet</td>
<td>7,600</td>
<td>None ...</td>
<td>1,200</td>
</tr>
<tr>
<td>Merced Lake</td>
<td>North Shore</td>
<td>7,200</td>
<td>1,000 ...</td>
<td>5,500</td>
</tr>
<tr>
<td>Rafferty Creek</td>
<td>100 yd above JMT crossing</td>
<td>8,790</td>
<td>None ...</td>
<td>300</td>
</tr>
<tr>
<td>Dana Fork</td>
<td>At Parker Pass Trail</td>
<td>9,500</td>
<td>None ...</td>
<td>600</td>
</tr>
<tr>
<td>Tuolumne River</td>
<td>Tuolumne Meadows</td>
<td>8,550</td>
<td>100</td>
<td>None</td>
</tr>
<tr>
<td>Tuolumne River</td>
<td>JMT upper bridge (Glen Aulin)</td>
<td>8,330</td>
<td>...</td>
<td>None</td>
</tr>
<tr>
<td>Tuolumne River</td>
<td>200 yd below Glen Aulin bridge</td>
<td>7,800</td>
<td>...</td>
<td>None</td>
</tr>
<tr>
<td>Tuolumne River</td>
<td>At Cathedral Creek confluence</td>
<td>5,600</td>
<td>...</td>
<td>500</td>
</tr>
<tr>
<td>Tuolumne River</td>
<td>Just above Pate Valley</td>
<td>4,832</td>
<td>...</td>
<td>None</td>
</tr>
<tr>
<td>Return Creek</td>
<td>At confluence of Tuolumne</td>
<td>6,200</td>
<td>...</td>
<td>200</td>
</tr>
<tr>
<td>Rogers Creek</td>
<td>At confluence of Tuolumne</td>
<td>5,350</td>
<td>...</td>
<td>None</td>
</tr>
<tr>
<td>Piute Creek</td>
<td>Pate Valley</td>
<td>4,365</td>
<td>...</td>
<td>None</td>
</tr>
<tr>
<td>Yosemite Creek</td>
<td>¼ mi. above Highway 120</td>
<td>7,474</td>
<td>100</td>
<td>None</td>
</tr>
<tr>
<td>Snow Creek</td>
<td>¼ mi. above Highway 120</td>
<td>8,430</td>
<td>None</td>
<td>1,000</td>
</tr>
<tr>
<td>Kibby Creek</td>
<td>Trail Crossing above Lake</td>
<td>4,700</td>
<td>...</td>
<td>250</td>
</tr>
<tr>
<td>Chain of Lakes</td>
<td>Outlet</td>
<td>8,900</td>
<td>None</td>
<td>...</td>
</tr>
<tr>
<td>South Fork Merced River</td>
<td>1 mi. west of Chain of Lakes</td>
<td>8,100</td>
<td>None</td>
<td>...</td>
</tr>
</tbody>
</table>

*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 2,000 CFU/100 mL, the highest found during this study.
5. Merced Lake (1,000 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 (1,000 CFU/100 mL)
Table 4. Aquatic bacteria cultured

Kings Canyon
- Achromabacter species
- Pasteurella haemolytica
- Rahnella aquatilis
- Serratia odorifera
- Yersinia intermedia
- Yersinia kristensenii

Sequoia
- Pseudomonas putida
- Pseudomonas species undetermined
- R. aquatilis
- S. plymthica
- 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. 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. 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 nutrient availability, pH, and water temperature. The number of years that E. coli can survive in aquatic environments has been debated. A recent study of the beaches of Lake Michigan suggests that E. coli sustains itself indefinitely in inappropriate environmental situations. 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. 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. Studies of this strain have also shown it to survive in cold water. 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.

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 ecological 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*. A previous study of wilderness water suggested a correlation between total bacterial counts and use by backpackers. 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 nutrients, elements, and organic compounds and are considered oligotrophic in scientific terms. This limits algae growth and may create an environment that supports only limited preservation of bacteria. Eutrophication (nutrient loading) of heavily used lakes is of concern, because it may lead to the formation of algae blooms and upset the natural ecological balance. This nutrient 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 *Campylobacter, Salmonella*, and *Y. enterocolitica*. 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 ecological environment, or they may be secondary to contamination from humans, pack animals, or natural wild animals. Further studies are necessary to answer this question.

**Acknowledgments**


**References**


