Ecology and Virulence of Legionnaire's Disease Bacteria in Aquatic Habitats Near Mt. St. Helens

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ABSTRACT

Illnesses among researchers exposed to lakes and streams in the Mt. St. Helens blast zone after the 18 May 1980 eruption prompted us to determine the occurrence and potential virulence of *Legionella* (Legionnaire's disease bacteria) in aquatic habitats near Mt. St. Helens during the summers of 1981 and 1982. Concentrations of *L. pneumophila*, *L. micdadei*, *L. gormanii*, *L. dumoffii*, and *L. bozemanii*, determined by microscopic counts using direct immunofluorescent staining, ranged from $<10^4$ to 10^5 cells/L in lakes and rivers outside of the Mt. St. Helens blast zone while the numbers of *Legionella* in aquatic habitats inside the blast zone were from 10^5 to 10^7 cells/L. *Legionella* numbers were consistently highest in N. Coldwater and Spirit lakes, which received water from hydrothermal seeps. *Legionella pneumophila* serogroups 4 and 6 were isolated from N. Coldwater Lake in 1981 and from S. Coldwater Creek in 1982, indicating that the potentially virulent strains of *Legionella* persist in aquatic habitats in the blast zone of Mt. St. Helens.

FOREWORD

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It is Institute policy to make available the results of significant water-related research conducted in Oregon's universities and colleges. The Institute neither endorses nor rejects the findings of the authors of such research. It does recommend careful consideration of the accumulated facts by those concerned with the solution of water-related problems.

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INTRODUCTION

The eruption of Mt. St. Helens on May 18, 1980 reshaped nearby pre-existing lakes and streams, created new lakes as a result of damming of drainages by debris flows, and produced numerous hydrothermal seeps. Tremendous quantities of pyrolized organic matter from the destroyed forest and inorganic nutrients from the ash and pyroclastics were added to previously oligotrophic sub-alpine lakes typical of those in the Cascade Range in the Pacific Northwest U. S. These aquatic environments progressed from oligotrophic to hypereutrophic in a matter of days; undisturbed, this progression would have taken centuries. Because of the proximity of academic research institutions to these unique environments, characterization of the chemical and biological properties of these habitats was initiated soon after the May 18, 1980 eruption (1, 7, 9).

Research personnel exposed to aquatic environments in the Mt. St. Helens blast zone reported illnesses with "flu-like" symptoms throughout the summer of 1980 and again early in the spring of 1981. The similarity of the reported symptoms to the "Pontiac Fever" syndrome cause by Legionella pneumophila and the nature of the aquatic environments produced by the cataclysmic eruption of Mt. St. Helens prompted us to initiate a study to determine the density and distribution of Legionella spp. (the etiologic agents of Legionnaires' Disease and Pontiac Fever) in the blast zone of Mt. St. Helens.

MATERIALS AND METHODS

The degree of impact by the May 18, 1980 eruption on lakes and streams in the Mt. St. Helens blast zone is shown in Figure 1. Sites sampled included Spirit Lake, North Coldwater and Castle creeks, which are in the scorch zone, and Ryan Lake, which is in the blowdown zone. South Coldwater Creek and hydrothermal seeps in the South Coldwater drainage area and sites outside of the blast zone including McBride Lake, Silver Lake (not shown) and the Muddy and North Toutle rivers were also sampled. Figures 2 and 3 illustrate the nature of the South Coldwater Creek drainage area.

The physical, chemical and biological characteristics of these sites have been described previously (1, 7, 9). In general, lakes in the blast zone were high in dissolved organic carbon (DOC), nitrogen-limited, and dominated by heterotrophic and metal oxidizing bacteria with total direct microscopic counts ranging from 10^6 to 10^8 bacteria per ml (1, 7, 9).

One-to-four liters of water were collected, concentrated by membrane filtration (6), and examined microscopically using direct immunofluorescence (3). Cells reacting with fluorescent antibody conjugates (Biological Products Section, Centers for Disease Control) specific for L. pneumophila serogroups 1-6, L. micdadei, L. gormanii, L. dumoffii, and L. bozemanii were quantitated. The virulence of presumptive Legionella was assessed by guinea pig innoculation (5).

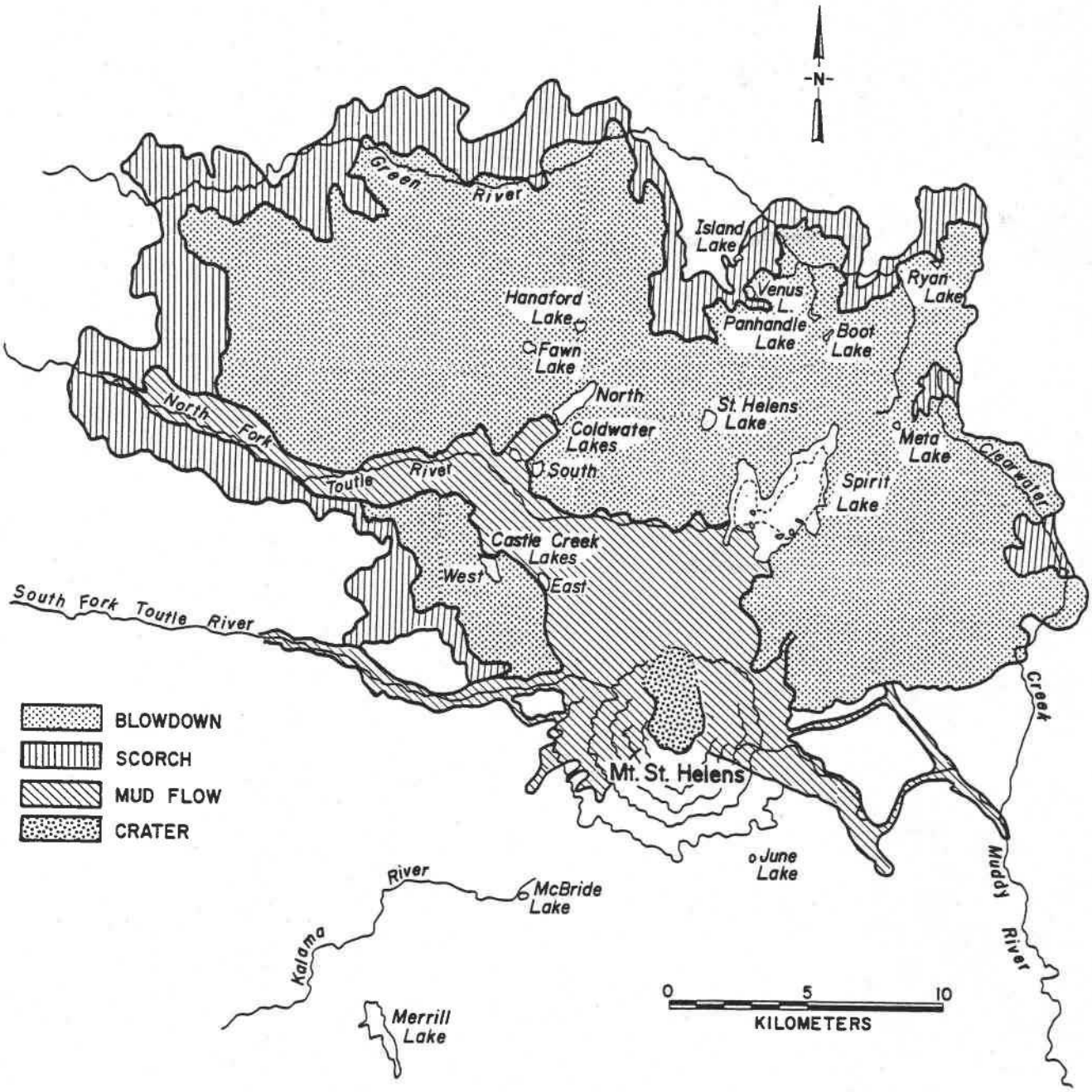

Figure 1. Mt. St. Helens and vicinity, showing rivers and lakes affected by May 18, 1980 eruption.

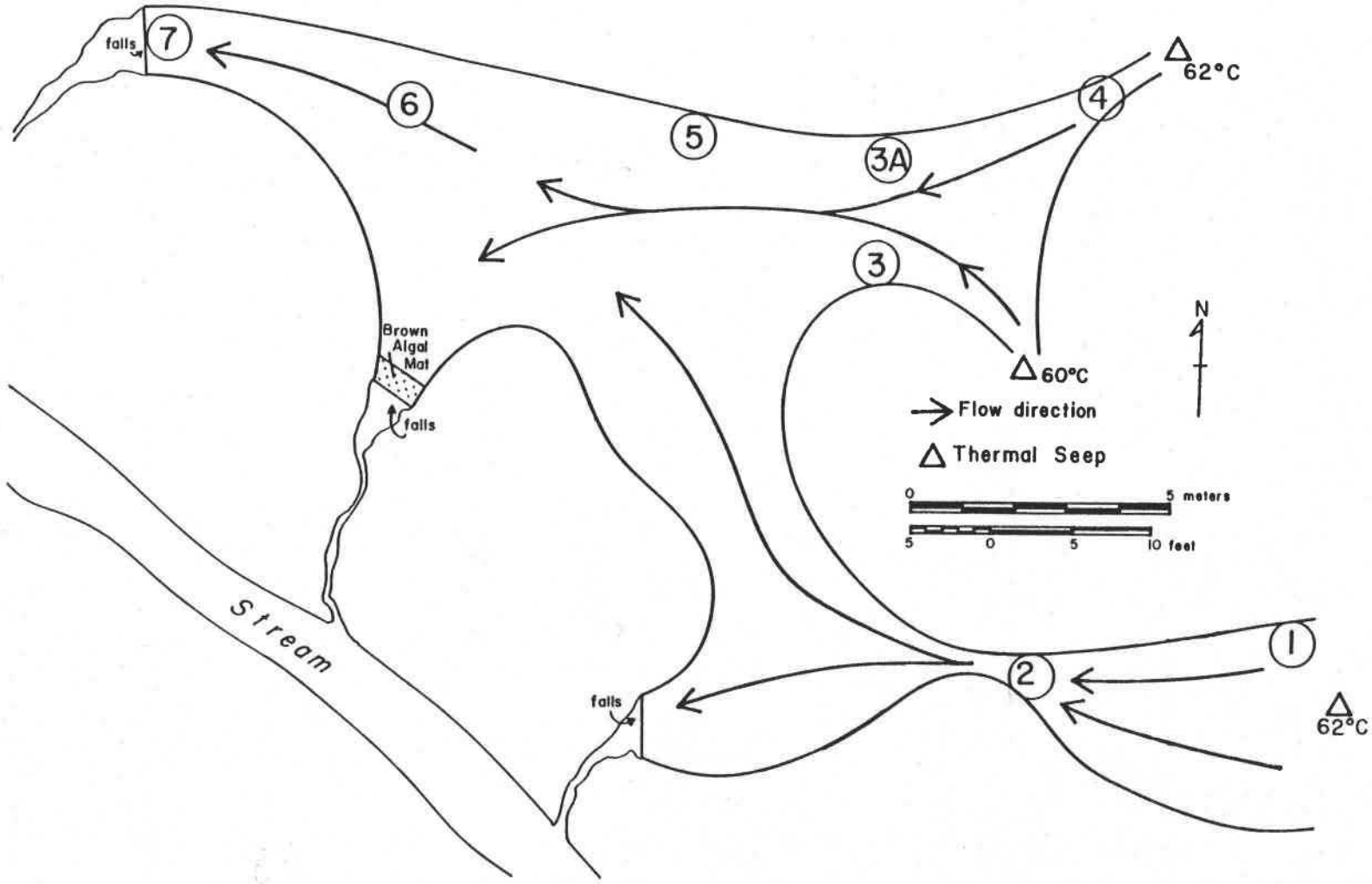

Figure 2. Diagrammatic representation of a sampling area in the S. Coldwater Creek drainage area. The thermal seeps located near sites 1 and 4 contained water of about 62°C. The seeps flow into S. Coldwater Creek which is illustrated on the left by the term "stream".

Figure 3. A portion of the S. Coldwater Creek drainage area illustrated in Figure 2. The photo was taken from sites 3, 3A (Figure 2) looking toward site 7. Note how the thermal seeps (light areas) flow off the bank ("falls") and into S. Coldwater Creek.

RESULTS

Concentrations of Legionella species from the most intensively sampled sites are presented in Table 1. The numbers of Legionella spp. in Ryan and Castle Creek lakes are of the same order of magnitude as those found for L. pneumophila in a survey of a number of lakes and rivers in the southeastern United States (4). Concentrations of Legionella spp. in North Coldwater and Spirit lakes were consistently high (10^6 to 10^7 cells/L) when compared to lakes outside of the blast zone (Table 1). These data may be attributed to the high concentrations of DOC and available metals in these lakes. Although the concentrations of Legionella spp. were particularly high in North Coldwater and Spirit lakes, the percentage of the total bacterial population these bacteria comprised (0.1% to 1%) was not unusual when compared to other aquatic habitats (4). The enrichment of these aquatic environments with DOC from pyrolysis of the productive coniferous forest and inputs of inorganic nutrients from the ash and pyroclastics have enriched for not only Legionella but the total bacterial population as well.

Legionella pneumophila serogroups 4 and 6 were isolated on BCYE agar inoculated with the liver, spleen and peritoneal fluid at necropsy from animals inoculated with concentrated water from North Coldwater Lake collected on April 30, 1981 and from South Coldwater Creek collected on June 15, 1982. These isolates were characterized biochemically and serologically as described previously (6).

Table 1. Numbers of cells per liter reacting with serogroup specific fluorescent antibody conjugates to *Legionella* spp.

Site & date sampled	*L. pneumophila*	*L. micdadei*	*L. gormanii*	*L. dumoffii*	*L. bozemanii*
Spirit Lake					
4/30/81	$1.3X10^7$	ND[a]	ND	ND	ND
7/22/81	$2.3X10^5$	--	--	--	--
8/16/81	--[b]	$1.7X10^7$	--	$7.1X10^6$	--
8/31/81	--	$9.9X10^5$	--	$2.3X10^5$	--
6/16/82	--	--	$1.2X10^6$	$5.2X10^6$	--
N. Coldwater Lake					
4/30/81	--	ND	ND	ND	ND
6/29/81	--	--	--	--	--
7/22/81	$1.1X10^5$	$1.1X10^7$	--	$3.0X10^5$	--
8/16/81	--	$3.1X10^6$	--	--	--
8/31/81	$4.5X10^4$	$1.9X10^6$	--	--	--
6/15/81	$7.7X10^4$	$1.9X10^6$	--	--	--
Castle Creek Lake					
4/30/81	--	ND	ND	ND	ND
6/29/81	--	--	--	--	--
7/22/81	--	$7.6X10^4$	--	$7.6X10^4$	--
8/16/81	$2.7X10^5$	--	--	$2.7X10^5$	--
8/31/81	--	$4.5X10^4$	$2.3X10^5$	$2.7X10^5$	$9.9X10^5$
Ryan Lake					
7/22/81	$1.1X10^5$	$1.9X10^5$	--	$3.8X10^5$	$1.5X10^5$
8/16/81	--	$2.7X10^5$	$4.5X10^5$	$1.8X10^5$	--
9/01/81	--	$2.3X10^5$	--	$1.2X10^5$	$6.0X10^4$
S. Coldwater Creek					
6/15/82	--	--	$2.3X10^5$	$1.6X10^6$	--
Merrill Lake					
6/15/81	$4.4X10^4$	$1.0X10^5$	$1.5X10^5$	$1.1X10^5$	--
Silver Lake					
6/10/82	--	$2.4X10^5$	--	--	--
N. Toutle River					
6/10/82	--	$4.4X10^4$	--	--	--

[a] ND = not determined

[b] below limit of detection ($<10^4$)

DISCUSSION

The concentrations of *Legionella* spp. in hydrothermal seeps in the South Coldwater Creek drainage particularly emphasize the enrichment potential of aquatic niches in this area. Thermal seeps eminating from the mud flow ranged in temperature from 75°C to ambient (18°C). Dissolved organic carbon concentrations ranged from about 700 to 900 mg C/L in seeps having temperatures above 65°C while concentrations in thermal seeps with temperatures from 40 to 60°C were about 250 mg C/L (C. Dahm, pers. comm.). Microbial communities similar to those in natural thermal springs (2) and man-made thermal habitats (8) were observed. At temperatures above 40°C these communities were comprised mainly of the cyanobacteria *Phormidium* sp., and to a lesser extent, *Oscillitoria* sp., and associated non-photosynthetic bacteria. Concentrations of *Legionella* spp. ranged from 10^5 to 10^7 DFA positive cells per cm . It is interesting to note that those lakes in the Mt. St. Helens blast zone found to harbor the highest densities of *Legionella*, North Coldwater and Spirit, were the only lakes receiving waters from thermal seeps. These observations suggest that enriched thermal waters which foster microbial communities with high concentrations of biomass may act as source of *Legionella* where these bacteria may proliferate during favorable seasonal conditions in non-thermal waters such as Spirit and North Coldwater lakes.

Legionella species were observed in rivers and lakes in the Mt. St. Helens blast zone in concentrations similar to those found in other aquatic habitats outside of the blast zone except at hydrothermal seeps in the South Coldwater Creek drainage, North Coldwater Lake and Spirit Lake. The concentrations of Legionella in those warmer waters were consistently one to two orders of magnitude higher than those found elsewhere. Virulent strains of L. pneumophila were isolated during both seasons sampled; however, illnesses among workers exposed to aquatic habitats in the Mt. St. Helens blast zone were not confirmed as legionnellosis. The high concentrations of Legionella in waters in the blast zone (10^7 cells/L) are diluted significantly as these waters flow to populated areas, as shown by the low numbers found in the North Toutle River (10^4 cells/L) outside of the blast zone.

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