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Project Summary

Investigation of *Legionella pneumophila* in Drinking Water

Linden E. Witherell, Robert W. Duncan, Kenneth M. Stone, Lori J. Stratton, Lillian Orciari, Steven Kappel, and David A. Jillson

An investigation of *Legionella pneumophila* in drinking water systems and home plumbing appurtenances was undertaken in two phases. In Phase 1, 68 water samples for *L. pneumophila* analysis were collected from hot and cold kitchen sink faucets in homes on 17 community water systems.

No *L. pneumophila* organisms were isolated from any of the samples in Phase 1. The relatively small sample size, the many variables in the sampling procedure, and potential limitations of the laboratory detection techniques employed may have contributed to the failure of significant recovery.

In Phase 1A, the kitchen sink faucets/aerators and showerheads/supply pipes were sampled with sterile swabs and a sample of hot water was collected from the drains of domestic hot water heaters in each home. A total of 184 samples (92 swabs, 92 hot water samples) were collected from homes on four community water systems. In addition, two samples (one swab, one hot water sample) were collected from a home with an individual, shallow, dug well.

Field analyses of pH, temperature, turbidity, and chlorine residuals and laboratory analyses for standard plate count (SPC), total coliform, and iron content were performed using *Standard Methods*, 15th Edition. *L. pneumophila* analyses were conducted using a modification of the Gorman and Feeley Direct Plating method developed at the Centers for Disease Control.

During Phase 1A, *L. pneumophila* organisms were recovered from two hot water heater flush samples; one from a home having a community water system and the other from a home having an individual, shallow, dug well. Both of the positive samples were taken from electric, non-recirculating, hot water heaters. This finding together with the lack of recovery in the home water distribution plumbing may indicate that *L. pneumophila* may be transported from natural aquatic sources in very low levels in drinking water systems to hot water heaters in homes where the organisms may increase to detectable levels.

The finding that approximately 2 of the 59 (3.4%) electric, nonrecirculating, domestic hot water heaters sampled contain *L. pneumophila* organisms may have public health significance. These sources may serve as a foci of the pathogen with resultant sporadic endemic legionellosis.

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This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction/Background

The investigation by the Centers for Disease Control (CDC) of the mysterious outbreak of pneumonia at the July 1976 annual convention of the Pennsylvania

Department of the American Legion led to the discovery of the pathogen, *Legionella pneumophila*. Investigations of subsequent outbreaks, together with review of stored samples from previous unexplained similar outbreaks, have added to knowledge of the pathogen and its role in human disease.

Acute human infection caused by *L. pneumophila* is now described as legionellosis. Legionellosis is presently recognized with two distinct clinical disease presentations: Legionnaires' disease and Pontiac fever.

Both Legionnaires' disease and Pontiac fever appear to be non-communicable; person-to-person transmission has not been documented. The only documented reservoir for *L. pneumophila*, and similar atypical *L. pneumophila*-like organisms, is environmental. Epidemiological studies show that the method of transmission from environmental sources to susceptibles is airborne with entry into the body via the respiratory system.

L. pneumophila is a rod-shaped bacillus measuring 0.3 to 0.4 μm in width and normally 2 to 3 μm long and even 50 μm in length. Electron microscopy studies have found the organisms to be atypical, gram negative bacilli, with double membranes and no cell walls. Some of the organisms have flagella.

Laboratory studies show that the organisms are extremely fastidious bacteria with unusual growth requirements. An interesting characteristic with possible public health implications is the ability of the *L. pneumophila* to persist in distilled or tap water. The organisms have been known to survive up to 139 days in distilled water and for up to 369 days in tap water. In fact, one report suggests that the organism may multiply in sterile water.

Ecological studies suggest that the organism is part of the natural aquatic environment and is capable of surviving wide ranges of environmental conditions. In one study of 793 water samples collected from 67 different lakes and rivers in the United States, virtually all samples were found positive for *L. pneumophila* when the direct fluorescent antibody (DFA) technique was used for detection. The water samples had the following characteristics; pH 5.5-8.1, temperature 5.7-63° C, dissolved oxygen 0.3-9.6 mg/l, and Secchi disk readings 1-4 m. Chi square analysis of the data found a significantly greater isolation efficiency in the 36-60° C range. These findings suggest that *L. pneumophila* may favor warm aquatic environments.

Another ecological study reported the isolation of *L. pneumophila* from an algal-bacterial mat community growing at 45°C in association with a blue-green algae over a pH range of 6.9-7.6. The researchers surmised that *L. pneumophila* was using algal extracellular products as its carbon and energy sources. These observations indicate that in this particular environment of a man-made thermal effluent, the temperature, pH, and nutritional requirements were not as stringent as those observed when the organism is cultured in the laboratory on complex media. The suggestion has been made in the literature that the association between *L. pneumophila* and certain blue-green algae could explain the apparent widespread distribution of the bacterium in the natural environment.

Another possible explanation of the widespread distribution of *L. pneumophila* is provided by the finding that the organism can grow within freshwater and soil amoebae. Because *L. pneumophila* thrives within ubiquitous soil and fresh water amoebae of the genera *Acanthamoeba* and *Naegleria*, it has been suggested that amoeba-associated *L. pneumophila*, rather than free *L. pneumophila*, could be the infective form for humans.

Because *L. pneumophila* species are neither new nor rare organisms in the natural environment, it seems probable that a low level of previously undetected human infection with the organisms from natural sources has occurred in the past and will continue to occur in the future. Reviews of human blood sera for DFA positivity have in some cases (Vermont studies) revealed relatively high prevalence rates of 8-26% (using a titer of 1:128 as a criterion for positivity), indicating at least a widespread human exposure to antigens consistent with those of *L. pneumophila*. Reviews have also shown seropositivity to *L. pneumophila*-like organisms among fatal and non-fatal clinical cases whose probable sources were aquatic (aspiration in a near-drowning, contaminated SCUBA equipment). Additionally, examination of blood sera from a large (2000) number of animals has shown evidence of widespread exposure in horses (>600 samples) to four serogroups of *L. pneumophila* (31.4% titer of >1:64 to at least one serogroup antigen).

As of September 30, 1979, 1005 confirmed cases of sporadic legionellosis in U.S. residents had been reported to CDC and 19% of these cases were fatal. In fact, it is estimated that there are approximately 25,000 sporadic cases of legion ellosis in the U.S. every year. Howeve outbreaks of legionellosis are relativel new phenomena. Widespread media cov erage in 1976 acquainted many with thi "new" disease, yet careful historica review revealed that the earliest outbrea occurred in 1965.

A review of epidemiological investiga tions of 17 outbreaks of legionellosi through 1979 finds that most occur i buildings: hospitals, hotels, a golf cours clubhouse, and a factory were among th first sites. Construction and excavatio activities have also been implicated i some outbreaks. Cooling towers an evaporative condensers have been as sociated with nine outbreaks. A history o recent travel or attendance at a conven tion were also factors associated wit legionellosis. Two outbreaks of legion ellosis in Vermont in 1980 were assoc iated with a hospital, a medical schoo and a cooling tower.

To date, no known outbreak of legion ellosis has been directly associated with a natural environment such as a lake, pond or stream. Apparently, in outbreak situa tions, human activities or man-made facilities which concentrate and/or dis seminate *L. pneumophila* organisms ir sufficient numbers and in a respirable form, are responsible for large numbers of disease cases. A review of the environmental aspects associated with pas legionellosis outbreaks may suggest ways to prevent future occurrences.

The role of buildings in legionellosis outbreaks is not clear. Certainly, buildings are necessary for bringing together large numbers of people, a critical requirement for an outbreak. There is close association between travel and/or conventions and buildings. Both of these activities usually require the use of buildings such as hotels and convention centers. In hospitals, of course, there are concentrations of people who are ill, and consequently, more at risk of contracting diseases such as legionellosis. However, not all buildings have been involved in outbreaks nor are all victims of Legionnaires' disease hospitalized. In addition to the recognition of buildings as areas of concentrations of people, consideration must be given to activities and/or facilities in and near buildings such as construction, cooling towers, and water distribution systems, which have been associated with legionellosis outbreaks.

Another environmental source of *L. pneumophila* organisms associated with nosocomial legionellosis is water distri-

bution systems in hospitals. The literature notes that the two largest sustained outbreaks of nosocomial Legionnaires' disease have been at hospitals in Los Angeles and Pittsburgh where *L. pneumophila* organisms have been isolated from the potable water supplies of each facility. Where *L. pneumophila* organisms have been isolated from hospital potable water distribution systems, the investigations were prompted by occurrence of nosocomial Legionnaires' disease cases.

The recovery of *Legionella* organisms in hospital water systems may have important public health significance because of the possible generation of contaminated aerosols from showerheads in buildings containing large numbers of immuno-compromised hosts. A possible explanation for the occurrence of *L. pneumophila* organisms in hospital water systems is the normal low operating temperature (43 to 49°C) of the hot water used in such systems. This temperature, used for patient safety, falls within the environmental temperature range at which optimal isolation is achieved. Worthy of note is the fact that many other hot water systems are now being operated at similar temperatures for energy conservation reasons.

L. pneumophila organisms have also been recovered from hot water systems in other institutions and homes. While the existence of these bacteria in other plumbing systems is not necessarily associated with an outbreak of disease, they could be a reservoir and means of transmission of legionellosis.

Legionella in drinking water systems has also been investigated. In a study of three municipal drinking water reservoirs, it was concluded that *Legionella* is either absent or present in undetectable numbers in these waters. In another study, 5 of 856 samples taken from chlorinated public water supplies yielded *Legionella* isolates.

Water is an interesting and perhaps very important element common to many of the ecological findings regarding *L. pneumophila* organisms and environmental health investigations involved with legionellosis outbreaks. *L. pneumophila* organisms are found in water in the natural environment and in water in cooling towers, evaporative condensers, hospital shower heads, and whirlpools associated with legionellosis disease outbreaks. In fact, except for one report of the isolation of *L. pneumophila* organisms from riparian mud of a thermally polluted stream, there are no known isolations from natural environmental sources other than water.

Water systems may play a role in the transmission of *L. pneumophila* organisms which cause legionellosis. *L. pneumophila* organisms common to the natural aquatic environment may be taken into water systems and delivered to devices such as cooling towers/evaporative condensers, whirlpools, showerheads, etc. In these water-using devices, *L. pneumophila* organisms may increase in numbers due to mechanical concentration or growth resulting from favorable environmental conditions such as elevated temperature, existence of algae, amoeba, and/or other factors not yet clearly understood. Such water-using devices generate aerosols which may contain *L. pneumophila* organisms possibly transmitted by airborne hosts entering the respiratory system. This scenario may explain the method of transmission of *L. pneumophila* organisms from the natural environment to the human host not only for outbreak situations but also for the estimated large number of sporadic cases which occur each year.

Obviously, more information concerning *L. pneumophila* in drinking water systems is required, especially the method of determining the presence of *L. pneumophila* organisms in drinking water. The methods commonly used for determining microbiological quality of drinking water are not suitable for determining the presence of *L. pneumophila* organisms. For example, a , study by the Vermont Department of Health has found no correlation between coliform and/or standard plate count results and the presence of *L. pneumophila* in water in cooling towers.

The literature identifies studies which determine the presence of *L. pneumophila* in drinking water using the DFA analysis technique. This method is limited, however, in that there is cross reaction between *L. pneumophila* and at least two *Pseudomonas* strains with the DFA procedure. Recent attempts to overcome another problem with DFA (staining of non-viable cells) by measuring dye uptake of the electron transport system (DFA-INT) did not overcome the problems of cross reactivity between *L. pneumophila* and *Pseudomonas*.

In view of the limitations of the DFA method, culture methods of analysis have been developed. The Gorman-Feeley culture method using synthetic media was developed at CDC. A recent study comparing the GPP method with direct plating on synthetic media have shown significantly greater sensitivity of the plating method ($p<0.01$) for environmental samples.

The mere detection of *L. pneumophila* in a drinking water system, in itself, provides little information concerning the public health significance. Epidemiological studies are required to determine the incidence of legionellosis on systems with and without *L. pneumophila* and other *Legionella* organisms. The present study was undertaken in an attempt to determine the prevalence of *Legionella* organisms in household plumbing fixtures and distribution systems. If a high prevalence rate was found, then an epidemiological study would be conducted to determine the significance of the organisms in home distribution systems.

Conclusions and Recommendations

In Phase 1, 68 water samples for *L. pneumophila* analysis were collected from hot and cold kitchen sink faucets in two homes on each of 17 community water systems in Vermont. No *L. pneumophila* organisms were isolated from any of these samples.

During Phase 1A, the kitchen sink faucets/aerators and showerheads/supply pipes were sampled with sterile swabs and a sample of hot water flushed from the drains of domestic hot water heaters in each home. A total of 184 samples (92 swabs, 92 hot water samples) were collected from homes on four community water systems. In addition, two samples (one swab, one hot water sample) were collected from a home with an individual, shallow, dug well. *L. pneumophila* organisms were recovered from two samples; one from a home on a community water system and the other from the home with the individual, shallow, dug well. Both of the positive samples were taken from electric, non-recirculating, hot water heaters.

Of the 93 samples collected from domestic hot water heater tanks, one had fungal overgrowth which prevented completion of *L. pneumophila* analysis. *L. pneumophila* organisms were recovered from two of the remaining 92 samples (2.17%). The recovery of *L. pneumophila* from the two domestic hot water heaters in Phase 1A is of interest. This finding together with the lack of recovery in the home water distribution plumbing may indicate that *L. pneumophila* may be transported from natural aquatic sources in very low levels in drinking water systems to hot water heaters in homes

where the organisms may increase to detectable levels. However, using the methods employed in this study, the occurrence of *Legionella* in home water systems was rare.

Continued study of this matter is recommended. Also, further information is needed regarding the sensitivity and specificity of the various laboratory methods for *L. pneumophila* analysis. An accurate method is needed for the isolation of *L. pneumophila* from environmental samples. In addition, further information regarding the epidemiology of legionellosis is required to determine the population size that must be sampled to obtain statistically significant results.

Linden E. Witherell, Robert W. Duncan, Kenneth M. Stone, Lori J. Stratton, Lillian Orciari, Steven Kappel, and David A. Jillson are with Vermont State Department of Health, Burlington, VT 05402-00070.

***Walter Jakubowski** is the EPA Project Officer (see below).*

The complete report, entitled "Investigation of Legionella pneumophila *in Drinking Water," (Order No. PB 85-237 733/AS; Cost: $10.00, subject to change) will be available only from:*

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5285 Port Royal Road
Springfield, VA 22161
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Health Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

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