Clinical and Epidemiologic Investigation of Two *Legionella-Rickettsia* Co-infections

Michael Huerta MD MPH[1], Hana Castel MD[2], Itamar Grotto MD MPH[1], Ofer Shpilberg MD MPH[1,2], Michael Alkan MD[2] and Ilana Harman-Boehm MD[2]

[1] Army Health Branch, Israel Defense Forces, Israel
[2]Soroka Medical Center and Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel

Key words: *Legionella*, *Rickettsia*, co-infection, epidemiologic investigation

Abstract

Background: We treated two patients diagnosed with legionellosis and simultaneous *Rickettsia conorii* co-infection.

Objectives: To report the clinical and laboratory characteristics of this unusual combination, and to describe the execution and results of our environmental and epidemiologic investigations.

Methods: Serial serologic testing was conducted 1, 4 and 7 weeks after initial presentation. Water samples from the patients' residence were cultured for *Legionella*. Follow-up cultures were taken from identical points at 2 weeks and at 3 months after the initial survey.

Results: Both patients initially expressed a non-specific rise in anti-*Legionella* immunoglobulin M titers to multiple serotypes. By week 4 a definite pattern of specifically elevated IgG titers became apparent, with patient 1 demonstrating a rise in specific anti-*L. pneumophila* 12 IgG titer, and patient 2 an identical response to *L. jordanis*. At 4 weeks both patients were positive for both IgM and IgG anti-*R. conorii* antibodies at a titer $\geqslant$ 1:100. Heavy growth of *Legionella* was found in water sampled from the shower heads in the rooms of both patients. Indirect immunofluorescence of water cultures was positive for *L. pneumophila* 12 and for *L. jordanis*.

Conclusions: Although most cases of community-acquired *Legionella* pneumonia in our region appear simultaneously with at least one other causative agent, co-infection with *R. conorii* is unusual and has not been reported to date. This report illustrates the importance of cooperation between clinicians and public health practitioners.

IMAJ 2003;5:560–563

Legionella species have been well documented as pathogens of respiratory illness in humans. The role of community-acquired legionellosis has been recognized in recent years as a topic requiring further study. We report two unusual cases of community-acquired *Legionella* and *Rickettsia conorii* co-infection in otherwise healthy young adults, present the findings of our environmental and epidemiologic investigations, and demonstrate the importance of cooperation between clinical and public health physicians.

Patient Descriptions

Patient 1, a 19 year old male soldier in good general health, presented at our emergency department with a history of fever, headache, malaise and rash, which had evolved over a period of 5 days. He denied a history of contact with dogs, consumption of milk products from sheep or goats, travel outside of Israel, and entrance into caves. On examination he was febrile (39.4°C) and plethoric. A blanching maculopapular rash was found on the trunk and limbs, including the palms and soles of the hands and feet. Laboratory tests revealed an erythrocyte sedimentation rate of 10 mm/hour, white blood cell count of 4,500 cells/cm^3, and mild thrombocytopenia. Liver function tests were normal. Blood cultures were negative and the chest X-ray was unremarkable. A presumed diagnosis of rickettsiosis was made and treatment with doxycycline was initiated. One day later the patient reported feeling better and the rash began to subside. Initial serologic tests were negative for rickettsiosis but elevated titers of immunoglobulin M antibodies directed against *Legionella pneumophila* serotypes 4, 7, 10, 11 and 14 were found. Since his clinical condition improved under doxycycline, this treatment was continued. The patient was discharged after 1 week of hospitalization and made a full, uneventful recovery. Follow-up examinations performed 2 and 4 weeks after discharge were unremarkable.

Patient 2, a 19 year old female soldier in good general health, presented with dyspnea, malaise, fever and a widespread rash. On examination she appeared severely ill and demonstrated tachycardia, hypotension, severe dyspnea and a temperature of 39.2°C. A widespread fine, blanching, maculopapular eruption was seen on the trunk and limbs, excluding the palms and soles. Auscultation of the chest revealed bilateral rales, and palpation of the abdomen found mild hepatosplenomegaly. The white blood cell count was 5,000/cm^3 and the platelet count 70,000/cm^3. Arterial blood gas analysis indicated hypoxemia, and chest X-ray showed diffuse bilateral reticulonodular infiltrates. Transthoracic echocardiogram was normal. The patient was admitted to the intensive care unit where she underwent endotracheal intubation due to respiratory failure, and was mechanically ventilated for 6 days. Treatment with doxycycline was initiated on the first day, and was subsequently replaced by intravenous erythromycin, ofloxacin and minocycline. Serologic tests for *Mycoplasma pneumoniae*, *Rickettsiae*, *Coxiella burnetti* and *Legionella* performed on admission were negative. The patient was extubated and transferred to a medical bed, where she improved slowly on a regimen of minocycline and ofloxacin. She was discharged from the hospital 2 weeks after admission.

Methods

We conducted epidemiologic, environmental and serologic investigations of the two cases. Epidemiologic investigation aimed to identify risk factors and exposures that were common for both

Ig = immunoglobulin

patients in terms of place and time. Environmental investigation included an on-site engineering analysis to identify potential points of exposure to *Legionella*. Water samples were drawn from points along the settlement's water distribution system using standard methods, and were cultured for *Legionella*. Follow-up cultures were taken from identical points at 2 weeks and at 3 months after the initial survey. Isolates were subjected to rapid latex slide agglutination testing and serogroup-specific indirect immunofluorescence. Serial serologic testing of both patients for anti-*Legionella* IgM and IgG antibodies with indirect immunofluorescence assay was conducted at weeks 1, 4 and 7. A positive result was defined as a fourfold or greater rise in specific IgG titers in samples obtained 3 weeks apart, or an IgM titer greater than 1:64 [1,2]. Sera from both patients were tested at week 4 for antibodies to *Rickettsia typhi*, *Rickettsia conorii* and *C. burnetii*.

Results

Epidemiologic investigation

The army base on which both patients were stationed was a residential settlement supplemented by Israel Defense Force soldiers assigned to agricultural duty. The population comprised approximately 15 civilian households and 30 soldiers. No cases of illness were reported among the civilian residents.

Patient 1, a non-smoking male, worked as a gardener. His tasks included maintenance of the on-site irrigation system. He reported no preexisting illness and denied taking any medication. Patient 1 spent the 3 weeks preceding the onset of illness on base. He also identified himself as the boyfriend of patient 2. Patient 2, a non-smoking female, was assigned to the same unit as patient 1. She worked as a teacher's assistant in the kindergarten of a neighboring settlement. She was in good general health, reported no previous illness and denied taking any medication. As the partner of patient 1, she spent most of her free time with him, on base and off. Like him, she had spent the 3 weeks preceding the onset of illness on the base.

Environmental investigation

Potable water was supplied to this settlement through a central water main that was maintained by a commercial water provider. The water was chlorinated at a treatment facility located along the distribution line approximately 50 kilometers proximal to the settlement. Approximately 3 weeks prior to this episode the water supply to the settlement had been turned off for 1 day due to routine maintenance of the water main. There were no reports of major construction or excavation during the preceding months. The soldiers were exposed to a single air-conditioning unit located in the recreation room. No other rooms housed cooling units. There were no cooling towers, Jacuzzis or swimming pools on site, and the soldiers had no access to devices such as ice machines or drinking fountains. Water for household use was heated either by solar energy or in the settlement's vertically aligned boiler, which was set to supply water at approximately 59°C.

Heavy growth of *Legionella* was initially found in water sampled from the shower heads used by both patients, as well as in specimens drawn from the upper opening of the community hot water boiler and from a sink faucet in the room of patient 1 [Table 1]. Shower heads were treated by immersion in a 10% chlorine solution. The community water supply system was subjected to hyperchlorinated flushing at a concentration of 150–300 ppm. Superheating could not be effectively carried out due to the fragile nature of the piping system. Specimens drawn 2 weeks after completion of disinfection were negative for *Legionella*. The third set, drawn 3 months later, showed re-emergence of *Legionella* in the shower heads.

Isolates cultured from the first set of samples were subjected to rapid latex slide agglutination testing. Results were negative for *L. pneumophila* serogroup 1, but were positive for *L. pneumophila* serogroups 2–14 (pool). There were no *Legionella* species cultured from set 2. Set 3 was subjected to serogroup-specific indirect immunofluorescence, which was positive for *L. pneumophila* 12 and for *L. jordanis*.

Serologic investigation

Results of serial serologic testing for *Legionella* species are presented in Table 2. Patient 1 demonstrated a steady rise in specific anti-*L. pneumophila* 12 IgG titer from 1:256 at week 1 to 1:2048 at week 7, while patient 2 demonstrated a similar response to *L. jordanis*, with an increase in titer from 1:32 at week 1 to 1:2048 at week 7. No other significant increases in IgG titer were noted for *L. pneumophila* serogroups 1, 4, 8 or 12. The evolution of the

Table 1. *Legionella* contamination of serial residential water specimens

	Legionella colony-forming units/L		
Site	**Sample 1**	**Sample 2**	**Sample 3**
Boiler – lower aperture	0	0	0
Boiler – upper aperture	23,000	0	–
Boiler – dump valve	0	0	0
Solar heater	0	0	0
Shower head – patient 1	17,000	0	6,000
Sink faucet – patient 1	23,000	0	0
Shower head – patient 2	20,500	0	6,000
Administrative office	0	0	0

Sample 1 was taken during the initial investigation, sample 2 two weeks subsequent to disinfection of the water supply system, and sample 3 three

Table 2. Serial titers of IgM and IgG anti-*Legionella* antibodies at weeks 1, 4 and 7

		L. pneumophila 1,8		*L. pneumophila* 12	
		IgM	**IgG**	**IgM**	**IgG**
Patient 1	Week 1	256	64	128	256
	Week 4	1,024	64	64	1,024
	Week 7	64	64	32	2,048

		L. pneumophila 1,4,12		*L. jordanis* 12	
		IgM	**IgG**	**IgM**	**IgG**
Patient 2	Week 1	32	32	32	32
	Week 4	256	64	128	1,024
	Week 7	0	128	0	2,048

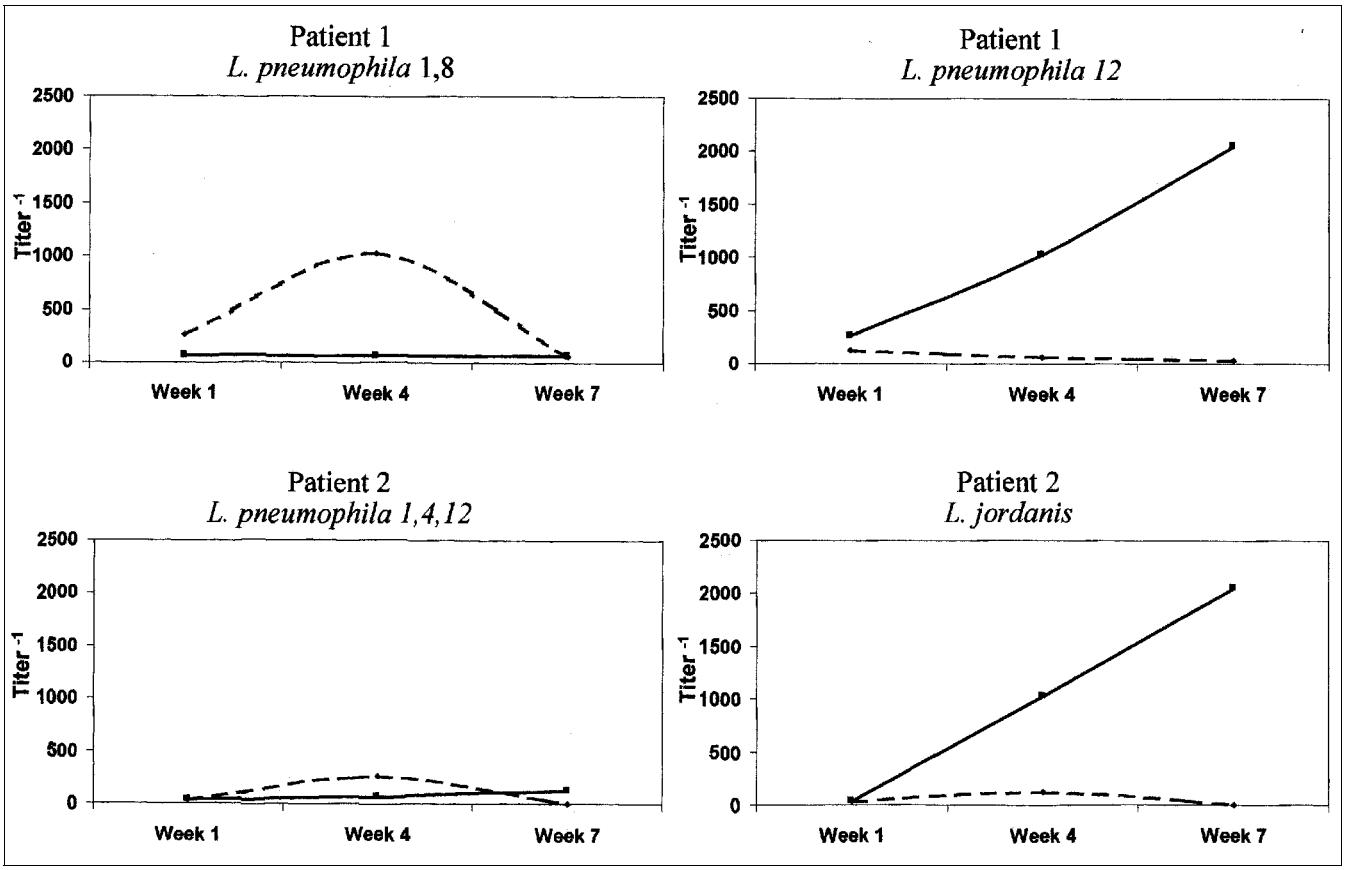

Figure 1. IgM (broken line) and IgG (solid line) anti-*Legionella* antibody titers for patients 1 and 2, as measured serially by indirect immunofluorescence assay at weeks 1, 4 and 7.

serologic response over time is shown in Figure 1. Both patients initially expressed non-specific rises in anti-*Legionella* IgM titers to multiple serotypes (left panels). As of week 4, however, a definite pattern of specifically elevated IgG titers became apparent (right panels).

Sera from both patients were tested at week 4 for antibodies to *Rickettsia typhi, Rickettsia conorii* and *C. burnetii*. Both patients were negative for *R. typhi* and *C. burnetii,* but were positive for both IgM and IgG anti-*R. conorii* antibodies at a titer $\geqslant$ 1:100.

Discussion

We report two cases of clinically and serologically diagnosed community-acquired *Legionella* and *R. conorii* co-infection in young adults. The findings of our epidemiologic and environmental investigations suggest that the hot water delivery system in the subjects' residence – specifically their respective shower heads – was the source of *Legionella* infection.

Potable water in the home has been shown to represent a significant source of community-acquired legionellosis [3–8]. While considerable investigation has been conducted on large-scale epidemics of legionellosis [7,9–12], much has yet to be learned about the sources, routes of transmission and epidemiology of sporadic, community-acquired disease [3,5,13–15], which constitutes the vast majority of legionellosis cases [2].

Environmental isolates in this case were positive for both *L. pneumophila* 12 and *L. jordanis*. The isolation of these two *Legionella* species from the subjects' residences strengthens the serologic findings of significant rises in IgG titers for the identical *Legionella* serotypes. Similar findings of multiple *Legionella* species in residential potable water have been previously described in the context of a community-acquired legionellosis investigation [2].

Simultaneous co-infection with another organism is not uncommon in cases of legionellosis and appears to have occurred in these two patients. A prospective study conducted in our region identified at least one other co-infective etiologic agent in 35 of 56 (62.5%) adult patients hospitalized over the course of 1 year due to community-acquired *Legionella* pneumonia [1]. The most frequent co-infectors were *Streptococcus pneumoniae, Mycoplasma pneumoniae, Chlamydia pneumoniae,* viruses, *Haemophilus influenzae,* and *C. burnetii*. Less common co-infections such as *Pneumocystis carinii* and *Rhodococcus equi* have been described in patients with human immunodeficiency virus [16]. To the best of our knowledge, there has been no previous description of *Legionella-R. conorii* co-infection.

It should be noted that an interspecies serologic cross-reaction may occur after infection with *Rickettsia* [17] and *Legionella* [18,19]. This phenomenon is due to a non-specific antibody response to polysaccharide surface antigens that are common to a number of organisms. This non-specific response is well documented and, in the case of certain rickettsial antigens and the polysaccharide

O-antigen of *Proteus vulgaris* OX-19, represents the principle underlying the Weil-Felix reaction, wherein a positive agglutination test for *Proteus* antigens is suggestive of a rickettsial infection [20,21]. A specific cross-reaction between *R. typhi* and *Legionella bozemanii* has been documented [22], and the cross-reacting antigen of *L. bozemanii* has been shown to be closely related to the O-antigen of *Proteus* OX-19 [20]. This cross-reactivity might seem to suggest that the *Legionella-R. conorii* co-infection presented here may be a false positive laboratory result rather than a true co-infection. This interpretation, however, does not seem to be the case. Of the *Legionella* species examined for rickettsial cross-reactivity, the offending antigen has been found only in *L. bozemanii*, while no cross-reactivity has been demonstrated between *Rickettsiae* and *L. jordanis* or *L. pneumophila* 12. Similarly, of the rickettsiae examined, the cross-reacting antigen has been found only in *R. typhi*, and not in *R. conorii* [20]. As such, we strongly believe that the serologic findings in our patients do not represent false positive results due to cross-reactivity but rather indicate a true co-infection. This interpretation is further supported by the clinical manifestations seen in patient 1, which were classically suggestive of a rickettsial infection.

Conclusion

We describe two cases of community-acquired legionellosis in previously healthy young adults. Epidemiologic and environmental investigation traced the source of *Legionella* infection to a residential potable water source. Both patients were found to harbor co-infection with *R. conorii*. Although most cases of community-acquired *Legionella* pneumonia in our region have been shown to appear simultaneously with at least one other causative agent, co-infection with *R. conorii* appears to be unusual, and to our knowledge, unreported to date.

References

1. Lieberman D, Porath A, Schlaeffer F, Lieberman D, Boldur I. Legionella species community-acquired pneumonia: a review of 56 hospitalized adult patients. *Chest* 1996;109:1243–9.
2. Straus WL, Plouffe JF, File TM Jr, et al. Risk factors for domestic acquisition of Legionnaires disease. *Arch Intern Med* 1996;156:1685–92.
3. Stout JE, Yu VL, Muraca P, Joly J, Troup N, Tompkinms LS. Potable water as a cause of sporadic cases of community-acquired legionnaires' disease. *N Engl J Med* 1992;326(3):151–5.
4. Stout JE, Yu VL, Muraca P. Legionnaires' disease acquired within the homes of two patients: link to the home water supply. *JAMA* 1987;257:1215–17.
5. Hood J, Hay J, Gillepsie T, Seal DV. A fatal case of community-acquired Legionnaires' disease acquired from potable water. *J Infect* 1994;29:231–3.
6. Marrie T, Green P, Burbridge S, et al. Legionellaceae in the potable water of Nova Scotia hospitals and Halifax residences. *Epidemiol Infect* 1994;112:143–50.
7. Hanrahan JP, Morse DL, Scharf VB, et al. A community hospital outbreak of legionellosis: transmission by potable hot water. *Am J Epidemiol* 1987;125:639–49.
8. Breiman RF, Fields BS, Sanden GN, Volmer L, Meier A, Spika JS. An outbreak of legionnaires' disease associated with shower use: possible role of amoebae. *JAMA* 1990;263:2924–6.
9. Garbe PL, Davis BJ, Weisfeld JS, et al. Nosocomial legionnaires' disease: epidemiologic demonstration of cooling towers as a source. *JAMA* 1985;254:521–4.
10. Dondero TJ Jr, Rentdorff RC, Mallison GF, et al. An outbreak of legionnaires' disease associated with a contaminated air-conditioning cooling tower. *N Engl J Med* 1980;302:365–70.
11. Mahoney FJ, Hoge CW, Farley TA, et al. Community-wide outbreak of legionnaires' disease associated with a grocery store mist machine. *J Infect Dis* 1992;165:736–9.
12. Fraser DW, Tsai TR, Orenstein W, et al. Legionnaires' disease: description of an epidemic of pneumonia. *N Engl J Med* 1997;297:1189–97.
13. Mermel LA, Josephson SL, Giorgio CH, Dempsey J, Parenteau S. Association of legionnaires' disease with construction: contamination of potable water? *Infect Control Hosp Epidemiol* 1995;16:76–81.
14. Rampling A, Butt CJ, West AA, Tully M, Palmer KT. Community-acquired legionnaires' disease following minimal exposure to a contaminated source. *J Infect* 1997;35(3):300–2.
15. Bhopal R. Source of infection for sporadic Legionnaires' disease: a review. *J Infect* 1995;30:9–12.
16. Blatt SP, Dolan MJ, Hendrix CW, Melcher GP. Legionnaires' disease in human immunodeficiency virus-infected patients: eight cases and review. *Clin Infect Dis* 1994;18(2):227–32.
17. Philip RN, Casper EA, Burgdorfer W, Gerloff RK, Hughes LE, Bell EJ. Serologic typing of rickettsiae of the spotted fever group by microimmunofluorescence. *J Immunol* 1978;121:1961–8.
18. Collins MT, McDonnald J, Hoiby N, Aalund O. Agglutinating antibody titers to the family *Legionellaceae* in cystic fibrosis patients as a result of cross-reacting antibodies to *Pseudomonas aeruginosa*. *J Clin Microbiol* 1984;19:757–62.
19. Wilkinson HW, Farshy CE, Fikes BJ, Cruce DD, Yealy LP. Measure of immunoglobulin G-, M-, and A-specific titers against *Legionella pneumophila* and inhibition of titers against nonspecific gram-negative bacterial antigens in the indirect immunofluorescence test for legionellosis. *J Clin Microbiol* 1979;10:685–9.
20. Sompolinsky D, Boldur I, Goldwasser RA, et al. Serological cross-reactions between *Rickettsia typhi, Proteus vulgaris* OX19, and *Legionella bozemanii* in a series of febrile patients. *Isr J Med Sci* 1986;22:745–52.
21. Weil E, Felix A. Zur serologischen diagnose des fleckfiebers. *Wien Klin Wochenschr* 1916;29:33–5.
22. Westfall HN, Goldwasser RA, Weiss E, Hussong D. Prevalence of antibodies to *Legionella* species in a series of patients in Israel. *Isr J Med Sci* 1986;22:131–8.

Correspondence: Dr. M. Huerta, IDF Army Health Branch, Military Post 02149, Israel.
Phone: (972-3) 737-6337
Fax: (972-3) 737-7260
email: mhuerta@netvision.net.il