10. Slack CL, Dahn KA, Abzug MJ, Chan KH. Antibiotic-resistant bacteria in pediatric chronic sinusitis. *Pediatr Infect Dis J* 2001; **20**: 247–250.
11. Gwaltney JM, Scheld WM, Sande MA, Sydnor A. The microbial aetiology and antimicrobial therapy of adults with acute community-acquired sinusitis: a fifteen-year experience at the University of Virginia and review of other selected studies. *J Allergy Clin Immunol* 1992; **90**: 457–461.
12. Gwaltney JM. Acute community-acquired sinusitis. *Clin Infect Dis* 1996; **23**: 1209–1223.
13. Wald ER, Milmoe GJ, Bowen A, Ledesma-Medina J, Salamon N, Bluestone CD. Acute maxillary sinusitis in children. *N Engl J Med* 1981; **304**: 749–754.
14. Orobello PW, Park RI, Belcher LJ *et al*. Microbiology of chronic sinusitis in children. *Arch Otolaryngol Head Neck Surg* 1991; **117**: 980–983.
15. Sener B, Hascelik G, Onerci M, Tunckanat F. Evaluation of the microbiology of chronic sinusitis. *J Laryngol Otol* 1996; **110**: 547–550.
16. Gehanno P, Lenoir G, Barry B, Bons J, Boucot I, Berche P. Evaluation of nasopharyngeal cultures for bacteriologic assessment of acute otitis media in children. *Pediatr Infect Dis J* 1996; **15**: 329–332.
17. Lindbaek M. Acute sinusitis: guide to selection of antibacterial therapy. *Drugs* 2004; **64**: 805–819.
18. Leung AK, Kellner JD. Acute sinusitis in children: diagnosis and management. *J Pediatr Health Care* 2004; **18**: 72–76.

ABSTRACT

The role of *Legionella* spp. in the aetiology of acute respiratory infections (ARIs) is largely unknown. In this case-control study, conducted in a general practitioner setting during 2000 and 2001, nose and throat samples from patients presenting with ARIs (n = 230) and controls (n = 200) were analysed for the presence of *Legionella* spp. by real-time PCR. *Legionella* DNA was not detected in any of the cases or controls. Thus, *Legionella* spp. do not seem to play a role in patients presenting with ARIs, nor were they present in patients who visited their general practitioner for complaints other than ARIs.

Keywords Acute respiratory infections, general practice, *Legionella* spp., real-time PCR, respiratory tract infection

Original Submission: 21 October 2004; **Revised Submission:** 28 December 2004; **Accepted:** 16 January 2005

Clin Microbiol Infect 2005; 11: 410–412
10.1111/j.1469-0691.2005.01112.x

RESEARCH NOTE

No evidence of *Legionella* infection in general practice patients presenting with acute respiratory infections in The Netherlands

B. M. W. Diederen[1], C. M. A. de Jong[1], I. Aarts[1], M. F. Peeters[1], A. B. van Gageldonk-Lafeber[2], B. Wilbrink[2] and A. van der Zee[1]

[1]Laboratory of Medical Microbiology, St Elisabeth Hospital, Tilburg and [2]National Institute of Public Health and the Environment, Bilthoven, The Netherlands

Corresponding author and reprint requests: B. M. W. Diederen, Laboratory of Medical Microbiology, St Elisabeth Hospital, PO Box 747, 5000 AS Tilburg, The Netherlands
E-mail: b.diederen@elisabeth.nl

Legionella spp. are an important cause of community-acquired and nosocomial pneumonia. Since the first description of *Legionella pneumophila*, more than 40 species of *Legionella* have been identified, approximately half of which have been isolated from patients [1]. Infection with *Legionella* spp. can present as a severe pneumonia, with or without multisystem disease and high mortality, but can also present as a self-limiting influenza-like illness. Many individuals who seroconvert to *Legionella* are entirely asymptomatic [1,2].

Acute respiratory infections (ARIs) are a major cause of morbidity and mortality worldwide. Various infectious agents, especially viruses, have been associated with clinical syndromes ranging from mild disease, such as the common cold, to more severe conditions, such as pneumonia. However, no aetiological agent is found in a large percentage of cases [3]. Although ARIs are very common, there is only one report on the prevalence of *Legionella* spp. as a cause of community-acquired infections in general practice [4]. This may be because the diagnosis is not considered, existing diagnostic tests are insensitive, or legionellosis is distributed unevenly across the world. In The Netherlands, *c*. 200 cases of severe legio-

nellosis are reported annually to the health authorities [5].

The aim of this study was to assess the frequency of *Legionella* spp. in nose and throat samples of patients presenting with ARIs in general practice. In The Netherlands, more than 100 general practitioners (GPs) participate in nationwide surveillance of respiratory pathogens and influenza-like illnesses, coordinated by the Netherlands Institute for Health Services Research (NIVEL, Utrecht, The Netherlands) [6]. The GPs register all patients with influenza-like illness and other ARIs. ARIs without influenza-like illness are defined as respiratory infections with acute onset (< 5 days) and coryza, sore throat and/or cough. Twenty GPs participated in the present study and were requested to collect a nose and throat sample from patients with ARIs without influenza-like illness (case). A corresponding control was also sampled within 7 days, defined as an individual who consulted the GP for a complaint other than ARIs, who was in the same age category as the case (0–4, 5–14, 15–24, 25–44, 45–64 or > 65 years), was not a member of the same household as the case, and had not received antibiotics or antiviral therapy in the preceding 14 days. A nose and a throat swab were placed together in 4 mL of Hanks' balanced salt solution containing gelatin, lactalbumin, yeast and antibiotics (GLY medium), and sent to the central laboratory by regular mail. Swabs for bacterial culture were also taken.

The primers and probes of the *Legionella* PCR assay were based on the 16S rRNA gene as described previously [7]. In brief, nucleic acid isolation was performed with a proteinase K lysis protocol, and primers Leg1 (5′-TACCTACCCTTGACATACAGTG-3′) and Leg2 (5′-CTTCCTCCGGTTTGTCAC-3′) were used to obtain a 200-bp amplicon. Real-time detection of the fragment was done with a *Legionella* genus-specific fluorescent probe conjugated to a minor groove binder (LSPP-VIC: 5′-GGTTGCGTCGTTACG-3′). An *L. pneumophila*-specific fluorescent minor groove binder probe was used on the complementary DNA strand (LPN-FAM: 5′-GAGTCCCCACCATCACATG-3′). Inhibition was monitored in a separate assay by amplifying a phocine herpes virus, and negative controls were included to monitor processing and cross-contamination. Sensitivity controls comprised ten-fold dilutions of *L. pneumophila* DNA (1000 fg to 10 fg), while 1000 fg of *Legionella bozemanii* DNA served as a control for discrimination between *L. pneumophila* and other *Legionella* spp.

Real-time PCR was performed with an ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA).

Nose and throat samples from 230 cases and 200 controls were collected between October 2000 and November 2001. The median age of the cases was 35 years (range 0–87 years), and 104 (45%) of them were male. The median age of the control patients was 36 years (range 0–86 years), and 76 (38%) of them were male. Evidence of at least one infectious agent was found by standard techniques in 146 (63%) of the cases, compared to 44 (22%) of the controls (Table 1). Real-time PCR failed to detect *Legionella* DNA in any of the samples from either the patients with ARI or the control group. Inhibition of the PCR occurred with one sample.

ARIs are the most common diseases seen in the community and a frequent reason to consult a GP. Knowledge of the causes of respiratory infections continues to evolve, with recognition of new pathogens [8], but no aetiological diagnosis is established in a large percentage of cases [3]. It might be expected that *Legionella* spp. could play a role in ARIs, as they are ubiquitous in the aquatic environment and artificial water systems, and can cause a variety of diseases, ranging from asymptomatic cases to severe life-threatening pneumonia [1,9]. Upper respiratory signs and symptoms, such as a dry cough and sore throat, have been reported in cases of Legionnaires'

Table 1. Frequency of detection of specific infectious agents in 230 cases of acute respiratory tract infection and 200 control patients

Pathogen	Cases *n* (%)	Controls *n* (%)
Influenza virus A	25 (10)	2 (1)
Influenza virus B	7 (3)	0 (0)
Parainfluenza virus 1	3 (1)	0 (0)
Parainfluenza virus 2	0 (0)	0 (0)
Parainfluenza virus 3	2 (1)	0 (0)
Rhinovirus	61 (25)	23 (11)
Adenovirus	2 (1)	0 (0)
Coronavirus	20 (8)	11 (5)
Respiratory syncytial virus	6 (3)	2 (1)
Human metapneumovirus	5 (2)	0 (0)
Enterovirus	7 (3)	3 (1)
Chlamydia pneumoniae	2 (1)	0 (0)
Mycoplasma pneumoniae	3 (1)	1 (0)
β-Haemolytic streptococci	20 (9)	4 (2)
One or more of the above	146 (63)	44 (22)
Negative	84 (37)	156 (78)
Total	230 (100)	200 (100)

disease [10], and a previous study [4] found serological evidence of infection with *Legionella* spp. in 11.2% of patients who presented with a febrile respiratory tract infection. However, observations based solely on antibody seroconversion should be viewed with scepticism, as the sensitivity and specificity of serology for *Legionella* spp. other than *L. pneumophila* have not been validated, and cross-reactions with other organisms may occur [1,11,12].

Culture on buffered charcoal yeast extract (BCYE) plates is the standard method for the laboratory diagnosis of *Legionella* infections, but has its limitations. Isolates are identified by a combination of colony and Gram's stain morphology, and reactions with specific antibodies. However, because of the fastidious nature of the organism and problems inherent in specimen collection, culture of clinical specimens may have a sensitivity as low as 10% [11]. Moreover, several non-*pneumophila Legionella* spp. tend to grow poorly on BCYE agar [13]. Nucleic acid amplification techniques are attractive tools for detection of *Legionella* spp. in clinical samples, as they are able to detect all legionellae and provide results rapidly. Several studies have found that PCR methods have a higher rate of detection than culture-based methods [14–16]. In the present study, the assay sensitivity was 10 fg for all *Legionella* spp., equivalent to two bacterial cells/reaction.

Throat swabs have been used successfully to detect respiratory pathogens such as *Mycoplasma pneumoniae* [17], *Chlamydia pneumonia* [17,18] and *Legionella* spp. [16,17] by PCR. In contrast to a previous report based on serological observations [4], *Legionella* spp. were not detected in the nose or throat of patients with ARIs in general practice, and no evidence was found for asymptomatic carriage of *Legionella* spp. in the upper respiratory tract.

ACKNOWLEDGEMENTS

Phocine herpes virus was kindly provided by H. Niesters, Erasmus Medical Center, Rotterdam, The Netherlands.

REFERENCES

1. Fields BS, Benson RF, Besser RE. *Legionella* and Legionnaires' disease: 25 years of investigation. *Clin Microbiol Rev* 2002; **15**: 506–526.
2. Boshuizen HC, Neppelenbroek SE, van Vliet H *et al.* Subclinical *Legionella* infection in workers near the source of a large outbreak of Legionnaires' disease. *J Infect Dis* 2000; **184**: 515–518.
3. Lieberman D, Shvartzman P, Lieberman D *et al.* Etiology of respiratory tract infection in adults in a general practice setting. *Eur J Clin Microbiol Infect Dis* 1998; **17**: 685–689.
4. Lieberman D, Lieberman D, Korsonsky I, Ben-Yaakov M, Lazarovich Z, Boldur I. *Legionella* species infection in adult febrile respiratory tract infections in the community. *Scand J Infect Dis* 2002; **34**: 1–4.
5. den Boer JW, Friesema IH, Hooi JD. Reported cases of *Legionella* pneumonia in the Netherlands, 1987–2000. *Ned Tijdschr Geneeskd* 2002; **146**: 315–320.
6. Heijnen MLA, Dorigo-Zetsma JW, Bartelds AM, Wilbrink B, Sprenger MJW. Surveillance of respiratory pathogens and influenza-like illnesses in general practices—The Netherlands, winter 1997/98. *Eurosurveillance* 1999; **4**: 81–84.
7. van der Zee A, Verbakel H, de Jong C *et al.* Novel PCR-probe assay for detection of and discrimination between *Legionella pneumophila* and other *Legionella* species in clinical samples. *J Clin Microbiol* 2002; **40**: 1124–1125.
8. van den Hoogen BG, de Jong JC, Groen J *et al.* A newly discovered human pneumovirus isolated from young children with respiratory tract disease. *Nat Med* 2001; **7**: 719–724.
9. Diederen BMW, de Jong CMA, Aarts I, Peeters MF, van der Zee A. Molecular evidence for the ubiquitous presence of *Legionella species* in Dutch potable water systems [abstract N-149]. In: *Abstracts of the 104th General Meeting of the American Society for Microbiology*. Washington, DC: American Society for Microbiology, 2004; 423.
10. O'Mahony MC, Stanwell-Smith RE, Tillett HE *et al.* The Stafford outbreak of Legionnaires' disease. *Epidemiol Infect* 1990; **104**: 361–380.
11. Murdoch R. Diagnosis of Legionella infection. *Clin Infect Dis* 2003; **36**: 64–69.
12. Harrison TG, Taylor AG. *A laboratory manual for Legionella*, 1st edn. Chichester: Wiley, 1988.
13. Lee TC, Vickers RM, Yu VL, Wagener MM. Growth of 28 *Legionella* species on selective culture media: a comparative study. *J Clin Microbiol* 1993; **31**: 2764–2768.
14. Hayden RT, Uhl JR, Qian X *et al.* Direct detection of *Legionella* species from bronchoalveolar lavage and open lung biopsy specimens: comparison of LightCycler PCR, in situ hybridization, direct fluorescence antigen detection, and culture. *J Clin Microbiol* 2001; **39**: 2618–2626.
15. Koide M, Saito A. Diagnosis of *Legionella pneumophila* infection by polymerase chain reaction. *Clin Infect Dis* 1995; **21**: 199–201.
16. Templeton KE, Scheltinga SA, Sillekens P *et al.* Development and clinical evaluation of an internally controlled, single-tube multiplex real-time PCR assay for detection of *Legionella pneumophila* and other *Legionella* species. *J Clin Microbiol* 2003; **41**: 4016–4021.
17. Ramirez JA, Ahkee S, Tolentino A, Miller RD, Summersgill JT. Diagnosis of *Legionella pneumophila*, *Mycoplasma pneumoniae*, or *Chlamydia pneumoniae* lower respiratory infection using the polymerase chain reaction on a single throat swab specimen. *Diagn Microbiol Infect Dis* 1996; **24**: 7–14.
18. Gaydos CA, Quinn TC, Eiden JJ. Identification of *Chlamydia pneumoniae* by DNA amplification of the 16S rRNA gene. *J Clin Microbiol* 1992; **30**: 796–800.