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ARTICLE

Diagnostic testing for Legionnaires' disease in the Netherlands between 2007 and 2009: a possible cause for the decline in reported Legionnaires' disease patients

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Abstract Legionnaires' disease (LD) is an acute pneumonia caused by the inhalation or aspiration of aerosols contaminated with the *Legionella* bacteria. In the Netherlands, around 300 LD cases per year were reported between 2000 and 2008, but in 2009, the number dropped to 251, which was the lowest number in the previous 5 years of surveillance. We investigated if this decrease could be explained by the number of performed *Legionella* diagnostic tests in this year. We analyzed the number of tests performed between 2007 and 2009 in three large microbiological laboratories in different geographical regions in the Netherlands. Our data showed that there was no decrease in the number of patients for whom a diagnostic test for *Legionella* was performed in this period. These results are not in line with our hypothesis that the decrease in reported *Legionella* pneumonia patients in 2009 would be due to a decrease in patients for whom a diagnostic test was performed. We conclude that it is more likely that other factors such as the influence of weather patterns might explain the sudden drop in reported *Legionella* pneumonia patients in 2009 compared to the previous years, and it would be interesting to investigate this for the period described.

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Introduction

Legionnaires' disease (LD) is an acute pneumonia caused by the inhalation or aspiration of aerosols contaminated with the *Legionella* bacteria. It was named after a point-source outbreak in a hotel that hosted the convention of the American Legion in 1976 [1, 2]. Legionnaires' disease is characterized by an acute pneumonia, a low attack rate (0.1–5%), and an average incubation time of 2–10 days, although it may extend to even longer than 10 days [1–4]. The disease proves fatal in about 6% of cases [5].

In the Netherlands, LD has been a notifiable disease since 1987. Treating physicians report cases of LD to the Municipal Health Services, who then report them to the Centre for Infectious Disease Control, where a national database is situated [6]. Around 100–300 LD cases per year were reported between 1999 and 2008 (with an unexpected peak of 436 reported cases in 2006) [7–10]. In 2009, the number of reported LD cases dropped to 251, which was the lowest number in the previous 5 years of surveillance [9]. This sudden drop gave rise to several hypotheses that might explain the decrease of reported LD cases, ranging from the influence of weather patterns, to the effect of the concurrent occurrence of the influenza A (H1N1) pandemic and the Q fever outbreak in 2009 [11–14]. Several studies have suggested that specific meteorological variables like relative humidity and temperature are related to the LD incidence, as these factors might influence the outdoor survival of the *Legionella* bacteria [15–17]. Weather conditions in 2009 may, therefore, have affected the LD incidence. Furthermore, the influenza A

(H1N1) pandemic was spread around the Netherlands from April 2009 onwards [11, 12] and competed for the daily headlines with the Q fever outbreak that started in the southern part of the country and peaked in the spring of 2009 [13, 14]. The nationwide increased attention for these two respiratory infectious diseases may have diminished the interest of both the public and health care employees for LD. As a consequence, changes in laboratory practices with respect to LD diagnostics could have resulted in a diagnostic bias and an underreported number of LD cases in 2009.

The aim of this study was to investigate if the number of requests for *Legionella* diagnostics in the Netherlands between 2007 and 2009 could explain the number of reported LD cases in these years. We, therefore, analyzed the *Legionella* diagnostics data from three, large, microbiological laboratories from different geographical regions in the Netherlands, between 2007 and 2009.

Methods

Patients

Legionnaires' disease has been notifiable in the Netherlands since 1987. Treating physicians are required to report cases of LD to a public health physician at one of the 28 Municipal Health Services within 24 h of diagnosis. The public health physicians are then required to report all confirmed and probable cases of LD to the Ministry of Health and, more recently, to the Centre for Infectious Disease Control within 24 h. The LD cases diagnosed in this present study were defined according to the standardized case definitions of the European Legionnaires' Disease Surveillance Network (ELDSNet) [18]. A confirmed case of LD is a patient presenting clinical and/or radiological signs of pneumonia with at least one of the following laboratory criteria: (1) isolation of *Legionella* spp. from respiratory secretions or any normally sterile site; (2) detection of *L. pneumophila* antigen in urine; or (3) *L. pneumophila* serogroup 1 specific antibody response. A probable case of LD is defined as a patient presenting clinical and/or radiological signs of pneumonia with at least one of the following laboratory criteria: (1) detection of *L. pneumophila* antigen in respiratory secretions or lung tissue, e.g., by direct fluorescent antibody staining; (2) detection of *Legionella* spp. nucleic acid in a clinical specimen; (3) *L. pneumophila* non-serogroup 1 or other *Legionella* spp. specific antibody response; or (4) a single high titer in specific antibody response for *L. pneumophila* serogroup 1, other serogroups, or other *Legionella* spp.

Laboratories

Three, large, microbiological laboratories in the Netherlands participated in this study and provided data on all requested diagnostic LD tests in 2007, 2008, and 2009. The Izore Centre for Infectious Diseases Friesland is situated in the city of Leeuwarden in the north of the Netherlands, and performs LD diagnostic tests for the 650,000 inhabitants of its adherence region. The Laboratory of Medical Microbiology and Immunology of the St. Elisabeth Hospital in Tilburg is situated in the southern part of the Netherlands, and performs LD diagnostic tests for the 800,000 inhabitants of its adherence region. The Regional Public Health Laboratory Kennemerland in Haarlem is situated in the western part of the country, and has an adherence region of about 700,000 inhabitants.

Diagnostic tests

Each laboratory performed a spectrum of different tests on the available patient materials for which *Legionella* diagnosis was requested by the treating physician of the patient.

Urinary antigen test

In all three laboratories, a commercial urinary antigen test (BinaxNOW, Portland, ME, USA) was used to test urine samples for the presence of *L. pneumophila* antigens. The BinaxNOW test has been recommended as a rapid specific test for LD caused by *L. pneumophila* serogroup 1, with several technical advantages over a conventional enzyme immunoassay test: there is no need for expensive laboratory equipment and the processing speed is higher [19]. However, the detection of non-*L. pneumophila* serogroup 1 cases is higher for the Binax EIA [19, 20].

Serological investigation

All laboratories used a commercial enzyme-linked immunosorbent assay (ELISA) to detect IgM and IgG serotype 1–7 antibodies to *L. pneumophila* in the acute- and (when available) in the convalescent-phase sera of patients (Serion ELISA; Institut Virion/Serion GmbH, Würzburg, Germany).

Culture

In all laboratories, available respiratory secretion, bronchoalveolar lavage specimen, or lung tissue of patients were used to culture *Legionella* spp. The available specimen was cultured on two media at 35°C, with increased humidity. In Haarlem and Tilburg, the two media used were buffered charcoal yeast extract supplemented with α-ketoglutarate (BCYE-α) and (1) the antibiotics polymyxin B, cefazolin,

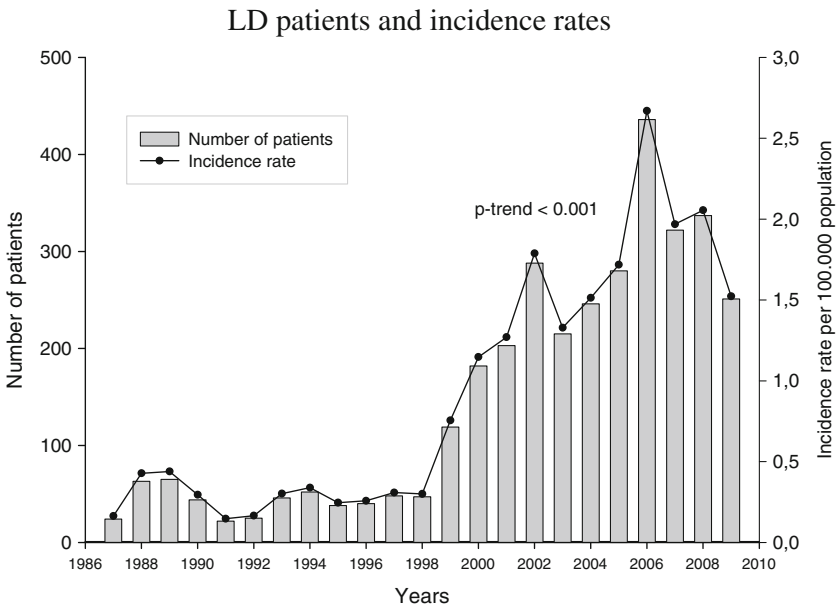

Fig. 1 Legionnaires' disease (LD) incidence rate and number of patients in the Netherlands 1987–2009. The *bars* represent the annual number of reported LD patients. The *line* represents the incidence rate per 100,000 population. The *p*-value reflects the trend of the incidence rate over time between 1999 and 2008 (Cochran–Armitage trend test)

and pimaricin or (2) the antibiotics polymyxin B, anisomysin, and vanomycin (Oxoid Ltd., Hampshire, UK). In Leeuwarden, the available specimen (bronchoalveolar lavage specimen or respiratory secretion) was cultured on two media at 35°C, with increased humidity. The two media used were buffered charcoal yeast extract (1) without antibiotics and (2) with the antibiotics polymyxin B and cefazolin (Mediaproducts BV, Groningen, the Netherlands).

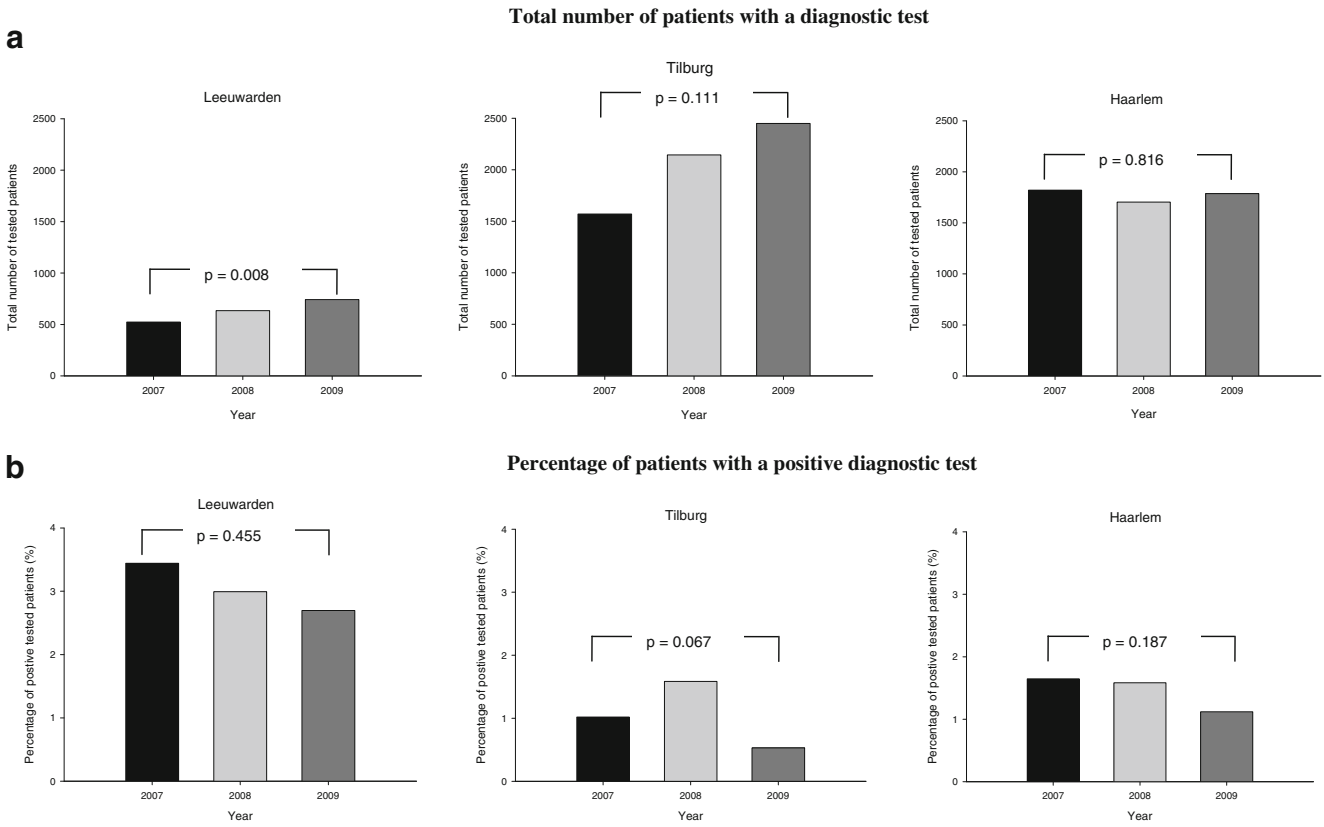

Fig. 2 Diagnostic tests for *Legionella* between 2007 and 2009. The top panel (**a**) shows the total number of patients for whom one or more diagnostic tests for *Legionella* were performed between 2007 and 2009 in the three participating laboratories. The *p*-values reflect the trend over time (linear regression analysis). The lower panel (**b**) shows the proportion of patients with a positive diagnostic test for *Legionella* between 2007 and 2009. The *p*-values reflect the trend over time (Cochran–Armitage trend test)

Table 1 Diagnostic tests performed between 2007 and 2009

Diagnostic tests performed	Year		
	2007	2008	2009
Leeuwarden			
PCR	88 (13)	137 (16)	117 (12)
Culture	118 (17)	146 (17)	110 (11)
Urinary antigen test	184 (26)	200 (23)	202 (21)
Serological test	313 (45)	393 (45)	534 (55)
Total number of tests	703 (100)	876 (100)	963 (100)
Tilburg			
PCR	60 (4)	88 (4)	72 (3)
Culture	160 (10)	218 (9)	191 (7)
Urinary antigen test	471 (28)	971 (42)	1,433 (52)
Serological test	970 (58)	1,049 (45)	1,066 (39)
Total number of tests	1,661 (100)	2,326 (100)	2,762 (100)
Haarlem			
PCR	78 (4)	55 (3)	81 (4)
Culture	94 (5)	53 (3)	81 (4)
Urinary antigen test	806 (42)	833 (46)	923 (49)
Serological test	961 (50)	859 (48)	806 (43)
Total number of tests	1,939 (100)	1,800 (100)	1,891 (100)

Data are displayed as numbers (%)

Polymerase chain reaction

In the laboratories of Haarlem [21, 22] and Leeuwarden [23], a polymerase chain reaction (PCR) assay that targeted the 16S ribosomal DNA gene was used to detect *Legionella* nucleic acid in the available patient materials. In Tilburg, samples were tested for *Legionella* spp. DNA in a 16S rRNA-based PCR and in an *mip* gene-based PCR for *L. pneumophila* [24].

Data collection

For each laboratory, we first calculated the total number of patients for whom one or more *Legionella* diagnostic tests were requested in 2007, 2008, and 2009. Additionally, the number of different diagnostic tests that were performed was calculated for the three laboratories over the same period.

Statistical analyses

The trend over time of the incidence rate of LD cases (between 1999 and 2008) was assessed by the use of the Cochran–Armitage trend test (R Foundation for Statistical Computing, Vienna, Austria) [25]. Incidence rates were compared between different years, using a two-tailed χ^2-test (PASW Statistics release 18.0, SPSS Inc., Chicago, IL, USA). The trend over time of the number of patients for whom a diagnostic test for *Legionella* was performed between 2007 and 2009 was assessed by the use of linear regression analysis (PASW Statistics release 18.0, SPSS Inc., Chicago, IL, USA). The trend over time (between 2007 and 2009) of the patients with a positive diagnostic test for *Legionella* as a proportion of the total number of patients with a diagnostic test was assessed by the use of the Cochran–Armitage trend test [25].

Results

From 1987 to 2009, a total of 3,393 LD cases (probable or confirmed) were reported in the Netherlands, which corresponds to an average annual incidence rate of 0.94 per 100,000 population (Fig. 1) [7–10]. Between 1999 and 2008, there has been a significant increase in the incidence rate ($p<0.001$, Cochran–Armitage trend test), with two peaks, one in 2002, with an incidence rate (95% confidence interval [CI]) of 1.79 (1.64–1.94), and one in 2006, with an incidence rate (95% CI) of 2.67 (2.52–2.82). In 2009, the lowest incidence rate was reported in 5 years of surveillance: 1.52 per 100,000 population (95% CI: 1.32–1.73). This rate was significantly lower ($p=0.001$, χ^2 test) compared to the other two years of the study period 2007–2009: in 2007, the incidence rate (95% CI) was 1.97 (1.76–2.17), and in 2008, the incidence rate was 2.05 (1.85–2.26).

Figure 2a shows the total number of patients for whom one or more diagnostic tests for Legionella were performed between 2007 and 2009 in the three laboratories. Overall, the number of patients for whom one or more tests were performed increased between 2007 and 2009: 3914 patients in 2007, 4484 patients in 2008, and 4978 patients in 2009 ($p=0.026$, linear regression). Figure 2a shows the data for the three laboratories separately.

Figure 2b shows the patients with a positive diagnostic test for *Legionella* as a proportion of the total number of patients with a diagnostic test. In total, there were 197 cases (96 probable; 101 confirmed) reported by the three laboratories between 2007 and 2009: 64 cases (32 probable; 32 confirmed) in 2007, 80 cases (35 probable; 45 confirmed) in 2008, and 53 cases (29 probable; 24 confirmed) in 2009. The corresponding incidence rates (95% CI) for the three laboratories were 2.98 (2.24–3.72) per 100,000 population of the adherence region in 2007, 3.72 (2.98–4.46) per 100,000 population in 2008, and 2.47 (1.73–3.20) per 100,000 population in 2009. The data from all three laboratories taken together showed that the proportion of patients with a positive diagnostic test (95% CI) decreased between 2007 and 2009: 1.64 (1.26–2.01) in 2007 (64 cases in 3,914

tested patients), 1.78 (1.43–2.14) in 2008 (80 cases in 4,484 tested patients), and 1.06 (0.73–1.39) in 2009 (53 cases in 4,978 tested patients) (p=0.019, Cochran–Armitage trend test). The data from Tilburg showed a slight increase in the proportion of patients with a positive test between 2007 and 2008, which was followed by a decrease between 2008 and 2009.

In Table 1, the data are shown for the number of different diagnostic tests that were performed in the three laboratories between 2007 and 2009. Several patients were diagnosed by more than one diagnostic method, which is reflected by the higher total number of tests that were annually performed compared to the total number of tested patients (Fig. 2a). In all three laboratories, the majority of diagnostic tests consisted of either a serological test or a urinary antigen test. Culture was the third most common test, followed by PCR. The increase between 2007 and 2009 in the total number of diagnostic tests that were performed in Leeuwarden and Tilburg was mostly due to the increase in serological tests (both laboratories) and in urinary antigen tests (Tilburg only), but remained the same when only the urinary antigen tests were considered: in total, 1,461 urinary antigen tests were performed in 2007, 2,004 tests in 2008, and 2,558 tests in 2009 (Table 1).

Discussion

Our data show that there was no decrease in the number of patients for whom a diagnostic test for *Legionella* was performed in three large microbiological laboratories from different geographical regions in the Netherlands between 2007 and 2009. On the contrary, the number of patients with a diagnostic test increased in two of the three laboratories in this period. There was an overall decrease in the proportion of patients with a positive diagnostic test for *Legionella*.

One of the strengths of our study was the large amount of diagnostic data provided by the three different laboratories. Their large adherence regions and the location of the laboratories in three different geographical regions in the Netherlands strengthens our belief that the available data on diagnostic tests formed a representative sample of the total number of performed diagnostic tests in the Netherlands.

The results are not in agreement with our hypothesis that the decrease in the number of reported *Legionella* pneumonia patients in 2009 could be due to a decrease in the number of patients for whom a diagnostic test was performed. We, therefore, conclude that it is more likely that other factors might explain the sudden drop in reported *Legionella* pneumonia patients in 2009 compared to the previous years, and it would be interesting to investigate this for the period described in this current study.

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Conflict of interest The authors declare that they have no conflict of interest.

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