Epidemiol. Infect. (2009), **137**, 1013–1018. © 2008 Cambridge University Press
doi:10.1017/S0950268808001684 Printed in the United Kingdom

Dynamics of *Legionella* antibody levels during 1 year in a healthy population

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(*Accepted 10 November 2008; first published online 23 December 2008*)

SUMMARY

A total of 522 Danish blood donors were followed during 2004–2005 to describe the seroepidemiology of *Legionella* infections in healthy individuals from a general population. Antibodies to *Legionella* spp. were measured by indirect immunofluorescence antibody test. The prevalence of *Legionella* antibodies (titre $\geqslant$1:128) was 26·8% and remained fairly constant during the year of follow-up. However, 6·9% of the blood donors developed a fourfold or greater rise in antibody titres. A history of visits to Danish summer cottages was associated with both *Legionella* seropositivity (OR 1·53, 95% CI 1·02–2·30) and seroconversion (OR 2·66, 95% CI 1·21–5·83). There were no consistent associations between either levels of antibody titres or seroconversion and self-reported health symptoms, absence from work due to illness, or to any risk factors. We conclude that community-acquired *Legionella* infections are frequent; however, they rarely result in severe illness.

Key words: Blood donors, epidemiology, *Legionella*, *Legionella* antibodies, prevalence.

INTRODUCTION

Legionella infection is associated with two well-recognized distinct clinical and epidemiological forms: Legionnaires' disease (LD), which is a severe type of pneumonia, and the self-limiting non-pneumonic disease Pontiac fever. LD is a notifiable condition in most countries, and community-acquired LD occurs both sporadically and in outbreaks. LD accounts for up to 8–14% of cases of hospitalized community-acquired pneumonia [1, 2]. There is a high degree of geographical variation in the incidence of LD [3]; some of this variation may be due to differences in surveillance and diagnostic methods [3]. Known sources of infective aerosols include evaporative cooling towers, fountains, showers, nebulizers, and whirlpool spas [3]. In addition to these exposures, host factors are important for LD; these factors include age, gender, smoking, underlying illness and general immunodeficiencies [4].

While the epidemiology of LD is fairly well understood, there is limited knowledge concerning less severe and subclinical *Legionella* infections. Seroepidemiology is a suitable methodology to address this question. The aim of the present study is to analyse the overall *Legionella* antibody prevalence and changes in antibody titres during 1 year in a healthy general population. To examine risk factors for a positive *Legionella* serology or seroconversion, we analysed the association between *Legionella* antibody

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titres and self-reported health or possible environmental exposures. To our knowledge, changes in *Legionella* antibody levels during 1 year in a healthy population and the possible association with health outcomes and exposure variables have not been previously described.

MATERIALS AND METHODS

Blood samples were collected from 708 healthy, unpaid volunteer blood donors aged between 18 and 65 years living in the towns of Randers and Vejle in Jutland, Denmark. Generally, most blood donors give blood at least once a year. We have previously shown that there were no differences in *Legionella* seroprevalence between the two towns [5]. For this study, the first sampling period was from late February to early June 2004 and the second one from February to June 2005. The median age of the blood donors was 45 years and 57% were males. There was no significant difference in age distribution (analysed in four age groups) or gender between donors in the two towns.

After 1 year, all returning blood donors ($n=522$) had a new blood sample taken and were asked to complete a questionnaire about socioeconomic variables, relevant exposures and health conditions during the previous year. A total of 27% of the blood donors had ceased donating blood because of age, pregnancy, health problems, moving to another area or unwillingness to continue to be a donor. The drop-out rates did not vary between the two towns.

The blood samples were analysed for *Legionella* antibodies by an indirect immunofluorescence antibody test (IFAT). Plate-grown, heat-inactivated *Legionella* (*L.*) *pneumophila* serogroups (sg) 1–6, *L. micdadei* and *L. bozemanii* were used as separate antigens. All sera were tested against all antigens. This assay is essentially the same as the well-characterized assay described by Wilkinson *et al.* [6], which follows the guidelines of the United States Centers for Disease Control and Prevention (CDC). Specifically, antibodies to *Legionella* were detected with a fluorescein isothiocyanate (FITC)-conjugated rabbit anti-human IgM, A and G antibody (Dako F0200, Dako, Glostrup, Denmark). An *E. coli*-blocking fluid was used to block cross-reacting antibodies to other Gram-negative bacteria [7]. The serum samples were titrated from 1:64 and upwards to end-point titre. A single titre of $\geqslant$1:128 was used to define a positive *Legionella* antibody response; titres below 1:64 were not considered meaningful due to false reactions and background staining. The selected antigens are the antigens used for routine serological testing for LD at the Statens Serum Institut reference laboratory in Denmark and include the serogroups responsible for more than 80% of diagnosed LD cases (serogroups 1, 3, 6). Serogroups 2, 4 and 5 were included mostly for historical reasons, although serogroups 4 and 5 are not uncommon causes of LD (especially nosocomial LD). *L. micdadei* and *L. bozemanii* were included as they are the most common causes of non-*pneumophila* LD. The limitations, including cross-reactivity with other bacteria, of our method have previously been discussed [5]. Cross-reactions between the *Legionella* antigens used are often seen; serological response to a *Legionella* infection is in general not serogroup specific. Infections with other serogroups than those used in the IFAT can on the other hand also be detected due to this cross-reaction. National laboratory test criteria for a confirmed diagnosis of *Legionella* infection include a $\geqslant$fourfold rise in antibody titre to $\geqslant$1:128 in IFAT (seroconversion) to *L. pneumophila* sg 1, 3, or 6. Seroconversion to other *Legionella* antigens and positive titres ($\geqslant$1:256) to any *Legionella* antigen are considered as presumptive of a recent or previous *Legionella* infection.

Risk factors examined included: type of residence; residence built before or after 1970; type of heating; presence of hot-water tank; hot-water tap-time (the tap-time was considered as slow if estimated not to be hot in ½-1 min); temporary stop in water supply; temporary uninhabited home; use of spa bath; showering elsewhere than at home; swimming pools, travel abroad; hotel stay in Denmark; summer cottage visits; and air-conditioning at work. Socioeconomic measures included previous education, job skills and total family income. Respondents were asked to provide information for the previous year including reporting the month of the possible exposure activity. Swimming pool use and showering outside the home were reported only if respondents engage in these activities regularly (several times a month).

A self-reported medical history for the past year was collected including questions about illnesses (influenza, pneumonia, common cold), hospitalizations, general practitioner (GP) consultations, absences from work due to illness and specific signs or symptoms (cough, fever, stomach pain, shiver, diarrhoea, headache, myalgia). The symptoms had to be present for at least 2–3 consecutive days in the previous year to be reported in the questionnaire.

Table 1. *Fourfold changes in 1-year prevalence of* Legionella *antibodies in numbers in 522 healthy Danish blood donors tested twice within a 1-year interval, 2004–2005*

	2005				
	<1:64	1:64	1:128	1:256	Total
2004					
<1:64	190	99	33†	2†	324
1:64	24	32	23	1†	80
1:128	7*	25	44	9	85
1:256	0*	5*	17	11	33
Total	221	161	117	23	522

* Indicates fourfold fall in titre.
† Indicates fourfold rise in titre.

The study was approved by the local scientific ethical committee (VF20030250) and the Danish Data Protection Agency.

Epi Data version 3 (Odense, Denmark) was used for data entry. Univariable analyses were conducted with antibody status as the dependent variable. Variables with a P value $<0{\cdot}2$ were included in further multivariable logistic regression analyses about health and risk factors adjusted for age, gender, current smoking and place of residence (town). The described multivariable models were reduced to include variables with P values $<0{\cdot}1$. The reference group was subjects with *Legionella* titres $<1:128$. The statistical analyses were done using Stata version 9.2 (Stata Corp, College Station, TX, USA).

RESULTS

In total, 522 subjects had blood samples available from 2004 and 2005 for testing and completed the questionnaire. One hundred and forty (26·8%) subjects had a *Legionella* titre $\geqslant 1:128$ in 2005. Seventy-one subjects (13·6%) had a titre $\geqslant 1:128$ to *L. pneumophila* sg 1. No subject had a titre $>1:256$. Thirty-six subjects had a $\geqslant$fourfold rise in *Legionella* antibody titre to at least 1:128, corresponding to a 1-year risk of seroconversion of 6·9%. Conversely, 12 (2·3%) had a $\geqslant$fourfold fall in titre from a titre of at least 1:128 after 1 year (Table 1).

In Denmark, LD is a notifiable disease. All donors with a titre $\geqslant 1:128$ were searched for in the register for notified LD cases. None were found to have been reported with LD.

Self-reported health

Neither self-reported illnesses (influenza, pneumonia, common cold), hospitalizations, GP consultations, absences from work nor specific symptoms (cough, fever, common cold, malaise, stomach pain, shiver, diarrhoea, headache, myalgia) showed any significant difference between control subjects and cases where cases were defined either by a seroconversion to any *Legionella* antigen, a single antibody titre $\geqslant 1:128$, or a single antibody titre $\geqslant 1:128$ to *L. pneumophila* sg 1 (Table 2).

There was no association between *Legionella* antibodies and a symptom complex of at least three, four or five symptoms of the eight common influenza-like symptoms (data not shown). In the multivariable models, no health-related variables were associated with being a case; this result was independent of the case definition (all P values $>0{\cdot}1$).

Risk factors

Positive *Legionella* antibody titres or seroconversion were not associated with age, gender or town of residence ($P>0{\cdot}2$ for all variables). Holidaying in Danish summer cottages was associated with an increased risk of *Legionella* seroconversion or a single positive *Legionella* antibody titre, but not for a positive *Legionella* sg 1 antibody titre $\geqslant 1:128$ (Table 2). In addition, current smoking was associated with increased risk of a positive *Legionella* antibody titre (Table 2).

The final multivariable model for *Legionella* seroconversion included visits to summer cottages [odds ratio (OR) 3·34, 95% confidence interval (CI) 1·48–7·55], showering outside the home (OR 0·41, 95% CI 0·19–0·88) and district heating compared with any form of domestic based heating (OR 0·75, 95% CI 0·59–0·96). The final multivariable model for a positive *Legionella* antibody titre included visits to a summer cottage (OR 1·61, 95% CI 1·07–2·43), and current smoking (OR 1·72, 95% CI 1·08–2·73). The final multivariable model for a positive *L. pneumophila* sg1 antibody titre only included visits to a summer cottage (OR 1·63, 95% CI 0·95–2·80) as a risk factor.

DISCUSSION

In 2005, the prevalence of a positive *Legionella* antibody titre in our study population was 27%. Thirteen percent of subjects were positive for antibodies to

https://doi.org/10.1017/S0950268808001684 Published online by Cambridge University Press

Table 2. *Univariable analysis of self-reported health and risk factors in the previous year in persons with antibodies to* Legionella pneumophila *in Denmark, 2005**

	Seroconversion in 1 year ($n=36$)			Titre $\geqslant$1:128 ($n=140$)			Titre $\geqslant$1:128 of *L. pneumophila* sg 1 ($n=71$)			Reference (<1:128) ($n=382$)
	No. (%)	OR (CI 95%)	*P*	No. (%)	OR (CI 95%)	*P*	No. (%)	OR (CI 95%)	*P*	No. (%)
Health										
Shivering (yes/no)	1/30 (3)	0·57 (0·07–4·42)	0·561	3/120 (2)	0·43 (0·12–1·48)	0·142	2/58 (3)	0·59 (0·13–2·61)	0·458	18/308 (6)
Headache (yes/no)	3/28 (10)	0·59 (0·17–2·02)	0·373	23/100 (19)	1·27 (0·74–2·19)	0·395	14/46 (23)	1·68 (0·86–3·28)	0·141	50/276 (15)
Hospitalization (yes/no)	4/31 (11)	2·77 (0·87–8·79)	0·114	5/131 (4)	0·82 (0·29–2·28)	0·697	3/65 (4)	0·99 (0·28–3·49)	0·987	16/343 (4)
Risk factors										
Type of residence; block of apartments, and other types		0·96 (0·67–1·38)	0·844		1·16 (0·96–1·42)	0·131		1·11 (0·86–1·44)	0·416	
Type of heating; district heating and different forms of domestic based heating		0·82 (0·65–1·02)	0·082		0·96 (0·83–1·10)	0·554		0·96 (0·80–1·16)	0·685	
Showering elsewhere than at home (yes/no)	12/23 (34)	0·50 (0·24–1·03)	0·053	58/78 (43)	0·71 (0·48–1·05)	0·087	33/35 (49)	0·90 (0·53–1·51)	0·681	185/176 (51)
Visit to swimming pool (yes/no)	4/31 (11)	0·54 (0·18–1·57)	0·223	18/117 (13)	0·64 (0·37–1·12)	0·108	12/56 (18)	0·89 (0·45–1·75)	0·735	70/291 (19)
Hotel stay in Denmark (yes/no)	11/24 (31)	0·68 (0·32–1·43)	0·297	47/89 (35)	0·78 (0·52–1·18)	0·237	21/47 (31)	0·66 (0·38–1·15)	0·138	146/216 (40)
Visiting a summer cottage in Denmark (yes/no)	26/9 (74)	***2·66*** ***(1·21–5·83)***	***0·010***	85/51 (63)	***1·53*** ***(1·02–2·30)***	***0·036***	42/26 (62)	1·49 (0·87–2·53)	0·139	189/174 (52)
Air-conditioning at work (yes/no)	9/25 (26)	0·82 (0·42–1·59)	0·557	32/94 (25)	0·73 (0·49–1·07)	0·105	13/50 (21)	0·65 (0·39–1·10)	0·102	114/225 (34)
Current smoking (yes/no)	9/26 (26)	1·36 (0·61–3·03)	0·460	39/97 (29)	***1·58*** ***(1·01–2·48)***	0·050	21/47 (31)	1·76 (0·99–3·12)	0·061	73/287 (20)

OR, Odds ratio; CI, confidence interval.

* The univariable analysis of the variables with $P<0·2$ in any of the three groups are reported.

L. pneumophila sg 1, the most common cause of LD. During the course of 1 year, 6·9% of healthy, volunteer blood donors acquired *Legionella* antibodies; 2·3% of previously antibody-positive donors became negative for antibodies to *Legionella*.

In our population, visiting a Danish summer cottage was a significant risk factor for *Legionella* seropositivity and seroconversion. Current smoking also increased the risk for a positive *Legionella* antibody titre. We found no significant association between *Legionella* seroconversion or a positive antibody titre and travelling abroad, hotel stays or visits to swimming pools and spa baths. A positive *Legionella* serology or serconversion was not associated with an increased risk of self-reported absences from work, self-reported sicknesses or specific symptoms.

One potential limitation of our study is recall bias. We asked about common signs and symptoms that occurred during the previous year and these may not be easy to remember, leading to possible underreporting of symptoms. As study subjects were not aware of their *Legionella* antibody status, recall bias should affect both groups equally, probably resulting in a non-differential misclassification. Symptoms lasting only a short time which did not result in sick-leave absence or medical care may have been forgotten. We are reasonably certain of detecting only those episodes of illness that were of sufficient severity to be remembered. To increase the chance of detecting symptoms relevant for *Legionella* exposure, we analysed combinations of symptom complexes; these analyses did not affect the interpretation.

The lack of association between a positive *Legionella* serology and health complaints or self-reported sicknesses is consistent with the results in a Dutch outbreak where no health complaints were found in the seropositive group except for stomach ache, which may have been a spurious observation due to the large number of statistical tests done [8].

Recall bias may have affected the collection of exposure variables as well, but probably to a lesser degree as most of the exposures are about everyday habits or activities that are easily remembered such as holidays and travel.

Our findings indicate that exposure to *Legionella* may be common. The possibility of repeated re-exposures in our population of continuously positive individuals cannot be ruled out, indicating that the yearly risk of exposure might be even higher than the number of seropositive individuals. Only a few subjects experienced a $\geqslant$fourfold fall in *Legionella* antibody titres during the course of 1 year. The low number of subjects with a fourfold antibody fall in our population compared with the number with a fourfold antibody rise may be due to a change in exposure during the year or to the 'trailing' of antibodies, as losing antibodies takes longer than acquiring antibodies if infected. It might also be a chance finding.

In an outbreak in The Netherlands, both the percentage of high-normal antibody levels (>75th percentile) and high antibody levels (>99th percentile) was found to increase with exposure to *L. pneumophila* [8]. Increased antibody levels were found in studies of exposed groups of hospital staff in nosocomial LD outbreaks as well [9]. These findings suggest a dose-dependent effect on antibody response, indicating a dose-dependency in getting LD [10]. However, a study has failed to show a consistent relation between duration of exposure, frequency of exposure, or distance from the source and risk of LD [11]. This study suggested that transient exposure was sufficient to cause infection [11]. Nevertheless, most studies indicate that there are associations with either duration, frequency, or distance from source [12, 13]. The present study corroborates the notion that exposure to and infection with *Legionella* is common. However, except for visiting summer cottages and current smoking, we were unable to pinpoint clear risk factors for *Legionella* serconversion or a positive *Legionella* antibody titre. *Legionella* are known to be widespread in the environment in Denmark [14, 15]. Enclosed or stagnant water systems are associated with *Legionella* growth and the risk for LD [16]; these conditions are often present in summer cottages.

To our surprise, we did not find an association with travelling abroad, even though travelling abroad accounts for about 20–30% of the notified LD cases in Denmark [17], and has been reported to be an independent risk factor in LD [18].

No socioeconomic risk factors were associated with a positive *Legionella* antibody titre. However, our study population consisted of a voluntary, healthy population that was mostly middle class and therefore not particularly socially or economically diverse. The present study also was not designed to identify comorbid illnesses as risk factors as blood donors tend to be a healthy population.

In conclusion, there was a large and fairly constant number of individuals with *Legionella* antibodies in Denmark, and a considerable proportion of healthy Danish people develop *Legionella* antibodies during the course of 1 year without any measurable illness.

These results indicate that environmental exposures to *Legionella* spp. in Denmark are common but seldom result in measurable morbidity in otherwise healthy adults.

ACKNOWLEDGEMENTS

We thank Helle Rieck and Marian Jørgensen for language revision.

DECLARATION OF INTEREST

None.

REFERENCES

1. **Braun JJ, *et al.*** Community-acquired pneumonia: pathogens and course in patients admitted to a general hospital [in Dutch]. *Nederlands Tijdschrift voor Geneeskunde* 2004; **148**: 836–840.
2. **Sopena N, *et al.*** Prospective study of community-acquired pneumonia of bacterial etiology in adults. *European Journal of Clinical Microbiology and Infectious Diseases* 1999; **18**: 852–858.
3. **Bhopal RS.** Geographical variation of Legionnaires' disease: a critique and guide to future research. *International Journal of Epidemiology* 1993; **22**: 1127–1136.
4. **Marston BJ, Lipman HB, Breiman RF.** Surveillance for Legionnaires' disease. Risk factors for morbidity and mortality. *Archives of Internal Medicine* 1994; **154**: 2417–2422.
5. **Rudbeck M, Molbak K, Uldum S.** High prevalence of antibodies to *Legionella* spp. in Danish blood donors. A study in areas with high and average incidence of Legionnaires' disease. *Epidemiology and Infection* 2008; **136**: 257–262.
6. **Wilkinson HW, Fikes BJ, Cruce DD.** Indirect immunofluorescence test for serodiagnosis of Legionnaires disease: evidence for serogroup diversity of Legionnaires disease bacterial antigens and for multiple specificity of human antibodies. *Journal of Clinical Microbiology* 1979; **9**: 379–383.
7. **Bangsborg JM, *et al.*** The *E. coli* immunosorbent as used in serodiagnosis of *Legionella* infections studied by crossed immunoelectrophoresis. *APMIS* 1988; **96**: 177–184.
8. **Boshuizen HC, *et al.*** Subclinical *Legionella* infection in workers near the source of a large outbreak of Legionnaires disease. *Journal of Infectious Diseases* 2001; **184**: 515–518.
9. **Saravolatz L, *et al.*** Prevalence of antibody to the Legionnaires' disease bacterium in hospital employees. *Annals of Internal Medicine* 1979; **90**: 601–603.
10. **O'Brien SJ, Bhopal RS.** Legionnaires' disease: the infective dose paradox. *Lancet* 1993; **342**: 5–6.
11. **Greig JE, *et al.*** An outbreak of Legionnaires' disease at the Melbourne Aquarium, April 2000: investigation and case-control studies. *Medical Journal of Australia* 2004; **180**: 566–572.
12. **Brown CM, *et al.*** A community outbreak of Legionnaires' disease linked to hospital cooling towers: an epidemiological method to calculate dose of exposure. *International Journal of Epidemiology* 1999; **28**: 353–359.
13. **Den Boer JW, *et al.*** A large outbreak of Legionnaires' disease at a flower show, the Netherlands, 1999. *Emerging Infectious Diseases* 2002; **8**: 37–43.
14. **Jeppesen VF, *et al.*** Occurrence of Legionella – risk analysis. Miljøprojekt no. 897, 1–49 [in Danish]. Department of the Environment. The Danish Ministry of the Environment, 2004.
15. **Brydov P, *et al.*** Content of Legionella in hot water systems. Identification and risk analysis [in Danish]. Department of the Environment. The Danish Ministry of the Environment, 2001.
16. **Straus WL, *et al.*** Risk factors for domestic acquisition of legionnaires disease. Ohio Legionnaires Disease Group. *Archives of Internal Medicine* 1996; **156**: 1685–1692.
17. **Uldum S, Krause T.** Legionella pneumonia. No. 36, 1–2, Epi-News, Statens Serum Institut, 2006.
18. **Den Boer JW, Nijhof J, Friesema I.** Risk factors for sporadic community-acquired Legionnaires' disease. A 3-year national case-control study. *Public Health* 2006; **120**: 566–571.