Seroepidemiological study after a long-distance industrial outbreak of Legionnaires’ disease

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Running title: Seroepidemiological Legionella study

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ABSTRACT

Following a long-range outbreak of Legionnaires’ disease from an industrial air scrubber in Norway in 2005, a seroepidemiological study measuring IgG and IgM antibodies to *Legionella pneumophila* with a polyvalent enzyme-linked immunosorbent assay was performed. One year after the outbreak, IgG levels in employees (n = 213) at the industrial plant, harbouring the scrubber, and in blood donors (n = 398) from the outbreak county were low but significantly higher (*P* ≤ 0.002) than in blood donors (n = 406) from a non-exposed county. No differences in IgM levels between the three groups were found after adjustment for gender and age. Home addresses of the seroresponders in the exposed county clustered to the city of the outbreak in contrast to the scattering of addresses of the seroresponding donors in the non-exposed county. Factory employees, who operated at an open biological treatment plant, had significantly higher IgG and IgM levels (*P* < 0.034) than those working > 200 m away. Most of the healthy seroresponders among the factory employees worked near this exposure source. Immunoblotting showed that IgG and IgM antibodies in 82.1% of all seroresponders were directed to the lipopolysaccharide of the *L. pneumophila* serogroup 1 outbreak strain. In conclusion, one year after the long-range industrial outbreak a small increase in IgG levels of the exposed population was observed. The open biological treatment plant within the industrial premises, however, constituted a short-range exposure source of *L. pneumophila* for factory employees working nearby.

(243 words)
INTRODUCTION

In May 2005, an outbreak of *Legionella pneumophila* serogroup 1 occurred from an air scrubber located at a large wood-based chemical factory in the south-eastern part of Norway. Legionnaires’ disease (LD) was diagnosed in 56 patients of whom 10 died (35). Fifty-one of the patients lived in the county where the industrial plant is located. The bacteria spread more than 10 km away from the scrubber probably due to the high emission velocity of the aerosols at the top of the scrubber and the prevailing weather conditions (35). Recently, the number of LD patients in this outbreak was adjusted to 103 based on antibody responses in patients with pneumonia from the region at the time of the outbreak (34). It is the largest outbreak of LD in Norway so far; only one outbreak in 2001 with 28 cases of LD was reported previously (5). In between these two outbreaks, there were yearly about 25 LD cases, about half of which were imported, corresponding to an incidence of 0.6/100 000 (34).

The prevalence of antibodies to *L. pneumophila* in healthy individuals following larger outbreaks has been described in several studies (7, 8, 13, 14, 19, 30, 36). The aim of our study was to determine whether the long-distance outbreak from the air scrubber in 2005 (35) had resulted in increased antibody levels to *L. pneumophila* in healthy blood donors residing in the exposed county compared with blood donors in a non-exposed county. Employees at the chemical factory were also included to investigate if they had been subjected to a higher exposure to the outbreak strain than blood donors in the same county. Antibody levels were measured in an enzyme-linked immunosorbent assay (ELISA) with a pool of *L. pneumophila* of serogroups 1 to 7 as antigen, and risk factors for LD, such as gender, age, smoking, and chronic lung diseases (16), were assessed from a questionnaire. In addition, the specificity of the antibody responses in participants, who showed increased antibody levels in ELISA, was examined by immunoblotting with the serogroup 1 outbreak strain.
(Parts of this work were presented at the 22nd and 23rd Meeting of the European Working Group for Legionella Infections (42, 43).
MATERIALS AND METHODS

Blood samples and questionnaires. Blood samples were collected 11-13 months after the industrial outbreak, which took place in Østfold county in the south-eastern part of Norway. A total of 1 017 volunteers were recruited: 1) 213 healthy factory employees working at different sites on the industrial premises, 2) 398 blood donors from the exposed county, and 3) 406 blood donors from Oslo, 80 km away, serving as a non-exposed group. After informed consent, the volunteers supplied a 10 ml blood sample. The participants answered an anonymous questionnaire about gender, age, address in form of postal codes, occupation, chronic lung diseases, and smoking habits to assess risk factors for LD (16). They were also asked whether they had experienced respiratory infections, such as dry coughing, bronchitis, or flu-like disease with muscle pain during the outbreak period in May 2005, as a possible indication of Legionella infection. In addition, factory employees were questioned where on the premises they worked. The study was planned with a power of 0.8 based on a 1.5 fold difference in antibody levels between exposed and non-exposed groups as shown in a previous outbreak (7). The study was approved by the Regional Committee for Medical Research Ethics.

ELISA. Sera were collected by centrifugation of the blood samples and stored in aliquots at -20°C until analyses. IgG and IgM antibodies to a pool of L. pneumophila of serogroups 1 to 7 as antigen were determined separately with a commercial quantitative ELISA test (40) in an ELISA robot (DSX Automated System, Dynex Technologies, Inc., VA, USA). Rheumatoid factors were adsorbed before the IgM analyses. This ELISA test performed well regarding sensitivity and false positive rates for LD compared with other commercial ELISA tests (15) and had been used in previous outbreak studies (7, 8, 14, 22). According to the kits’ manufacturer, negative, borderline, and positive values for L. pneumophila infection were <
50 U/ml, 50-70 U/ml, and > 70 U/ml for IgG, respectively, with corresponding values of < 120 U/ml, 120-140 U/ml, and > 140 U/ml for IgM (40). Single serum samples were analyzed twice at different dates, except for those of the factory employees, which were analyzed as duplicates the first time. Arithmetic means of these results were used as antibody levels. Neither the IgG nor the IgM levels of the duplicates showed significant differences, indicating good reproducibility of the ELISA test. Coefficients of variation for four standard samples per plate throughout this study were 6.9% for IgG (range 2.4 - 18.9%) and 4.1% for IgM (range 1.0 - 7.4%) compared with a maximal interserial coefficient of 16% given by the manufacturer. Individuals with IgG or IgM levels to \textit{L. pneumophila} in the positive and borderline range were defined in our study as seroresponders.

**Immunofluorescence assay (IFA).** Sera from the seroresponders were also tested in IFA with a pool of \textit{L. pneumophila} of serogroups 1 to 6 as antigen (Meridian Bioscience Europe, Milan, Italy) using conjugated anti-human Ig as secondary antibody.

**Strain characterisation.** \textit{L. pneumophila} strains were grown on buffered charcoal yeast extract agar plates at 35°C for 2 days followed by confluent growth for 24 h. Cells were harvested in phosphate-buffered saline (PBS), pH 7.2, with 0.02 % Na-azide, and inactivated by heating to 60°C for 1 h. The whole cell suspensions were used as antigens for dot blotting and immunoblotting. Serogrouping of \textit{L. pneumophila} strains was performed by dot blotting (46) with the Dresden panel of monoclonal antibodies (Mabs) directed to lipopolysaccharides (LPS) (20, 21). Two µl of the whole cell suspensions (0.5 mg/ml) were dotted on nitrocellulose strips and incubated overnight with the Mabs diluted in PBS with 3% bovine serum albumin. Antibody binding was detected with rabbit anti-mouse Ig conjugated to peroxidase (1:1 000) (DAKO AS, Denmark).
Immunoblotting. Whole cells (50 µg protein per gel) were boiled for 5 min in sample buffer with sodium dodecyl sulphate and mercaptoethanol (28), separated in 12% acrylamide gels and electrotransferred to nitrocellulose filters (7 by 9.5 cm; pore size 0.45 µm) as described (41, 45). Each blot was cut into about 25 strips which were incubated overnight at room temperature with human sera diluted 1:200 in PBS with 3% bovine serum albumin. IgG and IgM binding, respectively, were detected with 1:500 dilutions of rabbit anti-human IgG or IgM conjugated to horse radish peroxidase (DAKO AS, Denmark). Blotting was also performed with whole cells treated with proteinase K as antigen (Qiagen GmbH, Hilden, Germany) (23).

Statistical analyses. Data were analyzed with SPSS 14.0, Stata 10.0, GraphPad Prism 4.03, and SigmaStat 3.1 programs. P-values < 0.05 were considered significant. Proportions were compared with Fisher’s test instead of Chi-square test for numbers < 5. Arithmetic means of the IgG and IgM antibody levels were not normally distributed, nor were they after log-transformation except for IgM levels of factory employees and non-exposed blood donors. Non-parametric methods (Mann-Whitney rank sum test for differences between groups and Spearman rank order test for correlations) were therefore used for statistical analyses. Only variables that gave significant results in univariate analyses, were included in multivariate regression analyses.
RESULTS

Description of study participants. Table 1 shows the characteristics of the three groups of participants in the study, i.e. the employees at the factory, where the outbreak source was located, blood donors from the exposed county, and blood donors from a non-exposed county, based on information from the questionnaires. Response rates for the various questions ranged from 95% to 100%. Several significant differences ($P = 0.0001$ to 0.031) between the groups were observed. There were nearly twice as many men among the factory employees than in the two blood donor groups and also more men among the non-exposed blood donors compared with the exposed donors. The latter group was a few years older than the two other groups. Proportions of smokers among the factory employees and the exposed blood donors were 2-fold higher than in the non-exposed group. A daily consumption of > 10 cigarettes was reported by two to three times more factory employees compared with the blood donor groups. Similar differences were also observed for chronic lung diseases and airway infections during the outbreak period in May 2005.

Description of seroresponders. Measurements of IgG and IgM antibody levels to $L. pneumophila$ in the polyvalent ELISA showed that the numbers of seroresponders among the factory employees, the exposed blood donors, and the non-exposed blood donors were 9 (4.2%), 9 (2.3%), and 10 (2.4%), respectively, but the differences between the groups were not significant. Only one individual in each group had elevated IgM levels. About 60% of the seroresponders in the two blood donor groups were women, while all in the factory group were men. No significant differences in age, airway infections or smoking habits between the responders in the three groups were observed.
Of the nine seroresponders in the factory group, five worked at an open biological treatment plant, shown to emit aerosols with *L. pneumophila* and other *Legionella* species (3), two worked at the outbreak air scrubber 200 m away, and one at a combustion plant 125 m from the biodegradation plant. The remaining individual worked 2 km from the main premises. Of the 213 employees recruited, 63 worked at either the treatment plant, the air scrubber, or the combustion plant and 150 at other sites on the industrial premises. There was a highly significant association (*P* = 0.0003) between being a seroresponder and workplace at or near the biodegradation plant.

About 1/3 of the 398 exposed blood donors had home addresses within a radius of 5 km from the city where the outbreak occurred. Six of the nine seroresponders in this group resided within this range (Fig. 1). The remaining 2/3 of the exposed donors resided either in a larger city about 15 km southwest or elsewhere in the county. One of the remaining three seroresponders worked 10 km away from the outbreak city, while two resided further away. A clustering of addresses to the city of the outbreak was thus observed for the seroresponders in the exposed blood donor group. In contrast, home addresses of the ten seroresponders among the non-exposed blood donors were scattered all over the region (data not shown).

**Comparison of antibody levels of the seroresponders in ELISA and IFA.** When sera from all seroresponders were analysed in IFA with a *L. pneumophila* pool of serogroup 1 to 6, the median titre was 1:128 (range 1:32 to 1:1024, thus supporting the increased levels obtained in ELISA. According to the manufacturer of the IFA test, a titre of > 1:128 provides evidence of a recent *L. pneumophila* infection. Significant correlations were obtained between IFA titres and IgG levels in ELISA (Spearman rank order correlation coefficient 0.489; *P* = 0.006) or the sum of IgG and IgM levels (Spearman rank order correlation coefficient 0.495; *P* = 0.006).
172 **IgG and IgM antibody levels of exposed and non-exposed groups in ELISA.** Low median antibody levels to *L. pneumophila* were found in all three groups (Table 2). Although the differences were small with a statistical power in the lower range (0.63), factory employees and exposed blood donors had significantly higher IgG levels (*P* ≤ 0.002) than non-exposed blood donors. The median IgM antibody levels of the two blood donor groups were similar and significantly higher (*P* ≤ 0.005) than for the factory group. As reported below, this difference was mainly due to a gender difference in IgM levels.

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180 **Association of antibody levels in ELISA and risk factors for LD.** IgG levels to *L. pneumophila* of all participants remained constant over the age range of 18 to 69 years. In contrast, IgM levels showed a weak but significant decrease with age (Spearman rank order correlation coefficient – 0.177; *P* = 0.0001).

183 Men and women had similar median IgG levels (11 U/ml). In line with the higher IgG levels of the factory- and the exposed blood donor group shown in Table 2, also men in these two groups had higher IgG levels (12 and 13 U/ml, respectively) than men in the non-exposed group (11 U/ml; *P* ≤ 0.009). Exposed female blood donors also had significantly higher IgG levels than non-exposed females (12 vs 11 U/ml; *P* = 0.014).

191 A gender difference for IgM was observed as the median IgM levels in women were 1.5 fold higher than in men (19 vs 13 U/ml; *P* = 0.0001). The higher IgM levels of the blood donors groups compared with the factory employees in Table 2 were explained by the larger proportion of women among the donors (Table 1) and the age-dependent fall in IgM levels reported above. Multivariate regression analyses, adjusted for age and gender, showed no significant differences in IgM levels between the groups.
Airway infections during the outbreak period, a high cigarette consumption, and chronic lung diseases were reported by more factory employees than blood donors (Table 1), but no association between these variables and IgG or IgM levels to *L. pneumophila* was demonstrated.

Antibody levels of male factory employees according to the workplace at the industrial premises are shown in Table 3. Those who worked at the air scrubber, the biological treatment plant, or the combustion plant had higher median IgG and IgM levels compared with those working at other sites on the main premises or 2 km away, but significant differences (*P* = 0.013 to 0.046) were only found for employees at the treatment plant. Women were not included in this analysis as all but one of the 21 female workers were employed at other sites on the main premises.

**Antibody specificities of the seroresponders in immunoblotting.** The ELISA measurements were performed with a pool of *L. pneumophila* of serogroups 1 to 7 as antigen. To analyse whether the antibody responses of the seroresponders were directed to the serogroup 1 outbreak strain (35), the sera were immunoblotted with this strain as antigen. A *L. pneumophila* serogroup 4 strain, which had been isolated in air samples near the biological treatment plant just after blood sampling of the volunteers in our study (3), was used as a comparative antigen. Dot-blotting with LPS-specific Mabs demonstrated that the serogroup 1 and 4 strains belonged to subgroups Benidorm and Portland, respectively.

The general immunoblot pattern of all 28 seroresponders with the serogroup 1 strain (Fig. 2A-C) consisted of distinct IgG antibody binding to several protein bands in the molecular weight range of 80 - 15 kDa and a background staining including a strong to weak ladder-like pattern in the lower range that corresponded to the one given by the serogroup 1 LPS specific Mab.
Most individual sera bound with roughly the same IgG binding intensity to the proteins of the serogroup 4 strain as to the serogroup 1 strain (Fig. 2A-C). Few sera reacted with the serogroup 4 LPS of molecular weight about 20 kDa, as seen by the binding of the subgroup 4 Portland Mab Lp 4P in Fig. 2A. The position of this band and the ladder-like pattern of the serogroup 1 strain in the 25 - 15 kDa molecular weight range matched the LPS patterns in silver-stained sodium dodecyl sulphate gels (39) after proteinase K treatment of the two strains (not shown). LPS is considered to represent the serogroup-specific antigen in LD patients (9, 18). As seen in Fig. 2A-C, all IgG and IgM seroresponders, except for one individual in the exposed group and two in each of the other groups, showed the ladder-like LPS pattern with the serogroup 1 strain. In contrast, a total of 11 individuals in all groups had IgG and IgM antibodies to serogroup 4 LPS; six of these reacted with LPS of both strains and the remaining five with serogroup 4 LPS only. So, all seroresponders were found to have LPS-specific antibodies to one or both strains.

Additional blotting experiments were performed to study the specificity of the antibody activity (not shown). When cells of the serogroup 1 and 4 strains were treated with proteinase K before immunoblotting, sera from the responders showed the same LPS binding patterns as with untreated cells. The seroresponders demonstrated weak or no IgG binding to LPS of the serogroup 1 subgroup Philadelphia reference strain (ATCC 33152), indicating low levels of cross-reactive antibodies. Thirty randomly selected IgG negative sera (ten from each study group) generally displayed the same IgG binding intensity to the proteins of the two strains as those seen for the seroresponders in Fig. 2A-C. Only one serum with an IgG level near the borderline range (IgG = 42 U/ml) reacted with the serogroup 1 LPS and another serum with the serogroup 4 LPS. The blotting reagents did not contain Tween 20, because this detergent may abolish antibody binding to LPS (2) as previously demonstrated for meningococcal LPS (44). In our hands, no IgG binding to the serogroup 1 LPS was observed on blots with Tween...
249 concentrations greater than 0.1%. Taken together, the blotting results demonstrated that 23
250 of the 28 seroresponders (82.1%) in all three study groups had IgG or IgM antibodies that
251 reacted with LPS of the serogroup 1 outbreak strain. This reaction was not observed with sera
252 having antibody levels in the lower negative range as measured in the polyvalent ELISA.
DISCUSSION

In this study we measured antibody levels to *L. pneumophila* in exposed and non-exposed groups following a 10 km long-distance outbreak of LD from an industrial air scrubber in Norway (35). Healthy employees at the industrial plant, which harboured the scrubber, and blood donors from the same county had low but significantly higher IgG levels than blood donors from a non-exposed region despite the fact that the statistical power of the study was in the lower range. Although it cannot be excluded that the small differences may be due to cross-reactive antibodies to other bacteria (15, 16), our results suggested a higher exposure level of *L. pneumophila* in the county of the outbreak. IgG and IgM antibody levels were determined with a polyvalent ELISA test for LD used in a previous large outbreak study (7, 8). Interestingly, in that study the recognition of small boosting effects of *L. pneumophila* in seroepidemiological outbreak studies was emphasized (6, 7). The similar IgG levels among the factory group and the exposed blood donors were supported by the fact that no LD cases among the factory employees were reported during the outbreak. This observation was explained by both the height of the air scrubber and the high velocity of the emitted aerosols, leading to low bacterial concentrations on the industrial premises (35).

The blood samples were collected about one year after the outbreak, and higher IgG antibody levels might probably have been detected if the sampling had been performed closer to the outbreak period. IgG levels decrease with time, although levels in exposed healthy individuals may persist for some years after outbreaks (12, 30). The higher IgM levels to *L. pneumophila* in the two blood donor groups compared with the factory group were caused by the age-dependent decrease in IgM antibodies and the higher proportions of women among the blood donors, as no significant differences between the groups were found after adjustment for gender and age. Age-dependent IgM decreases in healthy exposed individuals and in LD...
patients have been reported previously (8, 37), as well as gender differences. Healthy women, exposed to *L. pneumophila*, tended to have higher IgM levels than men in the same ELISA test (8), and female blood donors had higher titres than men in microagglutination which measures IgM (17). The antibody levels in our study were in the lower negative range obtained by others with this ELISA test (7, 8, 22).

Proportions of seroresponders, i.e. about 4% among the factory employees and about 2% among the blood donors, were within the seroprevalence range of 1-16% in healthy individuals (10). Our seroprevalence results corresponded to those in Sweden (12), and both are lower than those in Denmark (38), suggesting different levels of exposure to *L. pneumophila* within the Scandinavian countries.

Our study pointed to the biological treatment plant as another exposure source at the industrial premises as eight of the nine seroresponders among the factory employees worked at or within a distance of 200 m from this plant. This plant contains two large open aeration ponds, shown to disperse aerosols with *L. pneumophila* and other *Legionella* species up to 200 m downwind from the ponds (3). Such ponds thus represent a potential occupational risk for *Legionella* infections (1), and similar transmission distances from biological waste water systems have been reported (26, 31, 32). The spread of airborne *L. pneumophila* from these ponds provided an explanation for the significant association of being a seroresponder and work at or near the biological treatment plant, as well as the higher IgG and IgM levels in employees at this plant compared with those working further away on the industrial premises. These findings corresponded with a preliminary study showing higher IFA titres among workers at such plants (27). It is possible that airborne *Legionella* species from the aeration ponds originally had infected the air scrubber (35).
While the outbreak strain of *L. pneumophila* serogroup 1 (subgroup Benidorm) was also isolated from or near the ponds closer to the outbreak period (4, 35), a *L. pneumophila* serogroup 4 strain (subgroup Portland) and various *Legionella* species predominated in aerosols from this site closer to the blood sampling period in our study (3, 4). The measured antibody levels in ELISA, based on a pool of *L. pneumophila* of serogroup 1 to 7, may thus result from exposure to several *Legionella* strains and possibly to other cross-reacting bacteria (15, 16). Immunoblotting was therefore used to study the reaction of sera from the seroresponders with the serogroup 1 and 4 strains. Nearly all seroresponders had IgG or IgM antibodies that bound to the serogroup-specific LPS antigen of the outbreak strain. Some of these sera also bound to serogroup 4 LPS, but few reacted with this LPS only, and none was without any LPS response. The demonstration of serogroup 1 LPS specific antibodies in sera with elevated antibody levels in ELISA was supported by another study showing that this ELISA test detected a high number of LD patients with serogroup 1 disease (15). However, the predominant LPS antibody reaction with the outbreak strain did not imply that all responders had been subclinically infected with this strain. Several subgroups within serogroup 1 present the same ladder-like LPS profile in silver-stained gels and in immunoblots (24, 33). Infection with such strains may possibly have induced corresponding LPS binding patterns, but the seroresponders gave only negligible antibody reactions with LPS of the serogroup 1 subgroup Philadelphia strain. Induction of cross-reactive LPS antibodies by other serogroups of *L. pneumophila* is disputed (11, 24, 33), and few other *Legionella* species have the same ladder-like LPS profile (25). The LPS response was probably not caused by exposure to other gram-negative bacteria, as their LPS is different from that of *Legionella* LPS (29).

The characteristics of the seroresponders in the three study groups may reveal the impact of the various long- and short distance infection sources. The scattering of the home addresses of
the seroreponders among the non-exposed blood donors suggested different domestic or workplace exposure sources. Such sources may also have contributed to the seroreponders among the exposed blood donors. However, their addresses clustered around the city of the outbreak despite the fact that the majority of the exposed donors lived or worked in the neighbouring city. The distribution of the seroreponders’ addresses was roughly similar to that of the LD patients in the outbreak (35), indicating the air scrubber as the exposure source. The short transmission distance of airborne *Legionella* strains from the aeration ponds (3) is less likely to account for this clustering, as are potential unfavourable weather conditions leading to a longer travel of the aerosols from the ponds, aerosols from the nearby river from which the outbreak strain was also identified (35), and later accidental unrecognized emissions of *L. pneumophila* from the air scrubber.

In conclusion, the long-distance outbreak of LD was reflected one year later in a small but significant increase in IgG levels of the exposed study groups. Within the industrial premises, a biological treatment plant was found to represent a short-distance infection source for factory employees who worked nearby. Most of the seroreponders in all three study groups had IgG and IgM antibodies directed to the serogroup-specific LPS antigen of the outbreak strain, but the possible contribution of cross-reactive antibodies from exposure to other *L. pneumophila* strains cannot be excluded.
ACKNOWLEDGMENTS

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REFERENCES


TABLE 1. Characteristics of the study participants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Factory employees</th>
<th>Exposed blood donors</th>
<th>Non-exposed blood donors</th>
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<td>N=213</td>
<td>N=398</td>
<td>N=406</td>
<td>N=1017</td>
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<td>Gender</td>
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<tr>
<td>Male</td>
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<td>188 (47.2)</td>
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<td>Female</td>
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<td>210 (52.8)</td>
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<td>113 (28.4)</td>
<td>64 (15.8)</td>
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<td>0-10 cigarettes/day</td>
<td>20 (27.4)</td>
<td>75 (66.4)</td>
<td>49 (77.8)</td>
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<td>&gt; 10 cigarettes/day</td>
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<td>38 (33.6)</td>
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<td>Chronic lung diseases</td>
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<td>385 (97.5)</td>
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<td>12 (5.6)</td>
<td>40 (10.2)</td>
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</tbody>
</table>

* Data are no. (%) of participants unless otherwise indicated.

* Significantly higher (P ≤ 0.015) than for the other two groups.
Significantly lower ($P = 0.0168$) than for non-exposed blood donors.

Significantly higher ($P \leq 0.031$) than for the other two groups.

Significantly lower ($P = 0.0001$) than for the other two groups.

One smoker did not specify the cigarette consumption.
TABLE 2. IgG and IgM antibody levels in ELISA to *L. pneumophila* in exposed and non-exposed study groups

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Factory employees</th>
<th>Exposed blood donors</th>
<th>Non-exposed blood donors</th>
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<td>ELISA IgG</td>
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<td>12 (3-372)</td>
<td>13 (1-109)</td>
<td>11* (1-120)</td>
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<tr>
<td>ELISA IgM</td>
<td></td>
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<td>Median U/ml (range)</td>
<td>12 b (1-238)</td>
<td>16 (1-124)</td>
<td>16 (1-124)</td>
</tr>
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</table>

*Significantly lower than for factory employees (*P* = 0.002) and exposed blood donors (*P* = 0.001).

b Significantly lower than for exposed (*P* = 0.005) and non-exposed blood donors (*P* = 0.001).
TABLE 3. Median IgG and IgM levels in ELISA to *L. pneumophila* in male employees working at different sites on the industrial premises

<table>
<thead>
<tr>
<th>Workplace</th>
<th>No. of employees</th>
<th>IgG U/ml</th>
<th>IgM U/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air scrubber</td>
<td>34</td>
<td>12.5</td>
<td>15.5</td>
</tr>
<tr>
<td>Biological treatment plant</td>
<td>21</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td>Combustion plant</td>
<td>7</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td>Other sites at main premises</td>
<td>108</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Plant 2 km away</td>
<td>22</td>
<td>10.5</td>
<td>10.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>192</strong></td>
<td><strong>12</strong></td>
<td><strong>12</strong></td>
</tr>
</tbody>
</table>

*Significantly higher compared with those working at other sites on the main premises (P = 0.034) or 2 km away (P = 0.028).*

*Significantly higher compared with those working at other sites on the main premises (P = 0.013) or 2 km away (P = 0.046).*
FIG. 1. Addresses shown as postal codes for the nine seroresponders to *L. pneumophila* among the 398 blood donors in the exposed county. Home and workplace addresses are shown as circles and squares, respectively. Seven of the nine seroresponders resided or worked within a range of about 10 km from the city where the outbreak took place (Sarpsborg), although 2/3 of the exposed blood donors lived in the larger neighbour city (Fredrikstad) or elsewhere in the county. Different home and workplace addresses for three of the seroresponders are shown by connecting lines. Seroresponders were defined as individuals with positive or borderline IgG and IgM levels in the polyvalent ELISA.

FIG. 2. Immunoblots demonstrating IgG and IgM binding of sera from all 28 seroresponders to *L. pneumophila* among the (A) exposed factory employees (N = 9), (B) exposed blood donors (N = 9), and (C) non-exposed blood donors (N = 10). Only one individual in each group was an IgM responder. The individual seroresponders are identified by numbers below the strips. Antigens for immunoblotting were whole cells of *L. pneumophila* serogroup 1 (*Lp* SG 1) of subgroup Benidorm and serogroup 4 (*Lp* SG 4) of subgroup Portland corresponding to 50 μg protein per blot. Binding patterns of the specific *Lp* 1 and *Lp* 4P Mabs are shown in A. IgG and IgM binding intensities of each serum to LPS of molecular weight 25 – 15 kDa are rated as +, strong; (+), weak; and -, none below the strips and are the results of five experiments of which a representative immunoblot is shown. All strips, incubated with the human sera, were stained for 10 min with peroxidase substrate. Adobe Photoshop CS4 was used for the figure preparation.