of P. jiroveci genotypes are related to underlying disease.

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References


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Viremia and Clinical Manifestations in Children with Rotavirus Infection

To the Editor—We read with interest the recent article by Fisher et al. investigating rotavirus antigenemia in patients hospitalized for acute diarrhea during a major outbreak of enteritis [1]. Antigenemia was demonstrated by EIA in 30 (42.8%) of 70 children and was confirmed by reverse-transcription polymerase chain reaction (RT-PCR) in 12 (66.6%) of 18 children. Rotavirus antigen levels were commonly detected on the first day of illness, peaked 1–3 days after symptom onset, and remained positive beyond 1 week only in a minority of convalescent children. Interestingly, an association between antigen detection in the serum and severity of clinical symptoms was suggested, since (1) there was a nonsignificant tendency for severity of illness to be associated with higher serum optical density values, as assayed by EIA; (2) in 1 of 2 deceased children, viral RNA was demonstrated in the serum; and (3) antigenemia was higher in children with primary infection than in those with subsequent infection, and it is well known that primary infection is usually more severe [2]. Unfortunately, no detailed data on extraintestinal involvement and its potential association with antigenemia were provided. This is an interesting issue, since the extraintestinal spread of the virus may occur through the blood [3].

In light of this, we would like to mention the results of our recent investigation on 54 immunocompetent children hospitalized for acute diarrhea who were prospectively evaluated by nested RT-PCR for the presence of rotavirus RNA [4]. Viral RNA was detected in the blood of 9 (64.3%) of 14 children with documented rotavirus infection but in no child with diarrhea of other origin. The presence of rotavirus RNA in the blood was associated with high fever and/or evidence of extraintestinal involvement. However, severe clinical manifestations were absent in all children. This finding suggests that viremia by itself is not the major determinant of severe illness.

Taken together, both the study by Fisher et al. and our study indicate that viremia is common in rotavirus-infected children. We suggest that future targeted studies should clarify whether specific rotavirus strains or a higher viral load are more frequently associated with severe and/or extraintestinal manifestations.

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References


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Legionella Bacteria in Aerosols: Sampling and Analytical Approaches Used during the Legionnaires Disease Outbreak in Pas-de-Calais

Between November 2003 and February 2004, an outbreak of legionnaires disease occurred in the Pas-de-Calais region of northern France. There were 18 deaths among the total of 86 cases found within a radius of ~10 km around a petrochemical plant that was the probable source of contamination [1].

From January to March 2004, we sampled aerosols produced by industrial cooling towers and by an industrial sludge water treatment basin to search for Legionella bacteria by use of a new experimental method. To date, management of this serious biological risk has relied solely on the monitoring of water quality. The only known contamination vector is the aerosol, and the transfer function between contaminated water and air remains unknown. Our contribution, which is based on the collection of large volumes of aerosols and the detection of Legionella bacteria by fluorescent in situ hybridization (FISH), offers an interesting new approach, leading to
more realistic information about human exposure to these pathogens.

Aerosol sampling was performed on 5 cooling towers from different plants and on an aerated sludge water treatment basin. Aerosol sampling was performed directly above the fan of the cooling towers, on the edge of the basin close to the aerators, and at some distance downwind and upwind of the basin. Two sampling series, 1 with and 1 without the aerators being operated, were performed.

An experimental bioaerosol collector (a cyclone-like device) was used to collect the airborne bacteria. The aerosols penetrated the sampler tangentially to the cyclone wall where they impacted. The wall was constantly washed by a filtered (0.2-μm pores) deionized water flow (160 mL) that circulated in closed loop, so as to concentrate the collected aerosol particles. The cutoff diameter of the experimental cyclone was 0.9 μm, with a collection efficiency of 100% for aerosols ≥1.2 μm. Sampling was done under controlled airflow conditions (1 m³/min during 40 min; total air flow, 40 m³).

Airborne *Legionella* bacteria were quantified by FISH [2, 3], as adapted from the method described by Manz et al. [4], using a mix of 3 specific probes validated for *Legionella* bacteria—LEG705 (5′-CTggTg- TTccTTcAgATc-3′), LEG226 (5′-TcgAggcTAATcT-3′), and LEGPNE1 (5′-ATc- TgAcccTccAggTT-3′)—that were labeled with CY3 dye (Eurogentec) at the 5′ end. This method yields results within 48 h (vs. 14 days for the standard method). In parallel with the FISH method, the cultivable fraction of aerosolized *Legionella* bacteria was evaluated using the standard method [5]. Last, 4,6-diamidino-2-phenylindol (DAPI) was used to evaluate the total number of airborne bacteria, by counting under an epifluorescence microscope (Olympus) [6].

All investigated sites generated bioaerosols with concentrations of bacteria ranging from 1 × 10³ to 1 × 10⁵ cells/m³ (DAPI staining). Among these airborne bacteria, *Legionella* bacteria were detected by FISH at all sites. Nevertheless, *Legionella* bacteria represented a small fraction—between 0.05% and 0.9%—of the total airborne biocontaminants. Moreover, no cultivable *Legionella* bacteria could be detected using the standard method, even when the use of FISH showed that there were concentrations of ∼1 × 10⁴ *Legionella* cells/m³ (table 1).

A remarkable result was obtained with the bioaerosols generated from the sludge water treatment basin operated with aeration; cultivable *Legionella* bacteria were detected at the same level as that found using the FISH method. This important finding enabled us to establish for the first time, to our knowledge, that there is a direct relationship between the epidemic *L. pneumophila* strain and that found in the aerosols collected close to the aerated basin: airborne *L. pneumophila* presented the same profile of macrorestriction of genomic DNA as the epidemic strain detected in patients [1].

Aeration of the sludge water treatment basin induced a diffusion of contaminated aerosols in the study zone, with, however, a decrease of the airborne *Legionella* concentration of a factor of 10 at 270 m downwind of the basin. At this distance, the air-

<table>
<thead>
<tr>
<th>Sampling site</th>
<th>Detection by FISH</th>
<th>Detected by FISH, cells/m³ (range)</th>
<th>Grown in BCYE culture at 37°C, cfu/m³</th>
<th>Total bacteria with DAPI staining, cells/m³ (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cooling tower</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>+</td>
<td>&lt;QL</td>
<td>&lt;DL</td>
<td>1.3 × 10⁴ (1.1 × 10⁴–1.6 × 10⁴)</td>
</tr>
<tr>
<td>B</td>
<td>+</td>
<td>5.9 × 10³ (1.4 × 10³–10.4 × 10³)</td>
<td>&lt;DL</td>
<td>8.5 × 10³ (6.7 × 10³–10.3 × 10³)</td>
</tr>
<tr>
<td>C</td>
<td>+</td>
<td>5.5 × 10³ (2.4 × 10³–8.6 × 10³)</td>
<td>&lt;DL</td>
<td>8.7 × 10³ (4.7 × 10³–12.7 × 10³)</td>
</tr>
<tr>
<td>D</td>
<td>+</td>
<td>3.3 × 10³ (2.6 × 10³–4.0 × 10³)</td>
<td>&lt;DL</td>
<td>1.6 × 10³ (8.0 × 10³–23.8 × 10³)</td>
</tr>
<tr>
<td>E</td>
<td>+</td>
<td>5.6 × 10³ (1.6 × 10³–9.5 × 10³)</td>
<td>&lt;DL</td>
<td>6.6 × 10³ (2.2 × 10³–10.9 × 10³)</td>
</tr>
<tr>
<td><strong>Sludge water treatment basin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Sampling series 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basin</td>
<td>+</td>
<td>4.9 × 10³ (2.8 × 10³–7.0 × 10³)</td>
<td>5.4 × 10³</td>
<td>8.2 × 10³ (5.5 × 10⁴–10.9 × 10³)</td>
</tr>
<tr>
<td>270 m downwind from basin</td>
<td>+</td>
<td>&lt;QL</td>
<td>3.3 × 10³</td>
<td>4.3 × 10³ (1.9 × 10⁴–6.7 × 10³)</td>
</tr>
<tr>
<td>60 m upwind from basin</td>
<td>+</td>
<td>&lt;QL</td>
<td>&lt;DL</td>
<td>3.7 × 10³ (1.1 × 10⁴–6.4 × 10³)</td>
</tr>
<tr>
<td><strong>Sampling series 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basin</td>
<td>+</td>
<td>&lt;QL</td>
<td>62</td>
<td>2.7 × 10⁴ (1.4 × 10⁴–4.2 × 10⁴)</td>
</tr>
<tr>
<td>200 m downwind from basin</td>
<td>+</td>
<td>&lt;QL</td>
<td>&lt;DL</td>
<td>1.1 × 10⁴ (0.3 × 10⁴–2.1 × 10⁴)</td>
</tr>
<tr>
<td>1500 m downwind from basin</td>
<td>−</td>
<td>&lt;QL</td>
<td>&lt;DL</td>
<td>1.2 × 10⁴ (0.4 × 10⁴–1.6 × 10⁴)</td>
</tr>
<tr>
<td>900 m upwind from basin</td>
<td>−</td>
<td>&lt;QL</td>
<td>&lt;DL</td>
<td>1.3 × 10⁴ (0.7 × 10⁴–1.7 × 10⁴)</td>
</tr>
</tbody>
</table>

**NOTE.** Cooling towers were sampled after completion of a weekly decontamination procedure that had been imposed by local authorities. BCYE, buffered charcoal yeast extract; DAPI, 4,6-diamidino-2-phenyl indol; DL, detection limit; FISH, whole-cell fluorescent in situ hybridization; QL, quantification limit (<1 cell/m³); +, hybridized *Legionella* species present in the 50 microscopic fields observed; −, hybridized *Legionella* species not detected in the 50 microscopic fields observed.
borne Legionella concentration, primarily L. pneumophila serogroup 1, still reached $1 \times 10^5$ cfu/m$^3$. Even with moderate aeration of the sludge water treatment basin (during the second sampling series), aerosols were generated with bacterial concentrations that were significantly higher (∼2-fold) downwind than upwind of the basin. However, compared with the concentrations that were found when the aerators were operating (during the first sampling series), a 2-log reduction in Legionella concentrations and an absence of cultivable Legionella bacteria, resulting in a lower dissemination of these bacteria in the air, was found in the aerosols when the aerators were not operating.

Our investigations during the outbreak of legionnaires disease in Pas-de-Calais enabled us to perform in situ sampling of aerosolized Legionella bacteria by use of novel methods and demonstrated, for the first time, that there was a relationship between the suspected contamination source and the epidemic strain. Sampling of airborne Legionella bacteria emitted by cooling towers and sludge water treatment basins, coupled with whole-cell FISH detection, offers more reliable information on human exposure to these bacteria than does standard water analysis.

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### References


### Issues in Chlamydia trachomatis Testing by Nucleic Acid Amplification Test

To the Editor—Brunham et al. [1] show that, since 1998, Chlamydia trachomatis infection prevalence rates in British Columbia have increased markedly and that case counts now exceed those reported before the advent of the province’s chlamydia control program. The authors attribute the cause of this resurgence to early treatment, because they hypothesize that early treatment can interfere with the development of natural immunity and, thus, enhance population susceptibility to infection. The authors speculate that treated individuals reenter unchanged sexual networks as susceptible and contribute to enhanced transmission.

Although this is an interesting and thought-provoking hypothesis, I have a few concerns. In figure 1 of Brunham et al.’s article, we observe that the resurgence in chlamydia rates started in 1998. In the mid-to-late 1990s, many laboratories had switched to nucleic acid amplification tests (NAATs), and, thus, it is possible that the increase in the number of cases reported by Brunham et al. could, in large part, be attributable to changes in laboratory testing techniques. For example, the C. trachomatis positivity rate in Philadelphia, Pennsylvania, increased by 46% when the clinics switched from a nucleic acid probe assay to a NAAT [2]. Thus, it is not easy to assess the validity and consistency of the results provided by Brunham et al. without factoring in the effect of switching laboratory tests and other confounding variables, such as an increased frequency of testing.

The results of their study depend on the assumption that NAATs detect current infection only and that NAATs are highly specific and reproducible. However, we have recently shown that the performance of NAATs has not been properly evaluated (published sensitivity and specificity estimates are optimistically biased) and that NAATs suffer from poor specificity (point estimates that range from 94% to 97.6%) and reproducibility [3]. This is consistent with the 2001 device correction memo [4] of Abbott Laboratories, the manufacturers of one of the most common NAATs for chlamydia, that demonstrated that the specificity of their test is as low as 92% in some on-market lots and recommended that all specimens with positive and gray zone results be retested. If the specificity of the NAATs is as low as 92%, then as many as 63% of the positive results in Brunham et al.’s study [1] may be false positives (assuming true chlamydia prevalence is 5%). If so, then all of the estimates of incidence rates, reinflection rates, and relative risks are biased, and the conjectures and speculations from the compartmental mathematical model can be distorted.

There is both empirical and biological