Risk assessment of *Legionella* and enteric pathogens in sewage treatment works

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**Abstract** Exposure of sewage treatment workers to *Legionella pneumophila* and enteric pathogens through aerosols at different sewage treatment installations was quantified. The presence of *Legionella* sp. and *L. pneumophila* in water and air samples at different sewage installations was determined by cultivation, Direct Fluorescence Assay and PCR. Simultaneously, coliforms, F-RNA phages and spores of sulphite reducing clostridia (SSRC) were enumerated in water and air. For enteric pathogens, the aerosolisation efficiency of coliforms, F-RNA phages and SSRC were combined with published data on the concentration of *Campylobacter*, enteroviruses and *Cryptosporidium parvum*, respectively, in wastewater to calculate the concentration of these pathogens in the air. The frequency and duration of the presence of sewage workers at or in the different installations was determined by a questionnaire. The concentration of micro-organisms in the air and the data on the presence of workers at these locations were combined into an estimate of the exposure to *Legionella* and the enteric pathogens. *Legionella* was detected in all wastewater samples (n=7) with PCR, in concentrations of approximately 10^5 per ml, but not with the culture method (<20–<200 /ml). *Legionella* spp. and *L. pneumophila* were detected in air samples of 10 m^3 with PCR at 3 of the 5 sewage treatment plants tested. Samples of air above trickling filters (2/2), aeration tanks (2/2), the screen (1/1) and the belt press (1/1) were positive for *Legionella*. The concentration ranged from 0.56 –56 per m^3 of air with PCR, but again no *Legionella* were detected in any of the air samples with the culture method. The *Legionella*-concentration (determined by PCR) in the air were low compared to published data on *Legionella* concentrations in the air at outbreak sites (determined by culture). Hence, the risk of legionellosis to sewage workers was considered low. The calculated concentrations in air ranged from very low (<0.001/m^3 up to 0.3–17/m^3 at sites with high aerosolisation). During cleaning activities, the concentrations rose up to 100/m^3. These concentrations were translated into the probability of infection using published dose-response models. The annual probability of infection for sewage workers was high (worker with average exposure 19%, high exposure worker (95-percentile) 100%). Therefore, measures to reduce aerosol formation or exposure to aerosols (both technical and protective) should be considered. Reduction of exposure to aerosols will not only reduce the risk due to enteric pathogens, but also to endotoxins, *Legionella* and other pathogens.

**Keywords** Aerosols; *Campylobacter*; *Cryptosporidium*; *Legionella*; risk assessment; viruses; wastewater

**Introduction** The outbreak of legionellosis that occurred in The Netherlands in 1999 has triggered research into various routes of exposure to *Legionella*. Sewage is reported to contain *Legionella* (Palmer et al., 1993; Roll and Fujioka, 1995; Pascual et al., 2001) and Pontiac fever has been associated with exposure to aerosols in (industrial) wastewater treatment (Gregersen et al., 1999). There is some evidence that employees with high levels of exposure to *L. pneumophila* through air in cooling towers result in an antibody response, but no clinical illness was observed in these studies (Buehler et al., 1985; Snowman et al., 1982; Goldman and Marr, 1980). An animal infectivity study showed that infectious *L. pneumophila* can be transmitted through the ingestion of wastewater, but found no infection after exposure of the animals to aerosols of the same wastewater (Northrop et al., 1980).
In addition to *Legionella*, sewage contains a variety of enteric pathogens (Medema et al., 2001; Koenraad, 1995) and sewage workers may be exposed to these pathogens through aerosols. Several epidemiological studies have been conducted on the health risk of exposure to enteric pathogens in wastewater aerosols (Camann et al., 1980; Johnson et al., 1980; Cliver, 1980), and suggest that the risk is insignificant. On the other hand, the occurrence of symptoms of intestinal illness in newly employed sewage workers is a well known phenomenon (Sekla et al., 1980; Clark et al., 1980).

Aerosols are produced in various steps of sewage treatment. The exposure depends on the concentration of pathogens in wastewater, the aerosolisation at the different steps in treatment and the frequency and duration of worker presence at the sites where aerosols are produced. A quantitative risk assessment requires quantitative information about all these factors.

The aim of this study was to quantify the exposure of sewage workers to *Legionella* and faecal pathogens through aerosols at the different sewage treatment installations. For faecal pathogens, this exposure was translated into a risk of infection using dose-response relationships. For *Legionella*, the significance of the exposure was assessed by comparing *Legionella* concentrations in the air at the sewage treatment with published data on *Legionella* concentrations in the air at other sites (showers, cooling towers, etc.).

**Methods**

To determine the installations that produced aerosols with micro-organisms, 5 sewage treatment systems were screened for the presence of heterotrophic plate counts (22 °C) in aerosols. The installations with relatively high concentrations of bacteria were selected for further study.

At these sites, *Legionella* concentrations in wastewater and aerosols were assessed by culture, Direct immunoFluorescence Assay (DFA: *Legionella* and *L. pneumophila*) and PCR (*Legionella* and *L. pneumophila*). Volumes of 250 ml water were collected in autoclaved glass bottles. The samples were homogenised before analysis to resuspend the sediment.

Air samples were taken with a MAS-100 air sampler (Merck, Germany). Preliminary experiments showed that 2000 litres of air could be taken without loss of viability of the micro-organisms in the air (due to desiccation). To sample 10,000 litres, 5 separate 2,000 litre-samples were taken, using Plate Count Agar plates. Micro-organisms were recollected from the agar plates by resuspension in sterile water. For cultivation, samples were filtered and pasteurised at 50° C for 30 min. to suppress growth of other micro-organisms. Filters were incubated on BYCE, with antibiotics (polymyxin B-sulphate 50 IU/ml and sodium-cefazoline 0.011 g/l) for 5–7 days at 37°C. For DFA, aliquots of the sample were filtered through 0.2mm and stained with monoclonal antibodies against *Legionella* spp. and *Legionella pneumophila* (all serogroups). For PCR, DNA was isolated from aliquots of the sample and 10 ml of undiluted and 10 and 100-fold diluted sample was used for *a Legionella* spp. PCR (primers LEG225 and 858 (Miyamoto et al., 1997)) and *L. pneumophila* (primers Lmip920 and 1548 (Mahbubani et al., 1990)). Heterotrophic plate counts (HPC22) were assayed on Plate Count Agar, incubated for 3 days at 22 °C.

Enteric pathogen data (enteroviruses, *Campylobacter* and *Cryptosporidium*) were collected from literature (Medema et al., 2001; Koenraad, 1995). Aerosolisation of coliforms (Lauryl-sulphate agar, 48 h at 37 °C), F-specific RNA bacteriophages (ISO 10905) and spores of sulphite-reducing clostridia (*Perfringens* agar base, pasteurisation 30 min at 70 °C) was assessed by measuring these organisms in the water and in air and used as indication of the aerosolisation of the faecal pathogens.

Data on the frequency and duration of the presence of sewage workers at the different treatment installations were collected with a questionnaire.
The aerosol and questionnaire data were used to calculate the exposure of sewage workers to *Legionella* and faecal pathogens at the different sites. For the faecal pathogens, published dose-response relationships were used to transfer these exposure data into the risk of infection.

**Results and discussion**

**Exposure sites**

Figures 1 and 2 show the concentration of HPC22 and faecal indicators respectively. The locations are sorted by the concentration of HPC22 (Figure 1) and the average concentration of the three faecal indicators (Figure 2). HPC22 were found in background samples, taken in the vicinity (upwind) of the sewage treatment works, but fecal indicators were not. Relatively high concentrations in air samples of both the HPC22 and one or more of the faecal indicators were found in the building with the intake screens for raw sewage, the buildings with sludge dewatering installations, above (covered) oxidation beds, in the vicinity of activated sludge basins and in the room for the preparation of poly-electrolyte.

**Figure 1** Heterotrophic plate counts in the air at different sites of 5 sewage treatment systems

**Figure 2** Concentration of coliforms, F-RNA phages and spores of sulphite-reducing clostridia in the air at different sites of 5 sewage treatment systems
During cleaning of installations with high-pressure sprayers, the concentrations were considerably higher. In the vicinity of diffused aeration at site 2, the air filter at site 4 and sludge dewatering at site 6 SSRC were detected in the air, but no coliforms or phages. The resistance of these spores to desiccation may render them the most persistent in air.

**Effect of aeration system**

The diffused aeration at site 2 yielded much less HPC22 and coliforms in the air than the diffused aeration at site 6. Mechanical aeration with mixers resulted in increased concentrations in the air for all micro-organisms, while in the air above covered trickling filters all micro-organisms were present in high concentrations.

**Efficiency of aerosolisation**

At the sites where micro-organism concentrations were available for water and air, the efficiency of aerosolisation ($C_{\text{air}} / C_{\text{water}}$) could be calculated (Figure 3). This was used as the basis for the exposure calculations, but also provides insight in the sites where aerosolisation and aerosol persistence is highest. The efficiency was highest during cleaning of the sludge-dewatering installation. Indoor sites (sludge dewatering (belt filter press), preparation of poly-electrolyte, above covered trickling filters) had a higher aerosolisation efficiency than outdoor sites.

**Legionella in water and air**

*Legionella* was detected in all wastewater samples ($n=7$) with PCR, in concentrations of approximately $10^5$ per ml, but not with the culture method ($<20$–$<200$ /ml). The culture method was also hampered by growth of other bacteria, despite the heat pre-treatment and the use of antibiotics. DFA was hampered by fluorescence of other bacteria present in wastewater samples. *Legionella* spp. and *L. pneumophila* were detected in air samples of 10 m$^3$ with PCR at 3 of the 5 sewage treatment plants tested. Samples of air above trickling filters (2/2), aeration tanks (2/2), the screen (1/1) and the belt press (1/1) were positive for *Legionella*. The concentration ranged from 0.56 – 56 per m$^3$ of air with PCR, but again no *Legionella* were detected in any of the air samples with the culture method.

The efficiency of aerosolisation of *Legionella* (as determined with PCR in air and water) was similar to the efficiency of aerosolisation of HPC22 at the same site.
Exposure of sewage workers to Legionella

The Legionella-concentrations (PCR) in the air and the data on frequency and duration of the presence of sewage workers at the different sites in sewage treatment works were translated into exposure data of sewage workers to Legionella at the different sites on a treatment works. These data indicated that a sewage worker is exposed to 2.3 Legionella per day on average, and 5% of the workers are exposed to 25 Legionella with a maximum exposure of 37 Legionella.

In the absence of good dose-response data for Legionella and with PCR-based Legionella concentrations of which the infectivity is unclear, the significance of exposure could not be determined by calculating a risk of infection or illness. To obtain an indication of the health significance of these findings, the concentration of Legionella detected in the air with PCR at the sewage treatment works was compared to published data on Legionella concentrations in the air at outbreak sites (with culture methods, Table 1).

Considering the concentrations in this study are obtained with PCR and as such a worst case estimate of the concentration of viable Legionella bacteria in air, these are relatively low compared with concentrations published in literature at other sites (showers, cooling towers, air-conditioned rooms). The risk of legionellosis for sewage workers was therefore considered to be low.

Enteric pathogens

The literature data on pathogens in wastewater in The Netherlands (Medema et al., 2001; Koenraad, 1985) were translated into concentrations in air using the aerosolisation of the model-organisms that were measured in this study. F-RNA phage data were used for enteroviruses, coliform data were used for Campylobacter jejuni and SSRC data were used for Cryptosporidium parvum oocysts. The calculated concentrations in air ranged from very low (<0.001/m³ up to 0.3–17/m³ at sites with high aerosolisation). During cleaning activities, the concentrations rose up to 100/m³. These concentrations were transformed into the exposure using the questionnaire data, the breathing volume and the assumption that 10% of the pathogens in air are ingested into the intestinal tract. The probability of infection was calculated using the dose-response models described by Teunis et al. (1997), Medema et al. (1996) and Haas et al. (1999). Figure 4 shows the resulting probability of infection for the three pathogens at the different sites where data on aerosolisation and presence of personnel were available. It is obvious that the sites with high concentrations of fecal micro-organisms (see Figure 2) and prolonged presence of sewage workers (indoor areas for sludge dewatering, preparation of PE (poly-electrolyte), above trickling filters) yield the highest probability of infection. As exposures at all sites should be added, the sum of the annual probability of infection for sewage workers was calculated. During normal operation the total probability of infection for the average sewage worker was 0.011 (P95% of sewage workers = 0.1) for enteroviruses, 0.18 (P95% = 1) for Cryptosporidium and

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<th>Location</th>
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Table 1 Concentration of Legionella in the air around water installations

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0.0032 (P95% = 0.03) for Campylobacter. When cleaning activities were included in the risk assessment the risk of infection rose to 0.055 (P95% = 0.46) for both enteroviruses and Campylobacter and 1 for Cryptosporidium.

The use of model-organisms rather than pathogen data may have overestimated the risk, especially in the case of Cryptosporidium, as the spores of sulphite-reducing clostridia are more resistant to desiccation.

The dose–response data did not include the presence of immunity in the exposed population. A relatively high immunity might be expected sewage workers. This is substantiated by some reports of high antibody titers against (enteric) pathogens in sewage workers. (Sekla et al., 1980; Dean, 1980), whereas others found no increase in antibody titers (Clark et al., 1980). There is evidence for increased risk for gastro-intestinal illness in inexperienced sewage workers (Sekla et al., 1980; Clark et al., 1980), that disappears after a few weeks of employment. This also suggests the onset of protective immunity among sewage workers.

Conclusions
L. pneumophila was detected by PCR in wastewater and aerosols at different sites of sewage treatment works. No culturable Legionella was found in wastewater or aerosols, partly because the culture methods were hampered by growth of other micro-organisms on the BYCE-plates. The concentration detected in the air were low compared to other water installations that have been implicated in outbreaks of legionellosis. The risk to workers was therefore considered to be low.

The risk assessment for enteric pathogens, using data on Campylobacter, Cryptosporidium and enteroviruses in wastewater and data on the aerosolisation of model organisms at sewage treatment plants yielded a high probability of infection for sewage workers, especially during cleaning activities. Therefore, measures to reduce aerosol formation or exposure to aerosols (both technical and protective) should be considered. Reduction of exposure to aerosols will not only reduce the risk due to enteric pathogens, but also to endotoxins, Legionella and other pathogens.

References


