Tracking pathogenic biological agents in air - A case study of the outbreak of legionellosis in Norway

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Pathogenic airborne microorganisms are of concern to public health especially those causing naturally occurring diseases and aerosolized biological threat agents (1, 2). Bioterror agents that might be dispersed in air are exemplified by *Bacillus anthracis*, *Francisella tularensis*, *Yersinia pestis*, *Brucella* spp. and *Coxiella burnetii* (1, 3). Pathogenic airborne agents may travel long distances in air and transmit diseases to humans as exemplified by legionellosis (4, 5, 6), tuberculosis and measles (7) as well as other bacterial pathogens such as *Escherichia*, *Neisseria*, *Burkholderia*, *Clostridium* and *Brucella* (8, 9) in addition to those stated above. However, the dispersion and transport of aerosols (droplets or solid particles) are determined by complex and mutual dependent physical processes also including weather conditions.

Legionella pneumophila is the ethiological agent of Legionnaires' disease and of the non-pneumonic legionellosis Pontiac fever. A *Legionella* infection results from inhalation of a contaminated aerosol rather than from ingestion (10). Exposure to legionellae is generally found in wet and humid environments such as air conditioning systems, humidifiers, cooling towers, wastewater treatment plants, sanitary landfill sites, whirlpools, spas, fountains, dental devices and shower heads (11).

In May 2005 an outbreak of legionellosis, caused by *L. pneumophila* serogroup 1, occurred in Sarpsborg/Fredrikstad, Norway. The wet scrubber at Borregaard Ind. Ltd. was identified as the source of the outbreak and the route of transmission was suspected to be respiratory (6). At this location, biological treatments plants tanks were shown to contain *Legionella* bacteria up to 10^{10} CFU/L.

To investigate whether legionellae could be dispersed as aerosols from the ponds and transported by the wind, the wetted-wall cyclone SASS 2000^{PLUS} and the impactors MAS-100 and STA-204 were used to collect air samples directly above, upwind, and downwind of the aeration ponds during a 4-month period. Computational fluid dynamics was used *a priori* to estimate the aerosol paths and to determine suitable air-sampling locations. Several *Legionella* species, including *L. pneumophila*, were identified in air samples at the biological treatment plant using microbiological and molecular methods. *L. pneumophila* was identified up to distances of 200 meters downwind from the ponds, but, in general, not upwind nor outside the predicted aerosol paths. The highest concentration level of viable legionellae was identified directly above the aeration ponds (3300 CFU/m^3) and this level decreased as the distance from the aeration ponds increased. The study demonstrated that aerosols generated at aeration ponds of biological treatment facilities probably contained *L. pneumophila*, which was transported by the wind to the surroundings (5).

A "worst-case scenario", in which air at certain locations containing high concentrations of bacterial interferences could have an impact on a real-time PCR analysis was addressed. We constructed an artificial air sample containing high concentrations of naturally occurring airborne bacteria and analyzed the detection limit of six potential bioterror agents in such a

complex environment. A high concentration of non-target DNA could challenge the PCR assay with respect to potential inhibition of amplification. Results showed that the six biological threat agents analyzed, including *B. anthracis*, *B. melitensis*, *C. burnetii*, *F. tularensis*, *Y. pestis* and *B. cereus* spores (simulant for *B. anthracis* spores) could be specifically detected within one hour even without the need for any DNA extraction (9). This suggested that real-time PCR analysis could be regarded as a robust and reliable tool for analyzing air with even with a high content of biological material.

Our work has demonstrated that mathematical modeling (CFD) can significantly contribute to trace aerosol pathways and to determine suitable locations for collecting airborne particles for specific detection. We believe that the modeling methods used in our work may generically be applied to trace biological, as well as chemical, aerosols that may pose a challenge to environmental occupational health. Also, our work has demonstrated that air containing high concentrations of diverse bacteria in general have minimal (no) impact on a real-time PCR analysis provided that a specific detection scheme is used. The presentation will demonstrate how this work can contribute in providing an overall generic tool to analyze the dispersion of and to specifically detect biological threat agents and airborne agents of public concern.

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