ESGLI

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Production by Amoeba of *Legionella*-associated particles and purification thereof

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Aims

A significant portion of legionellosis outbreaks is related to anthropogenic water ecosystem (industrial complexes, cooling towers, hot water system) all over the world, The risk to contract the disease mostly relies on the concentration of Legionella in water, the virulence of the strain, the individual susceptibility of the exposed persons and the existence of aerosols enhancing bacterial inhalation, However, the exact composition of this infective aerosol is not known and may explain some of the discrepancies observed between reported cases, Protozoans naturally present in water have been suspected since a long time to playa role in this infectivity, Several authors have reported the capacity of various protozoans to abridge and/or favor the growth of Legionella, as well as to generate smaller Legionella-containing entities named "vesicles", which become easily airborne if water droplets are formed,

Methods

The Vesicount project aimed to produce artificially, identify and numerate some of these entities expelled by protozoan, with the final objective to be able to recognize and count them easily in industrial water. The couple formed by *Legionella pneumophila* sg1 strain *Paris* (CIP 107-629-T) and *Acanthamoeba castellanii* (ATCC 30234) was used as a model system, Lp sgl strain *Corby*, as well as different amoeba strains were also used. Various protocols were tested in order to set up the most efficient production methodology. For the production of amoebal vesicles a robust and reproducible procedure was established and tested concomitantly by all participating laboratories, its accuracy was also verified using *L. pneumophila* strains expressing GFP.

Results

The effect of variation of different parameters such as temperature, MOl, pH, medium composition, etc was also tested… Together with the external vesicle-like structures which contain viable bacteria and were already observed by several authors, other types of *Legionellae* clusters associated most of the time to remnants of membranes were also characterized and may represent another potential danger for human health.

Conclusions

Data collected during this study have lead to a better knowledge of expelled elements produced, which may in turn be useful and generate new tools for water system monitoring, They also allowed us to better understand the conditions required for the infection of *Acanthamoeba castellanii* by *Legionella pneumophila*, opening the way to exciting perspective for water system control.

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