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Descrizione del progetto:

"Influence of compost facilities for the presence of Legionellae and amoebae in bioaerosols and the spread of community-acquired Legionnaires' disease"

Introduction

Legionella bacteria are the causal agent of the Legionnaires' disease [LD], a severe form of pneumonia, with a mortality rate of approximately 6.5%. Legionellae can parasitize human alveolar macrophages and amoebae. Free-living soil and water amoebae play an essential role in the ecology and epidemiology of *Legionella*. Many studies have documented the multiplication of *Legionella* in amoebae using co-cultural methods[1, 2, 3, 4]. To date, 13 species of amoebae, some of which human pathogens, were identified as reservoirs and vectors for *Legionella*[5]. Amoebae are able to survive in critical environmental conditions by forming cysts and may thus protect their endosymbionts[6]. These protozoa can also have an impact on the infectious capacity and the *in vitro* cultivability of *Legionella*. The role of amoebae as a vector during the aerosolisation of contaminated substrates[7](for example water, soil, potting mixes, composting material) has not yet been investigated in detail. Only little research has been carried out on the simultaneous presence of amoebae and *Legionella* in bioaerosols. In Ticino the incidence of LD is about 8.0 cases/100'000 habitants, four times higher than in the rest of Switzerland. Most of the cases are sporadic community-acquired infections, for which the source cannot be identified. This study investigates whether or not the composts and the bioaerosols developed from pile fermentation contain viable *Legionella* and amoebae.

Methods

Sampling Location

Seven green waste collection centers (composting facilities, or long term storing and short term storing centres) located in Ticino (figure 1) were sampled for bioaerosols and composts. (figure 2)

Samples collection and culture analysis

Compost was stored in plastic bags. Bioaerosol ($1m^3$) was collected by a Coriolis µ air sampler(figure 3) and concentrated in 10 ml saline solution PAGE. The protozoa were cultured from both composts and bioaerosols on non-nutritive agar plates covered with *E. coli* as a food source. *Legionella* spp. were cultivated on GVPC agar plates[8] and by co-culture with axenic *Acanthamoeba polyphaga* for two weeks.

Fig.1 Map of Ticino, showing the analysed location

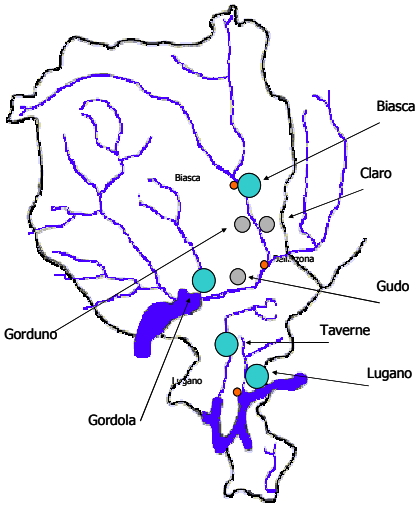

Fig.2 Composting station with released bioaerosol during fermentation of compost heap

Fig.3 Air sampler "il cigno" at work over a compost heap

Characterisation of amoebae

DNA from amoebae was extracted using a Qiagen tissue kit, performing the lysis with proteinase K overnight at 56°C. Amoebae were identified using the partial 18S rRNA sequence and the NCBI blast software.

Identification of *Legionella*

Colonies were tested for the presence of Legionellae with a GVPC/blood agar test. *Legionella pneumophila* was confirmed with a slidex test and further analysed with immunofluorescence. Species and fluorescent colonies were extracted by boiling colonies for 10 minutes and identification by *mip* gene sequencing. DNA was extracted directly from co-culture with the methods described for amoebae and analysed with a 16S semi-nested PCR specific for *Legionella*.

Results

Legionella pneumophila (Lp1, Lp3, Lp4, Lp5, Lp6, Lp10, and not characterizable Lp2-15) and *L.bozemanii* (figure 4) were found in the aerosols of six centres, but no *Legionella* could be isolated in Gudo with the co-culture methods.

L.bozemanii, L. cincinnatiensis, L.jamestowniensis, L.micdadei, L. jamestowniensis, L. oakrigensis and *L. pneumophila* were isolated from the composts of six centres.

The amoebae *Flamella arnhemensis, Hartmanella vermiformis* and *Heterolobosea* and were isolated from most composts facilities analysed but only from the aerosols in Lugano and Gordola. All results are summarised in table 1.

Fig.4 Biodiversity of *Legionella* from bioaerosols

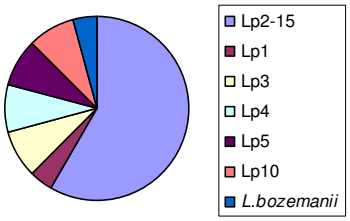

Table 1 All *Legionella* and amoeba isolated from composting facilities

Location	***Legionella* in compost**	***Legionella* in compost after co-culture**	**Amoebae in compost**	***Legionella* in aerosol**	**Amoebae in aerosol**
Biasca	*L. cincinnatiensis*	N.P.*	negative	Lp10, Lp2-15	negative
Lugano	Lp1, *L. bozemanii,* Lp2-15	Lp5, Lp6 Lp3, Lp4, Lp12, Lp2-15, *L. bozemanii*	*Stenoamoeba CRIB68* *Acanthamoeba* sp.	Lp6, Lp2-15, *L. bozemanii*	*Heterolobosea*
Claro	Lp3, Lp6, Lp10, Lp2-15, *L. bozemanii*	N.P.	*Singhamoeba horticola*	Lp1, Lp4, Lp10, Lp2-15	negative
Gordola	Lp1, Lp3, Lp4, Lp5, Lp6, Lp9, Lp10, Lp2-15, *L. jamestowniensis, L. micdadei*	Lp3, Lp5, Lp10, Lp2-15	*Vahlkampfia avara* *Naegleria CRIB*	Lp2-15	*Hartmanella vermiformis* *Flamella arnhemensis*
Gorduno	negative	N.P.*	*Naegleria CRIB*	Lp4, Lp5, Lp2-15	N.P.*
Gudo	Lp1, Lp10, Lp12, Lp2-15, *L. oakrigensis*	Lp6, Lp9, Lp2-15, *L. bozemanii*	*Acanthamoeba* sp.	negative	negative
Taverne	Lp1, Lp3, Lp4, Lp5, Lp6, Lp10, Lp2-15, *L. oakrigensis, L. micdadei*	Lp3, Lp4, Lp6, Lp10, Lp2-15, *L. bozemanii*	*Stenoamoeba CRIB68* *Tetramitus* *Hartmanella vermiformis* *Vahlkampfia inornata*	Lp3, Lp5, Lp2-15	negative

*N.P. = not performed

Discussion & Future perspectives

This study showed that viable amoebae and *Legionella* are both present in composts. Composting facilities are a possible reservoir for *Legionella* dispersion because *Legionella* and amoebae were cultivated from bioaerosols and could be potentially transported by air and wind. Composting and gardening could be considered risk activities.

Further studies are needed to demonstrate that amoebae can act as vectors for *Legionella* spread from composts to bioaerosols and how far vectors and bacteria can be transported. This year, four composting stations in Ticino (Gordola, Lattecaldo, Lugano and Taverne) will be monitored over one year period and the dispersion of bioaerosols will be evaluated. Composting facilities from northern Switzerland will be included in the investigation. Some experiments will be performed to quantify the enrichment of *Legionella* with the co-cultural method.

References

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