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Digital droplet PCR assay reveals an unexpected high prevalence of Legionella longbeachae in soils of Quebec, Canada: A possible emerging risk

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DD-PCR ASSAY REVEALS AN UNEXPECTED HIGH PREVALENCE OF *LEGIONELLA LONGBEACHAE* IN SOILS OF QUEBEC, CANADA

A POSSIBLE EMERGING RISK

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ABSTRACT

L.longbeachae (Llo) occurs mainly in Australia and New Zealand and, unlike other *legionella* species, is better adapted to soils. After two cases of occupational legionellosis attributable to Llo were reported in Quebec, the objective was to look at its prevalence in Quebec soils to assess a potential emerging occupational risk.

Samples of soil [n=56] were collected in four regions of Quebec and the DNA was analyzed with the QX200™ Droplet Digital™ PCR System (dd-PCR BIO-RAD)) using four systems allowing for the detection of *Legionella spp* (Lspp), *L. pneumophila* (Lp), *L. pneumophila Sg1* (LpSg1) and Llo.

The proportion of positive results (98%) is the highest reported in the literature. It is close to the 89% reported by Casati (4) in the soil, another study using PCR instead of culture. The use of dd-PCR may explain this difference because it is less subject to the inhibitions often observed in soil samples. Llo was detected in 68% of the samples. Few studies have specifically studied Llo and most of them have used culture detection; therefore, it is not possible to compare our prevalence with previous research.

We can assume that these results are inferable to the use of a more sensitive method, i.e. dd-PCR, but it could also highlight an unexpected distribution of this bacterium.

AIMS

- Develop a specific detection system of *Legionella longbeachae* using dd-PCR
- Investigate the prevalence of *L.longbeachae* in Québec soil
- Compare q-PCR and dd-PCR for the detection of *Legionella* in soil

REFERENCES

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2- Jonas, D., Rosenbaum, A., Weyrich, S. & Bhakdi, S. Enzyme-linked immunoassay for detection of PCR-amplified DNA of legionellae in bronchoalveolar fluid. J. *Clin. Microbiol*. 33, 1247–1252 (1995)

3- Mérault, N. et *al*. Specific Real-Time PCR for Simultaneous Detection and Identification of Legionella pneumophila Serogroup 1 in Water and Clinical Samples. *Appl. Environ. Microbiol*. 77, 1708–1717 (2011).

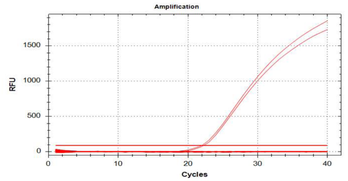
4- Casati, S., A. Gioria-Martinoni, et V. Gaia. Commercial Potting Soils as an Alternative Infection Source of Legionella Pneumophila and Other Legionella Species in Switzerland. *Clinical Microbiology and Infection* 15 (6): 571-75.(2009)

RESULTS

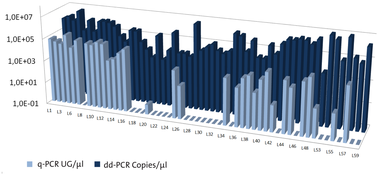
Llo PCR: System Performances

Demonstration of the Specificity

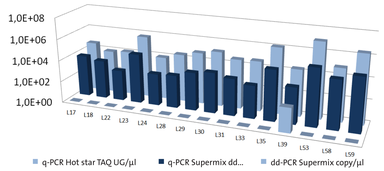
Bacteria	Nb. tested	Nb. positive
L. longbeachae	2	2
L pneumophila Sg1	32	0
Lp (Sg 2, 3, 5, 6, 7, 8, 9,)	30	0
Other Legionella	19	0
Others genus	62	0



Comparison of dd-PCR vs q-PCR

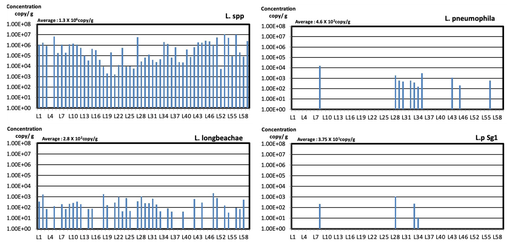


Comparison ot the Super mix for probes (BIO-RAD) and the HotStarTaq (QIAGEN)

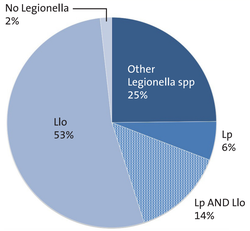


Soil analysis

Concentrations of *Legionella* in soil from Quebec

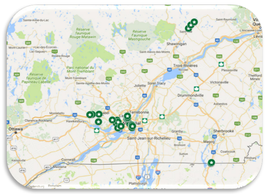


Percentage of soil samples with *Legionella*

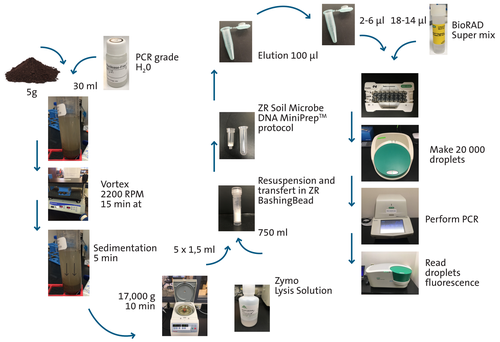


MÉTHODS

Soil sampling sites

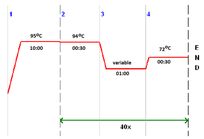


Soil DNA Extraction protocol



PCRs programs, primers and probes

PCR amplifications programs °C		
	dd-PCR	q -PCR
L.spp	51	60.5
Lp + sp1	58.5	60.5
Llo	51	54



Primers and probes sequences for the 4 *Legionella* detection systems		
Legionella spp. (1-2)	JRPmodif	CCA ACA GCT AGT TGA CAT CGT
	JFPmodif	GGAGG GTT GAT AGG TTA AGA GC
	LegLCmodif	TAC TGA CAC TGA GGC ACG AAA GCG T
Legionella pneumoniae (1)	PT70	GCT TTG CCA TCA AAT CTT TCT GAA
	PT69	GCA TTG GTG CCG ATT TGG
	LpneuFL	CCA CTC ATA GCG TCT TGC ATG CCT TTA
Legionella pneumoniae Serotype 1 (3)	P66	CAA ACA CCC CAA CCG TAA TCA
	P65	CAA AGG GCG TTA CAG TCA AAC C
	Sg1-PB	TCT TGG GAT TGG GTT GGG TTA TTT TAA CTC CT
Legionella longbeachae (IRSST)	Llo-272F	CAT TAC AGC GGG GAG TTG AT
	Llo-511R	CGG TAA CAT CCC GAG AGA AA
	Llo-380P	TAT TCA TTA CCT CAG GCG CC

CONCLUSION

- The detection system developed for *Llo* is very specific.
- The master mix and the PCR platform have a significant influence on the detection capacity of *Legionella* in soils.
- *L. longbeachae* was found in 3 times more samples than L pneumophila.
- Although common, *L. longbeachae* concentrations remain low and in most cases represent less than 1% of all *Legionella* spp in soil.
- Other species of *Legionella* should be searched for in soil samples.