Presence of *Waddlia chondrophila* in hot water systems from non-domestic buildings in France

Gemma Agustí [1,2], Thomas Le Calvez [3], Marie-Cecile Trouilhé [3,4], Philippe Humeau [3], and Francesc Codony [1,2*],

[1] Universitat Politècnica de Catalunya, Rambla Sant Nebridi 22, 08222 Terrassa, Spain

[2] GenIUL, Carrer de la Ciutat d'Assunción 4, 08030 Barcelona, Spain

[3] Centre Scientifique et Technique du Bâtiment, AQUASIM, 11 rue Henri Picherit, BP 82341, 44323 Nantes cedex 3, France

[4] Centre Scientifique et Technique du Bâtiment, Direction Opérationnelle HES – Division Canalisations, 84 avenue Jean Jaurès, Champs-sur-Marne, 77447 Marne-la-Vallée cedex 2, France

* Corresponding Author, fcodony@geniul.com

ABSTRACT

The presence of *Waddlia chondrophila* has been related to respiratory tract infections and human and animal fetal death. Although several sources of infection have been suggested, the actual source remains unknown and limited information exists on the prevalence of *W. chondrophila* in the environment. This pathogen has been previously detected in well water but its presence has not been confirmed in water networks. Since these bacteria have been detected in water reservoirs, it has been hypothesized that they can access artificial water systems and survive until they find appropriate conditions to proliferate. In this work, their presence in water samples from 19 non-domestic water networks was tested by quantitative PCR (qPCR). Approximately half of the networks (47%) were positive for *W. chondrophila* and the overall results revealed 20% positive samples (12/59). Furthermore, most of the samples showed low concentrations of the pathogen (< 200 genomic units/L). This finding demonstrates that *W. chondrophila* can colonize some water networks. Therefore, they must be considered as potential infection sources in future epidemiological studies.

Key words | hot water systems, human miscarriage, pathogen, *Waddlia chondrophila*

INTRODUCTION

In recent decades, the understanding of the role of free-living amoebae (FLA) in the transmission of infectious diseases has changed. Initial research data suggested a strong relationship between pathogenic bacteria and FLA, followed by experimental evidence that this interaction is responsible for most pathogen proliferation as endosymbionts (Greub & Raoult, 2004; Thomas *et al*., 2010; Codony *et al*., 2012a; Scheid, 2014). An example of an endosymbiont is *Waddlia chondrophila.* This microorganism is an obligate intracellular bacterium belonging to its own family, *Waddliaceae,* and to the order *Chlamydiales*. From its first detection in bovine fetal tissues (Rurangirwa *et al*., 1999) to date, evidence regarding its involvement as an agent of human miscarriages has been demonstrated (Baud *et al.*, 2004, 2007, 2011, 2014; Baud & Greub, 2011). Moreover, *Waddlia* is likely implicated as an agent of lower respiratory tract infection (bronchiolitis, bronchitis, pneumonia) since *Waddlia chondrophila* DNA was identified in respiratory tract samples from children with pneumonia (Lamoth & Greub, 2010). Their role in lung infection is further supported by a recent animal model demonstrating, in mice, the 3rd Koch postulate for this pathogenic bacteria (Pilloux *et al*., 2016).

Nevertheless, very little is known about the environmental distribution, prevalence, and infection source of *W. chondrophila*. The zoonotic transmission of *W. chondrophila* has been suggested as a potential infection source, in addition to the ingestion of contaminated water, meat, milk, and also sexual transmission (Corsaro & Greub, 2006; Vasilevsky *et al*., 2015; Lamoth *et al*., 2015). A previous study showed that *W. chondrophila* was present in 25% of well water samples (n=40) analyzed in a small study conducted in Spain (Codony *et al*., 2012b). In the same work, 30 domestic drinking water samples were tested for the presence of this pathogen and, in all of these cases, the results were negative. However, the presence of *W. chondrophila* in well water reinforces

the need for further evaluation of other water networks, in order to fully understand the potential risks associated with the proliferation of *W. chondrophila* in these artificial systems.

MATERIALS AND METHODS

Water samples were collected according to standard methods based on the ISO/CD 19458:2006 Standard. The samples were taken by the Centre Scientifique et Technique du Bâtiment (CSTB, France) in the context of a research program focused on detecting the risk associated with the presence of *Legionella* in public buildings. Samples were transported in the dark and at < 8°C and stored at 2–5°C until analyzed, within 24 h following sampling.

The *Legionella* analysis was done by culture according to ISO 11731:1998. On the other hand, *W. chondrophila* concentration was measured by qPCR. In this case, 500 mL of water was filtered through a 0.2 µm pore size PVDF filter, which was used for subsequent DNA purification using the High Pure PCR Template Preparation Kit (Roche Molecular Diagnostics, Mannheim, Germany) according to manufacturer's instructions.

The qPCR was done in the MSM-Lab at the Polytechnic University of Barcelona, in a blind mode, without knowledge of any data about the water source and other analytical results.

The qPCR procedure was based on previous work by Goy *et al.* (2009) and was performed on a LightCycler-1.5 PCR system (Roche Molecular Diagnostics). Briefly, the reaction mixture consisted of 10 µL Fast Start Taqman Probe Master (Roche Molecular Diagnostics); 0.4 U Uracil-DNA-glycosylase (UDG, New England Biolabs, UK); 9 µL DNA sample; 0.2 µM *W. chondrophila* specific primers WadF4 (5'-GGCCCTTGGGTCGTAAAGTTCT-3') and WadR4 (5'-CGGAGTTAGCCGGTGCTTCT-3'); and 0.1 µM probe WadS2 (5'-FAM-CATGGGAACAAGAGAAGGATG-BHQ-3'). The primers amplified a 101-bp DNA fragment of the 16S rRNA gene of *W. chondrophila*. The

probe contained locked nucleic acids (underlined in the sequence above). The qPCR conditions were optimized previously by Codony *et al.* (2012b) as follows: 2 min at 50°C, 10 min at 95°C as well as 50 cycles of 15 s at 95°C and 1 min at 60°C. A negative control (water, PCR-grade) and a positive control (DNA from *W. chondrophila*) were included in each run.

RESULTS AND DISCUSSION

The raw data are shown in Table 1 and summarized in Table 2. Briefly, a total of 59 samples from 19 different hot water networks were analyzed. The most significant finding was the high percentage of *W. chondrophila* in 47% of the analyzed networks, with 12 positive samples from a total of 59 samples. Furthermore, it should be noted that in most of the samples, the *W. chondrophila* numbers were below 200 GU/L. However, one sample with temperature lower than 40ºC showed the highest *W. chondrophila* numbers (1,000 GU/L). Additionally, seven samples were positive for *Legionellae* in four of the networks analyzed. Although both microorganisms need to interact with FLA, these results do not suggest a positive correlation between Legionellae and *W. chondrophila*. Interestingly, more positive samples (9 vs. 4) were colonized by *W. chondrophila* when compared with Legionellae.

Current knowledge about the prevalence of *W. chondrophila* in the environment is limited and a previous survey carried out in Spain was able to detect *W. chondrophila* in well water but not in domestic drinking water (Codony *et al*., 2012b). Now, this work demonstrates, in a different geographic area (France), that hot water systems from non-domestic networks can be colonized by this pathogen. It is well known that these types of systems, with low levels or absence of disinfectant, can easily support the proliferation of FLA and their endosymbionts (i.e. Legionellae). On the other hand, the previous data suggest, at least in Spain, that domestic drinking water systems are not colonized by *W. chondrophila* (Codony *et al*., 2012b). Many domestic water networks have a simple

structure, usually without water recirculation, which allows a minimum disinfectant level to be maintained. Maybe for this reason no pathogens were detected in those samples. These observations are in agreement with one previous epidemiological survey, conducted in Spain, that does not suggest domestic networks as the main potential infection sources for sporadic cases of legionellosis (Codony *et al.*, 2002).

The data from this work demonstrate that *W. chondrophila* can colonize hot water systems from non-domestic networks with higher prevalence than Legionellae. However, this trend needs to be confirmed and more prospective studies are needed in different geographical areas and with more samples. Similarly, with the limited number of positive samples detected here, the existence of an antagonistic relationship between Legionellae and *W. chondrophila* cannot be suggested.

More clinical and epidemiological information is available on *W. chondrophila* than environmental or ecological data. For this reason, the evaluation of environmental niches, as reservoirs of pathogens, can aid the actual understanding of potential infection sources. Similar to other members of the *Chlamydiales* order, *W. chondrophila* may use FLA for its proliferation and it is not surprising to find it in artificial water systems, such as hot water networks. In these systems, the low levels of residual chlorine, water recirculation, and the existence of dead legs can promote FLA growth and that of their endosymbionts.

CONCLUSIONS

Although the actual human infection pathway remains unknown, this is the first work demonstrating the existence of this pathogen in drinking water networks. Future epidemiological studies should take into account these results in order to evaluate the potential infection risk caused by FLA endosymbionts in hot water from non-domestic buildings.

ACKNOWLEDGEMENTS

We are most grateful to Gilbert Greub (Infectious Diseases Group of the Institute of Microbiology at the University of Lausanne) for kindly providing the purified DNA from *W. chondrophila*. We also thank the Polytechnic University of Catalonia for supporting this study. Financial support was provided by a grant to Gemma Agustí from this University (Convocatòria d'Ajuts per a la Iniciació i Reincorporació a la Recerca). The authors thank Dr. I. Douterelo from the Department of Civil and Structural Engineering, University of Sheffield, UK, for the kind review of this manuscript.

Table 1 | Water analysis results by building type.

Building	T (°C)	time (s)	L.pn cfu/L	L. spp cfu/L	W. c GU/L
Hospital	57.7	165	<250	<250	**+<200**
	38.5	240	**850**	**850**	<200
	43.5	195	<250	<250	<200
	53.5	75	**+<250**	**+<250**	<200
Locker Room	62.8	12	<250	<250	<200
	40.1	82	<250	<250	<200
Day Nursery	47	110	**+<250**	**+<250**	<200
	56.2	45	**2300**	**2300**	<200
	56.5	65	<250	<250	<200
	60	60	<250	<250	<200
School	53.1	60	<250	<250	**+<200**
Hotel	63.5	90	<250	<250	<200
	63.3	90	<250	<250	<200
Hospital	56	45	**+<250**	**+<250**	<200
	55.4	45	<250	<250	**+<200**
	55.8	30	<250	<250	<200
	54.1	45	<250	<250	<200
	40.8	30	<250	<250	<200
	53.2	60	<250	<250	<200
Kindergarten	43.3	15	<250	<250	<200
	57.8	65	<250	<250	<200
	62	15	<250	<250	<200
School	38.4	360	<250	<250	**1,000**
	54.7	45	<250	<250	<200
	57.7	55	<250	<250	<200
	53.8	60	<250	<250	<200
	34.5	45	<250	<250	<200
	50.8	30	<250	<250	<200
	53.8	30	<250	<250	<200
	39.5	25	<250	<250	<200
	61.6	20	<250	<250	<200
	62	30	<250	<250	**274**
	44.6	30	<250	<250	<200
Retirement Home	53.9	45	<250	<250	<200
	55.6	30	<250	<250	<200
	57.4	25	<250	<250	<200
Locker Room	40.4	120	<250	<250	<200
	38	95	<250	<250	<200
School	64.8	60	<250	<250	<200
	65.3	60	<250	<250	**269**

Locker Room	52.8	60	<250	<250	<200
Locker Room	32.7	120	<250	<250	<200
Locker Room	56.2	45	<250	<250	<200
	57.2	75	<250	<250	<200
	50.5	60	<250	<250	<200
	62.3	45	<250	<250	<200
Camping	40.2	65	<250	**+<250**	<200
	46.6	55	<250	<250	**+<200**
School	44.8	90	<250	<250	<200
	53.5	75	<250	<250	**+<200**
	44.8	50	<250	<250	**+<200**
	53.5	50	<250	<250	<200
Safe Houses	57	30	<250	<250	**+<200**
	42.2	25	<250	<250	**+<200**
Safe Houses	28	150	<250	<250	<200
	52.6	25	<250	<250	**+<200**
Safe Houses	45	20	<250	<250	<200
	52.4	80	<250	<250	<200
	52	40	<250	<250	<200

Note: Time indicates the water flowing previous to sampling. *Legionella pneumophila* (L.pn), *Legionella* spp. non *pneumophila* (L.spp), *Waddlia chondrophila* (W.c). Negative results (no detection) showed the quantification limit of the method. The symbol +, indicates qualitative positive detection at low levels, below the quantification limit. Positive results are marked in bold.

Table 2 | Microbiological results of Legionellae/*W. chondrophila* presence in hot water networks

		W. chondrophila		
		+	–	
Legionellae	+	3	1	4
	–	6	9	14
		9	10	19

Note: + detected; – not detected

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