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***Legionella* bacteria in shower aerosols increase the risk of Pontiac fever among elderly subjects in retirement homes**

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ABSTRACT

Background: 828 elderly subjects residing in nursing homes were followed-up during 4 months to ascertain incidence of symptoms associated with Pontiac fever (PF) in a non epidemic setting.

Methods: The exposure situation was inhalation of *Legionella* bacteria while showering. An audit of the hot water system in all institutions allowed ascribing each subject to a water quality area wherefrom one shower was sampled for *Legionella* assays at the end of the follow-up period. *Legionella* were detected in water and aerosols using the culture (CFU) and in situ hybridization (FISH) techniques.

Results: Among 32 Pontiac-like episodes, 29 cases complied with the operational definition of PF elaborated for this study. Incidence density was 0.11 case/person-year (95%CI [0.07-0.15]). Water concentrations greater than 10^5 *Legionella*-FISH/L and 10^4 *Legionella*-CFU/L were associated with an increased risk of PF (respectively RR=2.23, p=0.05 and RR=2.39, p=0.11, with significant dose-response patterns: p for trend <0.04). The condition also seems associated with aerosol concentrations above 10^3 *Legionella*-FISH/L of air. **A significantly higher risk of Pontiac-like episodes (RR=6.24, 95%CI [2.12-18.38]) was seen for elderly subjects receiving corticosteroid therapy.**

Conclusion: The water and threshold values identified in this research could be used to inform guidance measures aimed at protecting the institutionalised elderly from Legionnaires' disease. Immuno-suppressive therapy in the same population group can significantly enhance susceptibility to *Legionella* bacteria.

INTRODUCTION

Legionella may elicit infection with two clinical presentations: Legionnaires' disease, an acute pulmonary condition that may take the form of a serious pneumonia, and Pontiac fever (PF), a milder form that mimics influenza.[1-3] Epidemiology of PF is not as well characterized as Legionnaires' disease because of its benignity and lack of specificity.[4] The reasons why contaminated water may result in Legionnaires' disease or in PF are still not well understood;[3, 5] however episodes of PF may serve as an indicator for the risk of Legionnaires' disease.

Similar to many countries, regulations in France deal with *Legionella pneumophila* in water only.[6] However, *Legionella* infection results from inhalation of a contaminated aerosol, not from ingestion.[7, 8] No known relationship has been established between water and bacteria concentrations in aerosols. In the past, few works has been carried out on *Legionella* concentrations in aerosols generated by contaminated water.[9-13] A recent study conducted in the framework of a Legionnaires' disease outbreak in North of France, reported airborne *Legionella pneumophila* concentrations spanning from 3.3×10^2 to 5.4×10^3 culturable *Legionella* (CFU/m³).[14] To date, no dose-response relationship has been established among humans. Infectious or lethal doses for guinea pigs exposed through inhalation of *Legionella pneumophila* were highly variable across studies.[15-24]

The risk of PF associated with shower water and aerosols contaminated by *Legionella* among elderly subjects living in nursing homes has been assessed in the framework of an epidemiological study conducted in the Lorraine region, North-East of France.

MATERIAL AND METHODS

Study population

This retrospective follow-up study took place in 34 nursing homes from 2003 to 2006. The study population included 828 elderly subjects who were volunteers and signed informed consents. They

were followed-up during 4 months in each home. A set of predefined symptoms that have been associated in the literature with PF were registered daily by the nursing staff. Information was also collected on demographic characteristics, current and/or past smoking habits, relevant medical history (respiratory and immunity-related conditions, such as diabetes and cancer) and current prescription of immunosuppressive therapy and antibiotics.

Because taking a shower represented the key exposure determinant in this study, only volunteers who declared taking at least one shower a week were included in the study. Neurodegenerative disorders were not a cause of exclusion insofar as subjects could take showers alone or with the assistance of the staff. Data about showering were recorded by the nursing home staff (the day and the room where the shower was taken, including if volunteers used different shower rooms along the follow-up period). A trained nurse dedicated to the study surveillance periodically checked the quality of registers.

Case definition

In a previous paper based on preliminary data of this study, we have proposed an operational definition for epidemiological investigation and surveillance of PF.[4] According to it, the case definition of PF was based on two criteria: (i) occurrence within 3 days after a shower of at least one clinical symptom among headache, myalgia, fever and shivers, possibly associated with other 'minor' symptom; this event could last up to a maximum of 8 days (minimum: 2 days); and (ii) contamination of the water and/or aerosol in the shower enclosure by *Legionella* bacteria detected either by culture or the FISH techniques. No serological or microbiological evidence of infection was required as part of the case definition both for practical and ethical reasons because we could not take repeated sputum or blood or urine samples in this at risk population. Some recent papers showed that during epidemics, blood and sputum samples ~~for *Legionella* culture were negative,~~[25, 3] ~~as was *L. pneumophila* serogroup 1 urinary antigen.~~~~were for *Legionella* culture were negative, as~~ ~~*L. pneumophila* serogroupe 1 urinary antigen~~[26, 27]

Exposure assessment

The methodology and detection techniques used to assess exposure are detailed elsewhere.[28] Because all showers could not be sampled, for practical and financial reasons, the selected showers were chosen to be representative of exposure of a group of volunteers. To this end, hot water systems in each nursing home were evaluated in order to assess their criticality in terms of *Legionella* risk, and to choose the sampling points (showers) that best fitted volunteers' exposure. Sampling was undergone immediately after the 4 months follow-up period, meaning that exposure was assessed retrospectively, for evident ethical reasons. This study design was approved by the ethical committee of Nancy University Hospital.

One litre of hot-water was collected in sterile bottles after about 7 minutes of water flushing.[29] Airborne *Legionella* were collected using 2 bioaerosol samplers in parallel for each shower: a MAS-100[®] (impaction onto an agar medium: GVPC) and an SKC Impinger (impaction in liquid medium: sterile distilled water).[28]

Airborne and water *Legionella spp* were quantified by FISH using a mix of three probes validated for *Legionella* (LEG705, LEG226 and LEGPNE1).[30, 31] In addition, the culturable fraction of *Legionella* was evaluated according to the French standard method.[32] While only *Legionella spp* bacteria are identified using the FISH technique, culturable *Legionella* can be split according to species (*L. pneumophila* or *L. species*), as recommended by the French standard.[32]

Data analysis

A subject could take showers in locations with different microbial water qualities along the 4 months of follow-up, a feature that was accommodated through five exposure scenarios. Table 1 describes the key characteristics of the three scenarios which assessed exposure according to water *Legionella* data, and of the two scenarios for airborne *Legionella*. In order to study the dose-response relationship, the reference exposure level was chosen below the detection limit value,

which was 6×10^3 *Legionella*-cells/L of water for the FISH technique and 100 CFU/L for the culture technique, according to the analysed water volumes. In the aerosol, the FISH detection limit was 3.7×10^2 *Legionella*-cells/L of air. Data were input with EPI INFO and analysed with the SAS software.

RESULTS

Elderly population

The study included 828 subjects (29.9% males, 70.1% females) across 34 nursing homes. The total number of residents in the nursing homes was 2504, meaning that 33 % complied with the study selection criteria and volunteered (participation rate ranging from 13 to 78 % according to nursing homes). The average age was 81 years (standard deviation (SD) = 10; extreme values: 46-102), females being slightly older than males (respectively 83 and 78 years).[9, 10] Non smokers composed 81% of the population; subjects with neurological disorders and cognitive impairments (dementia, debility, Parkinson syndrome) represented 26%, diabetics 16% and those with co-morbidities (renal, liver, non cognitive neurological or psychiatric conditions) were 35%. Antecedents did not differ between males and females, except previous ($p < 10^{-4}$) and current ($p = 5 \times 10^{-4}$) smoking habits and alcoholism ($p = 2 \times 10^{-4}$), more frequent among men.

Table 1: Exposure of elderly residents in nursing homes to *Legionella* bacteria in water and aerosols while showering: five tested exposure scenarios.

	Description	Exposure calculation
Scenario 1 (Water quality)	For each subject, exposure (E_1) was the greatest <i>Legionella</i> concentration (C_{water}) among the showers (s) he/she utilized (not more than 4 have been recorded). E_1 represents the <i>worst case</i> exposure to water <i>Legionella</i>	$E_1 = \max(C_{water_s})$ for $s=1$ to 4
Scenario 2 (Water quality)	For each subject, exposure (E_2) was the greatest average of <i>Legionella</i> concentrations (C_{water}) across all the showers ($s = 1$ to 4) he/she utilized, based on the mean of water samples duplicates. E_2 represents the <i>average maximal</i> exposure to water <i>Legionella</i>	$E_2 = \max\left(\frac{C_{water_{s1}} + C_{water_{s2}}}{2}\right)$ for $s=1$ to 4
Scenario 3 (Water quality)	For each subject, exposure (E_3) was the weighted average of <i>Legionella</i> concentrations (C_{water}) (mean of water samples duplicates) across the different showers (s), where weights are the number of showers (N) taken with each shower. E_3 represents the <i>frequency weighted</i> exposure to water <i>Legionella</i> .	$E_3 = \frac{\sum_{s=1}^4 C_{water_s} \times N_s}{\sum_{s=1}^4 N_s}$
Scenario 4 (Aerosol quality)	For each subject, exposure (E_4) was the maximum <i>Legionella</i> concentration ($C_{aerosol}$) across the showers (s) he/she utilized. E_4 represents the <i>worst case</i> exposure to airborne <i>Legionella</i>	$E_4 = \max(C_{aerosol_s})$ for $s=1$ to 4
Scenario 5 (Aerosol quality)	For each subject, exposure (E_5) was the weighted average of airborne <i>Legionella</i> concentrations ($C_{aerosol}$), where weights are the number of showers (N) taken with each shower. E_5 represents the <i>frequency weighted</i> exposure to airborne <i>Legionella</i> .	$E_5 = \frac{\sum_{s=1}^4 C_{aerosol_s} \times N_s}{\sum_{s=1}^4 N_s}$

Pontiac fever cases

Among these 828 subjects, 32 met the clinical definition of PF; they will hereafter be referred to as ‘PF-like events’. No Legionnaires’ disease case was diagnosed during the study period. Contaminated shower water samples for *Legionella spp* were found among 29 of these 32 subjects, irrespective of the detection method (culture or FISH). Hence, these 29 subjects strictly comply with the study definition of PF.[4] Females composed 2/3 of these cases. The average age was 82 years (SD=9.3). The mean episode duration was 3.5 days and the average incubation period (i.e. the time elapsed after the most recent shower) was 2.1 days. Among the PF cases, the most frequent symptoms were fever (75.9%), cough (44.8%), diarrhoea (27.6%) and headache (17.2%) (Table 2). The median number of symptoms per case was 2. The overall incidence density of PF in this population was 0.11 case/person.year (95% CI [0.07-0.15]). Cumulative incidence over 4 months was 3.5 %. Among these 29 cases, 11 were associated with culturable *Legionella* and 21 with FISH *Legionella*. **In three instances, identifying bacteria was unsuccessful due to a high background fluorescence noise.** When only contaminated aerosols were considered, the number of cases reduced to 10 and only the FISH technique was able to detect the bacteria.

Table 2: Frequency of the clinical symptoms among the 29 cases of Pontiac fever.

Symptoms	Number of elderly subjects with clinical symptoms among the Pontiac fever cases (n=29)
Fever	22
Cough	13
Diarrhoea	8
Headache	5
Asthenia	4
Myalgia	4
Vomiting	3
Shiver	2
Agitation	1
Abdominal pains	1

Exposure characterization

The key exposure variable was “to take a shower”. The average number of weekly showers was 1.1 and the average follow-up 122 days. None of the risks are significantly in excess with the culture method (Table 3), but an increased risk of PF is suggested (although not significantly) for scenario 1, starting with a water concentration of culturable *Legionella* spp $\geq 10^4$ CFU/L (Rate Ratio, RR=2.39; p=0.11). On average, 78.3 % of the culturable *Legionella* bacteria present in hot water showers were *L. pneumophila*. All exposure scenarios for *Legionella* spp in hot water measured by the FISH technique show an increased risk over a concentration of 10^5 *Legionella*-cells/L, whereby subjects had at least twice a greater risk to develop PF than if exposure was lower (Table 3), with significant dose-response patterns (p for trend = 0.04 and < 0.001 for scenarios 2 and 3).

Dealing with bioaerosols, a borderline significant risk is associated with concentrations of hybridized (FISH) *Legionella* over 10^4 *Legionella*-cells/L of air according to exposure scenario 4 (RR=2.05; p=0.1), and over 10^3 *Legionella*-cells/L air with scenario 5 (RR=1.77; p=0.1) (Table 4).

~~When considering only the 11 cases that corresponded to contaminated shower aerosols with *Legionella* spp detected by FISH (i.e. discarding all cases with ‘positive’ water samples but ‘negative’ air samples), scenarios 4 and 5 suggest a (non-significant) increase of risk of PF at concentrations over 10^3 *Legionella* cells/L air (RR=2.35 with scenario 4, and RR=2.95 for scenario 5) (Table 4).~~

Table 3: Relative risk of Pontiac fever associated with *Legionella* concentrations in showers' hot water, according to the exposure scenarios and the detection methods.

Exposure scenarios	Culture method ¹					FISH method ²				
	Concentration (CFU/L water) ⁺	Cases or PF-like events	Person-days	RR (95% CI)	P	Concentration (<i>Legionella</i> - cells/L water) ⁺	Cases or PF-like events	Person-days	RR (95% CI)	P
Scenario 1 (worst case exposure to water <i>Legionella</i>)	< 100 (DL)*	21 ^o	73691	1.00	-	< 6000 (DL)*	8 ^o	31281	1.00	-
	≥ 10 ²	11 ^o	26477	1.46 (0.70 – 3.02)	0.20	≥ 6000	21 ^o	66388	1.24 (0.55 – 2.79)	0.39
	≥ 10 ³	5	16684	1.05 (0.40 – 2.79)	0.54	≥ 10 ⁴	20	62479	1.25 (0.55 – 2.84)	0.38
	≥ 10 ⁴	4	5872	2.39 (0.82 – 6.96)	0.11	≥ 10 ⁵	13	22788	2.23 (0.92 – 5.38)	0.05
	≥ 10 ⁵	1	2102	1.67 (0.22 – 12.41)	0.46	≥ 10 ⁶	5	7172	2.73 (0.89 – 8.33)	0.08
Scenario 2 (average maximal exposure to water <i>Legionella</i>)	< 100	21	73691	1.00	-	< 10 ⁴ §	9	31281	1.00	-
	≥ 10 ²	11 ^o	26477	1.46 (0.70 – 3.02)	0.20	≥ 10 ⁴	20 ^o	62479	1.11 (0.51 – 2.44)	0.48
	≥ 10 ³	5	13351	1.31 (0.50 – 3.49)	0.37	≥ 10 ⁵	13	22788	1.98 (0.85 – 4.64)	0.08
	≥ 10 ⁴	1	3019	1.16 (0.16 – 8.64)	0.59	≥ 10 ⁶	5	6558	2.65 (0.89 – 7.91)	0.08
	≥ 10 ⁵	1	1381	2.54 (0.34 – 18.89)	0.34					
Scenario 3 (frequency weighted exposure to water <i>Legionella</i>)	< 100	22	74069	1.00	-	< 10 ⁴ §	9	58536	1.00	-
	≥ 10 ²	10 ^o	26099	1.29 (0.61 – 2.72)	0.31	≥ 10 ⁴	20 ^o	57125	2.28 (0.89 – 7.91)	0.03
	≥ 10 ³	5	12063	1.40 (0.53 – 3.69)	0.32	≥ 10 ⁵	12	21901	3.56 (1.50 – 8.46)	3x10 ⁻³
	≥ 10 ⁴	1	2641	1.27 (0.17 – 9.46)	0.55	≥ 10 ⁶	5	4892	6.65 (2.23 – 19.84)	3x10 ⁻³
	≥ 10 ⁵	1	1257	2.68 (0.36 – 19.87)	0.32					

+ Concentration in *Legionella* spp CFU/L of water for culture and in *Legionella* FISH/L of water for FISH

* DL : Detection limit of the method

^o Subjects with PF-like clinical symptoms ; these are not PF cases, in absence of contaminated water or aerosol

¹ culture on agar media expressed in Colony Forming Unit (CFU) per litre

² fluorescent *in situ* hybridization (FISH) expressed in *Legionella*-cells per litre

£ Only the cases associated with contaminated water using the culture technique are here enumerated, which explains why their number is not 29

\$ Same comment as above, using the FISH technique; 3 events are missing because the slides could not be read.

§ No clinical event was detected for concentrations below DL; reference category is consequently $< 10^4$ *Legionella* FISH/L

Table 4: Risk of Pontiac fever according to *Legionella* concentrations measured by **fluorescent in situ hybridization** (FISH) technique on the shower aerosol samples collected by liquid impaction.

Exposure scenarios	Concentration (/L air) ‡	Cases or PF-like events	Person-days	RR (95% CI)	P*
Scenario 4 (worst case exposure to airborne <i>Legionella</i>)	< 10 ³	22 ^o	77594	1	-
	≥ 10 ³	10 [‡]	22574	1.56 (0.74 – 3.30)	0.17
	≥ 10 ⁴	6	10331	2.05 (0.83 – 5.05)	0.10
Scenario 5 (frequency weighted exposure to airborne <i>Legionella</i>)	< 10 ³	22	79664	1	-
	≥ 10 ³	10	20504	1.77 (0.84 – 3.73)	0.10
	≥ 10 ⁴	2	7258	1.00 (0.23 – 4.24)	0.61

* Incidence density Chi² test, one-sided p.

‡ Concentration in *Legionella* FISH/L of air

^o, [‡] and ^s : same comments as in table 3.

Predisposing factors

In table 5, immunosuppressive therapy is associated with a 6.2 fold increase in the risk of PF ($p=0.01$). Among females, a history of respiratory condition is mildly associated with a 2.5 greater risk ($p=0.1$), a result that does not stand for men nor on the total study population. Cognitive impairments seem to exhibit a borderline ‘protective’ effect ($RR=0.47$; $p=0.12$). This unexpected result is not due to cases taking a smaller number of showers than non cases ($p=0.45$). Also, incidence of PF did not differ according to whether subjects took more or less than 18 showers (i.e. the median value) over the whole study span ($RR=0.95$). These putative risk factors were included into a multiple logistic model, with age, gender, immunotherapy, cognitive conditions and water *Legionella* concentrations. Only two showed independent effects: water concentration threshold of 10^5 *Legionella*-cells/L detected by FISH ($OR= 2.3$; $p=0.03$) and immunotherapy ($OR= 4.0$; $p=0.04$) (data not shown).

DISCUSSION

The study relates to 828 elderly volunteers (82 years old on average, 2/3 women) residing in 34 nursing homes and followed-up during 4 months. Showering was the main route of exposure to *Legionella*, bacteria being measured both in hot water and in the shower aerosol. Among 32 subjects who exhibited Pontiac fever-like symptoms, 29 were exposed to *Legionella spp* in the shower hot water. The overall incidence density of PF was 0.11 case/person.year (95%CI [0.07-0.15]). Despite lack of specificity, the combination of symptoms we used to qualify episodes as “Pontiac-like” showed an association with contamination of the shower water: *Legionella spp* concentrations above 10^4 CFU/L (culture) (of which 2/3 were *L. pneumophila*) and 10^5 hybridized *Legionella*/L (FISH) were associated with an increased risk to develop PF. An increased risk was also suggested when airborne *Legionella* concentrations exceeded 10^3 *Legionella*-cells/L of air (FISH).

Table 5: Risk of Pontiac fever according to different factors, by gender

Risk factors	Females	Males	Males and females
	(RR [95%CI] and p value) n=581	(RR [95%CI] and p value) n=247	(RR [95%CI] and p value) n=828
Age (<75 years ; 75-90 ; >90 years)	(p=0.91)	(p=0.54)	(p=1)
- 75-90 vs <75 years	0.94 [0.31-2.82] (p=1)	1.34 [0.25-7.14] (p=1)	1.12 [0.45-2.80] (p=1)
- >90 years vs <75 years	0.72 [0.19-2.82] (p=0.72)	2.55 [0.38-17.30] (p=0.32)	1.09 [0.36-3.30] (p=1)
Gender (females/males)	-	-	1.12 [0.50-2.49] (p=1)
Number of showers (median cut off point)	0.79 [0.32-1.96] (p=0.64)	3.89 [0.46-32.71] (p=0.23)	1.08 [0.49-2.38] (p=1)
Immunosuppressive therapy	4.97 [1.30-19.00] (p=0.07)	11.62 [2.00-67.47] (p=0.09)	6.24 [2.12-18.38] (p=0.01)
Smoking habits (never ; past ; current)	(p=1)	(p=0.74)	(p=0.81)
- past vs never	1.05 [0.25-4.41] (p=1)	0.47 [0.06-3.81] (p=0.70)	0.75 [0.23-2.44] (p=0.79)
- current vs never	1.07 [0.15-7.73] (p=1)	1.11 [0.14-8.81] (p=1)	1.09 [0.27-4.50] (p=0.71)
Alcoholism	0.65 [0.04-10.42] (p=1)	1.54 [0.20-11.91] (p=0.51)	0.70 [0.10-5.04] (p=1)
Diabetes	1.23 [0.43-3.59] (p=0.76)	0.74 [0.09-5.85] (p=1)	1.09 [0.42-2.80] (p=0.8)
Cancer/ Hemopathy	0.52 [0.07-3.79] (p=1)	1.21 [0.16-9.48] (p=0.59)	0.72 [0.18-2.98] (p=1)
History of respiratory conditions	2.47 [0.99 – 6.18] (p=0.1)	0.28 [0.02-4.79] (p=0.36)	1.50 [0.62-3.60] (p=0.42)
History of cardiovascular conditions	1.14 [0.27-4.76] (p=0.69)	0.99 [0.06-16.25] (p=1)	0.92 [0.22-3.76] (p=1)
History of cognitive conditions [§]	0.47 [0.14-1.56] (p=0.31)	0.39 [0.05-3.12] (p=0.69)	0.44 [0.16-1.26] (p=0.14)
Other comorbidities	0.93 [0.38-2.27] (p=1)	0.28 [0.04-2.26] (p=0.27)	0.72 [0.32-1.61] (p=0.55)

[§] Dementia, mental retardation and Parkinson's disease

Pontiac fever: characteristics, incidence and risk factors

The incidence of PF is underestimated, with less than twenty epidemics described during the last 40 years, undoubtedly by lack of appropriate surveys and reporting definition. The clinical symptoms described, heterogeneous across studies, are those of a pseudo-influenza syndrome: nausea, myalgias, fever, shivers, headaches, cough.[1-3, 26, 27, 33, 34] While no single clinical definition is accepted to date, our work uses for the first time an operational definition of PF associating clinical symptoms and *Legionella* measurements in water and aerosols.

No other study has evaluated the incidence of PF in a non epidemic framework, in a nursing homes population. In this setting, cumulative incidence is low (3.6%) compared to other studies; the latter were all performed during epidemics, with incidences ranging from 11% to 94%.[1, 35] Hence, it is difficult to compare such different data, both in exposure settings and population characteristics. Our results reflect a background noise of PF within a sensitive population. Further, the relatively benign symptoms that are associated with PF episodes can remain unnoticed, leading to underestimation of incidence.[25] We accounted for possible inter-current epidemics during the follow-up. No influenza case was recorded in the nursing homes, nor did the French national institute for health surveillance report influenza epidemics during the study period.[36]

Analysis of risk factors showed that only cognitive disorders and corticotherapy were associated with Pontiac fever. No other studies have looked at the relationship between cognitive disorders and PF. Consequently, we cannot compare this finding and this requires further research. Our study also highlights a strong relation between corticotherapy and PF (RR= 4.0; 95%CI [1.1–14.9]) that had never been defined as a risk factor of PF, although already described during Pontiac epidemics.[33, 35]

No other health condition registered in this study proved a risk factor for PF; in accord with previous studies, gender was not found to be associated with the condition.[1, 35, 37, 38] Studies on smoking habits are contradictory, some suggesting that smoking does not influence the risk of PF[1, 37, 38], in contrast with what has been described for Legionnaires' disease[39], while other authors

report relative risks ranging from 1.2 to 5.1.[27, 33] In accordance with the former studies, our results do not show an association between smoking and PF.

Aerosols from showers: a source of exposure to airborne *Legionella*

In France, prevention aims to prevent *Legionella pneumophila* proliferation and to limit concentrations under a threshold value of 10^3 CFU/L in hot-water networks.[6] Few studies have tried to detect airborne *Legionella*,[9, 13, 40] but no relation between *Legionella* concentrations in water and counts of bacteria dispersed into aerosols has been yet established. As a consequence, the management of the hazard posed by *Legionella* faces serious limitations since inhalation of airborne *Legionella* is the route of exposure.

In our study, showering was the key exposure determinant. Other sources of *Legionella* dissemination, such as air-conditioning or surrounding cooling towers (up to a radius of 10 km) were looked for. None of the study nursing homes had air-conditioning during the study period. Cooling towers were present about most nursing homes, leading to potential sources of *Legionella* discharge; they could not be investigated, however. We nevertheless think that aerosol generated while showering is the main exposure route in these health facilities because (1) patients very seldom went out of their institution, preventing from direct outdoor aerosols exposure, (2) some of the nursing homes that presented several PF cases were not surrounded by cooling towers, and (3) the plausibility for cooling towers to be associated with increasing concentrations of *Legionella* in shower water and aerosol is low.

For ethical reasons, *Legionella* concentrations were only carried out at the end of the follow-up, encompassing, in each institution, one measurement for hot water, and one for bioaerosols. Using this point information to assess exposure relies on the assumption of the stability of *Legionella* levels in the hot water networks and in the generated aerosols. A follow-up of hot water quality was

undergone in two nursing homes, one with a high level of contamination (18 days, 21 water samples), the other one with less severe contamination (5 days, 42 water samples). In the latter, no cultivable or hybridized *Legionella* was detected during the follow-up whatever the point of shower (no graph presented). Figure 1 exhibits the results for the former (two showers). Shower point A was located close to the production of hot water; its temperature was on average 46.2°C. Shower point B was located further away of hot water production, with an average temperature of 50.1°C. This figure shows a variability of *Legionella* concentrations (FISH) about 1 log on average, which is close to the uncertainty of the detection method and in accord with microbial fluctuations usually found in water distribution systems.[41-43] One reason might be that all our water samples were taken after 7 minutes of flushing, meaning they represented water flowing in the drain, not merely water accumulated in the final shower pipe. Thus, the assumption of stability of the bacterial concentrations over the study period seems reasonable.

Figure 1: Repeated measurements of bacterial concentrations (total number of cells measured by DAPI staining, number of hybridized *Legionella* and number of culturable *Legionella* in the hot water of two showers (A and B) in one nursery home (follow-up of 18 days).

Another limitation of this study resides in the semi-ecological character of exposure assessment, since groups of elderly subjects were ascribed a ward or building estimate of *Legionella* exposure, after careful assessment of the hot water system homogeneity. This study design tends to lessen statistical power but does not bias point estimates when average group exposure is correctly represented by the measured concentrations.[44, 45]

Airborne *Legionella* exposure related to Pontiac fever

The originality of this study is to lean on a 4 months follow-up of an elderly population living in nursing homes rather than on an epidemic situation. To our knowledge, this is the first study which

assessed *Legionella* exposure in a non epidemic framework. Several scenarios were elaborated to assess exposure while showering. Scenarios with culturable *Legionella* detected in hot water showed no association with PF, except a non significant risk increase above a culturable *Legionella* spp exposure threshold of 10^4 CFU/L (RR=2.4). Among them, 78.3 % were *Legionella pneumophila*, the remaining 21.7% were non *pneumophila* *Legionella* species that we have not been able to identify ~~to not~~. This is in the scope of those described during PF epidemics, ranging from 9×10^4 CFU/L to 3×10^8 CFU/L (*Legionella pneumophila*).[3, 33, 38]

We chose to contrast increasing *Legionella* concentrations to a common “no exposure” level in a view to assess whether risks were augmented above a given threshold, information that would be meaningful in terms of risk management. A limit value of 10^3 culturable *Legionella* (CFU)/L of water has been set by the European Commission and the French Ministry of Health to prevent the risk of Legionnaires’ disease.[46, 47] Our results do not show an increased risk of Pontiac fever at this level, but –putatively- beyond 10^4 *Legionella* spp (CFU)/L of water. Because our population is composed of elderly subjects, one might infer that this *Legionella* level of 10^3 CFU/L of water protects the general population against the risk of PF. Whether this 10^3 *Legionella*-CFU/L threshold also offers a margin of safety for Legionnaires’ disease warrants further evaluation. To assess this hypothesis one would need, among others, information about the differential pathogenesis of PF and Legionnaires’ disease.

These *Legionella* thresholds are established on culture-based approaches. Even if culture is the standard method for environmental samples, numbers of studies have proved that it underestimates the true number of viable microorganisms in environments, including aerosols.[28, 31] The FISH technique, alternative to culture, was used to detect *Legionella*. An excess of PF fever incidence was obtained above 10^5 hybridized *Legionella*-cells/L of hot water, as it was suggested above 10^4 CFU/L for culturable *Legionella* spp. The 1 log difference between the values for FISH and culture is not surprising and highlights that (1) environmental bacteria have a low culturability, (2) a

fraction of the *Legionella* population in hot water are physiologically active (i.e. have enough intact rRNA to be detected by FISH), but are not culturable within 10 days. FISH might yield useful information on the *Legionella* physiology even if it is not considered as a viability marker.[28]

Of interest is the finding that incidence of PF might increase with airborne *Legionella* spp concentrations above 10^3 *Legionella*-cells/L of air (p for trend = 0.14, table 4). This analysis, strictly based on cases associated with aerosolized *Legionella*, backs the view that inhalation of aerosol is the true route of exposure.

To conclude, this study is the first one, to our knowledge, that quantifies the risk related to airborne *Legionella* produced by shower aerosols. According to the study definition of PF, it suggests excesses of PF incidence for *Legionella* spp concentrations above 10^4 CFU/L and 10^5 hybridized *Legionella*-cells/L (FISH) of water and, more putatively, above 10^3 hybridized *Legionella*-cells/L of air (FISH). However, extrapolation of these findings to Legionnaires' disease or to the general population should be proposed with caution. They need confirmation in various settings and for different populations.

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What does this study add ?

- Epidemiology of Pontiac fever (PF) is not as well characterized and the reasons why *Legionella* contaminated water may result in Legionnaires' disease or in PF are still not well understood.
- There is a risk of PF in a non epidemic framework in a nursing homes population to airborne *Legionella* produced by shower aerosols.
- The PF incidence increased for *Legionella* spp concentrations above 10^4 CFU/L (measured by culture) and 10^5 hybridized *Legionella*-cells/L (measured by in situ hybridization, FISH) of water, and, more putatively, above 10^3 hybridized *Legionella*-cells/L of air (FISH).
- A significantly higher risk of Pontiac-like episodes was seen for elderly subjects receiving corticosteroid therapy.

Competing interests

We declare that we do not have a commercial or other association that might pose a conflict of interest.

Authors' consent to publication

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