

Exposure to Legionellaceae at a hot spring spa: a prospective clinical and serological study

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SUMMARY

Following the occurrence of five cases of Legionnaires' disease among patients and therapists at a French hot spring spa, a series of cleansing procedures and an epidemiological study were undertaken. During a 3-month period, the spring water was repeatedly sampled. Serum samples were taken from 689 randomly selected patients, 230 therapists, 134 administrative staff and a control group of 904 blood donors.

Legionellaceae were present in the spring water at concentrations of 10^3 - 10^5 colony forming units/l. Fifteen different species or serogroups were isolated with *Legionella pneumophila* serogroups 3 and 1 predominating. No clinical cases of Legionnaires disease were observed during the study. However, 11% of the therapists and 5% of the patients either had a high titre of antibody (≥ 256) to at least one species or serogroup or seroconverted during the study. Mean antibody titres in the three study groups were significantly higher than those in the blood donors against 11 of the 32 legionella antigens tested. Nine of these 11 antigens corresponded to species or serogroups isolated from the spring water. The highest mean antibody titres in all three study groups were against *L. pneumophila* serogroup 3, the most common legionella in the spring water.

These findings have important implications for the maintenance of adequate standards of hygiene, bacteriological sampling and clinical surveillance in this and similar establishments.

INTRODUCTION

In 1986, five cases of Legionnaires' disease were diagnosed among patients and therapists at a hot spring spa in France. Three were due to *Legionella pneumophila* serogroup 3 and two to *L. pneumophila* serogroup 1. Bacteriological culture of the spring water subsequently yielded large numbers of Legionellaceae. Despite the mild nature of the clinical cases, a cleaning programme was immediately

undertaken and an epidemiological study carried out. We present the results of this 3-month study, which included systematic sampling of the spring waters and serological testing of patients, staff and a control group of blood donors. To our knowledge this is the first study of Legionellaceae in hot spring water used for therapeutic purposes.

MATERIALS AND METHODS

The spa was situated in the eastern region of France (Savoie), at an altitude of 260 m. The spring water had a temperature of 39–45 °C and contained mainly sulphates and thiosulphates as the calcium and sodium salts. Approximately 50000 patients, most with arthritis, receive treatments at the spa each year. Treatments last for 3 weeks and include aerosols, massages, swimming pool baths and mud baths.

Bacteriological sampling

Bacteriological sampling was carried out at the spa on eight occasions over a 3-month period. On each occasion, samples were taken at 9 representative sites, namely 2 springs, 2 holding tanks, 2 shower heads, 2 treatment room taps and 1 swimming pool. Depending on the site, 1–10 litres of water were taken and concentrated by continuous centrifugation and/or filtration (0.22 µm Nucleopore filter). Deposits were cultured on buffered charcoal yeast extract (BCYE) agar, either with or without antibiotics, or on glycine–vancomycin–polymyxin (GVP) agar (Bornstein *et al.* 1985).

Cleansing

Because of the waters' therapeutic function, it could not be chlorinated. All the equipment in the treatment rooms was thoroughly cleaned daily since the observations of cases of legionellosis. This included dismantling of the taps and shower heads for chlorination and heat treatment. On two occasions during the study, the springs were diverted and the tanks and piping drained and scoured.

Clinical and serological studies

With the help of local doctors, 689 patients were randomly selected. A clinical record sheet was completed at the beginning and the end of each patients' 3-week treatment period. Three serum samples were taken: on arrival, after 14 days and 3 or 4 weeks after departure. Seventy percent of the patients were women and 96% were aged over 40 years. Their place of residence and socio-economic status were varied. Only 17% had never had a course of treatment at a spa, whilst 65% had had at least two; 80% of patients had arthritis, mostly without other significant past medical history.

A single serum sample was taken from 364 members of staff at the spa including 230 therapists and 134 administrators. Seventy percent of the staff were women; their mean age was 33 years. Sera were also tested from a control group of 904 blood donors drawn from various regions including that of the spa.

All the serological studies were carried out by indirect immunofluorescence assay (IFA) using 32 antigens prepared in the laboratory (Taylor *et al.* 1979), from

Table 1. Percentages of the study groups with insignificant, borderline or positive titres of serum antibody to at least one legionella antigen

	Therapists	Patients	Administrative staff
Negative			
≤ 32 <i>L. pneumophila</i> } ≤ 64 other Legionellaceae }	57	85	94
Borderline			
64–128 <i>L. pneumophila</i> } 128 other Legionellaceae }	32	10	5
Positive			
≥ 256, single sample	11	1	1
Seroconversions			
≤ <i>L. pneumophila</i> } ≥ 256 other Legionellaceae }	—	4	—

both recognized species and serogroups and isolates from the spring water, including two isolates as yet unidentified: *L. pneumophila* serogroups 1 (Philadelphia, Pontiac, Albuquerque, Knoxville, Bellingham and Olda strains) 2, 3, 4, 5, 6, 7, 8 and 9, *L. micdadei*, *L. dumoffii*, *L. bozemanii* 1 and 2, *L. gormanii*, *L. longbeachae* 1 and 2, *L. anisa*, *L. jordani*, *L. parisiensis*, *L. erythra*, *L. rubrilucens*, *L. wadsworthii*, *L. sainthelensis*, *L. oakridgensis*, *L. feeleeii* 1 and *Legionella* spp.

To calculate the geometric mean of the antibody titres all sera with an antibody titre of < 16 were arbitrarily assigned a titre of 8. The results were analysed with the assistance of the computer research unit of the International Centre for Cancer Research (CIRC), Lyon. Bonferroni's test was used to determine the statistical significance of differences (Jones & Rushton, 1982).

RESULTS

The samples of spring water taken at the beginning of the study yielded legionellaceae at densities of 10^3 – 10^5 colony forming units (c.f.u.). Fifteen different species or serogroups were identified: *L. pneumophila* serogroups 1, 2, 3, 4, 6, 8 and 9, *L. dumoffii*, *L. gormanii*, *L. rubrilucens*, *L. erythra*, *L. anisa*, *L. oakridgensis* and two *Legionella* spp. The most common isolates were *L. pneumophila* serogroups 3 and 1 and *L. dumoffii*.

The cleansing of the spring water system led to an approximately 100-fold reduction in the density of legionella. However, these reductions were maintained for only about 1 month. The measures taken in the treatment rooms led to the disappearance of *L. pneumophila* serogroup 3, although this remained the most common isolate in the springs and tanks.

During the 3 months of the study, no clinical case of Legionnaires' disease occurred among the patients or staff in the study.

Using conventional criteria for a negative, borderline or positive (seroconversion or presumptive) serological diagnosis (Wilkinson, Fikes & Cruce, 1979), there were significant differences between the three study populations ($p \leq 0.05$) (Table 1). The highest frequency of positive or borderline reactions was seen in the therapists

Table 2. Relationships between the species and serogroups of Legionellaceae isolated from the spa water and geometric mean titres of antibody in the four groups

Species/ Serogroup	Isolated from the water	Geometric mean antibody titres			
		Therapists	Patients	Adminis- trative staff	Blood donors
<i>L. pneumophila</i> 1	+	9.1**	8.9	8.1	8.1
<i>L. pneumophila</i> 2	+	8.1	8.1	8.1	8
<i>L. pneumophila</i> 3	+	59.7**	20.5**	15.7*	8.1
<i>L. pneumophila</i> 4	+	9.1**	8.5	8.1	8.2
<i>L. pneumophila</i> 5	+	9.6**	8.4	8.4	8.4
<i>L. pneumophila</i> 6	+	11.1**	9.4*	8.1	8.4
<i>L. pneumophila</i> 8	+	18.1**	10.8	9.4	8.9
<i>L. anisa</i>	+	11.8**	8.4	10.5**	8.2
<i>L. rubrilucens</i>	+	8.2	8.1	8	8.4
<i>L. erythra</i>	+	8.5	8.3	8	8.7
<i>L. dumoffii</i>	+	9.2	8.4	8.2	9.1
<i>L. oakridgensis</i>	+	8.2	8	10.1**	8
<i>L. gormanii</i>	+	10.4	8.5	8	8.7
<i>L. saintelensis</i>	-	23.8**	8.4	19.8**	10.5
<i>L. wadsworthii</i>	-	10**	8	8.4	8.3
<i>L. spp</i> 1	+	9.5	8.8	8.8	8.4
<i>L. spp</i> 2	+	11.5**	10.2**	10.1**	8.4

Bonferroni's test. Significance: **1%, *5%.

Table 3. Titres of anti-*L. pneumophila* serogroup 3 antibody in the four groups (in percentages)

Antibody titre	Patients	Therapists	Administrative staff	Blood donors
< 16	87	41	89	99
16-32	4	25	7	1
64-128	7	26	3	0
≥ 256	2	8	1	0

and the lowest in the administrative staff. Among patients, there was no significant association between serological status and either the number of previous treatments at the spa or the type of treatment given.

Table 2 shows that the mean titres of antibodies among the therapists, the patients and the administrative staff were significantly higher than those of the blood donors against 10, 3 and 5 respectively of the 32 antigens tested. This represented a total of 11 different species or serogroups, 9 of which were among the 15 species or serogroups isolated from the spring water.

The highest mean titres of antibodies in all three study groups were against *L. pneumophila* serogroup 3, the serogroup most frequently isolated from the spring water. As for all but one antigen, the therapists had higher mean titres against *L. pneumophila* serogroup 3 than the other study groups (Tables 2 and 3).

The prevalence of antibodies against *L. pneumophila* serogroup 3 among first-

Table 4. Changes in geometric mean antibody titres against *L. pneumophila* serogroup 3 in first-time and other patients

Blood donors	Patients			
	First treatment		≥ Two treatments	
	1st serum	3rd serum	1st serum	3rd serum
8.1	8.1	23.7	10.9	24.1

time patients was extremely low with mean titres identical to those of the blood donors (Table 4). However, mean titres rose significantly ($p \leq 0.01$) between arrival and 1 month later among both first-time and other patients.

DISCUSSION

Legionellaceae can be found in all types of natural water except salt water; they are particularly prevalent in warm water (Fliermans, 1985). To our knowledge, however, this is the first study of Legionellaceae in hot spring water used for therapeutic purposes. We isolated a remarkably large number of species and serogroups from the spring water: 15 in all. It is probable that other species went undetected, owing to the practical difficulties of distinguishing them, especially when the density of Legionellaceae was high.

The demonstration of a direct link between the contaminated spring water and the appearance of cases of Legionnaires' disease was not possible: none of the initial clinical cases yielded a causative organism and no further cases were observed during the study. Our study therefore focused on serological comparisons. It showed a clear association between exposure to the hot spring water and antibody titres against the majority of the species and serogroups of legionella isolated.

The blood donors and the first-time patients on arrival rarely had raised antibody titres against the Legionellaceae in the spring water. In contrast, the therapists, the study group with the greatest exposure to the spring water, had raised mean antibody titres against the largest number of legionella antigens. They also had higher mean titres against individual antigens than the other study groups (Table 2). The highest antibody titres in all the exposed groups were against *L. pneumophila* serogroup 3, the most abundant legionella in the spring water (Tables 2 and 3). The mean antibody titres against serogroup 3 among patients also rose significantly following exposure (Table 4).

Despite the abundance and variety of Legionellaceae in the water, the clinically apparent cases which led to the study were few in number and mild. Furthermore, although several seroconversions were recorded during the study (Table 1), no further clinical cases were observed. Although we studied only about 1% of the annual number of patients attending the spa, the sample was large enough to indicate that the incidence of sporadic cases is low. This may be due to several factors: the strains of Legionellaceae in the spa water may be of low virulence; most of the subjects tested were women who may be relatively less susceptible

than men to the Legionellaceae; repeated exposure to the water may induce immunity. In addition, the cleaning procedures carried out after the cases of Legionnaires' disease were recognized, despite their relative lack of success bacteriologically, may nevertheless have reduced the risk of clinical infection.

In conclusion, this study casts further light on the ecology of the Legionellaceae and the epidemiology of human exposure to them. Although only mild cases of infection were encountered, the findings have important implications for the maintenance of adequate standards of hygiene and clinical surveillance in this and similar establishments.

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ADDENDUM

Since the submission of this paper, a few cases of legionellosis (one bacteriologically documented) have been observed in another French spring spa.

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