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An outbreak of pneumonia and meningitis caused by a previously undescribed Gram-negative bacterium in a hot spring spa

B. HUBERT[1], A. DE MAHENGE[2], F. GRIMONT[3], C RICHARD[3], Y. PELOUX[4], C. DE MAHENGE[5] J. FLEURETTE[6] AND P. A. D. GRIMONT[3]

[1] *Direction Générale de la Santé, Bureau des Maladies transmissibles, 1 place Fontenoy, 75350 Paris 07 SP, France,* [2] *Service de Médecine, Centre Hospitalier, Manosque,* [3] *Unité des entérobactéries, Unité INSERM 199 Institut Pasteur, Paris,* [4] *Laboratoire de Bactériologie Hôpital de la Conception, Marseille,* [5] *Laboratoire d'Analyses Médicales, Manosque,* [6] *Centre National de Référence des Légionelloses, Laboratoire National de la Santé, Lyon*

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SUMMARY

An outbreak of infection caused by a previously undescribed Gram-negative bacterium affected people attending a hot (37 °C) spring spa in France in 1987. Thirty-five case of pneumonia and two cases of meningitis occurred. None of these patients died. Attack rates were significantly higher for patients above 70 years old and for male patients. An epidemiological comparison of the 26 hospitalized cases with 52 matched controls suggests that spa treatment early on the first day (OR = 4·8) and attendance at the vapour baths (OR = 10·7) were significant risk factors for acquiring the infection. Person-to-person spread was not thought to have occurred. The same bacterium was isolated from the hot spring water. All strains studied shows a single rRNA gene restriction pattern. Epidemiological data indicated that the thermal water was the source of infection. This outbreak stresses the need for increased surveillance of infections in people attending hot spring spas.

INTRODUCTION

Investigation of epidemics usually contributes to understanding the transmission of infectious diseases. This is of particular importance when new organisms are identified. A recent example is the epidemic of Legionnaire's disease which occurred in Philadelphia in 1976 [1]. Since then, *Legionella* spp. have been found to be a common contaminant of hot water systems; aerosols from contaminated cooling towers, whirlpool baths and showers have been epidemiologically associated with legionellosis [2].

An outbreak of pneumonia and meningitis occurred in 1987 in Southern France among persons who attended a hot spring spa. This outbreak was caused by a bacterium which was not previously recognized as a cause of human disease. The features of this epidemic were a low attack rate and the difficulty in isolating the bacterium more than once from the thermal spring water despite intensive and prolonged investigations.

We report here the epidemiological investigations in order to document the epidemiology of this still unclassified bacterium (UCB) in the medical literature and to stress the need for an increased surveillance of disease associated with the use of hot water systems.

BACKGROUND

Gréoux-les-Bains is a village of 1800 inhabitants in Southern France (Alpes de Haute Provence), well-known for its hot spring spa. In 1987, 36400 patients attended the spa for rheumatic (80%) or respiratory tract (20%) afflictions.

Thermal spring care

The spring water is at a temperature of 37 °C and contains mainly sulphates and thiosulphates. The spa building comprises seven units on two floors. Thermal spring care consists of a combination of 3 to 6 treatments selected from 22 available treatments (aerosols, massages, swimming pool, mud baths, vapour baths). The treatment is prescribed for 3 weeks. During the first day of treatment, patients attend units in which places are available, but for the following days, they are given a timetable for attendance at the prescribed units. Daily duration of care is about 1 h for respiratory treatment and 2 h for rheumatic subjects.

Spa patients come from every part of France and they stay in hotels, camp sites or, more frequently, in rented accommodation.

Description of the epidemic

In October 1987, Manosque hospital (11 kilometres away from Gréoux-les-Bains) notified the public health authorities of an increase in hospitalization for pneumonia, originating mainly from Gréoux-les-Bains. A Gram-negative rod, originally identified as *Pseudomonas* spp. or *Flavobacterium* spp. and then identified as a still unclassified bacterium, was isolated from blood cultures of some of these patients and from cerebrospinal fluid of two patients with purulent meningitis. Preliminary investigations suggested that thermal spring care was a possible common source of contamination. The spa activities were thus suspended on 12 December 1987 by decision of the health authorities.

METHODS

Epidemiological surveys

Case definition

A definite case was defined as a person with an acute illness, pneumonia or meningitis, with isolation of the UCB from blood culture, CSF or respiratory secretions. A probable case was defined as a person (i) with clinical and radiological focal signs of pneumonia at chest examination, and (ii) no identified pathogens, and (iii) with onset of illness when staying in Gréoux-les-Bains during the epidemic period (1 August to 1 December 1987).

Case ascertainment

Manosque hospital survey: We first reviewed the records of all patients in the Medicine Unit since August 1986 to identify patients in whom a pulmonary infiltrate was diagnosed at the admission.

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Gréoux-les-Bains survey: To define the extent of the outbreak, the 31 physicians working in Gréoux-les-Bains were asked to identify and complete a form for each patient with clinical and radiological focal signs of pneumonia diagnosed between 1 April and 1 December 1987. To compare the rate of pneumonia between spa patients and other residents in Gréoux-les-Bains, an average daily number of persons was calculated for each month and for both of these populations. The average number of patients was calculated by the number of person-days of care in a month divided by the number of working days in that month. The average number of other residents was calculated by adding the population living in Gréoux-les-Bains and an estimated number of tourists not attending the spa (mostly spouses of patients).

The observed rates were compared to the results of a previously published survey of pneumonia in the city of Nottingham, United Kingdom, in 1984 in which an incidence of 4·7/1000 adult population per year was reported [3].

Case-control study

To determine the source of contamination, a case-control study was undertaken with the 26 definite and probable cases hospitalized in Manosque. Controls were chosen among subjects attending the spa at the same period without respiratory illness. Cases and controls were matched for date of beginning thermal spring care, purpose of care (rheumatic or respiratory), sex, and age within 5 years. Two controls were obtained for each case. A questionnaire was mailed to the cases and controls on 15 December, 1987. Information collected included place of residence in Gréoux-les-Bains, attendance at restaurant, spare time activities; occurrence of illness during the stay; date, duration and units of the prescribed care with particular emphasis on the first day; smoking history, medical history, and medication (including steroid therapy).

Statistical analysis

Probability values for two-by-two tables were calculated by two-tailed Fisher's exact test. Odds ratios and confidence intervals were calculated by the Taylor method. Continuous variables were compared by Student t test. All variables associated with pneumonia were assessed by unconditional logistic regression with BMDP software [4].

Environmental investigations

Changes in the internal and external environment of the thermal building and weather records for Gréoux-les-Bains were reviewed. During a 2-month period, multiple hot spring water samples were collected from representative sites.

Microbiological investigations

rRNA-gene restriction patterns were obtained for each isolate from patients and from water. Briefly, the cells were lysed, and DNA was extracted, cleaved by restriction enzyme *Eco* RI or by *Bam* HI. Restriction fragments obtained were separated by electrophoresis in 0·8% (wt/vol) agarose gel in Tris-borate buffer (tris-borate 0·089 M, boric acid 0·089 M, EDTA 0·08 M), and transferred into a nylon membrane [5]. The membrane was pre-hybridized, and hybridized at 65 °C for 17 h with acetylaminofluorene-labelled ribosomal 16+23 S RNA from

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Escherichia coli (Eurogentec S. A., Liège, Belgium), washed and revealed by immunodetection as described by Grimont and co-workers [6].

RESULTS

Clinical illness

The UCB was isolated from ten patients. Two clinical forms were observed among these patients. Pneumonia was observed in eight cases. Earliest symptoms were chills, rapidly rising fever above 39 °C, pleuritic chest pain, haemoptoïc and productive cough. Chest X-rays were abnormal in all cases and exhibited a lobar infiltrate or consolidation remaining unilateral. No cavitation was seen. Purulent meningitis was observed in two cases. One was associated with pneumonia, the other did not present respiratory signs. None of these ten patients died.

Laboratory analysis showed white-cell counts of over 10000 per mm^3. Isolates were recovered from two successive blood cultures (eight patients), respiratory secretions (one patient), and cerebrospinal fluid (two patients) by three different laboratories. For every case, serological tests for legionella, chlamydia, rickettsia and mycoplasma were negative.

Description of the UCB

A preliminary description of the bacterium has been published [7]. The main diagnostic features are as follow. The isolates of UCB grow on chocolate agar at 41 °C. The isolates have polar flagella, are yellow pigmented, Gram negative, strictly aerobic, oxidase positive and non-fermentative rods. Nitrates are reduced to nitrites and indole is produced in peptone water in 24 h.

A complete description of the organism, including its 16 S rRNA sequence and rRNA-DNA hybridization showing that the organism belongs to a new genus, will be published elsewhere.

Epidemiological findings

We first examined the reality and the extent of the outbreak. In 1987, 49 patients with pneumonia were hospitalized in Manosque. Of these, 37 cases (73%) clustered between 1 August and 1 December, 1987. During these 4 months, 34 cases (89%) originated from Gréoux-les-Bains. Only eight cases of pneumonia (four from Gréoux-les-Bains) were hospitalized during the previous year between 1 August and 1 December, 1986, thus ruling out seasonal variation.

The Gréoux-les-Bains survey identified 50 patients with pneumonia between 1 April and 1 December 1987, 44 of whom were spa attenders. The monthly incidence rate of illness was increased among spa attenders between 1 August and 1 December (Fig. 1). During this 4-month period, the incidence rate was 12·6/1000 population among persons attending the spa and 1·3/1000 among the other persons staying in Gréoux-les-Bains ($P < 0{\cdot}001$). The latter rate does not differ significantly from the average incidence rate previously observed in Nottingham in a 4-month period (1·6/1000).

During the epidemic period, 37 cases meeting the case criteria were identified among subjects attending the spa. Of these, 10 were definite cases and 27 probable cases. All 10 definite cases and 16 probable cases were hospitalized. Eight of these

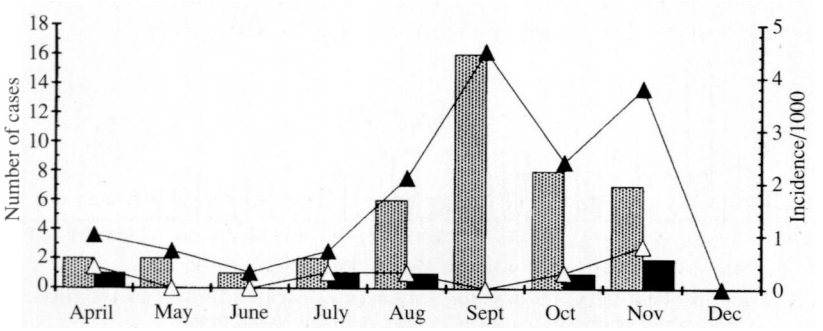

Fig. 1. Pneumonia in Gréoux-les-Bains residents by month of diagnosis (1 April to 1 December 1987). Number of cases and incidence rates per 1000 population in spa patients and Gréoux-les-Bains residents. Number of cases: ▨, spa; ■, community. Incidence: —▲—, spa; —△—, community.

Table 1. *Pneumonia cases among spa patients above 50 years old (August 1 to 1 December 1987)*

(Attack rates according to age, sex, and purpose of care.)

	No. of cases	Number of patients	Rate for 1000 patients	OR*	95% CI
Age					
50–59 years	10	4486	2·2 } 2·1		
60–69 years	14	6680	2·1 }	1	
> = 70 years	13	2815	4·6	2·1	1·0–4·2
Purpose of care					
Rheumatology	35	11915	2·9	4·7	1·1–20
Respiratory	2	2066	1·0	1	
Sex					
Male	32	4929	6·5	12·8	5·0–33·1
Female	5	9052	0·6	1	
Total	37	13981	2·6		

* Odds ratios adjusted for age, sex, and purpose of care.

16 probable cases were given antibiotic treatment before blood cultures were performed and the other eight had negative blood cultures but no cultures of respiratory secretions were performed. The other 11 probable cases were treated as outpatients and did not undergo microbiological investigation.

None of the 385 employees at the spa met the case criteria. During the epidemic period, 16465 patients attended the spa; no cases were observed among the 2484 patients under 50 years old. The age of the 37 cases ranged from 50 to 81 years, with a mean of 65·5 years. The attack rate was significantly higher for patients over 70 years. For patients over 50 years of age, the incidence rate was 6·5/1000 for men and 0·6/1000 for women. All but two were rheumatic patients (Table 1).

For the 37 cases from the spa, a comparison of the date of onset with the date of first treatment enables the possible range of incubation period to be estimated (Fig. 2). The overall range was 1–17 days and 48% of cases occurred in the first week of spa treatment. The range for definite cases was 2–17 days.

Comparison of the 26 hospitalized cases with 52 matched controls showed no

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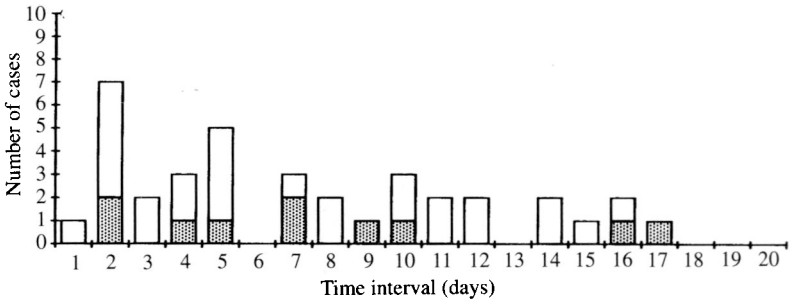

Fig. 2. Time interval between first day of thermal care and onset of the disease for probable cases (□) and definite cases (▨).

Table 2. *Comparison of cases and controls for attendance at vapour bath and for time of care*

	Cases (%)	Controls (%)	OR*	OR†	CI 95%†	p†
First day	$n = 23$	$n = 48$				
Attendance at vapour bath	74	46	3·4	4	1·2–13	0·01
Time of care before 09.00	26	8	3·9	4·8	1·5–15	0·03
Next days	$n = 26$	$n = 52$				
Attendance at vapour bath	84	48	5·9	10·7	2·5–45	< 0·001
Time of care before 09.00	50	33	2·1	2·7	0·9–8·5	0·07

* Odds ratios not adjusted.
† Odds ratios adjusted for age, attendance at vapour bath, time of care.

significant difference between these two groups for place of residence. No restaurants, organized tours or leisure centres were found to be significantly associated with occurrence of illness. Forty-six percent of the cases and 38% of the controls were accompanied – in most cases by their spouse. None of these persons presented respiratory ailments.

On the first day of spa treatment, 26% of the cases and 8% of the controls attended the spa before 09.00. In the following days, these percentages rose to 50% for the cases and 33% for the controls. Attendance at vapour baths was significantly more frequent for the cases (84%) than for the controls (48%). As the attendance at the vapour bath by the controls was associated with age and time of care, we performed a logistic regression including age, attendance at vapour bath and time of care (Table 2). No differences were found between the two groups for attendance at other treatments. The cases reported using the same units as the controls for attendance at the vapour bath and for attendance at other treatments. The average daily numbers of treatments were similar in both groups (4·4 for cases and 4·2 for controls, NS).

The cases did not have different medical history from the controls (at least one of the following: pneumonia, chronic bronchitis, asthma, emphysema, diabetes mellitus, neoplasm or tabagism) (42 *v.* 31%, OR = 1·6, CI 95% = 0·5–4·8, *P* value = 0·3). There was no difference in medications between cases and controls.

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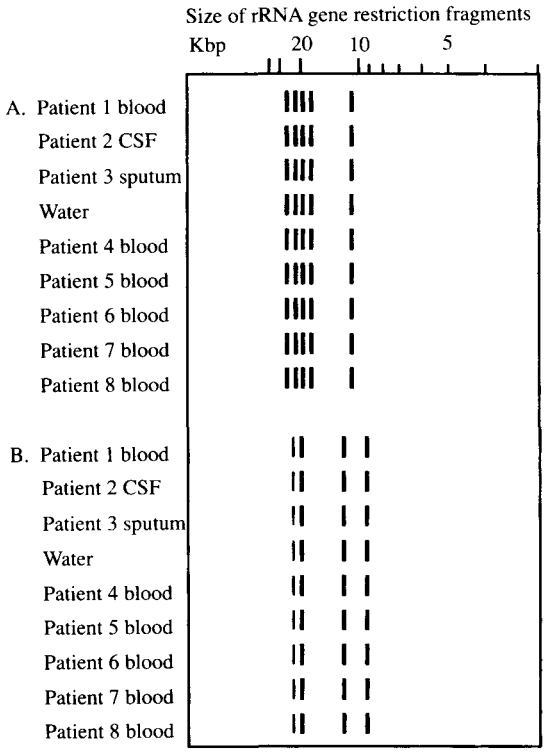

Fig. 3. Normalized graph showing migration patterns of rRNA gene restriction fragments after cleavage by restriction enzymes *Eco* RI (A), or *Bam* HI (B) and hybridization with acetylaminofluorene-labelled *E. coli* 16+23 S rRNA.

Environmental findings

Eight samples of water were collected in different pipes early in October 1987. *Pseudomonas cepacia*, *P. aeruginosa* and *Flavobacterium balustinum* were identified in six samples. In one of these samples collected in a pipe leading to a respiratory care unit, culture yielded the UCB. rRNA gene restriction patterns, obtained for each isolate of the UCB recovered from patients and from water, showed the isolates to be one strain whether DNAs were cleaved by *Eco* RI or by *Bam* HI (Fig. 3). Further samples taken after December 1987 yielded *Legionella* spp. and *Pseudomonas* spp. but not the UCB.

Within the spa building, we focused our attention on three main events. First, the central air-conditioning system had been functional since March 1987 but air sampling did not yield the UCB. During the summer, excavation was carried out near the water pipes. On 25 August a heavy rainfall (65 mm) led to a flash flood in the hall of the building. However, investigations into drinking water, rivers and soil surrounding the spa building did not detect the UCB.

DISCUSSION

Between 1 August and 1 December, 1987, an outbreak of pneumonia and meningitis occurred among persons attending the spa at Gréoux-les-Bains. The recognition of this outbreak was possible mainly because of the use of a single

county hospital for health care and the large number of people attending the spa. The attack rate was rather low but some cases might not have been attributed to the UCB infection, as the case definition for probable cases agrees with the clinical symptoms of the definite cases. As it is unusual for a general practitioner to prescribe bacteriological examinations to investigate pulmonary infection, mild disease may not have been identified. Some pneumonia cases may have been diagnosed after patients returned home following their stay in Gréoux-les-Bains. However, as onset of illness occurred mainly in the first two weeks of spa treatment, it was assumed that very few recognized cases were missing from this survey.

As the UCB has never been previously described, its mode of spread is unknown. The hypothesis of air-borne transmission is consistent with clinical features and activities in the place at highest risk of exposure. As none of the family contacts had any respiratory ailment and as detailed examination showed no space–time clustering of cases inside the building, it is obvious that secondary person-to-person spread was unusual or non-existent.

The accurate date of exposure for each case is unknown, but it is assumed that an increased susceptibility to respiratory infection during the first days of care was induced by the aggressiveness of sulphur water. This permits an estimate of the possible range of incubation period between 2 and 17 days for the definite cases.

The highest risks of exposure are likely to have been attendance at vapour baths and treatment early in the morning, mainly during the first day of care. No specific care unit could be incriminated, implying that it was the nature of the treatment and not its localization which was involved. Vapour baths are small rooms in which thermal spring water is vaporized by pressurized air from a tank of a capacity of 3 litres. Vapour baths are the only places in which water supply is turned off at night; the water is thus stagnating in the tanks all night. The water supply is switched on at 05.00, 1 h before the first patients arrive for treatment. This could explain the increased risk of disease when exposed early in the morning.

As the UCB was isolated in a pipe leading to a respiratory care unit which was not a risk factor for the disease, we can assume that these bacteria were present in the hot water spring spa, but favourable growing conditions were found only in vapour baths. No alternative hypotheses are consistent with microbiological and epidemiological data. The vapour baths were definitively closed and water pipes (but not thermal spring water) were disinfected weekly by chlorination. The spa was re-opened in March 1988 and no further cases of infection by the UCB have been observed.

The reasons for the sudden appearance of this bacterium in August remain unclear. Excavation near the water pipes or the flood may be the source of introduction of the bacteria in thermal spring water, but hot water is likely to be a natural reservoir of these bacteria. Failure to culture further the UCB from water may be due to the time of sampling after the pipes were chlorinated in late November. Other possible reasons are insensitivity of the culture method and presence of high levels of competing organisms.

The epidemiological features of this outbreak are similar to those of Legionnaire's disease: a low attack rate, an increased susceptibility to the illness or to a more serious illness among elderly people, a high male:female incidence

ratio (12·8:1), and an air-borne spread from hot water. Characterization of the UCB will permit the development of sensitive methods of detection; this will allow extensive research in other hot spring spas and hot water systems.

Hot spring spas have been used for therapeutic purposes in Europe since the Roman era. Exposure to this water is thought, without scientific evidence, to be of some benefit to chronic diseases such as rheumatism. However, by law, this hot spring water cannot be treated by chlorination and it does contain various microorganisms. An outbreak of legionellosis has been previously described in a similar establishment [8]. This stresses the need for an increased surveillance of infections associated with the use of hot spring spas.

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