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Legionella infections in Scotland

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SUMMARY

Four thousand two hundred and thirty-five sera from 2794 patients and 740 sera from 735 tourists domiciled in Scotland recently returned from abroad were examined between 1977 and 1981 for antibodies to *Legionella pneumophila* and related organisms. In addition, specimens were examined from some patients for cultural or serological demonstration of these organisms in lung or sputum. One hundred and ten cases were diagnosed, 104 serologically, five by immunofluorescence demonstration of legionellas in lung biopsy or autopsy specimens and *L. pneumophila* serogroup 1 was isolated from a further case. Of patients with pneumonia 6·7 % showed evidence of legionella infection. Only 0·4 % of healthy tourists had antibodies at a level of $\geqslant 256$. The majority of cases occurred in patients aged 50–69 and in 36 patients the infection was acquired outside the United Kingdom. Males predominated, the male : female ratio being 2 : 1. Cases were less frequent in the first quarter of the year but those originating in Scotland were equally common in the remaining three quarters. Cases with disease acquired abroad added to indigenous cases resulted in a summer peak occurring in July, August and September. There were no outbreaks of legionellosis in Scotland.

INTRODUCTION

The first known cases of infection with *Legionella pneumophila* in Scotland occurred in 1973 in a group of tourists who had been on holiday in Benidorm in Spain (Lawson, 1978). Three tourists died and the diagnosis was established in retrospect by examination of sera or tissues at the Center for Disease Control (CDC), Atlanta, as was the diagnosis in three subsequent cases, one occurring in 1976 (Lees, Tyrrell & Boyd, 1977) and two in 1977. Details of the pathological findings in the cases occurring in 1973 and 1977 have been published by Boyd *et al.* (1978). Following the isolation of *L. pneumophila* (McDade *et al.* 1977) a culture was kindly provided by CDC and an ether-treated antigen was made from this as described by Fallon & Abraham (1979). Subsequently, heat-killed antigens made as described by Wilkinson, Fikes & Cruce (1979) were combined into a polyvalent antigen (Fallon & Abraham, 1978) and further polyvalent antigens were made as new serogroups of *L. pneumophila* and new *Legionella* sp. were described (Fallon & Abraham, 1982). Although we have examined sera from patients in several countries, this paper details the results obtained with sera from subjects in Scotland together with the results of direct fluorescent antibody (DFA) examination of tissues and secretions from patients, plus attempts at culture of the infecting agents.

MATERIALS AND METHODS

Strains of organisms

The following strains were received from CDC, Atlanta:

L. pneumophila serogroup 1 – Philadelphia-1,
L. pneumophila serogroup 2 – Togus,
L. pneumophila serogroup 3 – Bloomington-2,
L. pneumophila serogroup 4 – Los Angeles-1,
L. pneumophila serogroup 5 – Dallas i-E,
L. pneumophila serogroup 6 – Chicago-2,
L. (Fluoribacter) *bozemanii* – WIGA,
L. (Fluoribacter) *dumoffii* – Tex-KL,
L. (Fluoribacter) *gormanii* – LS-13,
L. (Tatlockia) *micdadei* – Tatlock.

In addition, two strains of *L. pneumophila*, designated P183 and P185, isolated from environmental sources were received from Dr J. O'H. Tobin. They do not react with antisera to any of the *L. pneumophila* serogroups yet described.

Preparation of antigens for IFAT

Heat-killed antigens were prepared as described by Fallon & Abraham (1979) until late 1978 when the method of Wilkinson *et al.* (1979) was adopted. Quadrivalent antigen (*L. pneumophila* serogroups 1–4) (Fallon & Abraham, 1978) was used from December 1978. An additional bivalent antigen containing *L. pneumophila* serogroups 5 and 6 was introduced in January 1980. A further trivalent antigen comprising *L. bozemanii*, *L. dumoffii* and *L. micdadei* was introduced in March 1980 and another trivalent antigen containing *L. pneumophila* strains P183 and P185 and *L. gormanii* in February 1981.

Indirect fluorescent antibody test (IFAT)

This was performed and interpreted as described by Fallon (1981). Positive results with polyvalent antigens were checked using appropriate monovalent antigens.

Human sera

These were forwarded for examination from two classes of individual, first patients with respiratory tract infection and second tourists who had recently returned from holidays abroad and who were being surveyed as part of an investigation by the Communicable Diseases (Scotland) Unit. Patients came from the whole of Scotland with the exception of the areas served by bacteriology laboratories in Aberdeen.

DFA test

This was as described by Cherry & McKinney (1978). Rabbit antisera to all the listed *Legionella* sp. were prepared as described by Cherry & McKinney (1978) except latterly when 2 ml of a dense suspension of organisms, heat-killed as for the preparation of antigen for the IFAT, was mixed with an equal volume of Freund's complete adjuvant, 2 ml of the resulting emulsion being injected beneath each scapula. Rabbits were test bled six weeks after this injection and were

exsanguinated when the serum titre was 16000 or greater when tested in an IFAT, booster injections being given if necessary.

Examination of pathological material

Sputum and other unfixed material such as lung taken by biopsy or at autopsy was examined by the DFAT, by inoculation on to charcoal yeast extract (CYE) agar (Horwitz & Silverstein, 1980) and by intraperitoneal inoculation into pairs of pre-bled guineapigs. Cultures were incubated in an atmosphere of 5–10 % CO_2 in air at 36 °C for 3 weeks before being discarded. Guineapigs were observed and temperatures taken daily. One of each pair was killed if it fell sick or after 4–5 days if there were no signs of illness. Peritoneal swabs were taken together with liver and spleen. These were examined by the DFAT, by subculture on CYE agar and spleen and liver were also examined by passage (after storage at −70 °C until CYE agar subcultures failed to demonstrate the presence of *Legionella* sp.) to the yolk sac of embryonated hen's eggs. Yolk sacs of eggs which died following inoculation were subcultured on to CYE agar. The remaining guineapig in each pair was bled 6 weeks after inoculation and serum examined for the development of antibody to *Legionella* sp. in parallel with the serum taken before inoculation. Formalin fixed lung was examined by the DFAT. Smears of lung portions were made as described by Cherry & McKinney (1978). Paraffin sections were treated by removing the paraffin and then smearing the sections on slides and heat fixing before staining.

RESULTS

The numbers of sera examined are shown in Table 1, together with the numbers of patients from whom they were derived. Paired sera were obtained from a substantial proportion of the patients with clinical disease whereas only single sera were obtained from almost all of the tourists surveyed. The nature of the notified clinical disease in the patients examined (based on a 10 % sample) was as shown in Table 2.

According to the request forms accompanying the sera 74 % of patients were said to have pneumonia or chest infection (including patients described as having a 'flu-like' illness). Subjects noted in Table 2 as having 'no chest infection' had a variety of symptoms such as 'chest pain', upper respiratory infection, malaise and weakness.

Details of the numbers of patients showing a high ($\geqslant 256$) or $\geqslant$ fourfold rise in antibody titre to *L. pneumophila* are shown in Table 3. Not all sera from cases detected before 1980 were examined with a wide range of antigens but the numbers of patients, out of 86 whose sera were examined with *L. pneumophila* antigens of serogroups 1–6 and strains P183 and P185, with a monospecific response, including those previously reported (Fallon & Abraham, 1982), are shown in Table 4. Fifty-three had a monospecific response including 11 to serogroups other than 1.

Three cases were diagnosed by direct immunofluorescent examination of lung taken at autopsy, two by examination of lung biopsy and one by examination of tracheal secretions. The three latter cases were ultimately fatal. All except one were due to serogroup 1 infection, the other case was infected with serogroup 3 *L. pneumophila*.

Table 1. *Details of origin of sera examined and the individual from whom they originated*

	Number of sera examined		Number of individuals	
	Clinical disease	Surveys	Clinical disease	Surveys
1977	22	—	11	—
1978	552	203	440	198
1979	899	182	485	182
1980	1327	55	737	55
1981	1435	300	1121	300
Total	4235	740	2794	735

Table 2. *Clinical conditions of* 2794 *patients from whom sera were received* (% *of total patients*)

Pneumonia	50
'Chest infection'	24
Pyrexia of unknown origin	6·5
No chest infection	13
No information given	6·5
Total	100

Table 3. *Numbers of patients with high or* ⩾ *fourfold rising titres of antibody to* L. pneumophila (*only the major rise is shown**)

Data sera received	Serogroup								% with rising titre
	1	2	3	4	5	6	P185	Total	
1977	1	0	0	0	0	0	0	1	—
1978	24	1	0	0	0	0	0	25	(36)
1979	33	2	0	0	0	0	0	35	(60)
1980	17	3	2	3	0	0	0	25	(68)
1981	12	2	1	1	1	0	1	18	(61)
Total	87	8	3	4	1	0	1	104	(56)

* Where an equal rise in titre to more than one serogroup was found, infection is recorded as due to serogroup 1.

Table 4. *Nature of serological response in* 86 *cases of legionella infection*

Monospecific serogroup 1	42
2	3
3	2
4	3
5	1
6	1
P185	1
Reaction with two serogroups	17
Reaction with three serogroups	10
Reaction with four or more	6
Total	86

Table 5. *Year of occurrence of illness in patients with evidence of legionella infection, or year of receipt of specimen in those with no history of disease*

	1974	1975	1976	1977	1978	1979	1980	1981	Total
Patients with disease									
seropositive	2	2	1	3	17	32	28	16	101
organisms demonstrated by DFA	—	—	—	—	—	3	—	3	6
No disease									
seropositive	—	—	—	—	2	1	—	—	3
									110

Table 6. *Disease in subjects with serological or direct immunofluorescent evidence of* L. pneumophila *infection*

Disease	1974	1975	1976	1977	1978	1979	1980	1981	Total
Pneumonia	2	1	1	3	16	30	25	16	94
'Influenza'	—	—	—	—	—	2	2	1	5
Fever	—	—	—	—	—	—	1	—	1
Cough and rigors	—	—	—	—	—	1	—	—	1
Bronchiectasis	—	—	—	—	—	1	—	—	1
Emphysema	—	—	—	—	—	—	—	1	1
Myocarditis	—	—	—	—	1	—	—	—	1
Endocarditis	—	—	—	—	—	1	—	—	1
Pseudobulbar palsy	—	—	—	—	—	—	—	1	1
No information	—	1	—	—	—	—	—	—	1
Not ill	—	—	—	—	2	1	—	—	3
Total	2	2	1	3	19	36	28	19	110

Isolation of *L. pneumophila* was attempted in five of these six cases but was only successful in the case where serogroup 1 *L. pneumophila* was demonstrated in tracheal secretions by DFA examination and subsequently by guineapig inoculation followed by growth of the organism on CYE agar inoculated with guineapig spleen and peritoneal exudate.

The corrected figures for year of disease (if any) in both seropositive and DFA positive cases are shown in Table 5. Eight subjects whose sera were examined in 1978 gave a history of pneumonia in previous years: two in 1974, two in 1975, one in 1976, and three in 1977. These all had disease due to serogroup 1. A further two subjects were discovered as part of the tourist survey but gave no history of illness. Three cases were diagnosed by direct immunofluorescence in 1979 and three (one confirmed by culture) in 1981. The reported clinical disease is shown in Table 6. The majority of cases presented as pneumonia and in some cases where clinical information was scanty and did not include 'pneumonia' in fact pneumonia may well have either been present or may subsequently have been manifest.

The 94 cases of pneumonia represent 6·7% of all patients with pneumonia examined (1394 on the basis of data in Table 2). Three subjects with a titre of $\geqslant 256$ discovered as a result of surveys represent 0·4% of well patients – a very low background level of antibody. One of the three was a member of a group which stayed at a hotel in England for a golf tournament following which several members of the group (including one of the 94 cases of pneumonia in the present study) developed legionella pneumonia (Tobin *et al.* 1981) and hence may have

Table 7: *Countries visited within incubation period of legionella infection by* 36 *clinical cases of disease*

		Country visited							
		Greece	Spain	Portu-gal	Yugo-slavia	Canada	Italy	Holland	Total
Serogroup	1	0	24	2	1	2	4	0	33
	2	1	1	0	0	0	0	0	2
	3	0	0	0	0	0	0	1	1
Total		1	25	2	1	2	4	1	36

developed his antibody as a result of contact with *L. pneumophila* without resulting clinical disease. The nine seropositive cases with other respiratory tract disease represent 1·3% of the estimated 670 cases with 'chest infection' as their notified diagnosis. Four cases, one of whom stayed in the hotel described by Tobin *et al.* (1981) shortly after the groups who took part in the golf tournament, were diagnosed on the basis of DFA examination of postmortem tissue alone. Three of these cases were of serogroup 1 and one was of serogroup 3. One case was diagnosed both by DFA and an antibody titre of 128 and one other fatal case by DFA examination of tracheal secretions, an antibody titre of 128 and isolation of serogroup 1 *L. pneumophila* from tracheal secretions.

L. pneumophila was isolated by intraperitoneal inoculation of four guineapigs, two of which became ill five days after the injection and which yielded serogroup 1 *L. pneumophila* following culture of peritoneal exudate, spleen and liver on CYE agar. The other two guineapigs remained well and did not develop antibody. The identity of the organism as *L. pneumophila* was confirmed by gas liquid chromatography performed by the Bacterial Metabolism Research Laboratory, Colindale.

Thirty-six of the seropositive subjects (32% of all 110 positive subjects) gave a history of overseas travel within the incubation period of legionella infection. Details are given in Table 7. Two of the cases with presumptive infection originating in Spain fell ill after visiting a hotel previously associated with an outbreak of legionella infection (Reid, Grist & Nájera, 1978) and where serogroup 1 *L. pneumophila* was subsequently isolated from the water supply (Bartlett cited by Reid, 1981). The age and sex of those with evidence of legionella infection are shown in Figure 1. In addition to those shown in the figure, there were three males whose age was not stated. There were 74 males and 36 females, a male: female ratio of 2:1 and in both sexes infection was commoner in older subjects. The average age was 52·6 years, the mode being 59·8 years. Fifty-one per cent of cases occurred in the age group of 50–69. The attack rate by age is shown in Table 8. In general, the figures varied little between the years and hence have been averaged for the 4 year period. The only notable deviation was in 1981 where the figure for the 60–69 year age group fell to 0·6 cases per 100000. The youngest case in the series was aged 22 and those cases where *L. pneumophila* was demonstrated by DFA examination were aged from 43 to 61 years.

Fig. 2 shows the month in which cases infected both in Scotland as well as those infected abroad occurred. There were fewer cases in the first quarter of the year than in the other quarters and, if the cases infected abroad are excluded, there

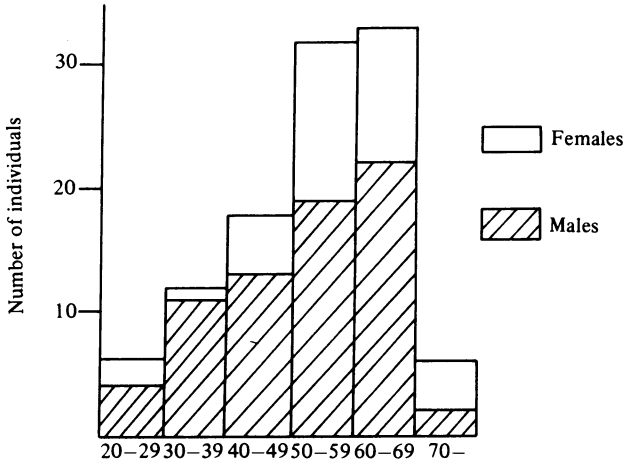

Fig. 1. Age and sex of 107 individuals with evidence of *L. pneumophila* infection in Scotland 1977–81.

Table 8. *Average age specific attack rate for legionella infection Scotland* 1978–81

Age	Average number of cases per year	Attack rate (per 100000)
20–29	1·2	0·16
30–39	3	0·46
40–49	4·75	0·8
50–59	8	1·3
60–69	7·5	1·5
70+	1·75	0·37
		0·73

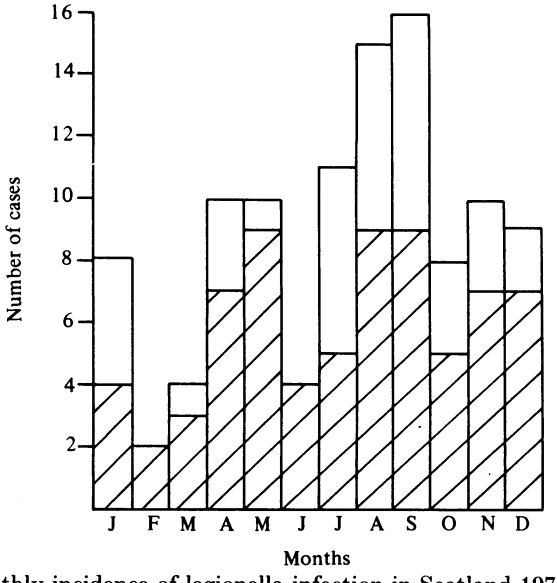

Fig. 2. Monthly incidence of legionella infection in Scotland 1977–81. Shaded areas represent numbers of cases who were not infected abroad.

was little difference between the remaining threequarters of the year. Taking all cases into consideration, the peak incidence was in July, August and September.

DISCUSSION

The development of diagnostic services for the detection of pneumonia and other infections due to an ever increasing number of legionella serogroups and related organisms has led to the discovery of a significant number of cases of disease due to these organisms, at least 6·7 % of cases of pneumonia in Scotland from whom sera or other specimens were submitted. Unfortunately, *L. pneumophila* and other legionellas and related species are seldom isolated from cases for a variety of reasons (e.g. lack of suitable culture media in many laboratories, scanty sputum production in many patients) so that confirmation of the serological findings by culture and characterization of the infecting organisms has seldom been possible. Nevertheless, the frequency of association of seropositivity with disease coupled with the infrequency of seropositivity in symptom-free individuals lends confidence that high and, more especially, fourfold or greater rising titres of antibody reflect legionella infection ($P < 0{\cdot}001$). The infrequency of antibodies in the tourists surveyed (who did not have clinical disease) contrasts with the high frequency of antibodies in normal subjects reported by some workers in the United States, for instance in Ohio (Snowman *et al.* 1982) where 19·2 % of healthy outdoor workers were found to have antibody titres $\geqslant 128$ to *L. pneumophila* serogroups 1 and 2 and 20·7 and 16·3 % of indoor workers to serogroups 1 and 2 respectively. The results in tourists agree well with surveys reported from England (Macrae, Appleton & Laverick, 1979) where 31 (1·5 %) of 2023 sera from healthy adults had antibodies to serogroup 1 *L. pneumophila* at a level of $\geqslant 32$ but only two (0·1 %) with a titre of 128. Although overall 6·7 % of cases of pneumonia showed evidence of legionella infection, the figure for any individual hospital may vary with higher figures obtaining in some instances.

Ninety-three (84·5 %) of the 110 cases produced antibody to, or showed evidence of infection with, serogroup 1 *L. pneumophila*. This figure is virtually identical with that quoted by England *et al.* (1981) for DFA examination of 57 fresh specimens in their survey of 1000 cases of sporadic legionellosis in the United States. Of 53 patients with a monospecific antibody response, 42 (79 %) were due to serogroup 1, three (5·6 %) to serogroup 2 and three to serogroup 4, two (3·7 %) to serogroup 3 and one (1·8 %) each to serogroups 5, 6 and P185. In the series of England *et al.* (1981) serogroup 2 was the second commonest infecting serogroup as determined by DFA examination. It is clear that a wide range of antigens must be employed in the IFAT if all cases of legionellosis are to be detected.

As noted by Harrison & Taylor (1982) an early low rise in antibody titre proved to be most helpful in initiating a discussion with the physician in charge of a case of pneumonia where the diagnosis of legionellosis had not seriously been considered. In a number of instances the change of therapy to high dose erythromycin (4 g/day) led to a significant and sustained improvement in the patient's condition. It is clear that as, after 1978, two thirds of cases have been diagnosed on the basis of a fourfold or greater increase in antibody there is now a greater awareness of the possibility of legionella infection, but it is significant that still

in one-third of cases there may have been some delay in arriving at the diagnosis. In some cases this is not surprising as the non-pulmonary features of legionellosis, such as renal failure or neurological involvement, may well confuse the diagnosis.

As in other series, males predominated and the median age was 52·6, the mode being 59·8, reflecting the fact that 55 (51 %) of the 107 cases whose age was known occurred in the 50–69 year age group. The age specific attack rates underline the higher prevalence of infection in the age groups 50–59 and 60–69, although there was a fall in the prevalence in the latter age group in 1981.

The attack rates are considerably higher than those reported for 1978 in the United States (England *et al.* 1981) but this may reflect differences in surveillance rather than a true difference. Nevertheless, the trend in age group prevalence is similar except that legionellosis seems to be much less frequent in Scots aged 70 or over compared with the American population. Cases occurred throughout the year but there were fewer in the first quarter than in the other three. Although cases infected abroad were most numerous in the summer, some late autumn and mid-winter cases were seen from this source reflecting the tendency for some tourists to take a winter holiday in the Iberian peninsula. The majority of patients who acquired infection abroad had visited Spain (including some cases from Majorca), but it must be acknowledged that large numbers of Scottish holiday-makers visit Spain in the summer. Nevertheless, some of the cases were associated with the hotel in Benidorm where the original Scottish cases of 1973 stayed.

Legionella pneumophila, especially serogroup 1, is a significant cause of pneumonia in Scotland. Apart from some Scottish tourists who acquired their infection abroad and four cases who acquired their infection in two small outbreaks in England, there have been no point source outbreaks. The origin of infection in sporadic cases remains unknown.

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