Legionella infections in Europe

The ninth meeting of the European Working Group on Legionella Infections, which was co-sponsored by WHO, was held in Viterbo, Italy, on 6–8 June 1994. A total of 67 participants from 20 countries attended and 47 papers were presented, mainly on the epidemiology, biology, ecology, laboratory diagnosis, and environmental control of legionellae. Below is a summary of the major topics discussed.

Epidemiology and travel-associated legionellosis. Ten papers were presented on surveillance of *Legionella* infections. In an overview of the epidemiology of legionellosis in Europe during 1993, it was reported that there were a total of 1203 cases of Legionnaires' disease, corresponding to an overall incidence of four cases per million inhabitants (range, 0.20–18.40 per million) (Table 1). This incidence reflects the level of surveillance rather than the true situation, and the disease is probably markedly underdiagnosed. The definition of Legionnaires' disease does not take into account cases of nonpneumonic *Legionella* infections.[b] Moreover, in some countries not all cases are notified to the national surveillance centre.

The reported mortality rate lies in the range 0–35%; this does not reflect disease prognosis since many countries did not report the mortality rate. None the less, deaths occurred among 5–10% of diagnosed cases in most countries; the male:female ratio was 1.9. Infections were mainly associated with hospital stay or travel. Laboratory diagnosis was mostly performed by serology (62%), followed by isolation of the microorganism (20%), urinary antigen detection (10.7%), and respiratory antigen detec-

[b] See: Epidemiology, prevention and control of legionellosis: Memorandum from a WHO meeting. *Bulletin of the World Health Organization*, 1990, **68**: 155–164.

Table 1: **Incidence of and mortality from Legionnaires' disease cases reported in Europe in 1993**

Country/area[a]	No. of cases	No. of deaths	Population ($x10^3$)	Incidence (per million)	Mortality rate (per million)
Austria	17	6	8 000	2.13	0.75
Czech Republic	21	3	10 500	2.00	0.29
Denmark	92	6	5 000	18.40	1.20
England and Wales	127	22	51 000	2.49	0.43
France	373	–[b]	57 000	6.54	–[b]
Germany (N and SE)	160	–[b]	10 000[c]	16.00	–[b]
Greece	11	0	2 000[d]	5.50	0
Italy	78	4	57 000	1.37	0.07
Netherlands	43	6	15 239	2.82	0.39
Northern Ireland	1	–[b]	1 610	0.62	–
Norway	3	0	4 300	0.70	0
Portugal	11	1	9 000	1.22	0.11
Scotland	18	6	5 100	3.53	1.18
Slovakia	1	–[b]	5 000	0.20	–
Spain	143	–[b]	39 166	3.65	–
Sweden	42	–[b]	8 644	4.86	–
Switzerland	62	4	6 872	9.02	0.58
Total	1 203	58	295 431	4.07	0.20

[a] Data from the Russian Federation have not been included because of intensive migration and administrative changes that make it difficult to estimate the population. A total of 94 cases and 5 deaths were reported for the Moscow area.
[b] Data not available.
[c] National population ca. 80 million.
[d] National population ca. 10 million.

tion (1.3%). Other diagnostic methods were less frequently performed.

The European surveillance scheme, introduced in 1987, permits rapid exchange of information among countries and facilitates identification of possible sources of infection; however, local microbiological investigations need to be carried out. The problem of liability and the legal aspects of the surveillance results were emphasized. In Norway, Legionnaires' disease is nominally notifiable, but control of *Legionella* in water is not compulsory. Sporadic cases and clusters of Legionnaires' disease in Scotland and Denmark caused by contamination of domestic and hospital water supplies and of ice-machines were reported. Surveillance of hospital-associated legionellosis in Spain was outlined and the epidemiological characterization of *Legionella* isolates from travellers' Legionnaires' disease was described.

Biology and ecology. Hydrothermal environments may constitute important natural reservoirs for dispersion of legionellae into other aquatic environments.

The presence of *Legionella* in thermal waters and the resulting risk for people attending therapy centres were discussed. In France, surveillance and control measures have been undertaken in centres where cases of legionellosis have occurred. Water from hot-spring tubs in public baths can be strongly contaminated by *Legionella* spp., and the Japanese Ministry of Health has therefore issued guidelines for control measures to be performed in public hot tubs. The hazard of *Legionella* infection on sailing yachts was discussed; the risk seems to be low, even if legionellae have been isolated from the water tanks of yachts. The usefulness of DNA amplification and hybridization in detecting the presence of noncultivable *L. pneumophila* located intracellularly within *Acanthamoeba* in water samples was outlined. Cloned and purified major outer-membrane protein (MOMP) gene fragments have been prepared, and use of the expressed proteins as antigens in serologi-

cal tests and for the production of antibodies against species-specific MOMP epitopes was proposed. The antilegionella activity of monensin, a lysosomotropic carbosylic ionophore, was reported.

New trends in molecular epidemiology. Results on the sequencing of the 16S rRNA genes from several members of the *Legionellaceae* family support the retention of a single genus for all *Legionella* spp. A method was described for the identification of blue-white autofluorescent *Legionella* spp. based on the amplification of the 16S rRNA genes via the polymerase chain reaction (PCR), followed by restriction analysis of the amplification product. Macrorestriction analysis of genomic DNA from many *L. pneumophila* strains obtained using pulsed-field gel electrophoresis can be used to subtype *L. pneumophila* and to establish epidemiological correlation among isolates. This approach has been used to subtype *L. bozemanii*, *L. micdadei*, and *L. longbeachae* strains and to study the transmission of some cases of non-*L. pneumophila* pneumonias. The use of arbitrarily primed PCR (AP–PCR) fingerprinting for epidemiological typing of *L. pneumophila* was reported; this method appears to be a rapid, simple, and discriminant analytical tool for clonal delineation in epidemiological investigations of *L. pneumophila* infections. In Finland the results of a pulsed-field gel electrophoresis study of the epidemiology of *L. pneumophila* serogroup 6 suggest that environmental isolates of this serogroup have a clonal homogeneity and stress the need for multicentre studies to define internationally the distribution of clonal types of serotype-6 *L. pneumophila*. A French–Belgian cooperative study has evaluated four epidemiological typing methods (monoclonal antibody subtyping, multilocus enzyme electrophoresis, macrorestriction analysis of genomic DNA, and random amplified polymorphic DNA fingerprinting) for the comparative analysis of *L. pneumophila* strains from different sources. The results indicate that unequivocal epidemiological correlations can be established, though over-restrictive differentiation criteria may fail to detect possible relationships between isolates.

Development and evaluation of diagnostic and typing methods. Detection of *L. pneumophila* serogroup 1 urinary antigen by enzyme-linked immunosorbent assay (ELISA) has proved useful in England and Wales for acute phase atypical pneumonia screening, although some changes are needed to simplify its format. Detection of *Legionella* DNA in urine samples by PCR is a promising, sensitive diagnostic method. Legionellae in lung tissue are usually detected using immunofluorescence; however, the failure to detect the microorganisms in tissue homogenates using monoclonal antibodies can arise because of blocking by patient antibodies. In clinical samples the detection of DNA coding factor for MOMP by PCR is as sensitive a method as culturing for *L. pneumophila* but is more specific than direct fluorescence staining; its value in diagnosing infection has, however, still to be defined. A nested PCR system for detecting *L. pneumophila* in respiratory secretions with primer directed towards the *mip* gene is a potential, rapid and sensitive diagnostic tool for legionella pneumonia. A 16S rRNA fluorescently labelled oligonucleotide probe has been developed that has proved useful for identifying metabolically active but nonculturable legionellae within cells and biofilms. *L. pneumophila* serogroups 1–8 and 10–12 have been identified using a panel of monoclonal antibodies. Because of the high reproducibility and specificity of the results achieved with these monoclonal antibodies, their use can be recommended to supplement or replace polyclonal antisera for *L. pneumophila* typing. Chemical modification and recognition with monoclonal antibodies has been used to identify a virulence-associated lipopolysaccharide epitope of *L. pneumophila* serogroup 1. Computer-assisted numerical analysis of the electropherograms of total proteins from *Legionella* species can be used to establish phylogenetic correlations between different strains, a method that could be used for epidemiological studies.

Laboratory environmental control and control measures. In France and England use of laboratory quality control methods has improved the performance of participating laboratories. A surveillance of hospital water systems for the detection of *L. pneumophila* indicated that DNA amplification and hybridization are less time consuming and more sensitive than culture methods, although such procedures should be limited to water samples that do not contain elevated quantities of rust, and in addition require considerable technical expertise. The application of the Weibull chart method permits more efficient use of data on legionellae concentration measurements, e.g., by evaluating the effect of water system management on the control of legionellosis. Use of electrolytically generated copper and silver ions is a highly promising method for controlling the growth of *L. pneumophila* in water systems. Legionellae that persistently colonized the water system of a hospital have been successfully eradicated through the use of chlorine dioxide; and *Legionella* contamination of the warm-water lines of a large surgical clinic was eliminated by making structural modifications to the water plant and by maintaining a temperature of 55–60 °C in the system.

Evaluation of serological tests in* L. pneumophila *non-1 infection. The indirect immunofluorescence

test (IFA) has been most widely validated and is a reference method. However, it has been validated only for *L. pneumophila* serogroup 1, and interpretation of antibody response against other *Legionella* species or serogroups is often difficult. The results of a retrospective study of patients with *L. pneumophila* non-serogroup 1 isolates using antigens prepared from ATCC-type strains indicated that serodiagnosis is useful for making a presumptive diagnosis of legionella infection, although serology is not conclusive for epidemiological investigations. Analysis of the serological results of 202 culture-confirmed cases caused by different serogroups of *L. pneumophila* and other *Legionella* species suggests that the serological response of patients is almost comparable with that observed for the *L. pneumophila* serogroup 1, and that as long as there is no common antigen it is important to use as many antigens as possible.

Differential diagnosis of atypical pneumonia. Diagnosis of atypical pneumonia is not easy. In Greece differential diagnosis of pneumonia caused by different agents indicated that out of 270 patients with a laboratory diagnosis, 10% had a *Legionella* infection. Laboratories should use a panel of viral and bacterial agents as a screening test for serological diagnosis, irrespective of the laboratory diagnosis that is requested by clinicians. Atypical pneumonia appears to be a problem in the Russian army. A differential laboratory diagnosis using serological and expressed methods developed at the Gamaleya Institute, Moscow, demonstrated that *L. pneumophila* community-acquired pneumonia accounted for 10.4% cases in 1990–92 and for 4% in 1993. An antigenic relationship has been described between *Legionella* and *Leptospira* spp., *Borellia* spp. and in part for *Coxiella* spp. A comparative study using sera from patients and rabbit immune sera suggests that this does not bias significantly the correct evaluation of serological tests, although more detailed investigations are needed. A case of hospital-acquired *Legionella* pneumonia involving a 6-month-old child who was receiving glucocorticoid therapy was described. After the failure of erythromycin and rifampicin therapy (although the patient's isolate proved to be sensitive to these antibiotics *in vitro*), the child was successfully treated with fusidic acid. The therapeutic problems were linked to the inadequate penetration of erythromycin and rifampicin into the lung abscesses.