LEGIONELLA INFECTIONS:

A REVIEW OF THE LITERATURE

AND A PROSPECTIVE SEROLOGICAL STUDY

OF THE INCIDENCE OF LEGIONNAIRES DISEASE

AT GROOTE SCHUUR HOSPITAL

Dr C de Goveia
Dept of Medical Microbiology
Medical School
University of Cape Town

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SUMMARY

A prospective study of patients with pneumonia admitted to Groote Schuur Hospital took place over a one year period in an attempt to assess the incidence of legionella pneumonia. Acute and convalescent serum samples were obtained from 113 patients. Eight patients (7,1%) showed a four fold rise in antibody titre against Legionella pneumophila group 1 antigen by indirect immunofluorescent test (IFAT).

The findings suggest that legionella pneumonia, although not common, should be considered in the aetiology of pneumonia at Groote Schuur Hospital.

The results are presented and a review of the literature is undertaken.

HISTORY

In July 1976, as part of the bicentennial celebrations, the Pennsylvania chapter of the American Legion held its annual convention in Philadelphia attended by approximately 4400 delegates.

By the 2nd of August, 182 delegates had been taken ill with an acute respiratory illness characterized by fever, headache, myalgia and non-productive cough. Of these, 147 required hospital admission and 29 died of severe pneumonia. (49,124) The epidemic curve suggested a common source outbreak and the hotel used as headquarters for the convention was strongly implicated.

Extensive investigations for the detection of toxins and infectious agents were undertaken. In January 1977, six months after the outbreak, the agent was identified by Joseph McDade of the Centers for Disease Control (CDC) as a gram negative bacillus, found to represent a new family and subsequently named Legionella pneumophila. (17,89,125)

By December 1978, cases of Legionnaires' disease had been reported from other states in America, from Europe, Israel and Australia. (23,64,91)

And so - a new killer organism was discovered - or was it? Subsequent evidence has shown that Legionella pneumophila is neither new, nor quite the killer it was thought to be.

Retrospective serological studies suggest that the organism was responsible for an outbreak of an influenza-like illness designated "Pontiac Fever" which affected 144 people in a single building in Pontiac in 1968. (57)

Serohistorical data suggest that an outbreak of pneumonia in a meat packing plant in 1957 was caused by Legionella pneumophila. (103)

Indeed, previously unidentified organisms isolated in 1947 and 1959 have subsequently been identified as legionella species. (90) By 1988, cases of legionella infections, both sporadic and epidemic, had occurred worldwide including South Africa (131), although no outbreaks had been as severe as the original.

To date 25 species of Legionella have been identified, of which 13 have been isolated from humans, suggesting a pathogenic role (Table 1). Almost all species have been recovered from environmental sites. Approximately 98% of infections are caused by Legionella pneumophila - particularly serogroup 1.

THE ORGANISM

Legionellaceae are aerobic, non-spore forming, gram negative, non-encapsulated bacilli. The guanine and cytosine content of DNA is 39-43%, which differentiates them from other gram negative organisms.

Electron microscopy studies have shown the presence of flagella and pili. (25,115) Ultrastructural features are similar to those of other gram negative bacilli. (24)

The bacterial cell wall is characterized by large amounts (>80%) of branched chain fatty acids containing ubiquinones with more than 10 isoprene units in their side chains - a relatively uncommon finding in nature. (81,95)

All species produce either catalase or peroxidase. (108) They are non-fermentative and produce neither urease nor nitrate reductase. Most strains are motile, liquefy gelatine and produce βlactamase. (143,148)

Metabolism depends largely on protein degradation, and carbohydrate degradation appears relatively unimportant. (9) The organism has an absolute requirement for l-cysteine, may utilize other amino

acids, and growth is enhanced by the presence of ferric ions, ∝ketoglutarate and charcoal. (55,82) The latter two substances may play a role in scavenging toxic oxygen radicals. (109)

The cell wall of Legionella species contains a lipopolysaccharide (LPS) with endotoxic function which differs in structure, function and biochemical characteristics from the LPS of enteric bacteria. (29,53) A major serogroup specific antigen carried on the side chains of the LPS may be responsible for the reactivity of sera in the IFAT.

The major outer membrane protein (MOMP) of Legionella pneumophila has a molecular weight of approximately 28kd and is immunogenic, although its role in immunity is not known. (53)

A genus-wide 60kd membrane protein has been isolated and shown to react with sera prepared against 23 Legionella species, and may prove useful as an antigen for serological tests. (105,110)

Immune response to the organism appears to be both cellular and humoral.

Some, but by no means all, isolates of Legionella contain plasmids (41,100), but their function has not been studied.

Legionella pneumophila 1, the serogroup most commonly implicated in disease, has been subtyped by a number of methods including plasmid analysis, monoclonal antibody typing and enzyme analysis in an attempt to identify virulent strains. (92,123,130) Monoclonal antibody typing studies suggest that there is an association between the presence of an antigen 2 marker and virulence. (34,130) Further studies are necessary to assess the specificity and sensitivity of these methods.

PATHOGENESIS

There are two major aspects to the pathogenesis of an infectious disease:- organism virulence factors and host immune response - both cellular and humoral.

Virulence factors: Legionella species produce a number of enzymes and toxins, including proteases, endotoxins and cytotoxins. (5,9,154) Proteases are thought to be important pathogenic factors in Legionella infections. This may be due to the low levels of antiprotease activity in the lung.(9) Animal studies have shown

that intranasal administration of proteases produces haemorrhagic pneumonia with degradation of collagen fibres. (6)

Legionella pneumophila is a facultative intracellular pathogen which multiplies within alveolar macrophages and monocytes, and can survive in human polymorphonuclear cells (PMNLs) after phagocytosis. (24)

Uptake of the organism occurs by bacteriopexis, a process whereby the organism binds via receptors and is surrounded by microvilli which fuse leading to engulfment of the bacterium. Multiplication occurs within ribosome-lined vacuoles, often leading to cell lysis. (101)

Antibodies enhance the uptake of Legionella pneumophila by macrophages, but do not inhibit intracellular multiplication. (75) There is however, no evidence that the presence of antibodies enhances the pathogenicity of the organism.

Virulent Legionella pneumophila strains inhibit phagolysosomal fusion and acidification of phagosomes in monocytes.

A cytotoxin of Legionella pneumophila inhibits oxygen dependent killing following phagocytosis by PMNLs. (51)

As yet, however, no single virulence factor has been identified. Legionella pneumonia can be produced in guinea pigs by intratracheal or aerosol inoculation. The initial site of bacterial replication in these animals is in alveolar macrophages. (30) Interferon activated macrophages inhibit intracellular multiplication. (13)

Host immunity: Mechanisms of immunity, and the relative roles of humoral and cellular immune defences are poorly understood. Most data suggest that phagocytosis by PMNLs and macrophages plays an important role.(30,75)

Resident alveolar macrophages are an inadequate defence and do not inhibit multiplication of the organism. In animal models, PMNLs are recruited within 24 hours and are the first effective immune response. After three days a macrophage influx occurs. Most viable organisms

are found in macrophages, while those in PMNLs are usually non-viable. (30)

The role of cell mediated immunity is not well defined. Lymphocyte blastogenic transformation occurs in lymphoid cells from sensitised, but not normal, guinea pigs. (162) It has been demonstrated that mononuclear cells from patients recovering from Legionella pneumophila infection respond to antigenic challenge by production of cytokines that activate monocytes, and by proliferation. Mononuclear cells from non-infected patients do not show this response. (163)

This data shows that cell mediated immunity develops in patients and animals infected with Legionella species.

It has been shown that passive transfer of antibodies may confer immunity. Antibody response to the organism is varied - antibodies may be of IgG, IgM and occasionally IgA classes, and may be specific for a single species or react with genus common antigens. (14)

Antibodies may persist for months or even years, and it has been suggested that the switch from IgM to IgG does not always occur, possibly because new clones of B lymphocytes may be stimulated to produce IgM by persisting intracellular antigen. (150) This

phenomenon does not occur with other intracellular organisms, so there may be another explanation.

Direct or haematogenous spread of organisms may occur, although this is an unusual event, occurring primarily in immunocompromised patients.

PATHOLOGY

Pulmonary pathology and sequelae: Lobular, often bilateral, pneumonia is the predominant finding, although a lobar pattern is also common. There is no predilection for one specific lobe. Small multiple abscesses may occur. Cavitation, although uncommon, has been described, particularly in immunocompromised patients. (59)

Serous or sero-sanguinous pleural effusions are not unusual, but are usually less than 200ml in volume.

Microscopically there is usually an acute fibrinopurulent pneumonia with exudate of neutrophils, macrophages, red blood cells and fibrin in alveoli and alveolar ducts. Leucocytoclasis is common and the extent is proportional to the number of organisms present. Clusters of bacteria are found within macrophages and leucocytoclastic foci. (152) The interstitium may be

inflamed and widened with oedema, fibrin deposition and cellular infiltration.

Electron microscopy shows organisms in cytoplasmic vacuoles of cells.

There may be organization and fibrosis. (15,26) Two patterns have been described:- one with interstitial fibrosis predominating, and a second in which there is also intra-alveolar organization and fibrosis. Disruption of alveolar epithelial lining and basement membrane has been demonstrated. (26) Bronchiolitis obliterans has been described in a compromised patient. (121)

Extrapulmonary: Lymph nodes are hyperplastic, or reactive with dilated macrophage filled sinuses.

Renal pathology includes acute pyelonephritis with organisms in tubules and abscesses, tubulo-interstitial nephritis, rapidly progressive glomerulonephritis with crescent formation, acute tubular necrosis and mesangio-capillary IgA nephropathy (1,32,47,61,144). Organisms have not been demonstrated in the lesions in the latter four conditions.

Focal myocarditis, with organisms in blood vessels, leukocytes and interstitium has been described. (146)

Organisms have been demonstrated in lung, blood, liver, brain, pleural and pericardial fluids, prosthetic valves, kidney, lymph nodes, spleen, bone marrow and myocardium, but not in skeletal muscle or cerebro-spinal fluid.

CLINICAL

Two major clinical syndromes are recognized: Legionella pneumonia and Pontiac fever.

Legionella pneumonia has an incubation period of 2-10 days and manifest as malaise, myalgia, headache, arthralgia and fever, usually with abrupt onset. Cough is non-productive, although small amounts of non-purulent sputum may be produced. Haemoptysis may occur in up to one third of cases. Dyspnoea and chest pain - often pleuritic - are found in about two thirds of patients.

Gastrointestinal symptoms such as watery diarrhoea, nausea and abdominal pain are common.

Temperature rise often occurs in a step-wise pattern with temperatures reaching 39-40^{0}C in two thirds of

patients. There may be a relative bradycardia. Convalescence is often prolonged, and there may be long term sequelae.

The disease cannot be distinguished from other pneumonias on clinical features alone, although multisystem involvement, high fever with rigors and pneumonia with lack of purulent sputum are highly suggestive of the disease.

Pontiac fever is an acute, short lived non-pneumonic illness with fever, headache, myalgia and cough lasting 2-7 days followed by complete recovery.

Asymptomatic seroconversion has been described. (62)

Laboratory findings: Leucocytosis due to a neutrophilia is common, and ESR is usually raised.

45-60% of patients have a hyponatremia (62,159) and about 50% have raised serum urea and creatinine. Liver enzymes are elevated in 50-75% of cases. (156)

Complications of Legionella pneumonia:

Pulmonary complications include respiratory insufficiency, cavitation, and progression to pulmonary fibrosis.

Cardiovascular system: Myocarditis, pericarditis, and prosthetic valve endocarditis have been reported.(50,138,146)

The most common neurological complication is a reversible encephalopathy manifested by confusion, disorientation and stupor. (79) Cerebellar dysfunction characterized by ataxia, gait disturbances, dysarthria and nystagmus have been described in 4% of 912 cases reviewed by Johnson et al. (79)

Other manifestations include peripheral neuropathy, and very rarely, encephalomyelitis, focal signs and seizures. (11,66,69,79) CSF is usually normal, with the most common abnormality being a moderate pleocytosis- neutrophilic, monocytic or mixed. (79) The aetiology of neurological derangements is unknown, and may be due to toxins or immunological factors. Legionella antigen has not been demonstrated in CSF and very rarely in brain tissue. (54)

Renal failure, although well recognized, is uncommon. Acute tubular necrosis - secondary to hypotension or myglobinuria; interstitial nephritis; and glomerulonephritis have been implicated. (47,61,144)

Myalgia, arthralgia and weakness are common symptoms in Legionella infections. A number of cases of rhabdomyolysis with resultant myoglobinuria have been described. (20,63) As organisms have not been demonstrated in skeletal muscle, direct muscle damage by a toxin, or ischaemia due to vasoconstriction may be responsible.

Miscellaneous complications: Skin rash (1), sinusitis (122), retinal abnormalities (50), Henoch-Schonlein purpura (22), autoimmune haemolytic anaemia (132), cutaneous abscess (2), haemodialysis fistula infections (80), wound infections (16), and appendix abscess (74) are very rare manifestations which have been described. In some cases the organism has not been demonstrated in lesions, and the association is tenuous.

RADIOGRAPHIC FEATURES

The radiographic patterns of Legionella pneumonia vary widely, with the most common being lobar or diffuse patchy infiltrates. (44) Small pleural effusions are common. There is no unique radiographic picture. The radiograph may remain abnormal for many months after clinical recovery. The most frequent residual abnormalities are pleural effusions or thickening

(33%), small irregular shadows (24%), and atelectasis (19%). (44)

DIAGNOSIS

Diagnosis may be made in three ways:- Demonstration of a specific immune response, demonstration of antigen or nucleic acid in clinical specimens or culture of the organism.

Serological Diagnosis: There are a number of limitations. Not all patients mount an antibody response. Diagnosis is retrospective as a fourfold rise in titre of specific antibodies must be demonstrated. Antibody production often occurs late, although seroconversion may occur within the first week in 27-40% of patients. (68,161) Finally, false positive results, although unusual, have been described in patients with gram-negative, rickettsial and chlamydial infections. (28,37,153)

Tests should measure IgG and IgM. Antibodies to several serogroups or species may be produced in an individual, therefore serology cannot be used to identify the infecting strain. (45,106) The immunofluorescent test (IFAT) is the most widely used and best evaluated test, with a specificity of 99% and sensitivity of 80% using

Legionella pneumophila serogroup 1 antigen. (68,147) Different methods of antigen preparation produce similar results if levels for determination of positivity are adjusted. (104,149) Reading and interpretation requires skilled personnel and results may be subjective. Methods to automate the test in order to overcome this problem have been described, but their efficacy has yet to be evaluated. (14,136)

Other serological tests, including agglutination, counterimmuno-electrophoresis and enzyme linked immunosorbent assay (ELISA), have not been as well assessed, although the ELISA appears to be a useful alternative to the IFAT. (68,72,73,106,120)

Detection of antigen or nucleic acid: The direct fluorescent antigen test, (DFAT) the first method described, is still the most commonly used. The sensitivity is low - 25 to 50%. Specificity is high, although cross-reactions of antisera with other bacteria including Pseudomonas species and *Bordetella pertussis* have been reported. (7,102) These cross-reactions are rare in clinical specimens, but have been described. (58,77) Use of monoclonal antibodies reduces the risk of cross-reactivity. (60)

A pseudo-epidemic of Legionella infections due to contamination of reagents by environmental Legionellae with resultant false positive results has been described. (114)

A nucleic acid probe has been produced. It is more sensitive than DFAT, and technically simple to use. A major drawback is the short half-life of the radioactive isotope in use at present. (107)

Legionella antigen in urine has been detected by radioimmunoassay, enzyme-linked immunosorbent assay and latex agglutination. (8,84,133) Antigen may be detected early in the course of disease, but prolonged excretion occurs, so a positive test may reflect prior infection. (85)

Culture: With the availability of commercial media, particularly buffered charcoal yeast extract agar with ketoglutarate (BCYE), culture should be the mainstay of diagnosis. Sensitivity may be as high as 80%, depending on the type of specimen cultured. The highest yields are obtained from transtracheal aspirates and bronchoscopy specimens, particularly bronchoalveolar lavage. (86) The organism can be readily cultured from sputum, especially with selective techniques such as acid or heat pretreatment; addition

of antibiotics to media; an antiserum-containing agar plate; and the use of discs impregnated with cysteine and ferric pyrophosphate on a deficient medium. (21,76,1126)

Colonies may be visible after two days, and most cultures are positive within 5 days, with colonies having a characteristic ground glass appearance.

Legionella pneumophila has been isolated from blood using a biphasic medium (12), and by the BACTEC radiometric system, although growth indices may not exceed the threshold limits. (113) Organisms, once isolated, may be identified by failure to grow on media lacking cysteine, DFAT, slide agglutination and the use of a DNA probe. (134,135,151)

Direct inoculation onto dye-containing medium may aid in the identification of species by pigment production or fluorescence. (140)

Legionella species are biochemically inert and traditional tests such as oxidase, catalase and gelatine liquefaction give variable results, and are not useful for identification. (153)

TREATMENT

During the 1976 outbreak, the drug found most effective was erythromycin. In vitro testing and animal studies supported this clinical finding. (97,98) Erythromycin is still the drug of choice for the treatment of Legionella infections, although relapses have been reported - usually following oral administration or inadequate doses.

In vitro, naturally resistant strains of Legionella have not been reported. However resistance to erythromycin has been induced by passage of organisms through drug-containing media. (36)

Erythromycin is bactericidal in vitro, but bacteriostatic in monocyte cell cultures. (141)

In vitro sensitivity testing suggests that Legionella pneumophila is sensitive to a number of antibiotics including the aminoglycosides, chloramphenicol and cefoxitin. The tetracyclines are inactivated by CYE medium. (38)

Clinical efficacy of drugs does not correlate with in vitro sensitivity results, probably due to the inability of certain drugs to penetrate macrophages. Disc diffusion

testing may be used to screen for antimicrobial resistance, but apparent sensitivity to certain drugs should be viewed with caution. (35) A cell model has been used to assess antibiotic activity against Legionella species multiplying in macrophages. This has led to the definition of a minimal extracellular concentration inhibiting the intracellullar multiplication (MIEC) of Legionella pneumophila. (141) There appears to be a good correlation between MIEC and clinical efficacy, and cell models allow the testing of drug combinations.

Rifampicin is useful in combination with erythromycin for severe infections, but resistance may develop rapidly if it is used alone. The tetracyclines and co-trimoxazole have been used to treat infections successfully, although their efficacy has not been fully evaluated. (118,158) The new quinolones such as pefloxacin(33), and the carbapenem, imipenem have been used successfully to treat patients. (46)

Prophylaxis of high risk patients with erythromycin has been shown to protect against infection in transplant patients in an environment contaminated with Legionella pneumophila. (139)

EPIDEMIOLOGY

There are three major factors in the epidemiology of Legionella infections:- an environmental source, transmission of the organism and a susceptible host.

Three disease patterns have been described:

1. Epidemics of Legionella pneumonia which have occurred worldwide. It has been suggested that the term "Legionnaires' Disease" be reserved for epidemic cases. The attack rate is low.

2. Sporadic cases of Legionella pneumonia. The incidence varies greatly, although it is increasingly being recognized as an important cause of community and hospital acquired pneumonia.

3. Pontiac fever. Due to the non-specific signs the incidence of sporadic cases is not known. The name "Pontiac fever" should be used only for outbreaks with a common source. The attack rate is about 95%. (31)

Environmental sources: Legionella species grow in water, are ubiquitous in nature, have been isolated from rivers, mud, excavation sites, cooling towers and potable water (4,88) and very rarely cause disease.

Their ability to survive so well in nature is in direct contrast to their fastidious nature in the laboratory, and it has been suggested that Legionella species have a symbiotic (or parasitic) relationship with algae and free-living amoebae (10,116,117), although Legionellae have been shown to survive and multiply in water free of these organisms.

Outbreaks of disease have been associated with infected water in cooling towers and the organisms may be carried long distances from the source in aerosols.(83,111) Originally attention was focussed on air conditioners as the main source of infection, but it was later shown that potable water could be a source. (65,71) Sporadic cases and outbreaks associated with whirlpools and residential water supplies have been described. (93,129,142) Nosocomial outbreaks have also been ascribed to infected showers and to contamination of humidifiers. (65,155) Legionella species are isolated more readily from hot water systems (153), and the presence of certain types of rubbers may enhance their growth. (27,99)

A number of methods have been used to identify the source of an outbreak by matching clinical and environmental isolates. These include plasmid profiles, restriction enzyme analysis, monoclonal antibody typing, outer

membrane protein profiles and alloenzyme analysis. (19,41,100,130,137)

The association between source and outbreak should be viewed with caution, as none of these methods are totally specific and there may also be more than one source of infection.

Transmission: The widely accepted mode of transmission is by inhalation of aerosols. The differences in clinical features and attack rates between Legionella pneumonia and Pontiac fever are not explained by this theory, although the relative size of particles within aerosols may play a role in the pathogenesis of the two diseases. Muder et al (96) suggest that Pontiac fever may be acquired by inhalation and Legionella pneumonia by aspiration or direct instillation via respiratory devices. They point to the association with surgery and intubation as evidence. However these theories do not explain the occurrence of community acquired pneumonia in previously healthy hosts. The same authors propose ingestion of organisms followed by bacteraemic spread as a mode of transmission, and cite the high incidence of gastrointestinal symptoms in patients with Legionella pneumonia. This seems an unlikely mode of transmission in a disease which primarily affects the respiratory tract.

There have been rare reports of pneumonic and non-pneumonic forms of disease acquired from a common source (56) suggesting organism load or host factors may be important in transmission.

A carrier state in man has not been convincingly demonstrated, although it has been suggested. (18) Indeed, the growth of Legionella species may be inhibited by pharyngeal flora (48). There is no evidence of person to person transmission.

Host factors: The majority of patients in the 1976 outbreak of Legionnaires' disease were healthy adults. Most cases of community acquired disease occur in immunocompetent hosts, although there are certain risk factors including smoking, ethanol abuse, chronic pulmonary disease, diabetes, age over 50 years and male sex. However these could apply to most causes of bacterial pneumonia. Children are less susceptible, although cases have been described, particularly in children with malignancies. One series (3) showed seroconversion in 52% of 52 children under the age of four years with no evidence of Legionella pneumonia, suggesting subclinical infection or atypical illness. The high incidence of seroconversion may have been due to cross reactions with antibodies against other

organisms -vide the much lower seroprevalence in most adult studies.

The majority of patients with nosocomial infections are immunocompromised, with renal and bone marrow transplant patients at particularly high risk. (87,94) There has been an increased incidence of Legionella infections in patients who have undergone surgery (78,96), probably due to greater awareness of the disease.

Incidence: The true incidence of Legionella infections is not known. In retrospective studies, serological methods only are used and often no records of other causes of pneumonia in the group studied are available. Prospective studies are difficult because a large number of diagnostic methods must be used, cases may be missed, and false emphasis may be placed on the isolation of potential pathogens such as Haemophilus influenzae or Streptococcus pneumoniae. The incidence reported varies from <5% to 24% of community acquired (52,157), and up to 25% of hospital acquired pneumonia. (78)

A prospective study of 142 cases of pneumonia by Yu et al (159) conducted over an 11 month period, showed that Legionella pneumophila was the most common single cause

of pneumonia (22,5%). However, in 14 of the 32 cases diagnosis was made on a single antibody titre of 128, with no 4 fold rise demonstrated. The study did not differentiate between nosocomial and community acquired cases.

In a study of 100 cases of pneumonia by Rudin and Wing (164), Legionella species were responsible for 7% of nosocomial infections, but no cases of community acquired legionellosis were detected. The authors did not indicate the total number of pneumonia cases admitted during the period of the study.

In a review of 92 patients with pneumonia in Denmark, 22 cases (24%) of Legionella infection were diagnosed. The group of patients studied was unusual because the majority (47%) came from a community of elderly people, many of whom had underlying disease. (52)

Control: The role of surveillance and decontamination measures in the prevention of nosocomial infections particularly, is controversial. (42,43,153) Some authors feel good housekeeping of water supplies is adequate, remembering Legionella species are resistant to temperatures up to 50°C, and to chlorine levels which eliminate enteric bacteria. Others suggest surveillance of high risk sites such as transplant and

intensive care units. A third approach is to carry out prospective surveillance of compromised patients with pneumonia to determine whether the hospital is a source of infection. One of the drawbacks of surveillance of water is that virulent strains cannot be differentiated from non-virulent strains, although monoclonal antibody typing may be useful in recognizing pathogenic subgroups. (34) Another problem is deciding when Legionella species are isolated from the environment in the absence of disease in patients, whether it is necessary to spend a great deal of time and money eliminating the organism from the environment.

The most practical approach appears to be the monitoring of water outlets and respiratory equipment in intensive care units, combined with surveillance of pneumonia cases in these units. Two methods of decontamination have been described. These are hyperchlorination using 20-30 ppm of free chlorine, which may cause corrosion of pipes; or "pasteurization" - flushing water of 70°C through the system several times. (42) Decontamination is often unsuccessful because organisms in blind ends of the water system or deep within sediment may not be reached.

STUDY

INTRODUCTION

Legionella pneumonia was first described in 1976. Since then the disease has been reported worldwide, with widely varying incidence rates. The prevalence of Legionella pneumonia at Groote Schuur Hospital is unknown. Knowledge of this may aid the clinicians in the initial treatment of patients with pneumonia and allow the microbiologists to assess the necessity to provide diagnostic tests and decide which should be routinely available.

The incidence of the disease is assessed and suggestions for further studies are made.

MATERIALS AND METHODS:

Specimens:

Pneumonia cases: Patients over the age of 16 years with clinical and radiological signs of pneumonia attending Groote Schuur Hospital during a one year period from July 1987 to July 1988 were included in this study. Medical staff in the Emergency Unit were contacted daily, and those on medical wards once or twice weekly to ascertain the presence of pneumonia cases within these areas. Blood culture request forms were checked daily for possible cases. These procedures were carried out as regularly as the routine workload allowed. Patients were included in the study even if an aetiology had already been found. The department of Radiology were asked to record the folder numbers of patients with chest radiographs suggestive of pneumonia, but it was felt that this would not be practical.

Those with underlying tuberculosis and acute exacerbations of chronic obstructive airways disease were excluded. Acute sera were collected on, or as soon as possible after the date of admission by the investigator. Convalescent sera were obtained 2-4 weeks after the acute specimens by the investigator, or at the patient's nearest day hospital. All patients were

given an envelope adressed to the sister in charge of the local day hospital. The envelope contained a labelled 10ml Vacutainer clotted blood tube, a note to the sister requesting that blood be taken from the patient, and an envelope addressed to the investigator for return of the blood sample. Patients were given a date on which to attend for a repeat specimen. 10-14 days prior to the date of the follow-up visit, a letter of reminder was sent to all patients. Patients were told that attendance was voluntary. The same method of follow-up was used for everybody. Informed consent was obtained from all patients.

Controls: Serum was obtained from 200 healthy blood donors to assess seroprevalence in the Cape Town population.

Antigen preparation: A reference strain of Legionella pneumophila serogroup 1-Philadelphia 1. (ATCC 33152) was grown for two days at 37°C on buffered CYE slopes. A suspension of the harvested organism was heat-killed at 100°C for 15 minutes. After titration against control sera of known titre, the antigen was used at a dilution of 1:20 in 0,5% buffered yolk sac.

Buffered yolk sac: Yolk sacs harvested from 12-14 day embryonated eggs were diluted in 0,02M phosphate buffered saline (PBS) to a concentration of 3% for serum dilution and 0,5% for antigen preparation.

Preparation of slides: Glass microscope slides were coated with teflon to make 14 well fluorescent slides. A 1:20 suspension of heat-killed antigen was used to cover the wells. Slides were air-dried, fixed in acetone and stored at -20°C. Frozen slides are stable for at least two months.

Indirect fluorescent antibody test (IFAT): All sera were screened at a dilution of 1:64, and any positive sera were then titred. Acute and convalescent sera were tested simultaneously. Fluorescent labelled rabbit antihuman globulin which detects IgG, IgM and IgA was used (Centers for Disease Control).

Slides were read on a Zeiss mercury vapour epifluorescent microscope with FITC filter and scored on a scale from 1+ (fluorescent organisms barely visible) to 4+ (brilliant yellow-green fluorescence). The serum titre was the reciprocal of the highest dilution giving at least 1+ fluorescence. Results were only accepted if negative controls were negative and positive controls

were no more than one dilution above or below the stated value.

Diagnosis of Legionella pneumonia: A fourfold rise in titre between acute and convalescent sera to a titre of at least 128. A single titre of 256 or greater was considered suggestive of infection at an undetermined time.

RESULTS:

Controls: Of 200 healthy blood donors (133 males, 67 females) examined, 8(4%) had measurable antibody titres against Legionella pneumophila 1 antigen (LP1). 3(1,5%) had titres of 256 or greater - suggestive of past infection. It was not possible to obtain histories from these donors, therefore recent infection could not be excluded. The majority of donors (70%) were between the ages of 20 and 39 years, and were not age matched to the pneumonia patients. Age distribution is shown in Table 2.

Pneumonia patients: Of 173 patients initially included in the study, 60 were lost to follow up, therefore the results reflect the remaining 113 (69 males, 44 females). No factors contributing to non-compliance could be identified as all patients were treated in the

same way and lack of compliance did not appear related to socioeconomic factors.

Age distribution is shown in Table 3. No single age group predominated.

One hundred and eight cases were community-acquired, 3 nosocomial (ie presented >72 hours after admission) and in 2 cases it was difficult to assess whether the infection was acquired in the community or in hospital.

Eighty seven had been ill for less than one week, 23 for 1-2 weeks, and 3 for more than two weeks before acute sera were collected.

Seventy one patients were treated in the emergency unit, and 42 as in-patients.

Eight patients showed a fourfold rise in titre of antibody to Legionella pneumophila 1, (Table 3) and were considered to have an acute Legionella infection. Six of these patients responded to conventional therapy and two were treated with erythromycin following failure of response to conventional antibiotics.

The incidence among patients treated in the emergency unit was 5,6% (4/71), while among admitted cases it was 9,5% (4/42). There was no significant difference. (Fishers Exact Test).

Two patients showed sustained antibody titres of 256 or greater with no rise in titre between acute and convalescent sera collected 4 weeks apart. (Table 3) In both cases acute sera were obtained less than one week after onset of symptoms, therefore it is unlikely that failure to demonstrate seroconversion was due to delay in obtaining acute sera. These 2 patients were not included as acute cases, as detectable antibody titres may persist for months or even years. (150) The Centers for Disease Control recommends that single or sustained high titres in patients with sporadic illness be considered only presumptive evidence of infection at some time in the past. (150)

DISCUSSION

Controls: The seroprevalence among healthy donors was 4% if a titre of 64 was considered positive. However at this titre a number of positive results may be due to cross reactions. The seroprevalence was 2,5% using a titre of 128, and 1,5% at a titre of 256. Other studies have shown a prevalence varying from 1,7% to 19% using a

titre of 128 or greater. (4,12,70,127,128) Cape Town therefore is at the lower end of the reported range.

Pneumonia patients: It is unlikely that all pneumonia cases admitted to GSH during the period of the study were included. However as many as possible were seen and hopefully these are a representative cross-section. Patients were not excluded if another infective aetiology was found; firstly because legionella may occur in association with other infections (52,157), and secondly because this would have resulted in a falsely high prevalence of legionella infections.

Cases may have been missed, as only a serological method of diagnosis was used, and the disease may occur without seroconversion.

The majority of patients in this study had community acquired disease. The incidence of legionella infection among the group tested was 7,1% (8/113), suggesting that the disease is not uncommon. Only a serological diagnosis was made, as most patients were already on antibiotics when seen by the investigator, and other methods of diagnosis of legionella infections are not routinely used in our laboratory.

In many instances no routine bacteriological specimens were submitted and in other cases only one sputum specimen without accompanying blood cultures were submitted . No meaningful data as to the alternative causes for pneumonia could be obtained. It was therefore not possible to assess the relative frequency of legionella infections in this group of patients. It is only possible to state that the disease was not rare amongst pneumonia cases admitted to GSH during that period.

Although numbers are too small to assess the significance of the age distribution of legionella infections, the majority of cases occurred in the 40 to 49 age group. This contrasts with other studies in which the majority of cases occurred in those over 50 years of age. (157,159)

The ratio of male to female was 5:3, and this probably reflects the proportion of males to females (69:44) in the study.

All 8 cases of legionella infection presented in a 3 month period from March to June 1988. This may be a reflection of the high incidence of pneumonia cases occurring in this period. (Fig 1) The incidence of sporadic cases of legionella pneumonia tends to peak in late summer and autumn.

Six of the eight patients responded to conventional therapy - supporting the observation that a large proportion of cases may recover without the appropriate therapy. (86)

As there was insufficient time to examine all 173 patients in the initial study it was not possible to ascertain whether the patients had distinctive clinical or laboratory features.

The results of this study suggest that the incidence of legionella infections is not high enough to warrant routine screening for this disease in all pneumonia cases. However, there should be suspicion particularly in cases who do not respond to, or relapse on conventional therapy - and erythromycin used early rather than late.

A further study of all nosocomial pneumonia cases, especially in compromised patients, would be of value to assess the incidence of legionella pneumonia and determine the necessity for surveillance of water supplies. Serological studies, cultures of sputum and blood, and more invasive specimens if warranted, should be undertaken. Anecdotally, there is no evidence of a high incidence of nosocomial legionellosis at GSH, but no studies have been carried out to confirm this. A limited study of post-surgical cases is in progress.

Liaison between clinician and microbiologist in suspected cases of legionellosis, either community or hospital acquired, would allow the laboratory to offer a more comprehensive diagnostic service including sputum and blood culture, and serology. It is suggested that a BCYE plate be put up on all bronchial brushings, particularly from patients with non-responding pneumonia.

TABLE1	LEGIONELLA SPECIES	
L. pneumophila	serogrps 1 - 13	*
L. bozemanii	serogrps 1 - 2	*
L. micdadei		*
L. dumoffii		*
L. gormanii		*
L. longbeachae	" 1 - 2	*
L. jordanis		*
L. oakridgensis		
L. wadsworthii		*
L. feelii	" 1 - 2	*
L. sainthelensi		
L. anisa		
L. maceachernii		*
L. jamestowniensis		
L. rubrilucens		
L. erythra		
L. hackeliae	" 1 - 2	*
L. spiritensis		
L. parisiensis		
L. cherii		
L. steigerwaltii		
L. santicrucis		
L. israelensis		
L. birminghamensis		*
L. cincinnatiensis		*

* isolated from man

LEGIONELLA INFECTIONS
SEASONAL DISTRIBUTION

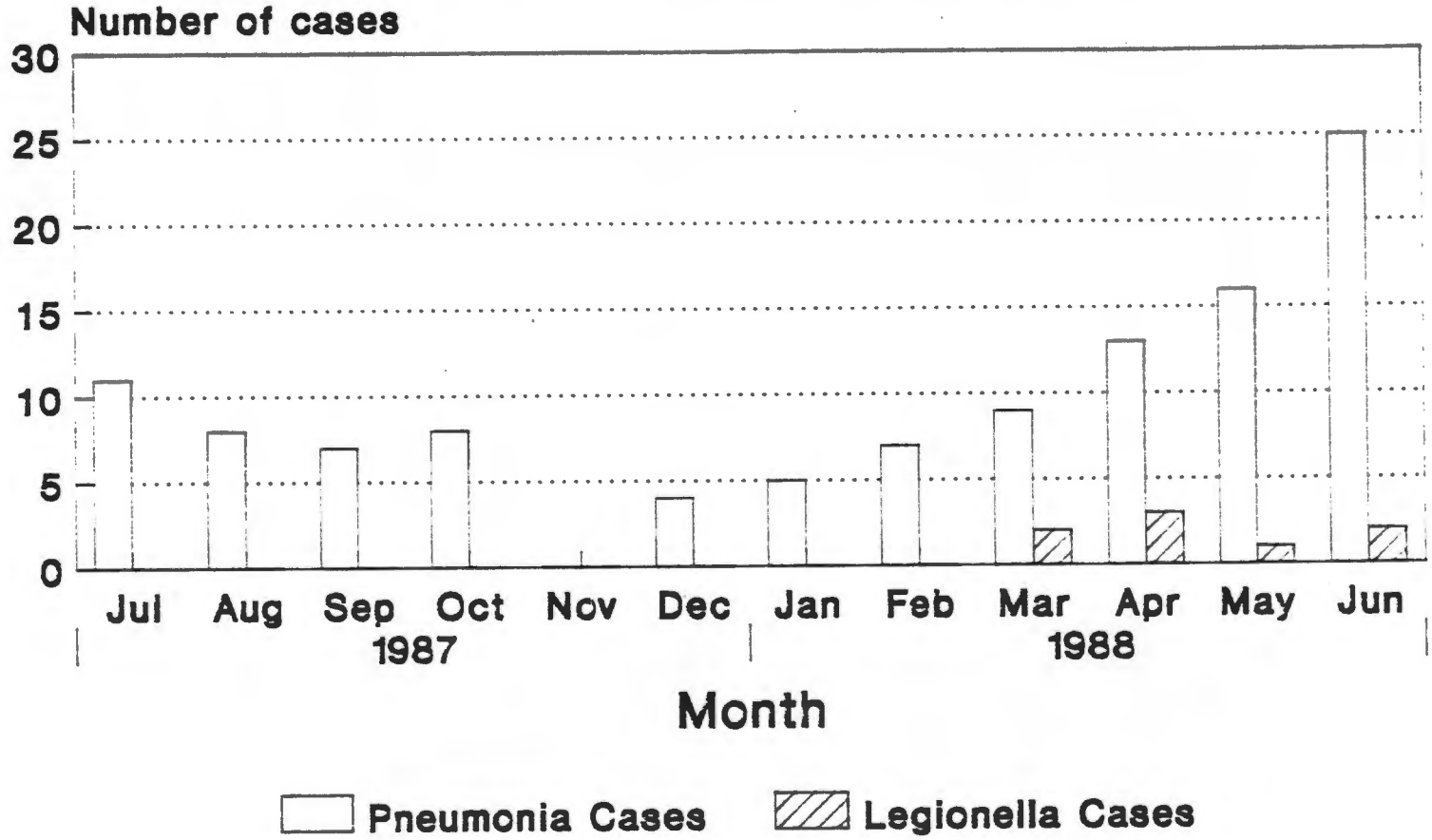

FIG 1

TABLE 2

BLOOD DONORS - antibody titres vs Legionella pneumophila

NUMBER OF PATIENTS

AGE (YEARS)	TITRES				TOTAL
	<64	64	128	>=256	
<20	6	0	0	0	6
20-29	79	1	1	0	81
30-39	57	1	1	2	61
40-49	37	0	0	0	37
50-59	9	0	0	0	9
>=60	4	1	0	1	6
TOTALS	192	3	2	3	200

TABLE 3

PNEUMONIA CASES - Antibody titres vs Legionella pneumophila

AGE (YEARS)	4-FOLD RISE IN TITRE	HIGH TITRE (>=256) (NO RISE)	NEGATIVE	TOTAL
		NUMBERS OF PATIENTS		
<20	0	0	6	6
20-29	1	0	25	26
30-39	0	0	13	13
40-49	5	0	19	24
50-59	0	2	14	16
60-69	1	0	20	21
>=70	1	0	6	7
TOTALS	8	2	103	113

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