First isolation of *Legionella pneumophila* in Sri Lanka

W M P B Wahala[1], and R S B Wickramasinghe[1]

(Index words: Cooling towers, past infection among staff, culture, fatty acid profile of isolates).

Abstract

Objectives To determine the presence of legionellae and species composition of the genus *Legionella* in Sri Lankan hotel cooling towers, and to determine the previous exposure of hotel workers to *Legionella pneumophila.*

Design Collection of water samples from 16 cooling towers of air conditioning plants from 7 representative hotels, and blood samples from hotel workers.

Setting Department of Bacteriology, Medical Research Institute, Colombo 8.

Results Water samples from 4 (57.4%) hotels selected were positive for legionellae. Five (38.4%) selected cooling towers yielded legionellae with viable counts ranging from 1 to 5 colony forming units (CFU)/ml. 93.7% of the isolates were *Legionella pneumophila.* Only one hotel worker had significant antibody levels denoting past infection to *Legionella pneumophila.*

Conclusion *Legionella* does occur in the Sri Lankan hotel environment and *Legionella pneumophila* appears to be the most common species.

Introduction

Legionella pneumophila which is an important respiratory pathogen world-wide, was first identified in 1976 in the USA as a result of an outbreak of acute pneumonia (1). To date a total of 42 *Legionella* species have been identified and 21 of these are pathogenic in humans, producing legionellosis in either the form of Legionnaire's disease or as Pontiac fever (2). At present *L pneumophila* contains 15 serogroups of which serogroup (sg) 1 is the most common clinical and environment isolate world-wide, followed by sg 6 (2).

Legionellae are ubiquitous in an aquatic environment including both natural habitats such as rivers, lakes and ponds, wet soils, and man-made environments such as cooling tower systems, hot-water tanks, evaporative condensers, shower heads, humidifiers etc (3). Legionellae live in close association with free living amoebae (eg *Acanthamoeba*), other protozoa and cyanobacteria, and survive in them as intracellular parasites. Viable counts of legionellae have been reported more in cooling towers than in natural habitats (4). Usually *Legionella* infection occurs through inhalation of aerosolised bacteria by susceptible persons. Man to man spread has not been reported (5) Cooling tower systems give a better chance for the organism to grow and multiply and disseminate the bacteria by formation of aerosols, thus producing epidemics. There are, however, many instances of legionellae isolated from the cooling towers without any resulting epidemics (6). Cooling tower and air-conditioning circuits are two separate entities. Usually cooling towers are located outside the building, and are used in a large air-conditioning system to cool the condenser of the air-conditioning circuit. When the tower is operational aerosols are generated and disseminated into the surrounding environment. If the water is contaminated with *Legionella* these aerosols can cause the disease.

The first serologically confirmed case of legionellosis in the country was reported in 1993 (7). Since then there have only been 4 cases (Unpublished data). Attempts at culture have not been successful. No epidemics have been reported to date. Numerous epidemiologic investigations done world-wide have convincingly shown that cooling towers can serve as a source of infection. In Sri Lanka man-made environment that favours the growth of legionellae such as cooling towers are now widespread, especially in hotels and some industrial complexes. So far no studies have been done, to the best of our knowledge, on legionellae in the environment. The present study was done to determine the presence and species composition of legionellae in selected cooling tower systems and the previous exposure of selected hotel workers to the bacterium.

Materials and methods

Seven hotels were selected from the western and central provinces for this study. A total number of 16 cooling towers were used for the sampling and 1 to 2 litres of water were collected from the bottom of the each cooling tower. Each sample was concentrated by centrifugation (6000 g × 30 min) and 0.1 ml of concentrated sample was inoculated on buffered charcoal yeast extract (BCYE) agar (8) and modified Wadowsky and Yee (MWY) medium (9). Part of the concentrated sample was treated with 0.2 m KCI-HCI acid buffer for 5 minutes at room temperature and 0.1 ml was inoculated as before. Acid treated water samples inoculated on MWY agar plates were used for counting the colony forming units (CFU/ml) of legionellae. All plates were incubated at 37°C for 10 to 14 days in the presence of 5% CO_2. Colonies that appeared after 4 days of incubation with a circular (1 to 2 mm in diameter), moist, smooth glistering surface, entire edge, grey-bluish in appearance, were subcultured on BCYE and 5 to 7% human blood agar plates. Those that grew only on BCYE agar and not on blood agar were considered as suspected legionellae, and presumptively identified by gram stain appearance, motility, gelatin liquefaction, positive catalase test and non-fermentation of sugars. Fatty acid profiles of some of these isolates were compared with one reference strain of *L pneumophila* by gas liquid chromatography (GLC) (10). All the profiles were similar to the profile of the reference strain (height of

[1]*Department of Bacteriology, Medical Research Institute, Colombo 8.* *(Revised version accepted 15 September 2000).*

the peaks varied due to the quantum of the acids produced by bacterial strains; identity of the acids are, however, proven by the retention time) suggesting that the isolates are *Legionella* (Figure). Presumptively identified strains were confirmed at St. Mariana University School of Medicine, Japan and Centres for Disease Control and Prevention (CDC), Atlanta, USA.

To determine past infection among the hotel workers, blood samples were taken from them and the indirect immunofluorescent test (IFA) was performed with Scimedx legionella (indirect) fluorescent test system (Scimedex Corporation, USA) for each sample according to the manufacturer's instruction. A titre of 256 or more was considered as evidence of past infection (11).

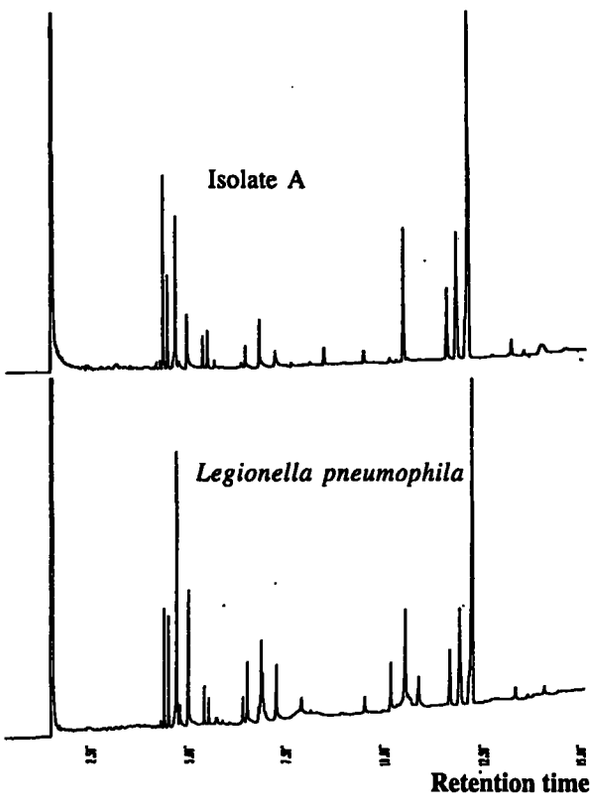

Figure. *Fatty acid profile of isolate by GLC.*

Results

Legionellae were isolated from 4 (57.4%) out of 7 hotels from 16 cooling towers. Five of them (38.4%) yielded legionellae. Viable counts of legionellae of these towers were in the range of 1 to 5 CFU/ml. Total number of isolates was 16. Most of these isolates (93.7%) were *L pneumophila*. Only one of these *L pneumophila* was serogrouped and proved to be sg 1, the most common clinical and environmental isolate recorded world-wide. One strain of these isolates was *L rubrilucens* which is a non-pathogenic species. Only one blood sample gave an antibody titer of 256 suggestive of past infection.

Discussion

This is the first study to explore the presence of legionellae in a Sri Lankan environment. The study area was limited to the western and central provinces where many tourist hotels with cooling towers are found. According to the results of this preliminary study *L pneumophila* does occur in the cooling towers in the country, and it was the most common *Legionella* species found.

Although 38.4% of the selected cooling towers yielded legionellae, evidence of past exposure to the organism among the hotel workers was low (1 of 25). One Japanese study reported that viable legionellae had been found in 63% of their cooling towers and average counts were recorded up to 100 CFU/ml (12), and 44% of cooling towers in the UK were reported to contain the bacterium (13). Legionellae counts of between 10 and 20 CFU/ml in cooling towers were reported by a recent study in Germany (14). Although viable counts of legionellae found in the present study is low (1 to 5 CFU/ml), and no epidemics have been reported in the country, further study is required to investigate the factors which lead to *Legionella* infection, as the various strains of *L pneumophila* differ in virulence, and multiple strains may colonise the cooling tower systems (6). Only a few of them may cause disease in humans exposed to the contaminated water.

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