

Evaluation of detection methods for *Legionella* in industrial cooling water systems

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DECLARATION

This is to certify that this dissertation, submitted for the degree of Doctor of Philosophy at the University of Pretoria, is my own work. It has not been presented before for any degree or examination at any other University.

DEDICATION

In memory of my late husband Cecil and
with love to Neill, Etienne and Mom

ACKNOWLEDGEMENTS

It is with appreciation that the following persons are acknowledged:

This is to certify that this dissertation, submitted for the degree of Doctor of Philosophy at the University of Pretoria, is my own work. It has not been presented before for any degree or examination at any other University.

Professor D Rees, the Director of the National Centre for Aquatic Toxicology and Hygiene, for the opportunity to do this study.

My colleagues and friends in the Immunology/Aquatic Toxicology Dept. for their support.

The management and staff of the various industrial works who provided access to the study and did the sampling on various occasions.

Catheleen Bartie

The Water Research Commission for funding the project.

The members of the Legionella Action Group for their valuable help.

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BACKGROUND

Legionella (plural: legionellae) is a type of bacteria that is found primarily in warm water environments. They are present in most natural water environments and are often present in man-made environments such as humidifiers or air-conditioning and ventilation systems and other humid environments.

ACKNOWLEDGEMENTS

Legionella was first identified in 1976 when a over 200 of veterans affected in an American city. It is with appreciation that the following persons are acknowledged for their contribution to the study. Thirty-four people died before the cause of the disease was identified by scientists of the Centres of Disease Control and Prevention. Today, legionnaires disease is reported more often in the world, in fact, even in the industrialized world. Legionnaires disease is a very serious multisystem disease with a potentially fatal outcome.

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A very special word of thanks to Tanusha Soogreem for her support and friendship throughout this study. The Legionella Action Group was formed to assist the medical profession to reduce the risk of legionnaires disease and to assist water treatment companies with the treatment of water distribution systems to decrease the risk of the disease for workers and for the public. This project was undertaken in part to assist the Legionella Action Group with this very important task.

TAB BACKGROUND INTS

Legionella (plural: legionellae) is a type of bacteria that is found primarily in warm water environments. They are present in most natural water environments and are often present in man-made water environments, like cooling towers, humidification, air-conditioning and ventilation systems and other humid environments.

Legionella was first identified in 1976 when a over 200 of veterans attending an American Legionnaires' Convention in Philadelphia, contracted a serious type of pneumonia. Thirty-four people died before the cause of the disease was identified by scientists of the Centres of Disease Control and Prevention. Today, serious outbreaks of the disease are still reported from all over the world. In fact, even more outbreaks are reported now than in the past.

Legionellosis is any form of illness caused by exposure to legionellae and include mainly two types of infections: Legionnaires' disease and Pontiac fever. Legionnaires' disease is a very serious multisystem disease with a potentially fatal pneumonia as the main symptom. Pontiac fever, on the other hand, is a mild disease, with flu-like symptoms, that mostly do not require medication. Many legionellosis cases go unreported because most people are not familiar with the symptoms. Although these diseases can be fatal, they can be treated successfully if treatment with the appropriate antibiotics is started early enough. Many health care professionals are under the false impression that legionellosis is rare, but the disease is actually quite common.

It is difficult to isolate legionellae from water samples and in South Africa, the methods used are not standardised. As a result, the prevalence of legionellosis in the country is not known. A Legionella Action Group has been formed to assist in the standardisation of the laboratory methods. One of their main functions is to create an awareness of the disease in the medical profession to reduce the risk of legionellosis and to assist water treatment companies with the treatment of water distribution systems to decrease the risk of the disease for workers and for the public. This study was undertaken in part to assist the Legionella Action Group with this, very difficult, task.

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2.	Identification methods for Legionella. University of Pretoria (1998).
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4.	Legionella identification from environmental samples. a progress report. FuturePact 199 Conference. (8 July 1998). Pretoria.
5.	Legionella detection from South African cooling water systems. 27th International Legionella Conference. (28-29 September 2000). New York, America.

CHAPTER 1

LITERATURE REVIEW

PUBLICATIONS AND PRESENTATIONS EMANATING FROM THIS THESIS

1.1 INTRODUCTION

PUBLICATIONS

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2. Bartie C and Soogreem T (2001). Legionellosis. *NCOH Occupational Disease Series Information Booklet* 1 (1): 1-23.
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5. Bartie C, Venter SN and Nel LH (2001). *Chapter 56: Legionella detection from South African cooling water systems. In: Legionella: Proceedings of the 5th Legionella Conference, 2000.* ASM Press, Washington DC.
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PRESENTATIONS

1. Identification methods for Legionella in industrial cooling water systems. *Annual microbiologists meeting, Eskom.* (25 July 1996).
2. Identification methods for Legionella. *University of Pretoria.* (1996),
3. Legionella identification from environmental samples. *Second South African Legionella seminar.* (16 February 1998, Johannesburg).
4. Legionella identification from environmental samples: a progress report. *FuturePath '98 Conference.* (3 July 1998, Pretoria).
5. Legionella detection from South African cooling water systems. *5th International Legionella Conference.* (26-29 September 2000, Neu-Ulm, Germany).

4.8.11 Presumptive identification

If single colonies are present, test all the characteristic colonies for cysteine dependence. In cases where no single colonies can be distinguished in any of the dilutions, stain a representative smear from each dilution by DFA. If positive, report as presumptive legionellae.

4.8.12 Cysteine dependence

Inoculate single colonies onto BCYE and nutrient- or blood agar and incubate as usual. Colonies that grow on the BCYE but not on the other media are considered cysteine dependent.

4.8.13 Confirmation

Cysteine dependent colonies are confirmed by DFA and/or latex agglutination.

4.8.14 Expression of results

Results are expressed as an estimated number of cfu/ml of original water sample or weight of original solid sample, taking the dilution factor into account. The highest dilutions yielding confirmed legionellae is reported.

4.9 THE WAY FORWARD

4.9.1 Surveillance and notification

South Africa has a well developed disease surveillance network. The prevalence of disease like tuberculosis, HIV/AIDS, malaria and cholera is well known. Unfortunately, the surveillance of other respiratory infections, including Legionnaires' disease and legionellosis has been neglected in the past, with the result that their prevalence is not known. The NCOH has embarked on a program of assistance in the reporting of legionellosis cases to the Gauteng Provincial Government. This program also includes a

continued effort to increase the awareness of this disease, not only in medical professionals, but also in workers, management and the general public.

Only one Legionnaires' disease outbreak has been reported in South Africa to date. However, there is always a possibility of outbreaks occurring in the country; in order to recognise such outbreaks and be able to respond timeously and efficiently to these threats, cases have to be reported to the proper officials. This program is designed to assist in this process.

4.9.3 Standardisation of detection methods

There is no standard method for the detection of legionellae from environmental samples in South Africa. This project served to point out some of the problems regarding legionella detection and propose a standard method for development and discussion. This method will be subjected to extensive interlaboratory testing before being standardised and used routinely.

4.9.4 Research

⇒ The presence of legionellae in dental unit water lines

The NCOH is currently involved in a research project, studying the presence of Legionella organisms in dental unit water lines at the Dental School, University of the Witwatersrand. This project will be expanded during 2002 to study the prevalence of antibodies in students and personnel at the Dental School. Preliminary results have shown a high proportion of dental unit water lines to contain legionellae. The findings from this study will be used to initiate appropriate prevention and control measures and will be of great value to increase the awareness of the risk of legionellosis in these systems.

⇒ Legionella infections in HIV positive mine workers

Pneumonia is one of the most frequent complications of HIV/AIDS patients. Apparently *Pneumocystis carinii* is the leading cause. The prevalence of *L pneumophila*, *C pneumoniae*, *C trachomatis* and *M pneumoniae* in this population is unknown. The reason for this may be the currently used diagnostic methods that make use of the measurement of antibody levels which is of limited value in HIV/AIDS patients due to their compromised immune response.

⇒ Legionella infections in miners with tuberculosis

Similarly, the high number of mine workers with tuberculosis increases the immunocompromised population in this sector. In order to provide the best primary and occupational health care, these patients should be tested for the presence of antibodies to respiratory pathogens like *C pneumoniae*, *L pneumophila* and other pathogens. To this effect, a research project is planned by the NCOH for the near future.

⇒ Legionella-like amoebal pathogens (LLAPs)

Intracellular replication within protozoa plays a major role in the ecology and pathogenesis of Legionella species. The recent emphasis on the presence of pathogenic free-living amoebae in water distribution systems, legionella-like amoebal pathogens and their potentially serious health risks in both the occupational and public health sectors have received much attention in scientific literature. A research project in collaboration with the Water Research Centre at the Tennessee Technological University is in the planning stage. A visit to the Centre has provided excellent information and training into the methodologies to be developed for this work, which will be a first in South Africa.

⇒ Molecular methods

Polymerase chain reaction (PCR)-based methods have been used fairly successfully in detecting legionellae in clinical specimens, but problems remain in environmental samples. Although an effective PCR method for Legionella has been developed in a recent South African study, the results obtained from using this method for Legionella detection from the samples evaluated in this study did not correlate with those obtained by culture. This was especially true for the samples from the underground areas of the mine (results not shown). Further research is therefore needed on this area of Legionella detection. Molecular typing methods should be developed for use in outbreak situations where it is essential to link isolates from patients and environments.

⇒ Immunomagnetic separation techniques

Immunomagnetic separation has been shown to have potential as a simple way of extracting bacteria from environmental samples prior to detection by other methods, by workers at the University of Pretoria. Research into this aspect of Legionella detection may prove useful.