MOLECULAR DETECTION OF PATHOGENIC LEPTOSPIRES FROM ANIMAL AND ENVIRONMENTAL SAMPLES

K.SUBASH
ID.No. MVM 08041

Thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF VETERINARY SCIENCE IN VETERINARY MICROBIOLOGY

to the

TAMIL NADU VETERINARY AND ANIMAL SCIENCES UNIVERSITY
CHENNAI - 600 051

DEPARTMENT OF VETERINARY MICROBIOLOGY
MADRAS VETERINARY COLLEGE
TAMILNADU VETERINARY AND ANIMAL SCIENCES UNIVERSITY,
CHENNAI - 600 007

2010

TAMILNADU VETERINARY AND ANIMAL SCIENCES UNIVERSITY
DEPARTMENT OF VETERINARY MICROBIOLOGY
MADRAS VETERINARY COLLEGE
CHENNAI – 600 007

CERTIFICATE

This is to certify that the thesis entitled "**MOLECULAR DETECTION OF PATHOGENIC LEPTOSPIRES FROM ANIMAL AND ENVIRONMENTAL SAMPLES**" submitted in partial fulfillment of the requirements for the degree of **MASTER OF VETERINARY SCIENCE** in **VETERINARY MICROBIOLOGY** to the **TAMILNADU VETERINARY AND ANIMAL SCIENCES UNIVERSITY, CHENNAI,** is a record of bonafide research work carried out by **K.SUBASH**, under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

Date: 31/7/10
Place: CHENNAI

Dr.G.RAVIKUMAR
CHAIRMAN

RECOMMENDED

Date: 4/10/2010
Place: Mannuthy

.....
Dr.M.MINI
EXTERNAL EXAMINER

APPROVED

Chairman : .....
Dr.G.RAVIKUMAR

Members :

1. .....
Dr.T.G.PRABHAKAR

Date: 25/11/10
Place:

2. .....
Dr. M.RAMAN

ABSTRACT

Title	:	**Molecular detection of pathogenic leptospires from animal and environmental samples**
Name of the student	:	**K.SUBASH**
Degree for which submitted	:	M.V.Sc (Veterinary Microbiology)
Name of the Chairman	:	**Dr.G.RAVIKUMAR, Ph.D.,** Professor, Leptospirosis Research Laboratory, Centre for Animal Health Studies, Madhavaram Milk Colony, Chennai – 600 051.
University	:	Tamil Nadu Veterinary and Animal Sciences University, Chennai – 600 051.
Year	:	2010

Leptospirosis is a zoonotic disease of worldwide importance, caused by pathogenic species of *Leptospira*, which affect almost all mammals. The infected animal, becomes renal carrier and excrete pathogenic leptospires in their urine and contaminate the environment. The disease is transmissible between animals and human through direct contact with urine and tissue fluids of infected animals and indirect contact with contaminated environment. Hence, detection and identification of pathogenic leptospiral DNA, in tissues of animals and environmental water and soil samples will give an idea about the occurrence of leptospirosis in animals and humans in correlation with dissemination of leptospires. In this study, a methodology was developed for detection and identification of pathogenic leptospires in animal tissues and environmental samples using, Polymerase Chain Reaction (PCR) and hybridization with specific probes.

The biological samples, like kidney and liver tissues were collected from various species of livestock and field rats. The environmental samples such as water and soil were collected from various lakes and ponds nearby human dwellings where, both animals and humans have contact with such water bodies for all purposes. The samples were processed by conventional method to extract genomic DNA.

The polymerase chain reaction with *lipL*21 and *lipL*32 primers, reported to be conserved only in pathogenic leptospires were employed to amplify the pathogenic sequences of leptospires in biological and environmental samples. Amplified products of 561 bp and 756 bp were obtained with *lipL*21 and *lipL*32 specific PCR respectively. The results were compared with widely used, 16S rRNA gene specific primers G1/G2, B64-I/B64-II and obtained an amplified product of 285 bp in all *lipL*21 and *lipL*32 PCR positive samples.

The *lipL*21 and *lipL*32 PCR products of *Leptospira* serovar *rachmati* were labeled with commercial biotin labeling kit, to develop specific probes. Hybridization of PCR positive samples was performed with specific synthesized probes, and then the hybridized DNA was detected using commercial chemiluminescence detection kit, to confirm the specificity of PCR. Spiking of biological and environmental samples with known serovars *rachmati* was done in PCR negative samples to detect the sensitivity of PCR and hybridization. PCR combined with hybridization using specific probes were found to be more sensitive than PCR alone for detection of pathogenic leptospiral sequences in biological and environmental samples.

(key words: Leptospirosis – biological samples – environmental samples - lipL21 – lipL32 – PCR – Hybridization – chemiluminescence)