

# A CARRIER STATE FOR HUMAN LEPTOSPIROSIS IN AN ENDEMIC TROPICAL REGION

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## INTRODUCTION

Proven leptospirosis in a patient in the immediate post-operative period following an open-heart surgery, after having received “fresh whole blood” was the trigger for this study. The objective of this study was to investigate the possible presence of a carrier state for leptospirosis in an endemic area.

## MATERIALS AND METHODS

### Serum samples

Between March 1997 and August 2002, serum samples from 20,455 blood donations were screened by dark field microscopy (DFM). Positive samples were subjected to additional tests like culture on EMJH medium, IgM antibody to leptospira and polymerase chain reaction (PCR) to validate the results of DFM. Arbitrarily primed PCR (AP-PCR) was done for serovar identification.

### Dark field microscopy

Aseptically-collected blood was centrifuged at low speed and a drop (50 µl) was viewed under DFM with a 40x objective. A grading system developed in-house was used to semi-quantify the load of leptospira in the samples (Table 1).

### Culture

EMJH (Ellinghausen, McCullough, Johnson, Harris) dehydrated medium commercially available from HiMedia/Difco was used. A selective agent 5 fluorouracil was added along with BSA and mineral solution as enrichment, and the pH was adjusted to 7.4 using 1N NaOH. Standard procedures were followed as per approved protocols.

### IgM antibody

IgM antibody to leptospira was detected using the Virion Serion GmbH kit procedure according to the manufacturer's protocol.

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## Molecular tools

Twenty-six randomly-selected culture-positive samples were sent to the Animal Biotechnology Department of the Madras Veterinary University for confirmation by PCR. Two sets of primers G1, G2 and B 641, B 651 were used. Serovar identification was possible using AP-PCR.

## RESULTS

Of 794 DFM positive samples, 155 were cultured on liquid EMJH medium. The results obtained are shown in Table 2.

## DISCUSSION

The presence of a carrier state for leptospirosis among healthy voluntary blood donors exists in a tropical endemic area. This underlines the need to screen the donated blood for leptospira, especially in these regions. Further studies are underway to determine if transfusion-transmitted leptospirosis also exists as a distinct entity.

Table 1  
Grading of DFM

Result	Observation
Negative	No spiral organism seen
Positive (occasional)	Occasional leptospira seen/slide
Positive (+)	1 – 2 leptospira seen / hpf
Positive (++)	3 – 7 leptospira seen / hpf
Positive (+++)	8 – 15 leptospira seen / hpf
Positive (++++)	> 15 leptospira seen / hpf

Table 2

	Culture	AP-PCR	IgM Ab
Positive	57% (88 / 155)	85% ( <sup>a</sup> 22 / 26)	12% (18 / 155)
Equivocal	-	-	13% (20 / 155)
Negative	43% (67 / 155)	-	75% (117 / 155)

<sup>a</sup>Organisms isolated : *L. louisiana* (15); *L. canicola* (6); and *L. pomona* (1).