ORIGINAL ARTICLE

Occurrence of leptospirosis among suspected cases in Chennai, Tamil Nadu

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ABSTRACT

Objective: To evaluate the serological profile of leptospirosis by microscopic agglutination test (MAT) and dark field microscopy (DFM) and to determine the serovar prevalence rate among patients with pyrexia of unknown origin. **Materials and Methods:** A total of 3830 blood samples were received from different hospitals and laboratories in and around Chennai. They were screened for leptospirosis by MAT and direct observation of live Leptospira by DFM. **Results:** A total of 748 (19.5%) Leptospira positive cases were identified; among these, 36.76% were *Leptospira australis*, 30% were *Leptospira canicola*, 14.57% were *Leptospira autumnalis*, 12% were *Leptospira icterohaemorrhagiae*, 4.68% were *Leptospira patoc* and 1.87% were *Leptospira grippotyposa*. Patients were in the age group of 1–86 years, with a median age of 43.5 years. 50% positive cases were in the age group of 10–35 years. Majority of the *Leptospira* infected cases were males (62.98%) than females (37.02%). **Conclusion:** Leptospirosis occurs in Chennai throughout the year although the number and positivity of cases increased during the monsoon season.

KEY WORDS: Dark field microscopy, leptospirosis, microscopic agglutination test, pyrexia of unknown origin

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INTRODUCTION

Leptospirosis is an emerging zoonotic disease of worldwide distribution and is endemic in Tamil Nadu, Kerala, and Andamans.[1,2] It is now being increasingly reported from other parts of India and has been reported throughout the year both from urban and rural centers, perhaps with better facility to diagnose the disease for better treatment. The disease is essentially spread by animal urine contaminating the environment. It is known that leptospirosis is widespread in farm and domestic animals in many parts of India, including the North-East, West Bengal, Bihar, Madhya Pradesh, Maharashtra, Andhra Pradesh, Karnataka, Kerala, Tamil Nadu, Punjab and Haryana.[3] It is a major public health concern. Early diagnosis and treatment of leptospirosis is essential, or else the infection can result in substantial morbidity and mortality. There is a need for a screening test for the early and rapid diagnosis of leptospirosis. Wolff noted that dark field microscopy (DFM), after differential centrifugation of Ruys, may enhance the chances of seeing Leptospira, and thereby makes an early diagnosis possible.[4] Microscopic examination of tissues or body fluids is not recommended as a single diagnostic procedure since the concentration of Leptospira in the blood may be too low and artifacts such as fibrils and extrusions from cellular elements can be easily mistaken for Leptospira by a novice.[5] However, DFM alone is not used by most of the lab workers for diagnosis.

In our laboratory, samples are received throughout the year and the data reveal that leptospirosis occurs throughout the year although the number may increase during the monsoon season (from September to December). Previous studies in Chennai revealed that the commonest serovars in Chennai were *Leptospira icterohaemorrhagiae* followed by *Leptospira australis* and *Leptospira grippotyphosa*.[6] Our objective was to assess the occurrence of Leptospira infection among suspected individuals.

MATERIALS AND METHODS

The department received blood samples from public, private hospitals and diagnostic laboratories, which are in and around Chennai. In some cases, patients were referred to the department; from whom 2–3 ml of whole blood was collected. Serum was separated for testing. All the serum samples were tested using microscopic agglutination test (MAT) and DFM for live Leptospira visible by naked eye in the blood of infected patients. Ten live pathogenic serovars, *L. icterohaemorrhagiae, L. australis, Leptospira autumnalis, L. grippotyphosa, Leptospira pomona, Leptospira sejroe, Leptospira ballum, Leptospira louisiana, Leptospira hebdomadis, Leptospira javanica*, and one *Leptospira patoc* nonpathogenic serovar were included for MAT preparation. The live antigens were cultured and prepared using standard methods.[4] MAT was done

at doubling dilutions starting from 1:20. A titer of 1:80 and above was considered positive. All the positive and negative samples were again observed under DFM.

RESULTS

A total of 3830 blood samples were tested for Leptospira using MAT and DFM. The patients were in the age group of 1–86 years, with a median age of 43.5 years. Leptospires were detected in 748 (19.5%) cases by MAT method. Among 748 positive cases, live leptospires were detected in 598 (80%) cases using DFM. The common serovars were *L. australis* (36.76%), *L. canicola* (30%), *L. autumnalis* (14.57%), *L. icterohaemorrhagiae* (12%), *L. patoc* (4.68%) and *L. grippotyposa* (1.87%) identified by MAT method [Figure 1]. Males were infected (62.98%) more than females (37.02%).

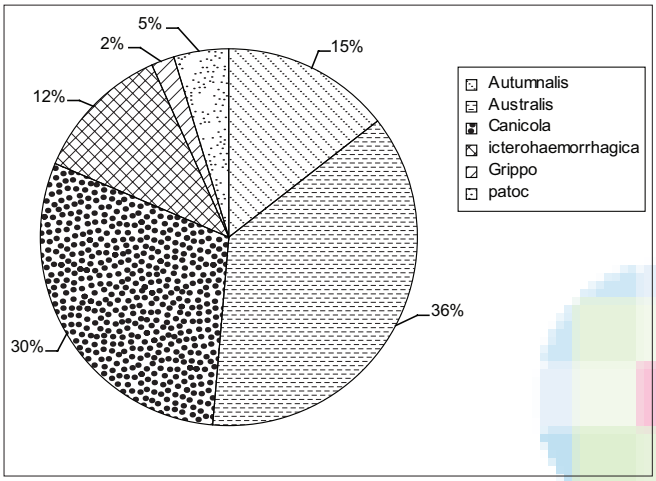

Figure 1: Show the prevalence rate of leptospira serovars in the year 2008

The number of samples were highest in the monsoon months (northeast monsoon: September–December) (1968 samples, 48.6%) and the average positivity was also high during this season (402 cases, 46.25%) [Figure 2].

DISCUSSION

There has been a dramatic increase in the number of samples and the number of Leptospira positive cases in our laboratory. This year's (2008) study shows *L. australis*, *L. canicola*, *L. autumnalis* to be the predominant prevalent serovars. The prevalence of leptospirosis reported from studies from different parts of India shows a wide variation.[6-10] In this study, it shows 19.5% positivity for leptospirosis which is equivalent or higher than the earlier studies. The age and sex distribution of patients in this study indicates that leptospirosis is the disease of occupationally active age group (44.25%), i.e., young to middle age adults (20–40 years) with a male preponderance. During monsoon, the infection rate is high. This shows that the polluted environment (exposure to overflowing sewage and stagnant rainwater on the roads) may play an important role for spreading this disease atory.

Our study shows 80% correlation between DFM and MAT; also, 80% of DFM positive cases show that they were recently exposed to Leptospira and the remaining 20% might have had current and/or past history of Leptospira infection. The original Faine's criteria have utilized MAT for diagnosis of leptospirosis on the basis of clinical, epidemiological and laboratory data. Though MAT is a specific test, its sensitivity may be low as the antibody titers rise and peak only in 2nd or 3rd week. A single positive

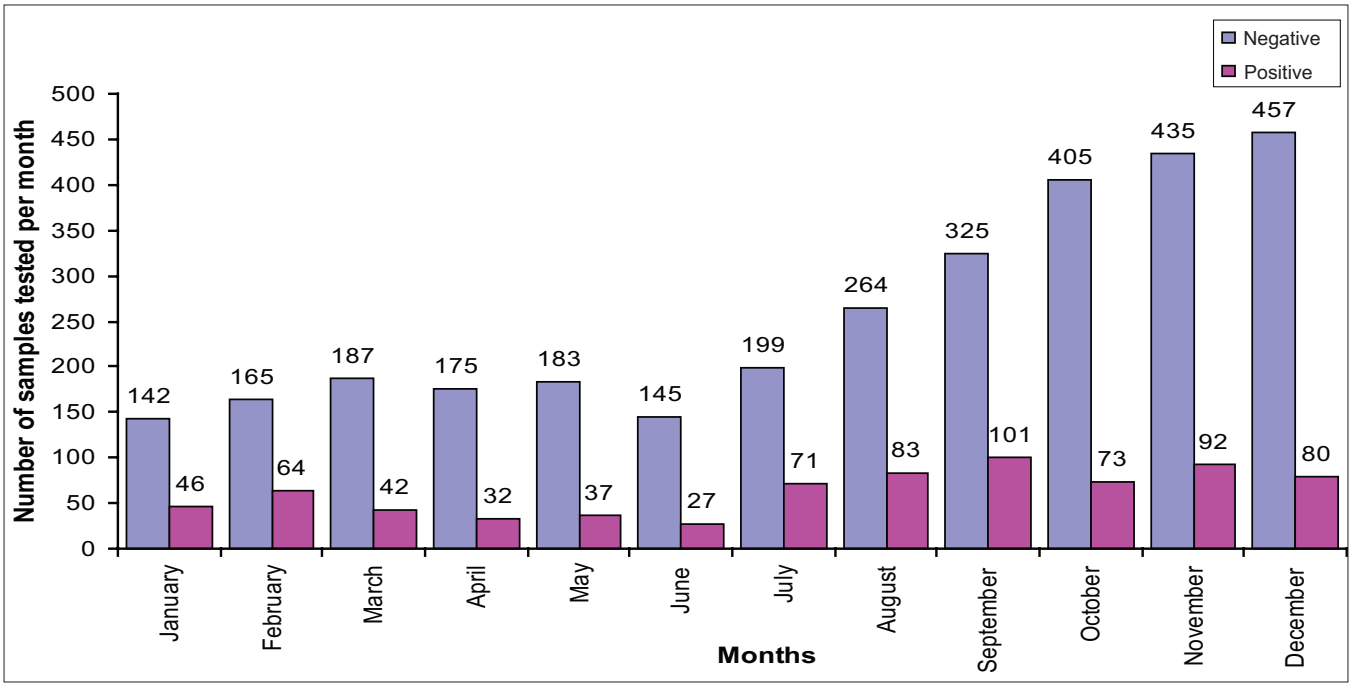

Figure 2: Shows blood samples tested month wise and shows number of negative and positive test results in the year 2008

titer may represent a rising titer of current infection or declining titer of past infection. However, in our laboratory, every sample was tested using MAT and DFM. As DFM detects live organisms, we were able to confirm our findings from MAT with DFM. Varying concentration of Leptospira, from one per field to 10–20 leptospires per field, was taken as DFM positive. In other studies from India and Africa, DFM exhibited greater sensitivity of 93.3% (56/60) than that of SERION ELISA for Leptospira IgM antibody (33.3%, 20/60) and the MAT.[11,12]

We conclude that leptospirosis occurs in Chennai throughout the year. There has been a dramatic increase in the number of samples and Leptospira positive cases, probably because of increased awareness of the illness. Samples were received throughout the year although the number and positivity of cases increased during the monsoon season.

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