

学 位 論 文 題 名

Leptospirosis infection among febrile patients at the
University of Peradeniya Teaching Hospital in Sri Lanka:
Clinical, epidemiological and laboratory investigations

(スリランカ・キャンディ市の大学病院における発熱外来患者を対象とした
レプトスピラ症に関する研究：臨床的・疫学的・生化学的アプローチ)

学位論文内容の要旨

Background and Objectives

Leptospirosis is a globally important zoonosis which has been recognized as an emerging infectious disease, especially in the tropical countries including Sri Lanka. The discovery of *Leptospira* was done 100 years ago but the understanding of its pathogenesis is still very poor and has subsequently hampered the development of new intervention strategies. A variety of wild and domestic animals form the natural reservoirs for pathogenic *Leptospira*. Leptospirosis is transmitted to humans by direct contact with reservoir animals or by exposure to environmental surface water or soil that is contaminated with their urine. Clinical symptoms of leptospirosis are protean leading to difficult diagnosis. Serological diagnosis is often used for confirmation of cases of leptospirosis and microscopic agglutination test (MAT) has been the gold standard.

The incidence of leptospirosis based on notifiable cases was 35.7 per 100,000 in 2008. In Kandy District, infections were reported to have also risen from 147 in 2007 to 501 in 2008. To help formulate local intervention, the study describes the socio-demographic characteristics and the circulating leptospiral serogroups and serovars among the clinically diagnosed cases; deduces the leptospiral species among laboratory-confirmed positive cases; and examines the characteristics between laboratory-confirmed positive and negative cases.

Methods

Patients were identified by physicians according to the surveillance case definition of the Epidemiology Unit of the Ministry of Health Sri Lanka from 1 April 2009 to 31 March 2010. We interviewed 97 patients clinically diagnosed with Leptospirosis and requested for blood samples from these cases. We examined serum samples for distribution of serovars using microscopic agglutination test (MAT) with a battery of representative leptospiral serogroups; and polymerase chain reaction test (PCR) was applied to the serum samples to detect *flaB* gene. Data were analyzed by SPSS version 14 and χ^2 test was employed for the test of significance.

Results

Among others, the 97 clinically diagnosed cases were mostly men (92.8%); nearly two-thirds were 35 years of age or older and had secondary or higher education (61.8%); half were farmers or laborers; and more than half were acute cases (57.7%). Of these 97 cases, 17 (17.5%) were MAT-confirmed positives, while 80 (82.5%) were negatives. The predominant serogroups among the confirmed positives were *Sejroe* and *Tarassovi*. Of 8 (8.2%) PCR-positives detected among 97 clinical cases, 1 PCR-positive occurred in the serum with MAT titre ≥ 200 but the remaining PCR-positives (7) were observed among those with MAT-negative. The 7 leptospiral species among

8 PCR-positives were *L. interrogans*. Having a pet (dog) seemed to be statistically associated with laboratory-confirmed positives.

Discussion

This study was undertaken in Kandy District, Sri Lanka for the purpose of collecting epidemiological and laboratory evidence on the socio-demographic profile of clinically diagnosed cases; the serogroups, serovars and species related to Leptospirosis; and the specific characteristics of laboratory confirmation status. Data are intended to help formulate strategies for the local control and prevention measures. Local data that are germane to the efficacious implementation of these measures, while critical, are sparse.

Our research findings suggest that *Leptospira* infection appears to be relatively common in Kandy District as borne out by our survey/clinical examination, and laboratory results. Our laboratory evidence particularly on serogroups (i.e., Sejroe and Tarassovi), is partly consistent with the findings from the same study site and source. All these studies have revealed Sejroe as the most predominant serogroup in humans and dairy cattle, suggesting that the reservoir of human Leptospirosis in Kandy is the smallholder dairy cattle. This information is useful for local control and prevention efforts.

Positive rate of the results by MAT also depends, to some degree, specimen-collection time during clinical course and chronic state of the infections. Further research is needed to validate this finding using, for examples, pairs of sera and/or serial blood samples of clinically diagnosed patients. Anyhow, in these circumstances, the choice of an appropriate assay(s) is not only critical but also vital in confirming *Leptospira* infections particularly in endemic areas like Sri Lanka.

Due to, among others, resource limitations and technical/logistical difficulties, clinical diagnosis and surveillance case definition do not accompany laboratory confirmation in Sri Lanka. The protean clinical manifestations of Leptospirosis tend to masquerade a true picture of Leptospirosis including fever and other signs and symptoms which are also common in many communicable diseases prevalent in Sri Lanka, such as dengue fever and hanta virus infections. Therefore, it is essential to employ laboratory support to confirm and/or differentiate Leptospirosis from other febrile diseases especially prevalent in the area.

Conclusions

Leptospirosis is relatively common in Kandy District and the most common leptospiral species is *L. interrogans*. The laboratory results by MAT and PCR depend on the time of blood collection after onset of fever. Laboratory-confirmed positives seem to be associated with those who played with a dog. Further studies are warranted for choice and development of appropriate assay methods as well as identification of potential reservoirs in the region.

学位論文審査の要旨

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(スリランカ・キャンディ市の大学病院における発熱外来患者を対象としたレプトスピラ症に関する研究：臨床的・疫学的・生化学的アプローチ)

レプトスピラ症は、熱帯諸国を中心に、世界的に流行している人獣共通感染症のひとつで、社会経済的要因や気候条件によって、保菌動物からヒトへ直接あるいは間接接触により伝播する。本研究ではスリランカ・キャンディ市において流行しているレプトスピラ症の血清型および種の同定とその分布の推定、およびそのリスクファクターの解明を行なった。

質疑応答では、副査の上田教授より、研究で用いられた質問紙の妥当性について、また、副査の藤田教授より、なぜ 2008 年に大流行したのかについて、さらに、副査の有川教授より、ヒトからヒトへの感染があれば疾患の再流行につながるが、その可能性について、質問があった。また、主査の寺沢教授より、ヒト臓器内でレプトスピラが引き起こす疾病の発症機序について質問があった。さらに副査の玉城教授より、本研究により明らかになった疫学的知見から提言できることがあればひとつ述べるようにとの質問があった。

申請者は、質疑の内容を十分に理解した上で、当該疾病に関する世界と地域の疫学的背景と研究結果を踏まえて的確に回答した。

上田教授の質問に対して、申請者は、質問紙の作成過程を述べながら適切に回答し、また、質問内容において考慮すべき地域の疫学的背景について把握するために、フォーカスグループディスカッションを効果的に実施したことを説明した。次に、藤田教授の質問に対しては、地域で発生した洪水や報告システムの修正などに伴い疾患報告数が増

加することを述べ、地域の実情を十分に踏まえて回答した。また、有川教授のヒトからヒトへの感染の可能性に関する質問に対しては、その可能性は否定できないものの、複数の先行研究の結果を照らせば現在までそれは立証されていないと回答した。しかしながら、流行国へのエコツアーのような海外旅行や流行国でのスポーツイベント、余暇活動は、感染の機会を増大させているという報告があることを述べた。寺沢教授のヒト臓器における疾病の発生機序に関する質問に対しては、先行研究や文献を適切に引用し、ワイル病の臨床的徴候としてよく知られている、レプトスピラが組織や臓器を貫通するために生じる影響について述べ、的確に回答した。最後に、玉城教授の質問に対しては、疾患対策として有効な方法を構築するために、本疫学研究から得られた知見は有意義なものであると回答した。

審査員一同は、これらの成果を高く評価し、大学院課程における研鑽や取得単位なども併せ申請者が博士（医学）の学位を受けるのに十分な資格を有するものと判定した。