学位論文内容の要旨

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学位論文題名

Surveillance and Epidemiology of Leptospirosis among Humans and Animals, Sri Lanka: Search for Important Reservoirs

(スリランカにおけるヒトおよび動物のレプトスピラ症に関する疫学調査:保菌動物の特定に向けて)

[Background and Objectives]

Leptospirosis, which is caused by spirochetes belonging to the genus *Leptospira,* is a worldwide public health problem. Annual incidence rates range from approximately 0.1–1 per 100,000 persons in temperate climate to 10–100 in humid tropical climate. The occurrence of the disease is usually related with socioeconomic and climatic conditions, which in turn favor animal vectors and human exposure.

Clinical diagnosis of leptospirosis is complicated due to the varied and non-specific manifestations of its symptoms. Poor and inadequate laboratory facilities tend to also hamper the accurate confirmation of leptospirosis, thus the disease remains largely under-estimated especially in resource-constrained settings.

Leptospires infection, one of the 28 notifiable diseases in Sri Lanka, is routinely reported to the Ministry of Health through the National Disease Reporting System. While the government's reporting system has captured an uptrend in the notifications of leptospirosis cases, it requires further clinical and epidemiological information with which to understand and control the disease dynamics. Thus, in 2004, in parallel to its notifiable diseases reporting system, the government started hospital-based sentinel surveillance for leptospirosis. The sentinel surveillance seeks to obtain information on clinical, epidemiological, laboratory and prophylactic treatment among those suspected of having an infection. The country's inadequate laboratory facilities suggest that the bulk of reported cases of leptospirosis remain unconfirmed. Due to the use of saprophytic leptospira in genus-specific micro-agglutination test to confirm the infection, the laboratory data in Sri Lanka are often incomplete. Thus, limited local information is available on circulating leptospiral serogroups and species, and status of reservoir animals. This epidemiological information is important for disease control and prevention.

This study aimed 1) to describe the dynamics of leptospira infection; 2) to identify circulating leptospiral serogroups and species; 3) to estimate the prevalence and carrier status of leptospirosis in smallholder dairy cattle and peridomestic rodents; and 4) to identify hantavirus infection among leptospirosis suspected patients.

[Materials and Methods]

Multiple research methods were employed to fulfill the research objectives. All research activities were approved by the Ethics Committee of the Faculty of Medicine, the University of Peradeniya.

Dynamics of leptospira infection: All variables from the Ministry of Health's nation-wide sentinel surveillance data on leptospirosis from 2005 to 2008 were analyzed using descriptive statistics.

Circulating leptospiral serogroups and species, and prevalence and carrier status: This particular research activity was undertaken in Kandy District because of our ongoing collaboration with the University of Peradeniya. Humans and animals were involved. In 2008, blood samples were collected from patients at the University of Peradeniya Teaching Hospital. These patients had acute febrile disease and fulfilled the case definition criteria for leptospirosis. In 2009, blood and urine specimens were obtained from smallholder dairy cattle and blood and kidney samples from peridomestic rodents in the Yatinuwara and Udunuwara divisional secretariats of the district. Both human and animal samples were serologically analyzed using microscopic agglutination test (MAT) with a battery of pathogenic leptospiral strains and genetically examined using polymerase chain reaction (PCR).

Hantavirus infection: The human blood specimens to detect anti-hantaviral antibodies were derived from the same samples used in the 2008 research to identify leptospira infection. This additional laboratory investigation was carried out to determine co-infections and/or differential diagnosis, since leptospirosis and hantavirus have similar clinical and epidemiological features. Serum specimens were screened by employing indirect immunoflourescence and Enzyme-linked immunosorbent assay.

[Results]

Dynamics of leptospira infection: Of 4000 suspected cases, approximately half (46.9%) and one fourth (26.8%) were recorded from Western and Sabaragamuwa provinces, respectively. Most were men (83.5%) and aged 30-49 years (45.6%). 16.5% were farmers and 16.1% were laborers and nearly half (44.8%) had no information on occupation. Half (53.9%) had an exposure to paddy lands. Almost all or most had acute fever (98.8%), myalgia (92.9%) and headache (92.7%); fewer had other related symptoms. Moreover, 2496 cases (62.4%) underwent a laboratory examination to detect proteinuria and blood urea nitrogen, complete blood cell count, and MAT, of which 1482 (59.4%) had laboratory test results. Merely 107 (2.7%) had MAT results and only 69 (1.7%) cases took an antibiotic prophylactic treatment. There were increases in the numbers of suspected leptospirosis deaths reported to sentinel surveillance sites from 19 deaths in 2005 to 108 in 2008. Of 108 deaths, 27, 26, 17 and 14 deaths were reported from Colombo, Kandy, Kalutara, and Kegalle districts, respectively.

Circulating leptosprial serogroups and species in febrile patients: Anti-leptospiral antibodies were found in 26 of 107 serum specimens studied (24.3%). The predominant reacting serogroups was Sejroe (9/26, 34.6%) followed by Icterohaemorrhagiae (5/26, 19.2%). Leptospiral DNA was detected in 3 (2 *L. interrogans* and 1 *L. kirschneri*) of the 107 serum specimens. Anti-leptospiral antibodies did not detect in PCR positive specimens.

Prevalence and carrier status of leptospirosis in smallholder dairy cattle and peridomestic rodents: The cattle in 19 (38.8%) of the 49 farms harbored anti-leptospiral antibodies. Out of 113 cattle serum specimens, 23 (20.3%) were positive; 17 (73.95%) and 6 (26.1%) reacted with serogroups Sejroe and Hebdomadis, respectively. Out of the 74 rodent specimens, 13 (17.5%) were positive; 8 (61.5%) and 4 (30.7%) had reactions to serogroups *Javanica* and *Icterohaemorrhagiae*, respectively. Leptospiral DNA was detected in one cattle urine sample and identified as *L. interrogans*.

Hantavirus infection: Among 105 patients suspected as having leptospirosis, eight had only IgG anti-hantavirus antibodies. Serotyping ELISA shows that eight patients had high optical density values for Thailand virus. Most of the sera indicated that the focus reduction neutralization test titer against Thailand virus was higher than the test titer against Seoul virus. A lack of significant association was observed between presence of hantavirual antibodies and socio-demographic characteristics.

[Discussion]

Among others, the results from the sentinel surveillance data analysis reveal that the suspected cases for leptospirosis mainly come from areas with high rainfall levels and from specific socio-demographic categories (e.g., men, aged 30-49 years, agricultural workers). Laboratory confirmation of suspected cases is not sufficient and needs to be institutionalized more routinely.

Serological and genetic analyses of human samples confirm the existence of a wide array of leptospiral species and serogroups in Kandy.

There was a high prevalence of leptospirosis in both smallholder dairy cattle and peridomestic rodents. The carrier status of dairy cattle in the area was identified by detecting leptospiral DNA in cattle urine using *flaB*-PCR. The dairy cattle in Sri Lanka are not vaccinated against leptospirosis; the detected anti-leptospirosis antibodies are attributed to natural infection. Thus, infected cattle could be a source of infection for the rest of the herd. Half of the dairy farmers allowed their cattle to free-roam in the area, suggesting that cattle could also contaminate the environment.

The study found IgG anti-hantavirus antibodies that are indicate prior infection of Thailand virus-related hantavirus among patients.

[Conclusion]

Our findings suggest that leptospirosis is indeed a re-emerging infectious disease in Sri Lanka. Control and prevention measures must be continued and improved (e.g. community-based education programs fostering awareness and hygiene practices among people at-risk). These interventions need to give due and special attention to geographic areas with a high rate of case fatality and to individuals of particular socio-demographic categories (e.g., men and agricultural workers).

Animal reservoirs must be provided with critical attention. Serological data indicate that the cattle, relative to rodents, constitute a more important reservoir for human transmission and a greater source of potential risk to local agricultural communities in the study area. However, further studies are needed to identify important reservoirs. These studies must isolate and characterize leptospires from domestic and feral animals, and humans in this area.