# Leishmaniasis: unravelling the mysteries of a new health issue in Sri Lanka

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## Abstract

Leishmaniasis has resulted in devastating epidemics and many deaths in the world. Sri Lanka has been identified as a newest focus for human leishmaniasis, in South Asia. This paper describes our efforts in different phases from the detection of the first case to the collection of essential information which facilitated the formulation of a national action plan for disease control.

Awareness campaigns in Northern war vacated areas and Southern areas in Sri Lanka resulted in increased case numbers. Initial and extended clinical studies carried out at different time points confirmed the presence of a cutaneous form of leishmaniasis (CL) with no evidence of visceralization. Biochemical, gene sequencing and micro satellite loci analysis identified the presence of a genetically distinct variant of *Leishmania donovani*. Four different field studies identified peri-domestic, outdoor transmission and evidence for case clustering. Further expanding the picture, other clinical forms (visceral and mucosal infection) have been detected recently (authors' unpublished data). Immunological studies suggested that there is a sero-conversion potential in local CL parasites. *L. donovani* is not known to be associated with animal reservoirs. Local animal study confirmed the potential of dogs as a reservoir. Diagnostic method comparison and clinical studies have established LM, PCR, cultures, clinical markers and a two staged clinical screening tool for field case detection.

Since 2001, our institution has provided free of charge diagnostic service to over 3000 patients through our Centre for Research, Training and Diagnosis of Leishmaniasis. Two international colloquia (held in 2009 and 2013) formulated a national action plan for disease control. Training workshops for government technical staff and many research projects were undertaken. Many international collaborations including support from the Third World Academy of Sciences (TWAS) have enabled our research activities and the visits of experts who guided us on laboratory development. Baseline information on the project is available at our website, http://www.med.cmb.ac.lk/index.php/services?index_php?option=com_content&id=504.

Leishmaniasis has resulted in devastating epidemics and many deaths in the world. Sri Lanka has been identified as a newest focus of human leishmaniasis, in South East Asia. This paper focuses on the University of Colombo efforts in different phases from detection of the first case to obtaining essential information which facilitated the formulation of the national action plan for disease control.

Leishmaniasis is a protozoan infection which draws much attention of the World Health Organization as a neglected disease . Leishmaniasis in the past has resulted in devastating epidemics and many deaths in the world. It places a huge disease burden especially in tropical and subtropical countries . Clinical manifestations of human illness range from skin infection that lead to disfiguring lesions to visceral disease which is often fatal, if untreated.

Human leishmaniasis is the newest vector-borne disease established in Sri Lanka. Nearly 3000 cutaneous leishmaniasis (CL) patients have been investigated by the Department of Parasitology, Faculty of Medicine, Colombo alone during the past decade. Other more debilitating clinical forms such as mucosal (MCL) and visceral (VL) have also been reported recently. More alarmingly, local disease is caused by *L. donovani* the most virulent, visceralizing species of the genus.

Before the year 2001, leishmaniasis in Sri Lanka was limited to few sporadic cases, mainly imported in nature. The recent outbreak was detected in year 2001. With the detection of first few cases, several awareness campaigns were held by some of us for medical, para-medical and non medical sectors in Northern/North-Central Sri Lanka which resulted in increased number of referrals providing prime evidence of local establishment of the infection. Characteristics of leishmaniasis are known to be complex and differ between the endemic settings. Therefore, the need for essential local scientific information for patient care and disease control was highlighted. Focused preliminary studies were begun immediately afterwards with limited resources.

Clinical arm of the project also commenced in year 2001. Patients referred with provisional CL were clinically evaluated and laboratory confirmation by

microscopy of aspirations/scrapings of skin lesions was done. Study identified the basic clinical profile . Majority was soldiers, and males serving Northern Sri Lanka and having an outdoor occupational pattern. Single skin lesions occurred mainly, on exposed body areas. Papules, nodules, ulcerating nodules and ulcers were seen in almost equal proportions. No evidence of visceralization was found during this early part of the outbreak as confirmed by negative formol gel test. Wider local scientific community and general community was informed via scientific presentations and further awareness programms, in Southern Sri Lanka resulting in an increased reporting of more patients with a wider spatial distribution, indicating the need for further studies.

Leishmaniasis is caused by several species of genus and the clinical picture and case management is mainly dependant on the causative species. What is the causative organism in Sri Lanka?

Parasitological studies were commenced in year 2003. Biochemical methods which remain as the gold standard in species identification revealed *Leishmania donovani* as the causative species for CL in Sri Lanka . *L. donovani* usually results in VL in other endemic sites. In spite of this finding, the local picture remained as CL with widening of its case distribution pattern. Further confirmatory genetic based studies were initiated in year 2005 and re-confirmed the finding of *L. donovani*. Infra-specific studies further identified it as a genetically distinct variant within *L. donovani* family. Therefore, by year 2007, molecular studies helped the confirmation of a dermotrophic genetic variant of *L. donovani* in Sri Lanka.

Further confirming this, cases of CL continued to be reported. Therefore, continued clinical studies were necessary. Clinical studies including larger data sets to cover a wider spatial and temporal distribution have further confirmed a clinical picture consistent with CL, without signs of visceralization. Second clinical study included 400 patients and further confirmed CL clinical picture, studied atypical manifestations and further details of different developmental stages .

By year 2008 all 9 provinces of the country reported cases, though initial cases were mainly from North. Last clinical study included 1200 cases. Laboratory diagnosis was established by light microscopy (LM) /culture/ PCR in 67% (n=804/1200) patients with 1108 lesions. Majority of confirmed cases were civilians (n=778, 96.8%) who contracted CL while based in the same province (n= 797, 99.1%) and belonged to only 2/6 districts (n=759, 94.4%). Proportion of males was only slightly higher than females (n= 477, 59.3%, P=>0.05) while 1/3 belonged to below 20 year age group (n=234, 29.1%). Most had single lesions (n=736, 91.5%). Most lesions presented within 3 months of onset (n=495, 44.7%), however, majority were ulcers (n=603, 54.4%). They were less than 1cm in diameter (n= 415, 37.5%). Head and face (n=384, 34.7%) and upper limbs (n= 501, 45.3%) were mainly affected. Bi-annual seasonal variation was observed in case presentation with 2 peaks in April-June and August-October.

Leishmaniasis is transmitted zoonotically or peri-domestically and possesses a range of risk factors depending on the endemic setting. Field studies were commenced in year 2002 and aimed at identifying the prevalence and risk factors of leishmaniasis. First study covered a war vacated area in North (Welioya Sampath Nuwara DS division). Socio-demographic and clinical data was gathered by house to house screening survey followed by case control study that revealed a prevalence of 2.4% and age between 21–40 years, male sex, and spending outdoor time as risk factors. This indicated outdoor/zoonotic nature of disease transmission. In year 2005, second study in Mamadala GN division in District of Hambantota covered 938 subjects, giving a point prevalence of 3.4%, age between 11 and 40 years, living in a household with five or more members, and the presence of cracks and crevices on the walls of the house as risk factors. This study indicated a peri-domestic transmission in Southern Sri Lankan disease foci. Third study which covered an area in Kataragama which has no detected cases of CL but gave a 0.5% prevalence, indicating the underreported status of CL. Fourth field study covered the whole district of Matara with a coverage of 52 GN divisions. These studies have shown evidence for regional variation and case clustering.

Multiple inputs have been made up to date with regard to the clinical and epidemiological profile since year 2003. CL remains as the main presentation and bi - annual seasonal variation remains constant. Different skin lesions are seen. Further expanding the picture, few VL (25) and MCL (3) suspected patients have been reported recently. Diagnosis has been confirmed in 4/25 VL and 2/3 MCL patients from Northern Sri Lanka. Three (¾) VL patients had a history of overseas

travel while fourth was a locally acquired infection (manuscript in preparation).

Early case identification and management is considered the mainstay of control of *L.donovani* infections due to it's absence of reservoir hosts in affected countries. Importance of case detection and management in Sri Lanka has been highlighted at several occasions. Diagnostic clinical markers were identified and a two staged screening method was developed for the use in the field setting .

Potential of the local parasite variant of CL to cause simultaneous or late onset VL or MCL is unknown. Therefore, immunological studies were started in year 2009 to examine this possibility. Twenty six patients with parasitologically confirmed leishmaniasis (24 with CL, one with VL, and one with MCL) were investigated by rK 39 rapid diagnostic assay which is the recommended serological assay for VL diagnosis. All the sera from CL cases were negative while ML and VL patients were positive. It is likely that local antibody profiles are different from that of known global endemic sites.

*L. donovani* are usually transmitted from human to human via sand flies and are not known to be associated with animal reservoirs. However, the suspicion of zoonotic transmission based on the detailed investigation in to the history and transmission patterns led us to examine the possibility of animal reservoir hosts in Sri Lanka. An area with highest incidence of human cases of leishmaniasis over the past 2 years was selected from Southern Sri Lanka. A sample of 51 dogs were studied and 7.84% (4/51) was found to be serologically positive for *L. donovani* specific rK39 antibodies. rK39 is a *L. donovani* specific antigen to which the visceralizing infection can exert a serological response. This confirmed the potential of dogs to harbor *L. donovani* in the study area.

Since year 2001, a weekly leishmaniasis clinic is being conducted by staff, Department of Parasitology and free of charge diagnostic service is provided for patients referred to the clinic across the country. The service and activities have now expanded and the Centre for Research, Training and Diagnosis of leishmaniasis was established in the Department of Parasitology, Faculty of medicine, Colombo. Culture, PCR and serology techniques have also been established and are in place for diagnosis. Website of the centre provides baseline information to the public as well as health care staff which also acts as a resource to acquire more information and details of the services available (*http://www.med.cmb.ac.lk/index.php/services?index_php?option=com_content&id=504).*

Many training workshops held ensured dissemination of this knowledge and skills to other Universities as well as medical and para-medical staff of the Ministry of Health.

There is a regional effort in Indian subcontinent for leishmaniasis control . University of Colombo, Sri Lanka has held two international colloquia with participation of many stakeholders in collaboration of Ministry of Health which lead to the formulation of a national action plan for leishmaniasis control .

Leishmaniasis caused by the most virulent species among the complex *L. donovani* is now widely prevalent in Sri Lanka. Team work with active participation of the treating clinician, epidemiologists, laboratory personnel and the researchers will ensure successful control of leishmaniasis in this island.

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