

## Summary

Cutaneous leishmaniasis (CL) is an increasing public health concern in Suriname, due to a considerable upsurge of economic activities such as gold mining, logging, and ecotourism in the forested hinterland where this disease is endemic. CL is caused by protozoan parasites of the genus *Leishmania*. The clinical manifestations of CL range from a single lesion to many large ulcers on the skin, but the mucosae of particularly the nasal and oral cavities including pharynx and larynx can also be affected resulting in mucocutaneous leishmaniasis (MCL). The healing of the lesions caused by CL can take months to years and often leaves behind lifelong disfiguring scars. Transmission of parasites between mammals occurs by the bite of an infected female sand fly of the family Phlebotominae (Diptera: Psychodidae). The sand fly species of medical importance belong to the genera *Phlebotomus* and *Lutzomyia*. Wild mammals such as sloths and rodents, but also domesticated animals such as dogs are incriminated as reservoir hosts. Humans get infected incidentally when they intrude in the reservoirs' habitat.

For a long time *Leishmania (Viannia) guyanensis* was believed to be the only infecting *Leishmania* species in Suriname. However, in the past decade, clinical forms of leishmaniasis have emerged in the country that could not be attributed *L. (V.) guyanensis*. Moreover, an increasing number of patients does not respond adequately to the standard treatment with pentamidine isethionate. These observations are consistent with the recent identification of *Leishmania* species other than *L. (V.) guyanensis* in Suriname including *L. (V.) lainsoni*, *L. (V.) naiffi*, and *L. (L.) amazonensis*. However, when considering the prevalence of other *Leishmania* species in Suriname's neighboring countries French Guiana and Brazil and the busy travel between these countries, it is conceivable that more *Leishmania* species are present in Suriname. It is clear that information on *Leishmania* species present in Suriname is essential for the proper management of CL in the country. For the same reason it is necessary to obtain updated data about the sand fly species and reservoir hosts involved in the transmission of this disease in Suriname.

This thesis describes the results from studies designed to improve the insight into these aspects of the biology of leishmaniasis in the country. For this purpose, a quantitative real time - polymerase chain reaction (qPCR)-based method was used to detect and quantify *Leishmania* DNA in samples from patients, sand flies, and dogs. This assay targets the 18S ribosomal gene that is specific for all members of the *Leishmania* genus. To differentiate among the various *Leishmania* species, the mini-exon PCR-restriction fragment length polymorphism (PCR-RFLP) method was used, targeting the spliced leader RNA gene.

Chapter 2 provides an updated list of sand fly species present in Suriname, including those of medical importance. Using CDC light traps, sand flies were collected at seven locations where increasing numbers of CL cases are being reported. These locations included gold mining areas and Maroon villages in the districts of Sipaliwini, Para, and Brokopondo. A total of 34 different sand flies species was captured including four *Lutzomyia* species (*Lutzomyia aragaoi*, *Lu. ayrozai*, *Lu. damascenoi*, and *Lu. sordellii*) that were not recorded before in Suriname. Using qPCR and PCR-RFLP, four species comprising together about 50% of the catch - *Lu. trichopyga*, *Lu. ininii*, *Lu. squamiventris (s.l.)*, and *Lu. umbratilis* - were found to harbor *Leishmania* parasites. One female *Lu. squamiventris (s.l.)* specimen was positive for *L. (V.) braziliensis* while the others contained too low amounts of DNA to enable identification of the infecting *Leishmania* species. Nevertheless, this study demonstrates the diversity of the sand fly fauna in Suriname and indicates which *Lutzomyia* species are capable of transmitting *Leishmania* parasites in the country.

In many South American countries, evidence has been gathered to incriminate the dog, among other mammals, as a reservoir for leishmaniasis. The canine reservoir brings parasites and vectors closer to domestic areas, increasing the chances for infection of humans. In Chapter 3, the possibility of dogs as reservoirs for leishmaniasis in Suriname has been investigated. To this end, blood samples from a total of 47 dogs from different locations in the rural-interior districts of Sipaliwini and Brokopondo where CL is endemic, and from a kennel near Paramaribo, were collected on filter paper, and assessed for *Leishmania* antibodies and parasite-specific DNA using serology (with a direct agglutination test; DAT) and molecular biology (by PCR), respectively. Two dogs appeared sero-positive with DAT (titre > 1:1600), three had a border line titre (1:800), while the other animals were DAT-negative. PCR analysis found *Leishmania* DNA equivalent to 1 parasite per mL in only one dog at the first round of analysis, but this animal was negative after re-testing. These data suggest that canine leishmaniosis is prevalent in Suriname and that dogs could play an important role in the

sylvatic cycle of the disease. However, additional studies are necessary to incriminate the dog with more certainty as a reservoir host for leishmaniasis transmission in Suriname.

Chapter 4 describes the dynamics of *Leishmania* parasites in patients' biopsies before as well as 6 and 12 weeks after treatment with pentamidine isethionate. Using qPCR, parasites were detected in 98% of the biopsies before treatment (a parasite count of 2 – 46,024,919 per mL). At week 6 after treatment, the overall parasite count had substantially declined, but the drop was significantly more pronounced in cured patients (parasite count 0 – 61,088 per mL) than in those who had failed the therapy (parasite count 0 – 28,078,627 per mL). Twelve weeks after treatment a similar pattern was noticed in both groups of patients. The results suggest that there is a strong correlation between parasite load at week 6 and treatment outcome at week 12. Parallel analyses using PCR-RFLP showed that more than 90% of the 143 patients had been infected with *L. (V.) guyanensis*, 2.6% with *L. (V.) braziliensis*, and 1.3% with *L. (L.) amazonensis*. About three-quarters of the patients infected with *L. (V.) guyanensis* were cured, but all of those infected with *L. (V.) braziliensis* or *L. (L.) amazonensis* had failed the therapy. These data underscore the value of qPCR for monitoring the response of leishmaniasis patients to pentamidine isethionate and indicate that re-evaluation at 6 weeks after treatment is suitable for adequate management of treatment.

Furthermore, infections with *L. (V.) braziliensis* and *L. (L.) amazonensis* not only require other medications which are currently not available in Suriname, but infections with *L. (V.) braziliensis* may also cause the muco-cutaneous form of the disease. This occurs in 3 to 5% of cases. In Chapter 5, the first case of CL caused by *L. (V.) braziliensis* in Suriname is described. This patient was included in the study described in Chapter 4 where not only parasite dynamics in patients' biopsies was assessed over treatment, but also molecular typing of *Leishmania* species was performed using PCR-RFLP. The patient travelled to the hinterlands when he got infected and developed three nodular lesions with central ulceration. Fortunately, no mucosal lesions were detected. This finding emphasizes the need for differential diagnosis, particularly in patients who respond poorly to pentamidine isethionate treatment.

In conclusion, *L. (V.) guyanensis* is the main infecting species of CL in Suriname, but other species including *L. (V.) braziliensis* - the causative agent of MCL - are also present in the country. Furthermore, the *Lutzomyia* species responsible for the transmission of all previously mentioned *Leishmania* parasites are abundantly present in high-transmission areas in the country. Although not incriminated, the data suggest that the dog might play an important role as reservoir of CL. Importantly, the implementation of molecular tools for differential diagnosis and treatment follow-up proved essential for the proper management of the disease. This information provides significant grounds for further studies and for the realization of a control program in Suriname.

