

ABSTRACT

Visceral leishmaniasis (VL) is widespread disease caused by protozoa *Leishmania donovani* and *Leishmania infantum*. Human visceral leishmaniasis caused by *Le. donovani* in India is considered an anthroponosis, however in East Africa, the role of animals as reservoirs remains unclear. The first part of this thesis demonstrated natural *Leishmania* infection in wild rodents and bats in Ethiopia. Overall, 8.2% rodents and 4.9% bats were positive for *Leishmania* spp. Subsequent sequencing revealed that 10% of *Leishmania*-positive rodents were infected by parasites from *Le. donovani* complex, on the other hand, no *Le. donovani* DNA was detected in bats. All *Le. donovani*-positive rodents were captured in the localities of southwest Ethiopia where human VL cases have been reported and potential sand fly vectors occur. Our findings indicate that rodents are likely to play a role in VL transmission in Ethiopia.

During blood feeding, sand flies inoculate into the host skin immunogenic salivary proteins which elicit species specific antibody response. Anti-saliva antibodies could be used as a marker of host exposure to sand flies and, in leishmaniasis endemic areas, also as risk markers of *Leishmania* infection. In order to find out if the domestic animals (dog, goat, cow, and donkey) from north and northwest Ethiopia are involved in VL cycle we measured antibodies against saliva of *Phlebotomus orientalis*, the implicated vector of VL in Ethiopia. A total of 23.1% of the animals were seropositive for anti-*P. orientalis* saliva IgG, with the highest seroprevalence observed in dogs (58.8%) and sheep (47.7%) followed by donkeys, goats and cows. These results indicate opportunistic feeding preferences of *P. orientalis* and also the possibility of zoonotic transmission of *Le. donovani*.

The third part of this thesis is focused on the use of recombinant salivary proteins instead of whole saliva in antibody detection assays. We have used, for the first time the *Phlebotomus perniciosus*, salivary recombinant proteins in a longitudinal field study on dogs from *Le. infantum* endemic locality in south Italy. We found a strong correlation between IgG antibodies recognizing 43 kDa recombinant yellow-related protein (rSP03B) and the whole salivary antigen. The kinetics of antibody response had similar pattern for whole saliva and for rSP03B and was clearly related to seasonal activity of *P. perniciosus*. We found association between canine leishmaniasis infection and antibodies against whole saliva but not against rSP03B. Moreover, in cross-sectional study from Portugal and Italy we proposed rSP03B protein as universal marker of canine exposure to *P. perniciosus* within its entire distribution area.