ABSTRACT

In the Mediterranean basin, human visceral leishmaniasis caused by the protozoan parasite *Leishmania infantum* is a zoonotic disease that gives rise to 1,200 to 2,000 new cases annually. The domestic dog constitutes its main reservoir, of which some may suffer from a severe chronic disease, canine leishmaniasis (CanL). The sand fly *Phlebotomus perniciosus* is considered to be the principle vector. Saliva of bloodfeeding vectors of diseases has been used in the past to assess host exposure to vector bites and to evaluate vector control tools. This Ph.D. focused on saliva of *P. perniciosus* to identify exposure markers that could be used in the preparation of a new vector exposure tool.

The first part of this Ph.D. aimed at validating the use of a recombinant salivary protein of *P. perniciosus* – rSP03B – in endemic settings of CanL. During a cross-sectional study, no significant differences between the antibody (Ab) response against whole saliva or the rSP03B were observed between different regions across the Mediterranean basin. Furthermore, the rSP03B was shown to resemble the native protein. During a subsequent study this protein was used to assess the seasonal dynamics of the canine Ab response to *P. perniciosus* in an endemic area of *L. infantum*. This study elucidated that also in a heterogeneous dog population both salivary gland lysate as well as the rSP03B ELISA followed expected trends of *P. perniciosus* activity, with significantly lower anti-*P. perniciosus* IgG levels in non-transmission season. These results validated the Ab response against the rSP03B protein as a universal exposure marker for *P. perniciosus* and encouraged its further use.

The second part of this thesis was aimed at accelerating the use of this salivary protein to allow a rapid evaluation of vector control programs in the future. Therefore, a rapid vector exposure test based on the rSP03B protein was prepared which detects with a high sensitivity (100%) and specificity (87%) canine exposure to *P. perniciosus*. This rSP03B sero-strip was verified to substitute the conventional ELISA by showing an almost perfect degree of agreement with the latter.

Finally, during the third part of this Ph.D. thesis the rSP03B sero-strip was successfully optimized, which resulted in a higher specificity (95%) of the test and ensured test suitability with whole canine blood. The main focus was to ascertain field applicability of the test. In order to do so, 186 dogs from different CanL endemic areas and 154 longitudinally sampled dogs were screened with the SGH-ELISA and the rSP03B sero-strip. Moreover, cross-reactions between non-vector species were excluded and favorable cross-reactions with other *L. infantum* vectors belonging to the subgenus *Larroussius* were confirmed. The results supported the use of the test in a field population of naturally exposed dogs and showed its ability to distinguish recent from past exposure. The presence of favorable cross-reactions between members of the *Larroussius* subgenus expands its use in the field.