Review of the Ph.D. thesis by Laura Willen entitled „Development of a new sand fly test to evaluate vector control tools“

The thesis deals with the important subject, human visceral leishmaniasis in the Mediterranean basin. The candidate designed and verified a new diagnostic test enabling simply and reproducibly assess host exposure to *Phlebotomus perniciosus* bites as a tool for evaluation of vector control measures. The thesis is based on three papers published in impacted journals and one manuscript. Before particular papers, Introduction is included covering epidemiology of canine leishmaniasis, its control by insecticides or vaccination and culling of seropositive dogs. Second part of introduction contains description of saliva of hematophagous insects, its effect on the host and its use as a marker of canine exposure to visceral leishmaniasis vector. Special attention is paid to lateral flow immunochromatographic assays as rapid tests in vector transmitted diseases.

Objectives of the thesis are clearly formulated and consist in validation of the use of the rSP03B yellow-related protein from *P. perniciosus* as a marker of exposure to sand fly bite, preparation of a rapid diagnostic test based on this protein and optimization of the test for use in field conditions.

The first paper published in Medical and Veterinary Entomology as a short communication deals with the validation of the recombinant SP03B yellow-related protein for screening dog exposure to *P. perniciosus* sand fly. The authors showed that above mentioned protein can be used as a valid replacement for salivary gland homogenate (SGH) because rSP03B and the native yellow-related protein share similar antigenic epitopes.

The second paper published in Parasites & Vectors verified the applicability of rSP03B for screening of dog exposure to *L. infantum* vectors in natural dog populations from an endemic area. Canine humoral response to this antigen was compatible with the annual sand fly activity dynamics in the area.

In the third paper, the rSP03B sero-strip, a newly proposed rapid test for canine exposure to *P. perniciosus* was published in highly impacted journal PLOS Neglected Tropical Diseases (IF=4.367). In this paper Laura Willen described in detail and validated the first immunochromatographic test (ICT) in the field of vector exposure. The test showed to be highly sensitive and specific in the detection of antibodies (IgG) against *P. perniciosus* saliva and its results correlated well with whole salivary gland homogenate ELISA.

In the fourth paper (manuscript), Laura Willen and co-authors further improved the immunochromatographic test increasing its specificity (95%) by using rSP03B protein expressed in a human HEK 293-cell line. Moreover, the test was optimized for use with whole canine blood. The test was shown user-friendly, reproducible and its results were in good correlation with results obtained by “classic” ELISA with the whole SGH. The test was able to distinguish recent from past exposure to *P. perniciosus* bites, excluded the cross-reactivity with non-competent *L. infantum* vectors while confirmed favourable cross-reactions with other *L. infantum* vectors belonging to the subgenus Larroussius. According to the structure of the manuscript I assume that it will be submitted to PLOS Neglected Tropical Diseases or similar journal.

The thesis is finished by a short chapter Summary and Conclusions in which the candidate highlights the main results published in above mentioned papers and discusses their significance for monitoring
In assessment of the thesis I must mainly appreciate development of simple diagnostic tool allowing screening dog exposure to *L. infantum* vector saliva in field conditions. Using immunochromatographic test, vector control measures can be evaluated. The test is now available to be used during dog exposure studies throughout the western part of the Mediterranean.

I think that Laura Willen has done a good job showing the ability to develop a simple, reliable and sensitive test for evaluation of vector control measures which is the first vector exposure test in the field of vector biology.

I have some questions and comments for the candidate.

1. Can dogs, who recovered from leishmaniasis, get infected again?
2. In the MS you claim that ICT enables to distinguish recent from past exposure to *P. perniciosus*. Is there any difference between primary and anamnestic response to *Phlebotomus* bite?
3. What are the possibilities of quantitative measurement of ICT (automated strip reader) in comparison with SGH ELISA?
4. Why have you determined only IgG antibodies to sand fly saliva antigens? How is it with IgM immune response in dogs?
5. Mice repeatedly bitten by sand flies are resistant to *Leishmania* pathogens transmitted via sand fly saliva. Can repeated feeding on dogs have similar effect?

**Conclusion:** The thesis meets all requirements established by the Faculty of Science, Charles University in Prague. I strongly recommend accepting the candidate’s thesis as the basis for obtaining the degree Ph.D.