Development of new screening tools to assess the risk of sand fly-borne pathogens transmission

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Abstract:
Sand fly females (Diptera: Phlebotominae) are blood-feeding insects and vectors of medically and veterinary important pathogens, such as *Leishmania* (Kinetoplastida) or phleboviruses. The risk of sand fly-borne pathogens transmission can be assessed by measuring host exposure to sand flies through the detection of host antibodies to vector salivary proteins. Anti-sand fly saliva antibodies are elicited by repeated exposure of the mammalian host to sand fly salivary proteins deposited into the host skin during blood feeding. These antibodies are species-specific and correlate with the intensity of host exposure to sand fly bites, hence serving as a marker of exposure and consequently as a risk marker for pathogen transmission. Anti-sand fly saliva antibodies are typically measured using sand fly salivary gland homogenate (SGH) prepared from glands dissected from sand fly females (https://youtu.be/CxIcOnORQU0). However, the development of recombinant salivary proteins offers more standardised approach, independent of having colonised sand flies.

The aim of our study was to develop a standardized ELISA assay based on recombinant sand fly salivary antigens with a focus on two Old World sand fly vectors: *Phlebotomus tobbi* and *P. papatasi*. Using dog sera from endemic areas in Türkiye, we detected the main salivary antigens of *P. papatasi* and *P. tobbi* in immunoprecipitation and immunoblot assays, followed by proteomic characterisation. Four candidate salivary proteins from each species were expressed in *Escherichia coli* and subsequently evaluated and validated in an ELISA as potential risk markers of dog exposure to sand flies. Furthermore, the recombinant salivary proteins were tested for vector and host specificity to provide epidemiologically relevant guidelines for assay results interpretation.

Among the eight tested recombinant candidates, *P. tobbi* rSP38 (a yellow-related protein), *P. papatasi* rSP36 (an apyrase) and *P. papatasi* rSP42 (a yellow-related protein) were identified as the most reliable antigens to replace salivary gland homogenate (SGH) in serological assays. They demonstrated high correlation with SGH and exhibited high sensitivity and specificity (Kolarova et al., Parasites & Vectors 2026). These three recombinant proteins also provided the highest species-specificity when tested with antibodies from mice experimentally-bitten by *P. tobbi*, *P. papatasi, P. perniciosus*, *P. sergenti*, *Sergentomyia schwetzi*, *Lutzomyia longipalpis*, or two mosquito species (*Culex pipiens molestus* and *C. quinquefasciatus*) (Pribylova et al., under review).

Serological assays based on PAP-rSP36, PAP-rSP42, and TOB-rSP38 can be utilised to measure dog exposure to sand flies in large-scale field studies since they can provide epidemiologically relevant data complementing other surveillance and control tools for sand fly-borne pathogens, including leishmaniasis. Within the CLIMOS project (https://climos-project.eu/), the three optimized ELISA assays (PAP-rSP36, TOB-rSP38, and *P. perniciosus* rSP03B) were used to monitor exposure to these sand fly vectors in sentinel dog populations in Portugal, Spain, Italy, and Türkiye, serving as an early

warning surveillance tool for circulating *Leishmania* infection (Oliva et al., under review) and to evaluate the efficacy of protective measures (Courtenay et al., in preparation).

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