LONGITUDINAL STUDY OF SAND FLY COHORTS FROM SEVEN ITALIAN REGIONS AND MOLECULAR DETECTION OF PHLEBOTOMINE-BORNE DISEASES AS BASELINE FOR RISK-MAP IMPLEMENTATION

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Keywords: sand fly, natural infection, molecular detection

INTRODUCTION: Phlebotomine-borne diseases caused by *Leishmania* and Phlebovirus are considered to be expanding their boundaries and burden in southern Europe as the result of climate and environmental changes, allowing the geographical expansion of sand fly vectors into novel territories and/or increasing their densities in endemic ones. Zoonotic leishmaniasis and summer aseptic meningitis caused by *Leishmania infantum* and Toscana virus (TOSV), respectively, are endemic in our country. In this study, we report results on sand fly identification, their distribution and in-progress examination for natural infection with the above pathogens.

MATERIALS AND METHODS: A longitudinal study in the frame of West Nile virus surveillance was carried out from 2017 to 2021 in seven Italian regions: Emilia-Romagna, Friuli Venezia Giulia, Latium, Piemonte, Sardinia, Tuscany, and Veneto. Sampling was performed twice a month, using CDC and BG-sentinel traps and keeping flies frozen pending subsequent analysis. Collected specimens were morphologically identified and monospecific pools were molecularly tested by RFLP and RT-PCR for pathogen detection, *Leishmania* spp. and TOSV respectively (Sánchez-Seco et al., 2003. J of Med Virol, 71:140-49; Di Muccio et al., 2015. PLoS One, 10: e0134885). To obtain live specimens and be able to dissect them for Leishmania spp. isolation and culture, hand captures were conducted in one Sardinian site (Olmedo, SS).

RESULTS AND CONCLUSIONS: A total of 24,153 sand flies were identified as *Ph. perfiliewi* (91.81%), *Ph. perniciosus* (6.40%), *Se. minuta* (1.61%), *Ph. mascittii* (0.17%), *Ph. papatasi* (0.01%), and one specimen as *Ph. neglectus*. Altogether 314 pools (no.= 9403) were molecularly analyzed so far, of which 25 (0.3%) tested positive for *Leishmania* in Veneto (6.5%), followed by Tuscany (0.2%) and Sardinia (1.9%) (Tab.1), with higher vector species prevalence for *Ph. perniciosus* (1.3%). Focusing on dissection analysis, isolation and culture highlighted presence of 3 *L. tarentolae* (10.0%) and 1 *Trypanosoma platydactyli* (3.3%) strains obtained from *Se. minuta*.
A total of 146 pools (no.= 2,761) were analyzed for TOSV, of which 3 (0.1%) from *Ph. perfiliewi* tested positive, in Latium (25.0%) and, for the first time, Piedmont (1.8%) (Tab.1).
The putative *Leishmania* and Phlebovirus vectors were differently represented in the investigated sites with markedly different densities. These preliminary analyses and further studies will improve knowledge of presence and distribution of phlebotomine-borne diseases in the Italian territory.

		% Reported positive pools for region (N tested)				
Region	Species	*Leishmania spp.*	*L. infantum*	*L. tarentolae*	*T. platydactyli*	TOSV
Latium	*Ph. perfiliewi*	0.0 (N=429)	NA	NA	NA	25.0 (N=240)
Sardinia	*Ph. perniciosus*	0.9 (N=565)	0.0 (N=61)	1.6 (N=61)	0.0 (N=18)	NA
	Se. minuta	1.9 (N=313)	0.0 (N=96)	4.0 (N=126)	3.3 (N=30)	NA
Tuscany	*Ph. perfiliewi*	0.2 (N=6198)	0.1 (N=730)	0.0 (N=730)	NA	NA
Piedmont	*Ph. perniciosus*	0.0 (N=67)	NA	NA	NA	0.0 (N=67)
	Ph. perfiliewi	0.0 (N=46)	NA	NA	NA	2.2 (N=46)
Veneto	*Ph. perniciosus*	7.0 (N=43)	NA	NA	NA	NA
	Ph. perfiliewi	0.0 (N=1)	NA	NA	NA	NA
	Ph. mascittii	0.0 (N=2)	NA	NA	NA	NA

Table 1. Prevalence of *Leishmania*, TOSV and *T. platydactyli* sand fly infections. NA= Pending for analysis.

This study was partially funded by Italian Health Ministry (CCM CODICE CUP: I85J20000320005).