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DOTT.SSA Alice Magri
TUTOR: PROF.SSE Marialetizia Fioravanti e Roberta Galuppi

Survey on the presence of *Leishmania infantum* in the wild and peridomestic fauna of the Emilia-Romagna region

Objective. In Italy, *Leishmania infantum* is the only species responsible for zoonotic visceral leishmaniasis, having dog as traditionally recognized reservoir in central and southern regions. In Emilia-Romagna (ER) recent studies have characterized human strains as genetically different from those found in dogs within the same area (Rugna *et al*., 2017). To identify any animal reservoirs other than dogs, a survey has been conducted from 2019 to 2021 on wild and peridomestic animals in the province of Bologna (ER), where foci of human visceral leishmaniasis are currently active.

Materials & Methods. Samples from 204 carcasses of different animal species (see Figure 1 alongside) collected from the provinces of Bologna, Ferrara, Forlì-Cesena and Ravenna (ER) were tested for *Leishmania* sp. by a real-time PCR (Tsakmakidis *et al*., 2017). For species/strains discrimination, a new nested PCR protocol targeting the cysteine protease B (CPB) was developed and optimized in collaboration with the Laboratory of Trypanosomatids Biology – University of Ostrava (Czech Republic) during a 6-months study/research period.

Figure 1. Number of specimens per species examined for *Leishmania* presence from ER. For every specimen at least 2 target organs were examined.

Results
In Figure 2 the percentage of positivity for *Leishmania infantum* is shown for each species examined.

Figure 2. Species tested positive for *Leishmania infantum*

Discussion & Conclusions

The prevalence observed in rodents is similar to the one reported in Montecristo island (Tuscany) in absence of domestic carnivore (Zanet *et al*., 2014). Findings in hedgehog, despite the small number of specimens examined is remarkably higher than those reported in literature (Alcover *et al*., 2020). Interestingly, 33.3% of roe deer resulted positive at earlobe skin level and, in one sample, also in spleen. Along with the blood meal preferences of sand flies from ER (Calzolari *et al*., 2022), these findings indicate the possible involvement of roe deer in the epidemiology of zoonotic visceral leishmaniasis in the ER.

Other Research. Although sandflies are the only proven vectors of *L. infantum*, some researches on the possible role of ticks as vectors were carried out in Bologna province, where active foci of Leishmaniasis are ongoing. Questing ticks were collected in 4 sites from natural pathways and parks. Overall, 236 DNA extracts were screened: 151 larvae pool (10 larvae/pool), 72 nymphs pool (5 nymphs/pool) and 13 adults. A real-time PCR targeting the *Leishmania* kDNA was performed. All the collected ticks were *Ixodes ricinus*. From 2 of the 4 locations examined, 4 (1.7%) extracts tested positive: 1 larvae pool, 2 nymphs pool and 1 adult. To the best of our knowledge this is the first report of *Leishmania* kDNA in unfed questing *I. ricinus*, supporting the hypothesis that *Leishmania* spp. could have in ticks both transstadial and transovarial transmission.