Acta Tropica 125 (2013) 339–348

Contents lists available at SciVerse ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Entomological and ecological studies in a new potential zoonotic leishmaniasis focus in Torres Novas municipality, Central Region, Portugal

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ARTICLE INFO

Article history:
Received 2 July 2012
Received in revised form 5 December 2012
Accepted 8 December 2012
Available online 19 December 2012

Keywords:
Phlebotomine sand flies
Leishmania
Bioecology
Blood-meals
Torres Novas
Portugal

ABSTRACT

In Portugal human and canine leishmaniasis are caused by *Leishmania infantum*, and *Phlebotomus perniciosus* and *P. ariasi* are the proven vectors. Three main foci were identified in eighty's decade: Trás-os-Montes and Alto Douro region, Lisbon region and Algarve region, but according to OnLeish observatory data, canine leishmaniasis cases have been reported from several other regions, for which sand fly species and their infection rates are unknown. This study is the first phlebotomine survey in Torres Novas municipality, Santarém District, Portugal. The main objectives were to identify the phlebotomine species, their bioecological aspects, Leishmania infection rate and the risk factors for the presence of phlebotomine species in the municipality. From June to November, 2010, 275 biotopes were surveyed with CDC light-traps. Captures covered the 17 parishes of the municipality and included domestic, peridomestic and sylvatic biotopes. Specimens were identified morphologically and females were used for molecular detection of Leishmania and bloodmeal identification. Simple and multiple logistic regression analysis were used to identify risk factors for phlebotomine presence. Nonparametric tests were used to compare densities of independent groups. A total of 1262 sand flies were captured and identified, and four species detected: *P. perniciosus* (73.69%), *P. ariasi* (8.16%), *P. sergenti* (6.58%) and *Sergentomyia minuta* (11.57%). In 71.4% localities at least one *L. infantum* proven vector species was present. Risk factors were identified as: high average temperatures and low relative humidities, sheltered locations and absence of strong wind, presence of pine trees as dominant vegetation, peridomestic biotopes, particularly sheep pens or proximity of sheep, poultry and house martin nests. *L. infantum* infection rate was 4% for *P. ariasi* and 0.48% for the total of *Larroussius* females. *P. perniciosus* females exhibited an opportunistic behavior, feeding in a wide variety of vertebrate hosts. The high abundance and distribution of proven vector species, together with a canine leishmaniasis seroprevalence of 7.93% in the District, and the capture of a gravid infective sand fly female, suggests that Torres Novas municipality is a potential zoonotic leishmaniasis focus in the country.

1. Introduction

Leishmaniasis are neglected parasitic protozoan diseases endemic in 98 countries or territories, transmitted by females of species of the Phlebotominae subfamily (WHO, 2010). In Portugal, both visceral and cutaneous forms are caused by *Leishmania infantum* (Campino & Abranches, 2002; Campino et al., 2006) and the domestic dog (*Canis lupus familiaris*) is the main reservoir host, with an overall national *Leishmania* seroprevalence of 6.31% (Cortes et al., 2012). Cats (*Felis catus*) are strongly suspected of also acting as a reservoir host in domestic areas (Maia et al., 2010; Maia & Campino, 2011) while wild canids (*Vulpes* sp.) are considered the main sylvatic reservoir host (Abranches et al., 1983).

Five phlebotomine sand fly species have been detected in the country: *Phlebotomus* (*Larroussius*) *perniciosus* Newstead, 1911,

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http://dx.doi.org/10.1016/j.actatropica.2012.12.008

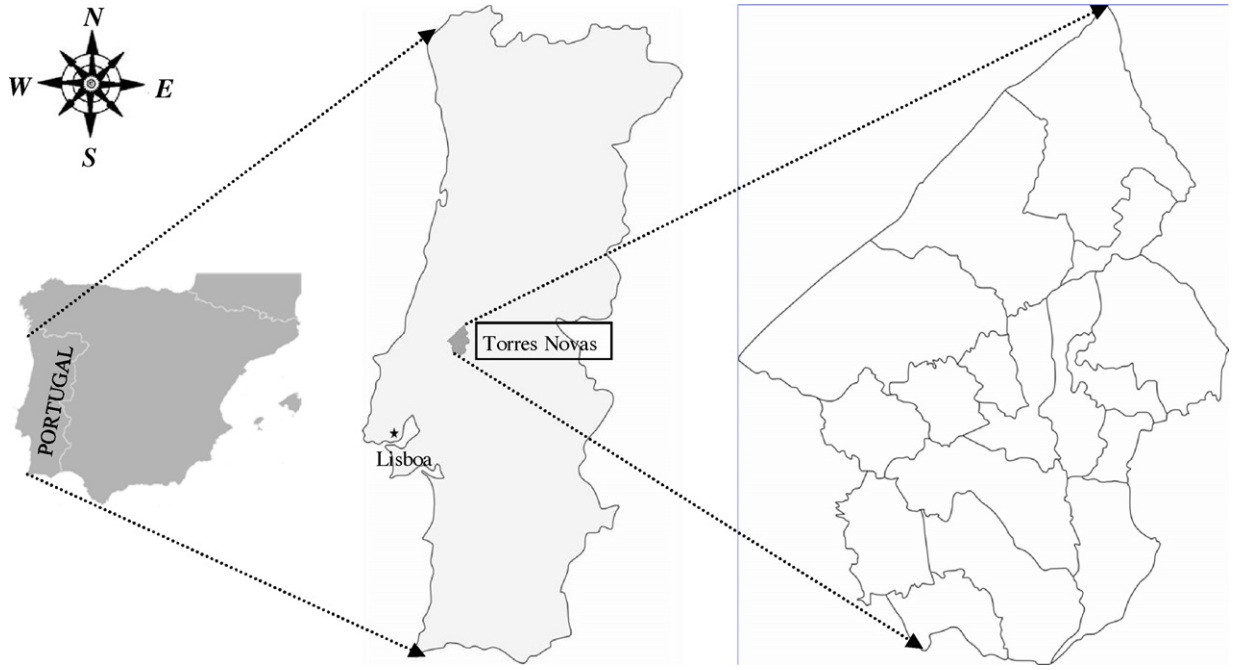

Fig. 1. Portugal map with the location of the study area: Torres Novas municipality, and its parish's boundaries (39°24–39°40'N, 8°27–8°40 W).

P. (L.) *ariasi* Tonnoir, 1921, *P.* (*Paraphlebotomus*) *sergenti* Parrot, 1917, *P.* (*P.*) *papatasi* (Scopoli, 1786) and *Sergentomyia* (*Sergentomyia*) *minuta* (Rondani, 1843). *P. perniciosus* and *P. ariasi* are the proven vectors of the parasite in Portugal (Pires, 1984). *P. sergenti* and *P. papatasi* are vectors of *L. tropica* and *L. major*, respectively in North Africa, Asia and Middle East, and have been captured mainly in South Portugal (Rés, 1957; Semião-Santos et al., 1995; Alves-Pires et al., 2004; Afonso et al., 2005; Maia et al., 2011) but, to date, no cases of autochthonous infection by *L. major* or *L. tropica* have been reported in the territory. *S. minuta* has been found in all studied regions of the country but generally with higher densities at lower latitudes. All these sand fly species present marked seasonality, with imagos emerging in April/May and with activity until October/November, ceasing with temperatures below 12 °C (Alves-Pires & Ribeiro, 1991; Pires, 1985, 2000). According to species, region or year of study, the cycles vary from monophasic to biphasic (one or two peaks of imago emergence) (Meira & Ferreira, 1944; Rés, 1957; Pires, 2000; Afonso et al., 2005).

Three classic zoonotic foci are recognized in the country since the 80s (Trás-os-Montes and Alto Douro Region in the North, Lisbon Region in the Centre and Algarve Region in the South) (Abranches & Pires, 1980; Campino & Maia, 2010). However, according to OnLeish data (Cortes et al., 2012), canine leishmaniasis (CanL) cases have been reported from several other regions, for which the sand fly species, their bioecology and infection rates are largely unknown. Among these regions, recently (2009), was found a global seroprevalence of 7.93% in dogs in the Santarém district (Cortes et al., 2012), supporting the need for vector surveillance.

This study is the first phlebotomine survey in Torres Novas municipality, Santarém District, in central area of Portugal. Our objectives were to identify the phlebotomine species in its 17 parishes and in different biotope types, to determine their relative abundances and densities, to determine the risk factors for phlebotomine presence and finally to determine *Leishmania* infection rates and blood-meal preferences of females by molecular techniques.

2. Material and methods

2.1. Study area

Torres Novas municipality is located in Santarém District, in Central Portugal (Fig. 1). It is composed of 17 parishes occupying an area of 279 km^2, approximately between 39°24′–39°38′N, and 8°27′–8°40′W, and has a total population of 36,925 inhabitants (Município de Torres Novas, 2009).

The mean annual temperature is 16 °C, with the lowest average monthly temperatures occurring in December and January (9.1–9.2 °C) and the highest in July and August (22.8–22.9 °C), the annual temperature range being moderate (13–14 °C) (Simões, 2003). The average annual rainFall is 730 mm. According to Köppen's classification the municipality's climate is classified as temperate Mediterranean, with hot Summers and rainFall in Winter (CMDFCI, 2004). The municipality is bordered to the north by the Serra de Aire, which rises above 600 m, along which extends a plateau of about 170 m. The remaining area is between 25 and 100 m high.

Vegetation wise, the main soil use is agricultural, with olive trees (*Olea europaea*) the dominant culture, followed by fruit trees (*Ficus carica*, *Vinis* sp., *Citrus* sp. and several kinds of nut trees) and extensive areas occupied by grain cereal meadows (family Poaceae). The forested zones are composed mainly by pine (*Pinus* sp.), eucalyptus (*Eucalyptus globulus*) and oaks (*Quercus* sp.). In the domestic and peridomestic areas ornamental gardens, crop plants and/or fruit trees are usually present, while in sylvatic biotopes, in addition to *Quercus* and *Pinus* trees, several other species coexist, mainly *Rubus* sp., *Erica arborea*, *Pistacia lentiscus*, *Arbutus unedo*, *Cistus ladanifer* and *Ulex* spp. (Simões, 2003).

2.2. Sand fly sampling, biotopes surveyed and morphological identification of phlebotomine species

Sand fly captures were performed from 11th June to 21st November, 2010 (average of 12 days each month and 4 traps per night, in a total of 46 traps per month from June to October and 45 traps in November) with CDC miniature light-traps, set out from