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Natural *Leishmania* infection of *Phlebotomus sergenti* (Diptera: Phlebotominae) in an endemic focus of cutaneous leishmaniasis in Şanlıurfa, Turkey

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ABSTRACT

Sand flies (Diptera: Phlebotominae) were surveyed for *Leishmania* in various villages of Şanlıurfa in southeast Turkey. A total of 474 sand flies were collected by CDC light traps. *Phlebotomus sergenti* Parrot (49.6%) and *Phlebotomus papatasi* (Scopoli) (48.1%) were the most abundant species, followed by *Phlebotomus alexandri* Sinton (1.05%), *Phlebotomus perfiliewi* Parrot (0.4%), *Phlebotomus* (*Adlerius*) sp. (0.2%) and *Sergentomyia theodori* Parrot (0.4%). 196 female sand flies were grouped in 34 pools of max 10 specimens each and 4 pools of *P. sergenti* were found positive for *Leishmania* DNA, detected by using ITS-1 primer set. This is the first molecular detection and identification of *Leishmania tropica* within naturally infected *P. sergenti* from the most important focus of anthroponotic cutaneous leishmaniasis in Turkey. The high frequency of *P. sergenti* together with natural infection by the parasite makes this species the probable vector of *L. tropica* in Şanlıurfa. The data obtained from this study could be used in strategic planning for the control of leishmaniasis in the region.

1. Introduction

Members of the genus *Phlebotomus* (Diptera: Psychodidae) transmit a protozoan parasite *Leishmania* (Kinetoplastida: Trypanosomatidae) along with a group of viruses called phleboviruses in Turkey (Özbel, 2013). Zoonotic visceral leishmaniasis (ZVL) is caused by *Leishmania infantum* in Ege and Mediterranean regions (Özbel, 2013) and by *L. donovani*/*L. infantum* in southern Anatolia (Koltas et al., 2014). Cutaneous leishmaniasis (CL) is caused by *Leishmania tropica*, *Leishmania major and L. infantum* in southern Anatolia (Svobodová et al., 2009; Koltas et al., 2014) and *L. tropica*/*L. major* in south eastern Anatolia (Özbel, 2013; Zeyrek et al., 2014). CL is a disease characterized by long-term sores on the skin. Although the infection is painless and self-healing, patients with CL have disfiguring scars and experience social stigmatization which cause anxiety, depression, decreased body satisfaction and quality of life (Yanık et al., 2004). Şanlıurfa -a provincial capital near the Syrian border -is the largest focus of typical anthroponotic cutaneous leishmaniasis (ACL) in Turkey with about 2000 new cases annually (Zeyrek et al., 2014). The only causative agent used to be *L. tropica* zymodeme MON-53 (Gramiccia et al., 1984; Akman et al., 2000). However, 3 new cases due to *L. major* (2 autochthonous, 1 imported from Syria) were also reported recently from Şanlıurfa (Zeyrek et al., 2014). Svobodová et al. (2009) isolated *L. infantum* both from CL patients and sand flies and proved the vectorial status of *Phlebotomus tobbi* in Çukurova region of South Anatolia. This was the first sand fly species incriminated as a vector of *Leishmania* in Turkey. Vectors of *L. tropica* have been investigated in Şanlıurfa, however, sand flies infected with promastigotes could not been found by microscopic examination although the most frequently encountered species, *Phlebotomus sergenti* is associated with CL in this region (Alptekin et al., 1999; Volf et al., 2002). Serological techniques such as ELISA were used for diagnosis of *Leishmania* infection in 83 sand flies of Şanlıurfa and infection rate was found to be 3.6% (Pazarbaşı et al., 2006).

CL have long been a serious problem despite all efforts of the Cutaneous Leishmaniasis Diagnosis and Treatment Center which was established in Şanlıurfa in the early 1980s. In addition to already existing CL problem, ongoing Syrian civil war also had impacts on epidemiological situation of the disease in south eastern cities of Turkey. In Syria the incidence of CL has dramatically increased since the beginning of the war in 2011 due to breakdowns in public health infrastructure and failure to implement public health control measures (Hayani et al., 2015). Hundreds of thousands of civilians have been obliged to leave their country

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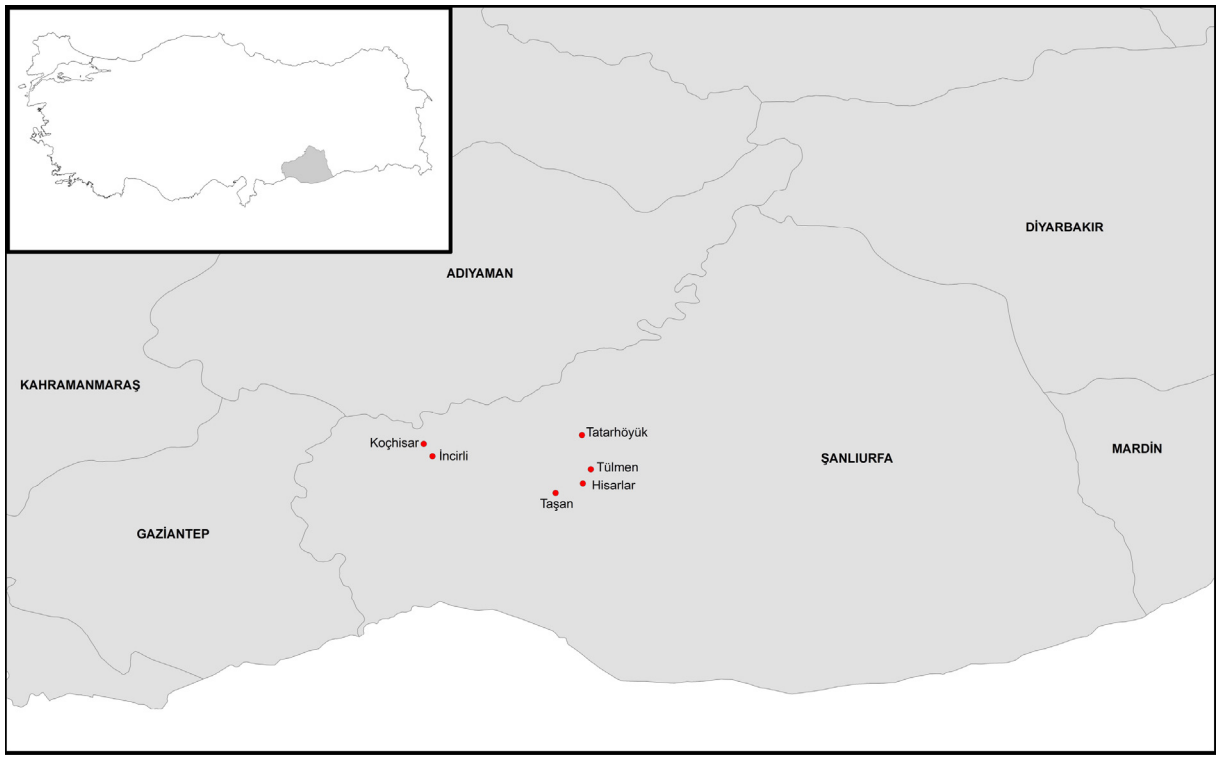

Fig. 1. Map of the collecting sites in 6 villages of Şanlıurfa.

to safer places including Lebanon, Iraq, Jordan and Turkey. This has resulted in the spread of the disease to new previously non-endemic parts. For example in 2013, Lebanese Ministry of Public Health reported 1033 confirmed cases in Lebanon which had no cases of CL before 2008, 998 (96.6%) of these cases were among Syrian refugees (Sharara and Kanj, 2014). According to the UN High Commissioner for Refugees, more than 900,000 civilians moved to south eastern cities of Turkey since the start of the civil war in Syria. Most of the refugees live in the cities and villages around them although there are refugee camps near the border. Koçarslan et al. (2013) notified a significant increase in the number of CL cases most of which were Syrian refugees in Şanlıurfa hospitals. It is expected that CL cases will increase in this area for years to come.

Previous studies on the sand fly fauna of Şanlıurfa were restricted to the central areas or districts around the city (Alptekin et al., 1999; Volf et al., 2002; Toprak and Özer, 2005; Toprak and Özer, 2007). However, CL is also a problem in some villages and rural areas. Cutaneous Leishmaniasis Diagnosis and Treatment Center officials reported 130 ACL cases only from villages of Şanlıurfa in 2013 and 74 cases for the first half of 2014 (personal communication). Sand fly species acting as vectors and the ecology of transmission in this area is poorly understood. We performed a study in order to determine the sand fly fauna and to study the occurrence of *Leishmania* infection in sand flies in the villages of Şanlıurfa which has active or past ACL cases.

Molecular biological methods which are sensitive and require less time and effort were used in the detection and identification of *Leishmania* species in sand flies of Şanlıurfa where the vectors are still unknown.

2. Materials and methods

Şanlıurfa, located in south eastern Anatolia is one of the oldest settlements in the world. The city is situated on a semi-arid plain at 550 m above sea level (Alten et al., 2003). The Turkish–Syrian border passes through the south of the city. Atatürk dam -one of the largest dams in the world- lies on the Euphrates River flowing through the west border of the city.

Sampling was performed at 12 sites in 6 villages most of which have had ACL cases in the last two years (2013, 2014) (Fig. 1). Villagers were mostly farmers living in stone, brick or cement houses with barns for poultry or livestock animals. The habit of sleeping outside or on the flat roofs during the hot summer nights and not using any protective materials like bednets were factors permitting sand flies' easy access to humans.

Study was performed in 7–14 August 2014 which is the hottest and dry season. Sand flies were collected with CDC miniature light traps which were placed approximately 1.5 meters above ground of the animal shelters near the houses. The traps were set in the afternoon and were collected following morning. On each catching night one light trap was set in each of the selected localities. The trapped sand flies were immobilized in deep-freeze and then transferred to 96% ethanol. In the laboratory the head and genitalia were mounted on permanent microscope slides for identification and the body of the females were examined whether they were engorged or unfed and kept for molecular investigation. Keys and descriptions of Perfil'ew (1968); Lewis (1982); Artemiev and Neronov (1984) were used for species identification.

After dissection and identification of sand flies, body parts were pooled according to the collection site and species each with a maximum number of ten. Pooled sand fly bodies were smashed with MagNa Lyser® using ZR Bashing Bead™ tubes. A total of 34 tubes and one negative control using male bodies were generated and incubated at 56 °C for 24 h with 500 μl volume of Qiagen® Tissue lysis buffer. DNA extractions were made using Qiagen®DNeasy kit by following the manufacturer's instructions.

Ready-to-use PCR master mixture (Helixamp® T500N) was used with ITS-1 primer set (LITSR/L5.8S) and amplification was carried out as described by El Tai et al. (2000). PCR products were analyzed by electrophoresis in 1.2% agarose gel stained with GelRed™