Veterinary Parasitology 209 (2015) 179–187

Contents lists available at ScienceDirect

Veterinary Parasitology

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

Seroepidemiological and entomological survey in a new focus of zoonotic visceral leishmaniasis in Kars province, Northeastern Turkey

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ARTICLE INFO

Article history:
Received 22 November 2014
Received in revised form 17 January 2015
Accepted 18 February 2015

Keywords:
Leishmaniasis
Visceral
Sand flies
Kars
Turkey

ABSTRACT

Visceral leishmaniasis (VL) has now been recorded from 38 provinces of Turkey. Twenty-one VL cases were reported within six years from settlements located in most northeastern Turkey and we therefore aimed to carry out an entomological and seroepidemiological survey in this new focus for clarifying risk factors. Blood samples from 290 children and 165 dogs were collected. Sera samples were investigated for anti-*Leishmania* antibodies using indirect fluorescent antibody test. Sand fly collection for determining the fauna and seasonal activity was performed in all settlements by CDC light traps between June and September 2006. Although no seropositive child was detected during the survey the overall seroprevalence rate of canine leishmaniasis was found as 7.2%. A total of 4154 sand flies were collected and 10 species of genus *Phlebotomus* were identified belonging to *Adlerius*, *Larroussius*, *Paraphlebotomus* and *Phlebotomus* subgenera. Among them *Phlebotomus kandelakii s.l.* (55.44%), *Phlebotomus balcanicus* (12.62%) and *Phlebotomus neglectus* (4.40%) was detected as probable vector species for this new focus. The poor sanitation, very high population size of sand flies, probably because of very short season, no control measures for sand flies as well as dogs, and presence of microclimate suitable for sand flies were considered as main risk factors in the area.

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http://dx.doi.org/10.1016/j.vetpar.2015.02.021
0304-4017/

1. Introduction

Leishmania protozoan parasites, transmitted by the bite of Phlebotomine sand flies (Diptera: Psychodidae), cause visceral, cutaneous or mucocutaneous leishmaniasis. Visceral leishmaniasis (VL) is the most severe form of leishmaniasis and if not treated, can potentially be a lethal disease. Zoonotic VL caused by *Leishmania infantum* is endemic in all countries bordering the Mediterranean Basin WHO (2010). *L. infantum* is responsible for human VL and canine leishmaniasis (CanL), while *Leishmania tropica* is mainly responsible for cutaneous leishmaniasis (CL) in Turkey. So far, all *Leishmania* strains isolated from VL patients were identified as *L. infantum* MON-1 or from dogs as *L. infantum* MON-1 and MON-98 in Turkey (Özbel et al., 2000; Ok et al., 2002) based on multi-site DNA polymorphism analyses. In addition to this, two strains isolated from two VL patients were identified as "*Leishmania major* variant" four differing from Friedlin strain of *Leishmania major* in the sequences of N-acetylglucosamine-1-phosphotransferase (NAGT) genes with four bases substitutions (Akman et al., 2000). Recently *L. major* and *L. donovani* were also reported as causative agents of CL in East Mediterranean Region of Turkey (Koltas et al., 2014). South and Southeast Anatolia regions are endemic for CL, while human VL and CanL have been mainly seen along the Aegean, Mediterranean coasts and Central Anatolia Region (Ok et al., 2002).

Dogs are recognized as the main reservoir hosts of zoonotic VL and the prevalence of CanL in Mediterranean countries varies from 1.1% to 48.4% (Dujardin et al., 2008). Sand flies belonging to subgenera *Larroussius* and *Adlerius* are proven or suspected vectors of *Leishmania* in the Mediterranean Basin and in some Eurasian countries (Killick-Kendrick, 1990; Sadlova et al., 2003).

In Turkey, the first epidemiological study for CanL showed a seroprevalence rate of 1.6% among 1150 dogs from Ege and Mediterranean Regions in 1981 (WHO, 1993). In the epidemiological studies carried out in 22 different provinces between 1993 and 2006 in Turkey, the average prevalence of CanL was reported as 15.7% (changing between 1.45% and 27.5%) (Dujardin et al., 2008). Zoonotic VL has been reported from all geographical regions of Turkey with low numbers. The total official VL cases are between 30 and 40 per year (Dujardin et al., 2008; MoH records) but these numbers are believed to be underestimated even the disease has compulsory notification in Turkey.

Seroepidemiological studies were previously carried out in Turkey in different endemic regions for VL (Özensoy Töz et al., 1998 and Özbel et al., 2000). Visceral leishmaniasis cases have reported from the eastern part of the country by Büyükavcı et al. in 2005 and the authors were reviewed 21 cases of childhood VL reported between 1996 and 2002 from eastern Turkey, 60% of whom were from Kagizman town and its villages. In all 21 patients, the disease was diagnosed by demonstrating the amastigote forms of *Leishmania* in bone marrow aspiration smears. We therefore aimed to carry out entomological and seroepidemiological survey on human and dogs in Kagizman town and the villages where the VL patients were diagnosed for finding risk factors in this geographical area located in high altitude and most northeastern part of Turkey.

2. Materials and methods

2.1. Study area

Kagizman town (40 09 N; 43 08 E) belongs to Kars province and is located in most northeastern part of Turkey. The town is divided into 62 villages covering an area of 1972 km^2. Eleven and 10 VL cases were reported from Kagizman town and its 5 rural villages (Yukarikaraguney, Gunindi, Karabag, Kuloglu and Karakus) between 1996 and 2002, respectively. These five villages and Kagizman town, representing active foci of VL, were selected as study sites. A village (Kotek) where no VL cases reported so far was also included in the study as control area only for sand fly seasonal activity survey. Physical examination and all samplings were done between July and September 2006. The geographical features and other information about the sampling sites were given in Table 1.

All of the villages are in high altitude (>1.300 m asl) and located near the Aras River that also crosses through Kagizman town and all settlements have similar ecological aspects. The climate is classified as a humid continental and meadows and grasslands are the dominant flora. Kagizman town has a temperate climate compared to other districts of Kars; the minimum mean temperature is ~2.6 °C in January and the maximum mean temperature is ~23.1 °C in July; the average annual rainfall is 425.5 mm. The major economic activity is cattle/sheep breeding. People are also engaged in orcharding and vegetable gardening.

2.2. Physical examination and sampling

2.2.1. Human

A total of 290 children aged between 2 and 14 years old were randomly selected (Table 1). After receiving the written consent form from their parents, children underwent a physical examination for detection of clinical symptoms by authorized physicians. Five ml of venous blood sample was collected to the blood tube and centrifuged for obtaining sera.

2.2.2. Dogs

A total of 165 household dogs from five villages were also selected randomly (Table 1). Dog sampling could not be performed in the districts of Kagizman town because of the all dogs were stray dogs. Prior to sample collection, the dogs were examined for clinical signs of CanL including weight loss, skin lesions, hair loss, local or generalized lymphadenopathy, epistaxis, onychogryphosis and kerato-conjunctivitis. Peripheral blood was taken from a femoral or brachial vein in 5 ml blood tubes and centrifuged for obtaining sera. Popliteal lymph node aspirates were obtained only from 6 dogs using 21 G needles and used for Giemsa-stained smears and parasite culture. Parasitological examination was regarded as positive on the basis of smear and/or culture positivity. Dog owners provided the consent form for sample collection from dogs.