INTRODUCTION

Phlebotomine sandflies (Diptera: Psychodidae) are responsible for the transmission of Leishmania infantum, the protozoan agent of human and canine leishmaniasis in the Mediterranean area. Review data on the phlebotomine sandfly fauna of Albania show that P. neglectus is the most abundant species being widespread in almost all Albanian territories (Adhami & Murati, 2000; Velo et al., 2003). Recent results from a retrospective analysis of human visceral leishmaniasis (VL) cases occurred in Albania, have shown an increase in the disease morbidity, resulting 20-40-fold higher than in other southern European countries (Velo et al., 2003). During the period 1997-2001, 867 parasitologically confirmed VL cases have been recorded in 35 out of 36 Albanian districts.
with an average of 173 cases/year and a cumulative morbidity of 2.8/10,000 population. VL cases have almost doubled during the past 10 years and a high proportion of patients (67.6 %) was represented by children below five years of age. An entomological study was carried out during June-October 2002 in two Albanian districts most involved in this VL recrudescence, with the aim to study sandfly fauna composition, the insect feeding habits and natural Leishmania infections.

MATERIALS AND METHODS

STUDY AREA

The entomological survey was carried out in the districts of Kruje and Lezhe, where high VL morbidity levels (5.1 and 12.5/10,000 population, respectively) were recently reported. The two districts, located in central and northern part of Albania (Fig. 1), respectively, consist of hilly territories (Kruje 600-658 m a.s.l, Lezhe 180-208 m a.s.l.). Kruje district, which has a surface of 333 km² and a density of 193.3, inhabitants/km² is located in the inland part of Albania. The annual rainfall in this district averages 900-1,700 mm and temperatures 5-16° C. Lezhe district, which has a surface of 142.3 km² with a density of 142,3 inhabitants/km², is very close to the Adriatic coast, with an average annual rainfall of 1,500-1,800 mm and an average annual temperature of 8-25° C. Both districts are in a geographical zone that belongs to the mesolithic layer. The sediments of this layer are composed mainly of lime rock, carbon and magmatic rocks.

COLLECTING SITES

Six collecting sites were selected, three in Kruje and three in Lezhe, showing a variety of phlebotomine sandfly diurnal resting sites. Characteristics of each site, collecting methods used and presence of domestic animals within a range of 50 m, are presented in Table I.

SANDFLY COLLECTION

Adult sandfly collections were carried out three times a month from June through October 2002. CDC miniature light traps, sticky traps and mechanical or hand aspirators were used outside peri-domestic sites, and inside cow barns, chicken coops and pigpens. Mechanical or mouth aspirators were used in diurnal collections inside bedrooms and cattle sheds in Kruje. An average of 10 sticky traps were used in each station for two consecutive nights. After capture, living flies were transported to the laboratory and anesthetized with low temperature; blood-fed females were then isolated and stored at -20° C pending further analysis. Males and unfed females were cleared prior to identification to species level. Specimens were identified by their morphological characteristics, according to Theodor (1958) and Léger et al. (1983).

TESTING OF BLOOD MEALS

Blood meal origin could be determined only for females caught from Kruje. Before testing, blood-fed females were identified to species level by removing the head and the terminal segments of the abdomen containing the spermathecae. Specimens were then classified into freshly fed, partially fed and late fed according to the amount and colour of the blood in the intestine. The blood origin was determined by a direct ELISA on nitro-cellulose membrane according to the method previously described (Bongiorno et al., 2003). Five peroxidase-labelled anti-animal IgG antibodies (Sigma) were tested, namely anti-human (A-8667), anti-dog (A-6792), anti-chicken (A-9046), anti-rabbit (A-6154), anti-bovine (A-7414). The anti-cat serum was a gift from the University of Camerino, Italy. Moreover, flies resulted negative to the above hosts were also tested for anti-sheep (A-3415), anti-mouse (A-4416) and anti-horse (A-9292) sera. An anti-pigeon serum was not available.

To determine the sandfly preference for hosts, forage ratios (FRs) were calculated by dividing the percentage of females feeding on a given host by the frequency at which that host was represented in the total census.
at the collecting site (Hess et al., 1968). According to the method described by these authors, an FR of 1.0 indicates neither preference nor avoidance of a given host animal, FRs significantly > 1.0 indicate selective preferences and values < 1.0 indicate avoidance in favor of other hosts.

Since the FR method does not consider relative body mass of the host, the host selectivity index (HSIx) was also evaluated according to Agrela et al. (2002). This index estimates the average weight of each individual animal present at the collection site. The available biomass for each host species was calculated by multiplying the number of such hosts counted in the census by its estimated average weight. HSIx was then calculated by dividing the number of sand flies that fed on a given host by the available biomass of such hosts.

**DNA EXTRACTION AND AMPLIFICATION**

Search for leishmanial DNA by PCR could be performed only for females caught from Lezhe. Sandfly species identification was carried out by removing only the genitalia. Genomic DNA was extracted from single female homogenized in 1.5 ml sterile tubes using a plastic pestle. 40 µl lysis buffer (100 mM TRIS-HCl, 100 mM NaCl, 25 mM EDTA, 0.5 % SDS, pH 8) was added and the homogenate was digested overnight at 37°C by 2 µg/µl of proteinase K (Promega). The DNA was extracted by phenol-chloroform and precipitated with 100 % ethanol then centrifuged for 30 min at 13,000 x g. The DNA pellet was resuspended in 50 Bl of sterile water and stored at −20°C until use. Small-subunit ribosomal DNA was amplified by PCR technique using the Kinetoplastida-specific primers R221 and R332 (van Eys et al., 1992). Negative (no DNA) and two positive (L. infantum DNA and Phlebotomus plus L. infantum DNA) controls were used in all experiments. Finally, the PCR product was electrophoresed through a 1.5 % agarose gel and visualized under UV transillumination.

**RESULTS AND DISCUSSION**

**SANDFLY FAUNA**

All collecting sites monitored were positive for phlebotomine sandflies. The numbers of sandfly specimens collected and the prevalence of the species identified in the two districts are presented in Table II. Five species belonging to Phlebotomus genus were identified among the 849 caught sandflies. Phlebotomus neglectus was the most abundant species followed by P. perfiliewi, P. papatasi, P. tobbi and P. similis. The highest number of sandflies was collected in Kruje (79.1 %), where all the above species were recorded, while only P. neglectus, P. perfiliewi and P. tobbi were present in Lezhe, being P. neglectus the most abundant (92.6 %).

In general, our survey confirms what previously observed on the sandfly fauna composition of Albania, and particularly on the distribution and abundance of P. neglectus (Adhami & Murati, 2000; Velo et al., 2003). The three Phlebotomus (Larroussius) species, i.e. P. neglectus, P. perfiliewi and P. tobbi, could play a role in the transmission of VL in the area studied, being proven L. infantum vectors (Léger et al., 1988, 2000; Maroli et al., 1987).

As for the seasonal trend of adult phlebotomine fauna, it could be determined only for P. neglectus, the most widespread species. The first adult appeared on June 11 and the last one was collected on October 16, the highest number of specimens being collected at the end of July. A similar seasonal trend has been reported for P. perniciosus and P. perfiliewi in Italy (Maroli & Bettini, 1977).

**BLOODMEAL IDENTIFICATION**

Among 66 fed females caught in Kruje district, P. neglectus was the prevalent species (56.1 %) followed by P. perfiliewi (30.3 %) and P. tobbi (10.6 %);
P. papatasi was represented by only two specimens. Table III shows the results of blood meal analysis. The identification was possible in 45/66 of females tested (68.2 %). Undetermined blood meals were due to small amount of fresh blood or to advanced blood digestion. By adjusting blood origin prevalence of P. neglectus according to FR and to HISx based on the available biomass, this species results in a quite opportunistic feeder rather than exhibiting preferences for any specific animal. Similar feeding habits are also known for other Leishmania vectors, both in the Old and New World (Killick-Kendrick, 1999; Morrison et al., 1993; Agrela 2002; Bongiorno et al., 2003). In Spain, France and Italy, phlebotomine vectors have been observed to feed on a wide range of domestic animals, with varying degrees of anthropophily (De Colmenares et al., 1995; Guy et al., 1984; Killick-Kendrick et al., 1977; Bongiorno et al., 2003).

Search for Leishmania Infections

P. neglectus, collected in Lezhe, was the only species investigated for the search of leishmanial DNA by PCR technique. Of the 39 female specimens examined, 36 were blood fed, at different stages of blood digestion. None of them was found positive.

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