

Survey for Canine Leishmaniasis

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Abstract: Leishmania parasites cause a group of diseases collectively known as Leishmaniasis. For a long time dogs have been implicated as the main domestic reservoirs of *Leishmania infantum*, the aetiological agent of zoonotic visceral Leishmaniasis. There are the same data about canine Leishmaniasis in other Mediterranean countries. The study investigates the level of prevalence into important areas in South Albania. Serologic methods are considered as the most efficient methods for the early diagnosis of Leishmaniasis. ELISA test is necessary to make the final serological diagnosis of most frequently parasitic diseases of dogs. The use of serological methods determinate in accurate and rapid way the diagnosis of Leishmaniasis infection. Cross-sectional investigation was done on seroprevalence of *Leishmania* sp. infection among apparently healthy dogs in an area where canine Leishmaniasis is endemic. Each dog was clinically examined for the presence of some discrete signs compatible with Leishmaniasis. The seroprevalence at the nonendemic area resulted 10.6%. A few of the seropositive dogs had moderately enlarged lymph nodes and some clinical signs. Trombocytopenia in animals with clinical signs can be used as a first indicator for leishmania diagnosis.

Key words: *Leishmania infantum*, dog, prevalence, ELISA, Albania

INTRODUCTION

Leishmaniasis is a complex of diseases caused by parasitic protozoans known as *Leishmania* sp. *Leishmania* sp. (Kinetoplastida: Trypanosomatidae) are protozoan parasites of great medical and veterinary significance which are transmitted to a susceptible host by phlebotomine sand flies (Diptera: Psychodidae) of the genera *Phlebotomus* in Europe (Ashford, 2000; Dujardin *et al.*, 2008). Leishmania parasites cause leishmaniasis, a group of diseases with diverse epidemiological and clinical patterns (from self-healing skin ulcers to severe, life-threatening visceral disease). The leishmaniasis are zoonoses. Seroprevalence of Leishmaniasis in Mediterranean countries ranges from 10-37% (Sideris *et al.*, 1999; Koutinas *et al.*, 1999; Pratlong *et al.*, 2009). The most important is canine Leishmaniasis, caused by *Leishmania infantum*. Recent studies have demonstrated that *Leishmania infantum* is endemic in the Mediterranean basin areas and a high percentage of dogs (60-80%) have come into contact with the parasite without exhibiting any signs of the disease (Ferrer, 1999). Since, clinical manifestations including weight loss, elongated and keratoconjunctivitis, dermatitis and lymphadenopathy (Ferrer *et al.*, 1988; Ciaramella *et al.*, 1997; Koutinas *et al.*, 1999; Miranda *et al.*, 2008) are observed only in a low

proportion of the infected dogs, serodiagnosis has been considered essential for evaluating the prevalence of the infection (Gradoni *et al.*, 1988; Ferrer, 1999). Seroprevalence studies of canine leishmaniasis have revealed a large number of asymptomatic seropositive animals (Zivicnjak *et al.*, 2005; Antoniou *et al.*, 2008; Athanasiou *et al.*, 2012). The ability to infect sand flies was similar in both asymptomatic carriers and animals with different degrees of signs of disease (Kellick-Kendrick, 1999). The current level known of prevalence of Leishmaniasis was about 3.3-43% in some different areas in Albania (CICKO, 1999). In the south parts of Albania, canine leishmaniasis was recognized as a problem for the first time in the first part of 20th century in humans (Petrela *et al.*, 2010). The aim of the study was to determine the prevalence of *Leishmania infantum* at owned dogs with a combination of hematological examination, Leishmaniasis-Dipstick test and ELISA Method. The survey was performed in non endemic areas where vectors have optional conditions and sometimes dogs have clinical signs.

MATERIALS AND METHODS

The cross-sectional survey was carried out during 2005 to 2012 in South Albania. The area was divided into two main parts: elevated hinterland and seaside coastal

strip. Even parts of the mountain areas are included like Gjirokastra, Permeti, Tepelena and Erseka while Saranda is a seaside area. The altitudes of the areas of included in this study varied from sea level to 750 m above sea level. In this survey were included dogs living under human care. In this area, stray dogs are not numerous because of the small urban areas and other conditions minimized their presence. The yearly precipitation pattern is maritime in character with dry summers and maximum precipitation during the cold months of the year. The geophysical and climaterically conditions create optimal biological conditions for the vector biology (Aransay *et al.*, 2004; Dujardin *et al.*, 2008). The target dog populations included in this study were owned dogs because in these small urban areas the street dogs are not in high numbers. Based on the record from the veterinary service in the study area are 6500 owned dogs and in this survey were included 151 dogs.

The survey was focused on owned dogs considered as clinically healthy, aged 6 months or more where 76 were males and 75 females. Clinical status was evaluated according to criteria suggested by Amela *et al.* (1995). After clinical evaluation, dogs with obvious two or more clinical signs of Leishmaniasis (weight loss, dermatitis, hair loss, mouth and skin ulcers, enlarged lymph nodes, arthritis and keratoconjunctivitis) were included in the survey. In the survey were included all dogs with or without any signs (asymptomatic) and those with moderately enlarged lymph nodes and/or discrete skin changes such as dull coat or exfoliative dermatitis without hair loss. Epidemiological data on age, sex, dog utility and location were recorded in a questionnaire filled out by the owners and the participating veterinarians. Blood was collected by cephalic venipuncture and kept and transported under standard conditions. For each sample were made blood smears for hematological examination. Subscapular and popliteal lymph node aspirates were taken from the dogs with enlarged lymph nodes and skin changes and from the seropositive dogs. Smears were prepared immediately after aspiration, fixed in methanol, stained with Giemsa and examined under a microscope for the presence of amastigotes form of leishmania. The amastigotes isolated from subscapular lymph node of positive dogs. In this survey, researchers used a rapid and economical ELISA (dot-ELISA) to determine the presence of anti-Leishmania antibodies. Basically, the technique is a combination of methods earlier described (Athanasίου *et al.*, 2012). Epidemiological data on age, sex, dog utility and location were recorded in a questionnaire filled out by the owners and the participating veterinarians.

RESULTS AND DISCUSSION

From a total of 151 samples of serum analyzed by ELISA, 16 resulted positive with clear blue dots on nitrocellulose strips. The seropositive dogs found were: 7 dogs in Saranda, 5 dogs in Gjirokastra, 3 dogs in Permet and Tepelena and only 1 dog in Erseka. In total resulted 16/151 seropositive dogs with a seroprevalence of 10.6%. The results for seropozitive dogs, examined with Leishmaniasis-Dipstick test from serum samples analyzed in the laboratory of the Parasitological in the Faculty of Veterinary Medicine in Tirana, Albania were 10/151 or 6.62%. From the 10 positive dogs, 4 dogs were from Saranda, 3 from Gjirokastra and 3 from Permet. With this rapid dipstick test resulted suspicious 3 in Saranda, 1 in Gjirokastra, 1 in Permet-Tepelene and 1 in Erseka. All these samples was reevaluated positive with ELISA test. All samples were analyzed for full hematological profile such as: number of Red Blood Cells (RBC), White Blood Cells (WBC), Hemoglobin level (Hgb), leukocytes subsets and trombocythes. One of the study purposes was to examine if infected macrophages (amastigote) were present. The difference between samples was not significant with a change in the number of WBC present only on 7 samples. From these 7 samples in 5 samples was identified a light leucocytosis and in two others leucopenia. The level of monocytes was increased in all the samples. The anemia was detected in 40 samples. The monocytosis and anemia are a target indicator for presence of Leishmaniasis. The infected macrophages were not present. Thrombocytopenia was observed tendency (7/16) at seropositive dogs with leishmania. From two dogs with both seropositive and clinical signs were prepared microscope smears. One of smears was positive for presence of infected macrophages. The 151 samples were analyzed by ELISA test (dot-ELISA) and 16 samples were positive. The positive samples belong different districts of Albania such as: Gjirokastra 5/38 samples, Saranda 7/35 samples, Permet and Tepelene 3/39 samples and Erseke 1/39 samples. In total the prevalence of leishmania infection was 10.6%. Researchers compared the prevalence of infection according to the geographic areas. The datas showed that the prevalence of infection is higher on seaside and areas with warm climate. This is associated with optimum ecological condition for the vector (Lukes *et al.*, 2007).

Seropositive results were founded to be higher on dogs aged 0.5-2 years old. These results are in accordance with some other publications. This has been reported by different studies in various Mediterranean countries: France (Marty *et al.*, 2007), Portugal (Campino *et al.*, 2006), Spain (Martin-Sanchez *et al.*, 2009), Algeria

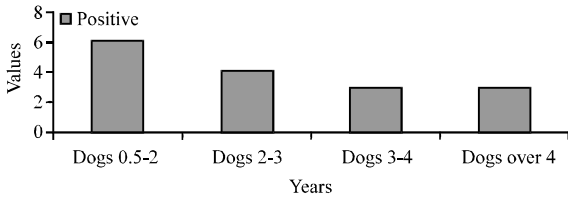


Fig. 1: Results of ELISA test according to ages of dogs

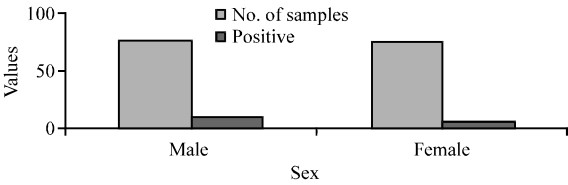


Fig. 2: Results of ELISA test according to sex

(Harrat *et al.*, 1996), Tunisia (Belhadj *et al.*, 2002), Cyprus (Deplazes *et al.*, 1998) and Italy (Gramiccia and Gradoni, 2007) (Fig. 1 and 2).

The prevalence was higher on male dogs compare with female. The reason, researchers think is that more female dogs have been more in contact with parasite and sometimes died before the reproduction process or during the pregnancy. This is probably attributed to the fall of the resistance during pregnancy and bad breeding conditions. The dogs belong two groups: in the first group there are included hunting dogs, the second group are included mix race. In South of Albania does not exist the pure dog race. The reasons why the prevalence of infection is higher in mix dogs is related to the higher exposition with the source of infection (Lukes *et al.*, 2007). Hunting dogs are not very common in the study area. The difficulty that we met during our study was related to the impossibility of controlling the number of hunting dogs. The results show that affected many from the breeding specific conditions in this region. This result think that affected primarily features included in the study areas. The dogs in this area almost only to protect livestock herds from wild canids very present in these areas. There are also smaller urban centers and only a very limited number of indoor living dogs. Some dogs had hair loss and moderately enlarged lymph nodes and lymph node aspiration was done. No dog was found serologically negative and parasitologically positive. Among the seropositive animals, 5/16 or 31% had some signs compatible with Leishmaniasis whereas 11/16 or 69% were entirely asymptomatic. Only in one case (from 5) researchers get amastigote form of *Leishmania infantum* (Zivicnjak *et al.*, 1998). Similar to other countries in the Mediterranean region (Koutinas *et al.*, 1999; Cardoso *et al.*, 2004; Dujardin *et al.*, 2008), the prevalence

of infection in non endemic areas of Albania show to be widely variable. The highest prevalence of infection was in Saranda district. Researchers think that it is related with the better climacteric conditions for growing of vector (phlebotomies). It makes sense because the prevalence decreasing in mountains areas. The difference on prevalence of infection among districts such as: Gjirokastra, Permet and Erseke. These areas expect Saranda did not differ significantly by the altitude. Three of four areas with low canine Leishmaniasis are situated at the altitude of 200-600 m or more. In Gjirokastra, Permet and Erseke-Albania, 600 m altitude is the limit where the Mediterranean climate switches to a cooler one, associated with changes in ecological conditions. According to Ashford (2000), the distribution of Leishmaniasis is determined by its vector, reservoir host and limiting factors, i.e., specific environmental requirements that lead to focal distribution of disease. In an entomological survey performed in 1999 in the middle region of Albania, the vectors were found in coastal cities of Albania but such survey was not performed deeper in the hinterlands and at higher altitudes. Further entomological investigations should be done to investigate if the vector occurs at higher altitudes. The distribution of seropositive dogs within the canine population showed significant heterogeneity according to the age and sex however, it was not relevant whether the animal was kept mainly indoors (pets) or outdoors (guard and hunting dogs). The results are in accordance with other study published by Zaffaroni *et al.* (1999) and lifestyles have been shown its impact on prevalence of infection. The increasing of seropositivity was found to be on dogs which belongs 2 years old group. This is related with low resistance on this group. This could be related to the cumulative increase of the time of exposure of dogs to phlebotomines and the increase of the death rate in old animals. As in some other Mediterranean foci (Cruz *et al.*, 1990; Zaffaroni *et al.*, 1999), researchers found a higher prevalence among males than in females. In the study, researchers could not find any gender-associated differences in exposure to the parasite; males and females were kept in the same manner. One possible explanation could be an increase in female mortality in which pregnancy and nursing may play an important role (Cardoso *et al.*, 2004) but gender-related differences in the host immune response might play a role in the resistance and susceptibility to infection. The ELISA, used for screening purposes, could detect subclinical and clinical Leishmaniasis at sera dilution. This result was confirmed by pathogen isolation in one case. In many dogs researchers find low titer (under cut off) indicate that they have been exposed to the infected phlebotomies. When

researchers deal with asymptomatic animals in endemic areas, immunological techniques do not discriminate between infected and resistant animals (Cardoso *et al.*, 2004). Leishmaniosis in Albania is higher in areas considered endemic. According to the data in Tirana which is conceived as endemic area, living a third of the country's population are over 22 veterinary clinics. From the data collected from them by January 2000 to December 2012 Leishmaniosis prevalence ranges from 16-36%. At the seroprevalence has a great influence animal race, place of residence and manner of holding. With regard to the breed of diseased dogs it was seen a predominance of medium and large sized breeds such as Boxer, Illyrian shepherd dog, German shepherd, Greyhound, English setter, Pit bull, Rottweiler, Pointer, Siberian husky, Napolitan hound and mixed breed who used to have a life style more exposed to the phlebotome bites. This data also goes well along with the results of the Spanish study (Miranda *et al.*, 2008) where positive dogs of large and medium size were over represented versus small sized dogs. From the preliminary data at the Petlife hospital (one of the largest in Albania) for the period 2010 to 2012 the prevalence of leishmaniosis results 18%. While at the German pastor, Rottweiler, Boxer and Illyrian shepherd the seroprevalence results 22%. About this data the seroprevalence values at the Pointer dogs at the same time period resulting in 42%. The relatively high number of owned dogs found positive for Leishmaniasis although, seen in a confined environment of Tirana, indicated that Leishmaniasis should be taken seriously into consideration as a disease of zoonotic importance and proper measures should be taken.

CONCLUSION

The epidemiology of Leishmaniasis at Albania involves a dynamic and complicated network of highly complex interactions among *Leishmania* parasites, phlebotomine sand fly vectors and susceptible hosts. These in a given moment in their life cycle are linked by chance by necessity or both. For most of the species of *Leishmania*, humans and domestic dogs are merely accidental hosts which are unfortunate enough to be exposed to an infected female phlebotomine sand fly. This study show that *Leishmania* infection is spread in South of Albania. Prevalence of infection at the nonendemic area resulted 10.6%. The dogs are sources and reservoir of this important zoonosis. This is why the cooperation between human medicine and veterinary medicine must be in high level and must undertake joint study for survey and control of this disease. In the clinical cases thrombocytopenia might be consider for making of

diagnosis so, serological tests must apply for confirmation of diagnosis in both clinical and asymptomatic dogs. The disprict kits can use in the veterinary routine of diagnosis but ELISA is one of the best serological methods to confirm diagnose the Leishmaniasis. Researchers can conclude that *Leishmania* infection is present in above areas. Researchers suggest that further study will be undertaken to establish a real prevalence of infection. This infection is a threat for humans in these habitats and both veterinary and public health services must put in place a survey and control program. Retesting of dogs will allow to find out the phase of infection and will help in discover the early infection.

Diagnostic confirmation could be established after a combination of the clinical examination and other relevant diagnostic methods. Results of haematological examinations and especially thrombocytopenia can use be as the first indicator in asymptomatic dogs.

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