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Canine Leishmaniasis in Yerevan

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Abstract **– Research performed from 2019 to 2021 revealed stationary foci of canine leishmaniasis in Yerevan suburbs: Avan, Nor Nork Block, Kharberd, as well as in one of the central districts of the city - Arabkir Community. The disease has been observed in purebred dogs at the age of 2 to 6 years, regardless of gender and specific breed, and proceeded with clinical signs of dermatitis (cutaneous leishmaniasis) or fever, lethargy, anemia, ascites, and conjunctivitis (visceral leishmaniasis). Microscopy of conjunctival smears in visceral, and in some cases, in cutaneous leishmaniasis detects amastigotes of the causative agent of the disease that are located in both cytoplasm of tissue macrophages, and beyond.**

Keywords **– Leishmaniasis, Dog, Sand Fly, Smear, Diagnosis.**

I. INTRODUCTION

Canine leishmaniasis is acute or chronical seasonal protozoal infection of animals and humans transmitted by bite of Phlebotominae subfamily sand flies - blood-sucking arthropods. Causative agents of leishmaniasis are unicellular parasites belonging to type Sarcomastigophora, class Kinetoplastida, order Trypanosomatida, genus *Leishmania* Borovsky, 1898 (Ross, 1903). Currently, about 20 species and subspecies of *Leishmania* are known. Parasites enter the blood of animals and humans from the salivary glands of sand flies during the bite. In mammal host, parasites affect the tissue phagocytes in the liver, spleen, vessels, lymph nodes, skin, as well as blood macrophages (monocytes) in which their reproduction occurs [1, 10, 11]. Causative agents of the canine leishmaniasis in our region are as follows:

1. *Leishmania infantum* Nicolle, 1908, that is usually registered in Mediterranean Region and Middle East; it infects macrophages in liver, spleen, blood, bone marrow, and causes visceral leishmaniasis.
2. *Leishmania major* Yakimoff and Schokhor, 1914, that is typical for Northern Africa, Middle East, and Central Asia, and causes zoonotic cutaneous leishmaniasis [1, 9, 10, 12, 23, 24].

Leishmania spp. are characterized by a complex life cycle with the participation of two hosts -vertebrate host (canids, rodents, hyraxes or humans), and invertebrate host (Phlebotominae subfamily-*Phlebotomus*, *Lutzomyia* and *Sergentomiya spp.* sand flies living in hot and warm areas of the Earth). There are almost 600 species of Phlebotominae subfamily sand flies living in Africa, Central Asia, Kazakhstan, Southern Caucasus, the Crimea, southern part of Ukraine, Moldova etc. The main vectors of *Leishmania* in Armenia are *Phlebotomus balcanicus* Theodor, 1958, *Ph. (Adlerius) halepensis* Theodor, 1958, *Ph. (Larroussius) kandelakii* Shchurenkova, 1929, *Ph. sergenti* Parrot, 1917, and *Ph. wenyoni* Adler et Theodor, 1930 [1, 3, 7, 12, 17, 18, 21].

In the organism of vertebrate host, *Leishmania* develops intracellularly as amastigote – in tissue or free circulating macrophages, and reticuloendothelial cells. Amastigotes are small round or oval cells having a size of 1-3 to 2-6 μm with clearly visible oval nucleus and kinetoplast. By Giemsa, cytoplasm is stained blue, nucle-

-us is red-purple, and kinetoplast is dark red. Amastigotes multiply by simple division [1, 14].

First case of visceral leishmaniasis in Armenia was registered in 1913, and cutaneous leishmaniasis was detected in Armenia for the first time in 1920 [14]. In Armenia it is currently characterized as re-emerging vector-borne infectious disease [12, 14, 22]. Although dogs are the main sources for human infection with *Leishmania infantum* in Mediterranean Region and Middle East [4], data about canine leishmaniasis in Armenia are single and very old [13].

II. Material and Methods

Taking into account the fact that there are limited data on canine leishmaniasis in Armenia in the available special literature, the goal of our research, covering the three-year period (from 2019 to 2021), was the study of the dynamics of epidemic process, and characterization of clinical manifestation of canine leishmaniasis in separate districts of Yerevan. At the same time, all cases of the disease registered by the Department of Epidemiology and Parasitology of the National Agrarian University of Armenia, were monitored. The final diagnosis for leishmaniasis was made taking into account epidemiological data and characteristic clinical signs of the disease, as well as on the basis of the detection of specific antibodies to the pathogens of leishmaniasis in blood serum of dogs using rapid immunochromatographic diagnostic test with blood serum of dogs [16, 20]. We also proposed a method for the diagnosis of canine visceral leishmaniasis based on the detection of the causative agent of the disease in tissue macrophages in microscopy of conjunctival smears stained by Giemsa. The pure glass was touched to the surface of the conjunctive, then the sliding movements up and down were performed in order to obtain conjunctival smear-imprints. The smears prepared were fixed with 96^0 ethanol for 10 minutes, stained by Giemsa for 20 to 45 minutes, depending on air temperature (longer in winter, shorter in summer), and examined with immersion system of microscope (1000-1600x).

Skin smears were also obtained from patients with characteristic skin lesions. Crusts were removed, and skin was scraped till the firs drop of blood appeared. Thin smears were prepared from blood drops, then fixed and stained as described above.

III. Results and Discussion

Leishmania amastigotes having a size of 4 to 5 micrometers, were located in an amount from 2 to 10 in cytoplasm of tissue macrophages, rarely – beyond (see photos 1 to 4).

In total, 67 dogs with skin lesions were examined. 26 cases of canine leishmaniasis (38.81% of examined dogs with skin lesions) were detected during the specified period of time. 6 of them were registered in Kharberd and Dzoraghbyur (southern and eastern suburbs of Yerevan), 5 cases were found in dogs in Nor Nork and Avan Communities, and 4 more cases were detected in one of the central regions of the city – Arabkir community.

Thus, leishmaniasis is characterized by certain focality determined by biotopes of sand flies, specific vectors of the causative agents of leishmaniasis.

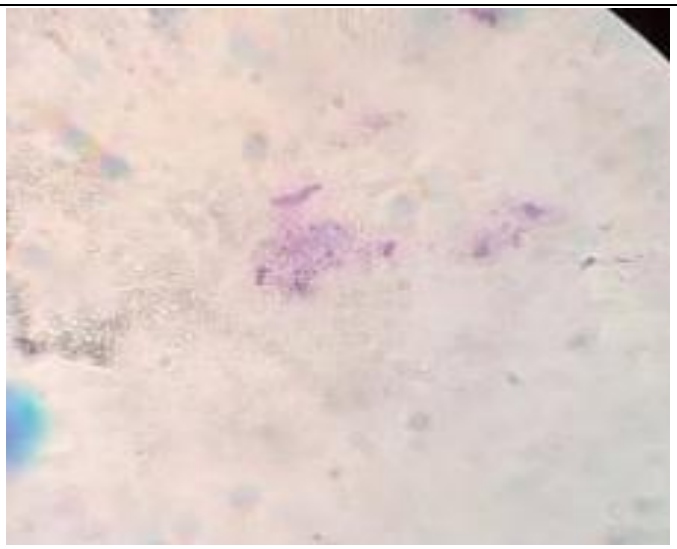

Photo 1. Multiple amastigotes in the protoplasm of a tissue macrophage. 1000x.

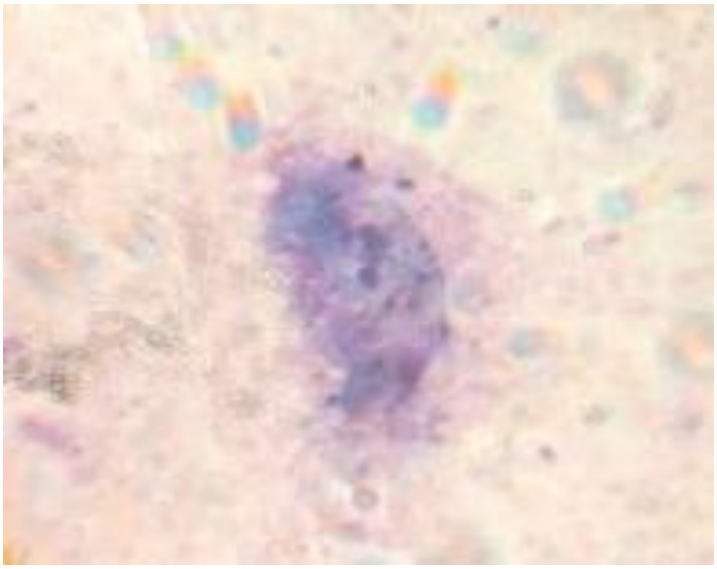

Photo 2. Single amastigotes in the protoplasm of the tissue macrophage and beyond. 1600x.

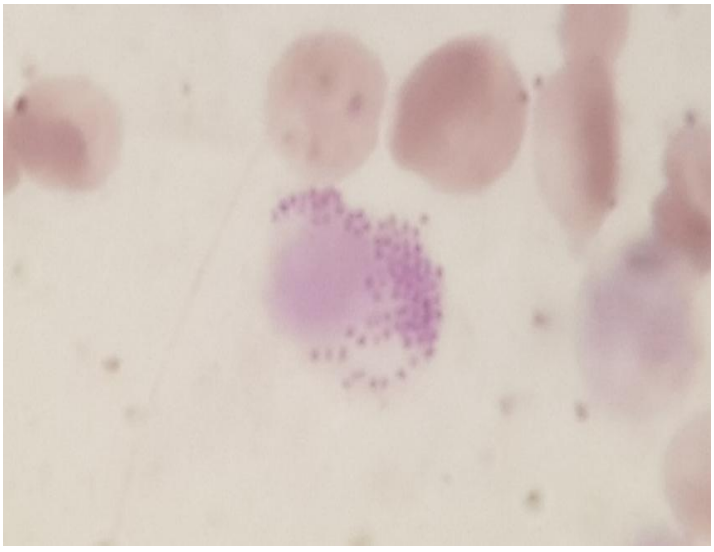

Photo 3. *Leishmania* amastigotes in blood macrophage (monocyte). 1600x.

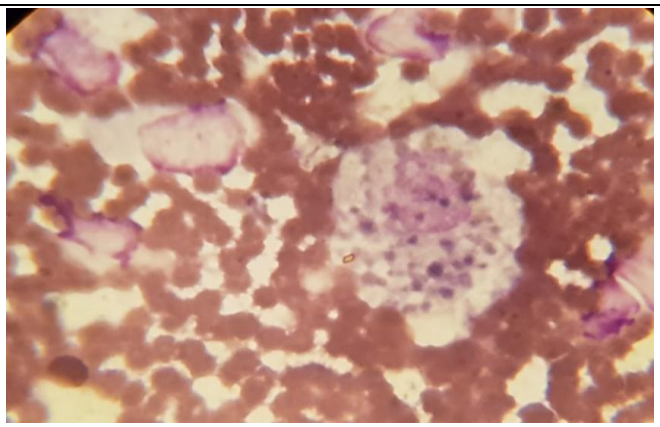

Photo 4. *Leishmania* amastigotes in skin macrophages. 1600x.

The earliest case was observed on June 1, 2019, and the latest one was on December 03, 2021, with a highest incidence registered in late summer and early autumn. The breed structure of dogs infected with leishmaniasis, was as follows: 12 - Caucasian Shepherd, 6 – Doberman Pinschers, and 8 - German Shepherds. The age of sick dogs ranged from 2 to 6 years, and the ratio of gender was as follows: 24 males (10 Caucasian Shepherds, 6 Doberman Pinschers, and 8 German Shepherds), and only 2 females (Caucasian Shepherds). Such pattern is probably explained by specific purpose and exploitation of certain breeds kept outdoors, that are more exposed to the bites of the sand flies.

All the diagnosed cases of leishmaniasis were chronic. 22 cases of cutaneous leishmaniasis in male Dobermans, Caucasian and German Shepherds were registered, while only two cases of visceral leishmaniasis in two Caucasian Shepherd females were detected. This fact is also explained by preferred keeping and exploitation of males as security guard dogs that are kept mainly outdoors, and exposed to the sand flies attack more frequently than females.

The diagnosed cutaneous leishmaniasis was manifested by nodular rash in various parts of the body, mainly on the hind limbs and face (see photo 5), pustules and purulent fistulae in the joint areas, alopecia with hyperkeratosis around the eyes (the so-called “glasses”), superficial, long term non-healing ulcers on the back of the nose. At the same time, the general condition of patients was satisfactory, they maintained activity, the average body temperature was about 39.5^0C, with no signs of anorexia. In 5 dogs with pronounced signs of cutaneous leishmaniasis, the symptoms of purulent-catarrhal conjunctivitis were observed.

Photo 5. Skin lesions of the back of the nose in dog with leishmaniasis.

Cases of visceral leishmaniasis were characterized by lack of mobility and general oppression of sick animals, the average body temperature was 40.5^0C and higher, with a sagging belly as a consequence of splenomegaly, and enlarged lymph nodes. Dogs died within two months after the first manifestation of signs of visceral leishmaniasis.

Our data mainly correspond to observation of numerous researchers [2, 5, 8, 10, 15, 19].

Examination of skin and conjunctival smears stained by Giemsa, with oil immersion system of microscope, allowed to detect amastigotes in macrophages. The amastigotes were 4 to 5 micrometers in size, and both cytoplasmic and extracellular localization. The number of amastigotes located inside macrophages varied from 2 to 10.

It should be noted that, for the cytological diagnosis of leishmaniasis, it is mandatory to investigate the punctate of bone marrow, lymphatic nodes or spleen. However, the fact that the puncture is a rather painful procedure for the animal, and technically complicated for a doctor, frequently requiring general anesthesia, makes this procedure limited in use for routine clinical practice. At the same time, the method of microscopic examination of smears, prepared from the surface of the conjunctive, and skin lesions, can be characterized as more promising for rapid diagnostics of canine leishmaniasis. The proposed method is easily performed and very efficient to diagnose both visceral and cutaneous leishmaniasis with corresponding clinical manifestations.

In addition, in available special literature we have not found references to a similar method for diagnostics of leishmaniasis in dogs.

From a clinical and epidemiological points of view, change in the geography of canine leishmaniasis should be emphasized: if previously the foci of the disease were registered mainly in southern suburbs of Yerevan, in Ararat Valley, now canine leishmaniasis is detected in central and northern regions of the city, as well as in foothills of Geghama Range (eastern part of Yerevan). Such a change in the geography of the disease is probably explained by global climate warming and, as a result, expansion of the habitat of blood-sucking dipterans – sand flies, vectors of *Leishmania* [6, 8, 12, 23, 24]. The pattern of infection in different breeds of dogs breeding composition of patients with leishmaniasis of dogs, as well as preferential infection of males has obviously a random character, however, the presence of visceral leishmaniasis only in females can be probably associated with the peculiarities of their hormone metabolism, which makes the reticuloendothelial cells of the internal organs more predisposing to be infected with *Leishmania.*

The average age of dogs infected with *Leishmania*, according to results of our research, was from 2 to 6 years. It generally corresponds to the data in special literature, according to which the disease affects animals in age primarily from 1.5 months to 15 years [4].

IV. CONCLUSIONS

Summarizing the results of clinical and epidemiological research, we can draw the following conclusions:

1. Canine leishmaniasis is recorded in the warm season: from the first half of June to the second half of September, which is associated with the activity of sand flies, vectors of causative agent of the disease. However, the cutaneous leishmaniasis that is generally characterized by chronic current, can be registered all year round, depending on the degree of manifestation of clinical signs.

2. Stationary foci of canine leishmaniasis have been detected in the suburbs of Yerevan: Kharberd, Avan, Nor Nork Block, as well as in Arabkir Community. Leishmaniasis is clinically manifested in thoroughbred dogs with 2 to 6 years of age, regardless of gender and a particular breed.

3. More often there is A chronic form of canine leishmaniasis characterized by signs of skin lesion of skin, is registered more often. An acute form characterized by hyperthermia, signs of as cites and a conjunctivitis, is observed more rarely.

4. Microscopic examination of conjunctival and skin smears during visceral and, in some cases, cutaneous leishmaniasis, allows to detect the causative agent of the disease as amastigotes, located in tissue macrophages.

5. To prevent the canine leishmaniasis, it is recommended to treat animals with insecticides in the form of sprays, drops, shampoos or collars that prevent sand flies attack, starting from the beginning of the activation period of sand flies (first half of June).

6. All the dogs with skin lesions should be also mandatory examined for leishmaniasis.

REFERENCES

[1] J. Alvar *Las Leishmaniasis*, Dela Biologia al Contro Laboratorios Intervet S.A., Salamanka, 2001 (in Spanish).
[2] S. Boutsini, M. Ptakakis, *Dog leishmaniasis in Attiko between 1995-2000*, in Proceedings of the 6-th Hellenic Symposium of Small Animal Practitioners, Athens, 2001, March 2001, p. 117.
[3] D. Carlotti D, *Canine European Leishmaniasis*, in Scientific Proceedings of the 9th FECAVA Congress, Estoril, Portugal, 2003, p. 82.
[4] F. Dantas-Torres, Miro G., Baneth G. et al., *Canine leishmaniasis control in the context of One Health*, in Emerging Infectious Diseases, 2019, vol. 25, N12. DOI: https://doi.org/10.3201/eid2512.190164
[5] Deplazes, F. Grimm, M. Papaprodromou et al., *Canine leishmaniasis in Cyprus due to Leishmania infantum*, in Acta Trop., 1998, vol. 71, pp. 169-178.
[6] M. Diaz-Espineira, R. Slappendel, *A case of autochthonous canine leishmaniasis in The Netherlands*, in Vet Q, 1997, vol. 19, pp. 69-71.
[7] N. Faghih, M. Mohebali, E. Javadian, *Epidemiology of visceral leishmaniasis in Kordan*, Tehran, 2002. http://www.sbmu.ac.ir/Journal/ Pejou handeh/Summer2002/pj28_13.htm.
[8] M. Gállego, *Emerging parasitic zoonoses: leishmaniasis*, in Rev. Sci. Tech., OIE, 2004, vol. 23, issue 2, pp. 661-676.
[9] A. Garifallou, M. Hadziantoniou, L. Schnur et al., *Epidemiology of human and canine leishmaniasis on the Island of Zakinthos*, in Leishmaniasis. Plenum Publishing Corporation, 1989, pp. 1011-1015.
[10] V. Kontos, A. Koutinas, *Old world canine leishmaniasis*, in The Canine Leishmaniasis. The Compendium of Continuing Education, 1993, vol. 15, pp. 949-959.
[11] A. Koutinas, Z. Polizopoulou, M. Saridomichelakis et al., *Clinical considerations on canine visceral leishmaniasis in Greece: a retrospective study of 158 cases (1989-1996)*, in J. Am. Anim. Hosp. Assoc., 1999, vol. 35, pp. 376-383.
[12] K. Kuhl, O. Moskalenko, A. Sukiasyan et al., *Microsatellite based molecular epidemiology of Leishmania infantum from re-emerging foci of visceral leishmaniasis in Armenia and pilot risk assessment by ecological niche modeling*, in PLOS Neglected Tropical Diseases, 2021, vol. 15, N4. https://doi.org/10.1371/journal.pntd.0009288.
[13] M. Mamikonyan, *Canine leishmaniasis in Erivan*, in Bulletin of Veterinary Research Institute, Armenia, 1935, vol. 1, pp. 109-115.
[14] Y. Ozbel, N. Turgay, S. Ozensoy, *Epidemiology, diagnosis and control of leishmaniasis in the Mediterranean region*, in Ann. Trop. Med. Parasitol., 1995, vol. 89, suppl 1, pp. 89-93.
[15] L.V. Paronyan, H.V. Apresyan, *Leishmaniasis in Armenia*, in Proceedings of the International Conference "Biological Diversity and Conservation Problems of the Fauna of Caucasus - 2", Yerevan, 2014, pp. 287-292.
[16] V. Sideris, G. Papadopoulou, E. Dotsika, E. Karagouni, *Asymptomatic canine leishmaniasis in Greater Athens area, Greece*, in European Journal of Epidemiology, 1999, vol. 15, issue 6, p 271.
[17] R. Slappendel, *Canine leishmaniasis. A review based on 95 cases in The Netherlands*, in Vet Q, 1998, vol. 10, pp. 1-16.
[18] B. Sotira, *Zoonotic diseases of major concern in Albania. Epidemiological overview*, in Inf. Circ., WHO Mediterr. Zoon. Control Cent., 2000, vol. 49, pp. 6-8.
[19] D. Strauss-Ayali, G. Baneth, *Canine Visceral Leishmaniasis*, in Recent Advances in Canine Infectious Diseases, Carmichael L. (Ed.), 2001. http://www.ivis.org/advances/Infect_Dis_Carmichael/baneth/chapter_frm.asp
[20] D. Strauss-Ayali, B. Sandler, C. Jaffe et al., *A seroepidemiological study of canine visceral leishmaniasis in Israel*, in Abstract book, Worldleish 2, Crete, Greece, 2001, 20 to 24 May, p. 41.
[21] M.V. Strelkova, E.N. Ponirovsky, E.N. Morozov et al., *A narrative review of visceral leishmaniasis in Armenia, Azerbaijan, Georgia, Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, Uzbekistan, the Crimean Peninsula and Southern Russia*, in Parasites and Vectors, 2015, vol. 8, p. 330.
[22] A. Sukiasyan, A. Keshishyan, D. Manukyan et al., *Re-emerging foci of visceral leishmaniasis in Armenia – first molecular diagnosis of clinical samples*, in Parasitology, 2019, vol. 146, issue 7, pp. 857-864.
[23] A. Trees, P. Howman, P. Bates et al., *Autochthonous canine leishmaniasis in the UK*, in Abstractbook, Worldleish 2, Crete, Greece – 20 to 24 May, 2001, p. 38.
[24] I. Tsachev, I. Kyriazis, E. Karagouni, S. Boutsini. E. Dotsika, *First Report of Canine Visceral Leishmaniasis in Bulgaria*, in Turk. J. Vet. Anim. Sci., 2010, vol. 34, issue 5, pp. 465-469.

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