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Histo-pathological Detection of Leishmaniasis in Dogs Tissue

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Abstract: The present research project was designed to calculate the Cutaneous Leishmaniasis infection in dogs few districts of Sindh, Pakistan. Dogs were screened for Cutaneous Leishmaniasis and potential Leishmaniasis positive cases were identified on the basis of active skin lesions. Affected tissues were collected and processed for Histo-pathological analysis. Dermal tissues of 35 domestic and 10 stray dogs were collected from Dadu, Hyderabad, and Jamshoro revealed that out of 45 dog samples, 30(66.66%) were positive for Cutaneous Leishmaniasis. The percentage of infection was 15(33.33%) positive in district Dadu, 10(22.22%) from district Hyderabad and 05 (11.11%) from district Jamshoro.

Keywords: Cutaneous Leishmaniasis, Cannine Leishmaniasis, Leishmaniasis, Dog, Tissue, Histopathology/Biopsy, Pakistan

1. INTRODUCTION

Cutaneous Leishmaniasis represents a major public health problem**.** It is one of the highly significant zoonotic parasite but very neglected disease in animals as for research is concerned. The infection is caused by more than 25 species of obligatory intracellular protozoans belonging to the genus Leishmania (Aneela *et al*., 2011). First time in Pakistan Cutaneous Leishmaniasis was reported in Afghan refugee's living in camps and Balouches living in 19 neighboring villages of refugee's in Baluchistan, Pakistan, (Brooker *et al*., 2004). The disease has great importance in veterinary and medical sciences. The vector of the disease is female sandfly which transmit the infection to a susceptible host through the bite (Torres, 2006 and Shakila *et al*., 2006. Cutaneous Leishmaniasis menace 12 million peoples and 350 million peoples are at risk in 88 countries including Pakistan around the world. It is estimated that one tenth of the population of world is at risk of this infection **(**Brooker *et al*., 2004**)**. Cutaneous Leishmaniasis showed variety of clinical signs and has severe socio-economic consequences (W.H.O 2007). The disease is frequently reported in people and Dogs. However, in human male more intensity of disease and infection rate was reported as compared to females (Weigle *et al*., 1994). The present study was designed to assess the infection rate of Cutaneous Leishmaniasis infection in dogs in few districts of Sindh, Pakistan.

The present study was focused on (a) Disease status of Leishmaniasis in dogs of in few districts of Sindh and (b) Histopathology of suspected Leishmaniasis cases.

2. MATERIALS AND METHODS

Study Area:

Sampling were collected from Dadu, Hyderabad and Jamshoro districts of Sindh Province, Pakistan.

Source and Collection of Tissue Samples:

The tissue samples were collected from face, eyes, ears, muzzle, brisket and legs of the Leishmaniasis affected dogs during entire study period.

Sample Size:

Tissue samples of 3-7 micron in thickness were collected from ulcerative area's washed thoroughly with 70 percent alcohol of forty five (45) Leishmaniasis suspected dogs. The tissue samples were , Bijou bottles that containing 3-5 ml of 70 percent alcohol to keep tissue for further processing.

Procedure:

The patient was comfortably restraint manually on the ground. Initially, the lesion was marked for the tissue specimen collection and then xylocaine, 2%, (2ml) was infiltrated around the lesion. Tissue sample by cutting with sterile scalpel blade was collected from infected area after 2-3 minutes of administration of xylocaine. The selected samples were then placed into Bijou bottle containing 3-5 ml of 70% alcohol for preservation. The selected samples of infected tissues were refrigerated for 24 hours and then processed for dehydration in the graded series of alcohols (60%, 70%, 80%, 85%, 90% upto 100%). The samples were shifted into cedar wood oil for clearing (6 to 12 hrs). Cedar wood

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oil was removed by placing the samples into the mixture of equal amounts of xylol and cedar wood oil and then washed with pure xylol. Slow penetration of paraffin wax was made possible (Akpulu, 2015).

3. RESULTS

Leishmaniasis cases were recorded in study area in dogs. Suspected dogs were restrained and body parts were thoroughly investigated for the presence of skin lesions. The tissues from the lesions were examined through histopathological evaluation for the confirmation of parasite **(Table-1).**

The presence of leishmaniasis in districts Hyderabad, Dadu and Jamshorois is presented in table-1. Out of total 45 observed cases 30 (66.66%) dogs were found positive for Cutaneous Leishmaniasis. Area wise data showed that the highest (33.33%) were found positive in dogs of district Dadu followed (22.22%) in Hyderabad and lowest infection rate (11.11%) was recorded from Jamshoro. In order to see the effect of leishmania parasite on the skin lesions samples were collected and analyzed **(Plate-1 and 2).**

Micro-histophotograph of the muzzle lesion showed non confluent granulomas composed of histiocytes and occasional multinucleated giant cells surrounded by numerous plasma cells, lymphocytes and macrophages **(Palte-3).** The epitheloid histiocytes were found loaded with Leishman bodies.

Micro-histophotograph of face area of skin, Unbillicated papule, nodule infiltrated plaque on the face presence of a dot-like structure reminiscent of Kinetoplast which conforming the diagnosis of Cutaneous Leishmaniasis **(Plate-4).**

Micro-histophotograph of brisket lesion, showing neutrophils and lymphocytes **(Plate-5).** The histiocytes is a large cell with plenty of light blue cytoplasm and a large round nucleus with clumped chromatin. The lymphocytes are round cells with small rim of basophilic cytoplasm and a large round nucleus. Neutrophils are seen as small cells with two types of granules are also obvious.

Micro-histophotograph of leg tissue lesions showed swollen macrophages, containing numerous Leishmania amastigotes **(Plate-6).**

The results presented from plate-3-6 indicated that the leishmania parasite severely affects the normal histology of invaded area.

4. DISCUSSION

Leishmaniasis is not a life threating disease, but it affects hundreds of people and causes both personels and social problems in the endemic regions. In the present research we confirm the present status of leishmaniasis infection in dogs in different districts of Sindh, Pakistan. Tissue samples that were collected from different body part's of dogs during the period, 2014-15 from few districts of Sindh, Pakistan. The potential clinical symptoms of suspected dogs were observed in domestic & stray dogs with active infection, the lesion samples were collected.

The present disease status in districts of Hyderabad was also under investigated and data was recorded through histo-pathological diagnosis. The epitheloid histiocytes were loaded with Leishman bodies. Our findings are in the agreement of Verham *et al.,* 1993, who reported the same findings of histology of muzzle lesions showing swollen macrophages containing numerous Leishmania amastigotes in Spain. Similarly, (Schraner., 2005) also reported after Histopathological examination of skin specimens that sub-epidermal infiltrations had lymphocytes, Plasma cells and histiocytes and small inclusion bodies representing amastigotes in Switzerland. (Ferrer *et al.,* 1988) also reported similar findings after histopathological examination of brisket lesion in which nodular form with numerous amastigotes of Leishmania inside the macrophages. Some multinucleated giant cells were also present in dogs of Spain.

(Machado *et al.,* 2002), also reported the similar clinical picture and histopathological findings for, the diagnosis of Cutaneous Leishmaniasis (CL) patients which were in early stage of (CL), 20 days after the appearance of first lesion. The majority of the patients included in this study was in the pre-ulcerative stage of, Cutaneous Leishmaniasis and presented with popular or nodular lesions. It was observed that the disease was affecting equally the adults and children in Brazil.

5. CONCLUSION

This current study shows that Canine Leishmanisis is a major health problem in Sindh province of Pakistan and dogs playing a key role in the incidence of Cutaneous Leishmaniasis in human beings infection. It is strongly suggested that strict hygienic measures should be taken in endemic regions to eradicate the leishmaniasis infection.

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Table-1. Infection rate of Cutaneous Leishmaniasis detected through Histopathology in dogs in division Hyderabad Sindh.

District	Observed	Infected	Percentage (%)
Dadu	24	15	33.33%
Hyderabad	14	10	22.22%
Jamshoro	07	05	11.11%
TOTAL	45	30	66.66%

Plate -1Cutaneous Leishmaniasis (Shallow ulcers) over left side of the dog.

Plate-2 Cutaneous Leishmaniasis (Ulcers) over head & whole body of the dog.

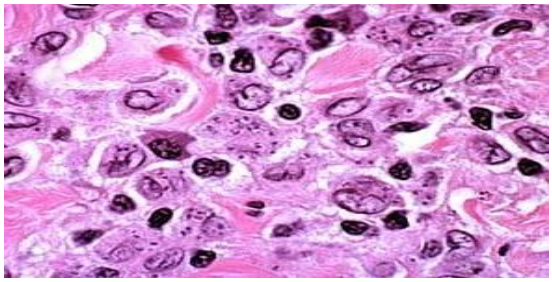

Plate-3 Non-confluent granulomas (Gr) composed of histiocytes and occasional multinucleated giant cells (Gc) surrounded by numerous plasma cells, lymphocytes and macrophages (Mac). The epitheloid histiocytes were loaded with Leishman bodies (H. E. Stain. Original magnification X 100).

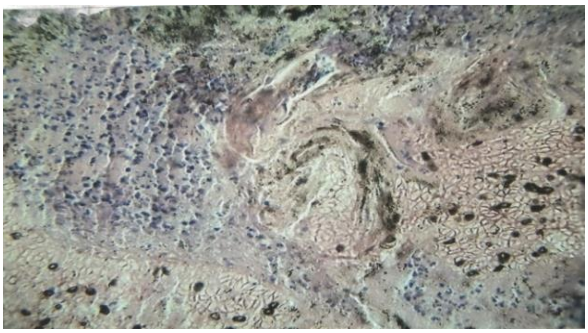

Plate-4 Unbillicated papule (Up), nodule infiltrated plaque on the faced (Nod) presence of a dot-like structure reminiscent of Kinetoplast (K) conforming the diagnosis of Cutaneous Leishmaniasis. (H. E. Stain. Original magnification X 40).

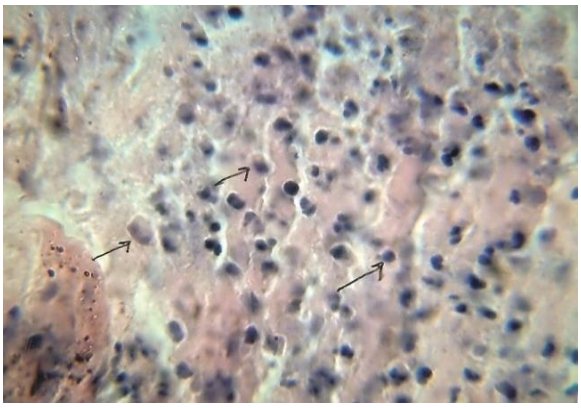

Plate-5 Histiocytes (H) a neutrophils (N) and lymphocytes (L). The histiocytes is a large cell with plenty of light blue cytoplasm and a large round nucleus with clumped chromatin. The lymphocytes are round cells with small rim of basophilic (Bas) cytoplasm (Ct) and a large round nucleus. Neutrophils are seen as small cells with two types of granules are also obvious (H.E. Stain Original magnification. 40 X).

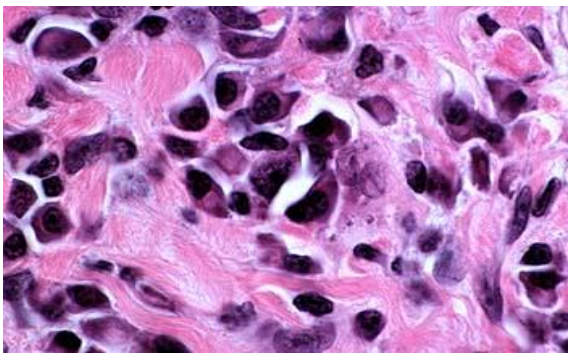

Plate-6 Histology of leg tissue (L), showing swollen macrophages (Mac), containing numerous Leishmania amastigote (Am) (H.E, Stain. Original magnification X 100).

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