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NEW REPORTS OF ACANINE CASE OF LEISHMANIA TROPICA INFECTION IN HAWIJA DISTRICT , KIRKUK , IRAQ

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Abstract
Canine leishmaniasis has been reported as the first autochthonous case in AL –Hawija district ,Kirkuk province ,Iraq . The use of PCR and DNA sequences provided evidence that *leishmania tropica* is the only etiological agent of cutaneous canine leishmaniasis .

Keywords : canine leishmaniasis , leishmania tropica , AL –Hawija , Iraq .

Introduction

-Canine Leishmaniasis is a vectors borne disrase ,caused by various members of hemoflagellate protozoan parasite of the genus leishmania that belong to the order kinetoplastida and the family Trypanosomatidae (Ciaramella et al ., 1997). The disease is transmitted by the bite of the infected female Phlebotomine sandfly (Soiano-Galego et al.,2011) .The reservoris of the disease are dogs and other wild animals (Kawamura et al .,2010). Canine leishmaniasis displays wide spectrum of clinical manifestations including visceral and cutaneous infection (Cleare et al ., 2014) . Although dogs remain to be asymptomatic despite a confirmed infection .

-In this investigation ,we describe for the first time an autochthonous case of canine leishmaniasis broke out in the region of AL-Hawija district ,Kirkuk province and the risk to the public health are discussed.

Materials and Methods

- Stray dogs : stray dogs were handled in accordance with good anomal practicp by animal welfare regulations . The exfliative dermatitis , alopecia ,and skin ulcerative lesion are the most frequently clinical features observed with stray dogs and further suggested a leishmanial infection (Figure 1) . Skin biopsies were fixed in formalin , embedded in paraffin and stained with haematoxylin – eosin . The smear of skin nodules scraping were stained by Giemsa stain .

- Isolation of Leishmania and strain typing : Skin and nodule samples were cultured at 25C for two week in two weeks . Tag Man - based kinetoplastic DNA minicircle targeted polymerase chain reaction (PCR) was purchased on skin samples and isolated promastigotes as described previously (Hassan and

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Shahab, 2022) using primers forward 5'GAC GGTGCCTGCCTACTTCAA – 3' and reverse 5' CCGCCCATGCTCTGGTACATC-3' .

- Standard sequencing : The purified PCR product was sequenced by Macragen Corp . Korra using * Sanger sequencing (ABI 3730XL Automated DNA sequencer) . The results were recieved by email then analysed using Geneious software.

Figure (1) leishmania tropica infected stray dogs showing alopecia exfoliative dermatitis and skin lesions .

Result and Discussion

-The examination of nodules and skin histopathology confirmed granulomatous nodular parasitic dermatitis that is consistent with Canixe cutaneous leishmaniasis as shown in Figures(2) and (3) . On the other hand Promastigotes were isolated from culting the skin lesion samples suggesting an evolving leishmanial infection .

- The extracted DNA was used as templates in PCR reaction . Molecular characterisation of the isolates revealed amplification of the characteristic 422bp minicircle band as represented in Figure (4) . The amplified PCR products of 422 bp were sequenced and analysed by alignment's with reported references sequences of Leishmania genotypes using Gene bank . The result of phylogenetic analysis showed that the Leishmania genotype was due to *L. tropica* strain . Analysis of alignment through online blast demonstrates correspondence of 97 % , 98 % and 99 % of an examined sequence fragment when compared with the data base of genbank of Leishmania accessible data . Also , it was evident and interesting that there are five codons transvertion and transition mutations between nucleotides (Tables 1and 2) .

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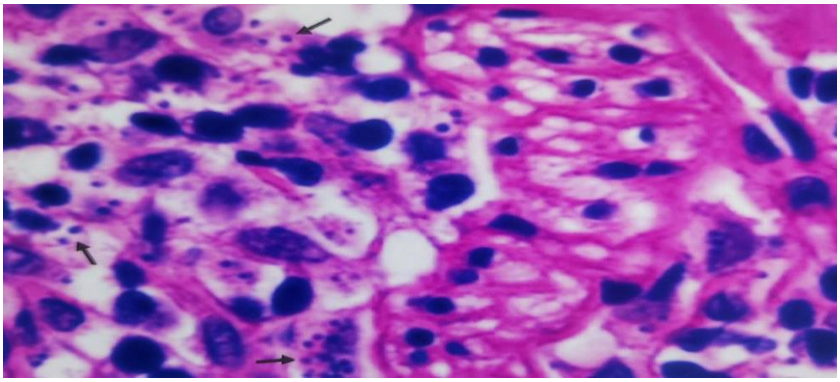

Figure (2) Intracellular amastigotes within macrophages of skin biopsy from stray doges

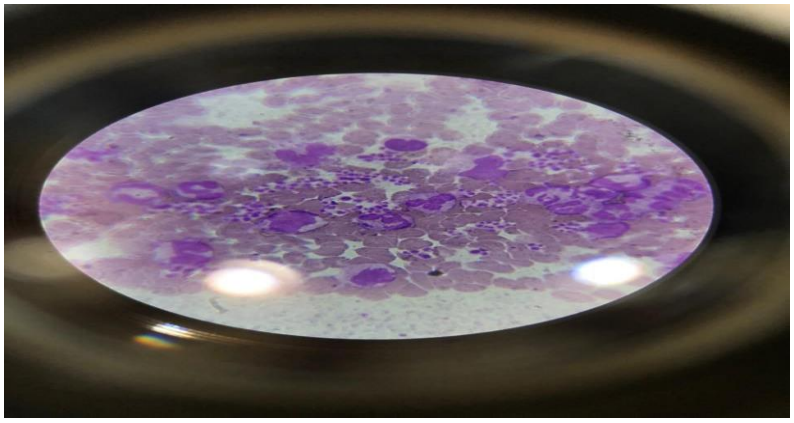

Figure (3) Intracellular leishmanial amastigotes in smear of skin nodules scraping from stray dogs .

M 1 2 3 4 5 6 7 8

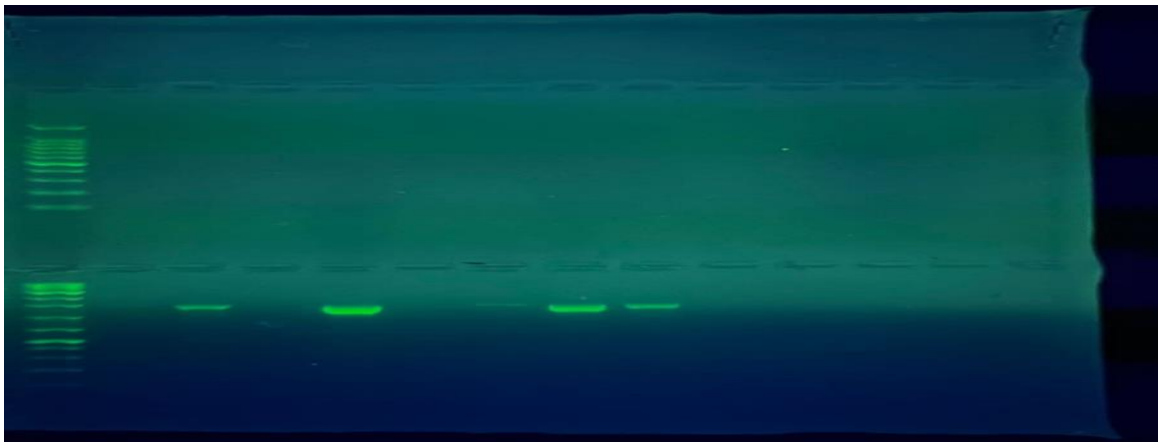

Figure (4) Agarose gel electrophoresis of PCR products obtained from skin biopsy samples at 422bp with primers specific for L.tropica . M represent 1500bp DNA ladder ;lane,1,3,5 represents negative controls ;lane 2,4 represents positive controls of isolated L.tropica ; lane 7,8 represents isolate from dog biopsy samples .

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Table (1) shows the percentage of concordance with cutaneous leishmaniasis when compared with the genetic bank.

L- tropica						
No.	Type of substitution	Location	Nucleotide	Sequence ID	Source	Identities
1	Transversion	800	G/T	ID:Y08020.1	Leishmania tropica hsp70 gene	%95
	Gap	819	A			
	Gap	839	G			
	Transversion	854	G/C			
	Transition	910	A/G			
	Transition	914	T/C			
	Transversion	983	C/A			
	Transition	989	C/T			
	Transversion	1016	G/C			
	Transversion	1019	G/C			
	Transversion	1022	C/G			
	Transition	1058	C/T			
	Transition	1062	G/A			
	Transversion	1068	T/G			
2	Transition		T/C	ID:Y08020.1	Leishmania tropica hsp70 gene	92%
	Transversion		C/A			
	Transversion					
	Transversion		G/C			
	Transversion		G/C			
	Transversion		C/G			
	Transition		C/T			
	Transition		G/A			
	Transversion		T/G			
	Gap		G			
	Gap		C			
	Transversion	1094	c/T			
	Transversion	1103	c/G			
	Transversion	1118	c/G			
	Transversion	1133	c/G			
	Transversion	1134	g/T			
	Transition	1148	A/G			
	Transversion	1175	C/G			
	Transversion	1181	G/T			
	Transversion	1196	G/C			
	Gap	1203	C			
	Gap	1210	C			
	Transition	1233	T/C			
	Transversion	1246	G/C			
	Transversion	1253	C/G			
	Transition	1260	A/G			
	Transversion	1275	C/G			
	Transition	1280	A/G			
	Transition	1287	A/G			
	Transition	1305	A/G			
	Transversion	1308	T/G			

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	Transversion	1310	C/G			
	Transition	1314	T/C			
	Transversion	1317	T/G			
	Transversion	1328	C/A			
	Transversion	1350	A/T			
	Transversion	1351	G/C			
	Transversion	1354	G/T			
	Transversion	1355	G/C			
	Transversion	1361	C/G			
	Transversion	1410	C/G			
	Transversion	1415	G/C			
	Transversion	1427	G/T			
	Transversion	1428	C/G			
	Transversion	1430	T/G			
	Transversion	1439	G/C			
	Transition	1445	T/C			
	Transversion	1457	A/C			
	Transition	1464	G/A			
	Transversion	1465	C/G			
	Transversion	1468	C/G			
	Transversion	1476	C/G			
	Transversion	1480	C/G			
	Transversion	1483	C/G			
	Transition	1498	G/A			
	Transversion	1500	T/G			
	Transversion	1513	T/G			
	Transversion	1515	T/A			
	Transition	1517	T/C			
	Transition	1523	G/A			
	Transversion	1529	A/C			
	Transversion	1544	T/G			
3	Error			ID:Y08020.1	Leishmania tropica hsp70 gene	
4	Transversion Transversion Transversion Gap Gap Transversion Transversion	788 800 819 837 839 854 914	A/C G/T T/A T G G/C T/A	ID:Y08020.1	Leishmania tropica hsp70 gene	95%
	Transversion	983	C/A			

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Table (2) shows the matching percentage of cutaneous leishmaniasis when compared with the LITSR/L5.8S initiator gene bank.

Rhizobium leguminosarum						
NO.	Type of substitution	Location	Nucleotide	Sequence ID with compare	Source	Identitie
1	Gap	83	A	ID: MT071547.1	Leishmania tropica strain AA1 internal transcribed spacer 1	97%
	Transversion	88	G/T			
	Gap	90	G			
2	Gap		T	ID: MT071547.1	Leishmania tropica strain AA1 internal transcribed spacer 1	98%
	Transversion		G/T			
3	Gap		T	ID: MT071547.1	Leishmania tropica strain AA1 internal transcribed spacer 1	96%
	Transversion		G/T			
	Gap		G			
	Transversion		A/T			
4	Gap		A	ID: MT071547.1	Leishmania tropica strain AA1 internal transcribed spacer 1	99%
5	Transversion		A/C	ID: MT071547.1	Leishmania tropica strain AA1 internal transcribed spacer 1	98%

Conclusions

1- For the first time, it was found that loose dogs are a stocking host for the cutaneous leishmaniasis parasite in Hawija.
2- It was found that the sand fly responsible for transmitting Leishmania parasite in Hawija district is Phlebotomus Papatasi.

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3- PCR purification is the best method for diagnosing leishmaniasis because it is the most sensitive and gives the best and most accurate results compared to traditional methods.
4- Through DNA sequences, it was found that the parasite responsible for the infection of cutaneous leishmaniasis in Hawija district, and in the main is tropical leishmaniasis *L .tropica*.

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