MOLECULAR CHARACTERIZATION OF K26 GENE OF LEISHMANIA INFANTUM, ISOLATE BY HUMAN PATIENTS FROM SICILY REGION

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Abstract:

Human Leishmaniasis is an emerging problem in Italy and increase in the Sicily region.
In the present work, we explored the genetic polymorphism of Leishmania isolates from twenty-five cases of human Leishmaniasis: two cases of visceral Leishmaniasis (VL) and twenty-three of cutaneous Leishmaniasis (CL). The characterization is carried out in comparison with twenty five human isolates of leishmania and one reference strain, *L. infantum* MHOM/TN/80IPT1 (MON-1). MON-1 is the most common zymodeme responsible for Leishmaniasis in Italy. The aim of the study is to genotype Leishmania isolates from Sicily by PCR ,analyzing size polymorphism of K26 gene to discriminate between MON-1 and non MON-1 zymodemes. K26 is a protein belonging to the Hydrofilic acylated surface protein B (HASPB) family. It is characterized by repeated aminoacidal domains and shows polymorphisms. The k26 polymorphism of MON-1 zymodeme is determinate in the size of 626 bp. The analysis show that all the 25 isolates belong to the *L. infantum* species, in particular the product size of 626 bp is detect in six patients affected by cutaneous Leishmaniasis. The molecular tools applied in this study can constitute a helpful support for parasite tracking and for a better understanding of the epidemiological evolution of Leishmaniasis.

Introduction:

Leishmaniasis is an anthropo-zoonosis caused by protozoa of the genus Leishmania transmitted to the host through the sting of insects phlebotomists is responsible for different clinical manifestations both in dogs and in humans. Based on multilocus enzyme electrophoresis (MLEE), the current reference method for characterizing and classifying Leishmania strains (1), *L. infantum* MON-1 is the predominant zymodeme in all Mediterranean countries. Each endemic area presents not only different species of the parasite, but also different MON strains. Many strains are associated only with VL (MON-27, 28, 72, 77, 187), others only with CL (MON-11, 29, 33, 78, 111) and others have been isolated in both forms of the disease (MON-1, 24, 34, 80). In particular, the MON-1 strain appears to be the prevailing zymodemes in the Mediterranean area with an incidence of more than 50% of cases in Italy.The aim of the study is to genotype Leishmania isolates from Sicily by PCR ,analyzing size polymorphism of K26 gene (figure 1) to discriminate between MON-1 and non MON-1 zymodemes.

K26 is a protein belonging to the hydrophilic acylated surface protein B (HASPB) family and is expressed only in methacyclic promastigotes and in Leishmania amastigotes. The protein is characterized by repeated amino acid domains and shows both inter-specific and intra-specific polymorphisms; The reference strain *L. infantum* MON-1 has an amplicon of the K26 gene of 626 bp. It is used as a reference marker in electrophoresis: it allows to identify the Leishmania strain (MON-1 non MON-1) that infected the patient.

ID SAMPLE	CLINICAL FORM OF	LEISHMANIA SKIN LESION	REAL TIME (Leish/mL)	K26 PCR (size)
MON 1	Reference strain of K26			626 bp
1	VL	Visceral	23000	Not amplified
2	CL	Headset region	330	**626 bp**
3	VL	Visceral	5700	388 -730 bp
4	CL	Frontal region	200	**626 bp**
5	CL	Left zygomatc region	15000	388 bp
6	CL	Region under orbital	100	**626 bp**
7	CL	Nose	110	**626 bp**
8	CL	Right arm	12000	680 bp
9	CL	Neck	10500	696 bp
10	CL	Right zygomatic region	8000	**626 bp**
11	CL	Left leg	6300	700 bp
12	CL	Forearm right	1160000	325 bp
13	CL	Right neck	75000	680 bp
14	CL	Under mandibolar region	210	Not amplified
15	CL	Right hand	1830	400 bp
16	CL	Zygom	5	720 bp
17	CL	Forearm left	730	750 bp
18	CL	Zygom	180	681 bp
19	CL	Right leg	100	370 bp
20	CL	Subscapular region	1100	388 bp
21	CL	Leg	2900	716 bp
22	CL	Hand left	1600	598 bp
23	CL	Front	11200	375 bp
24	CL	Nose	23000	696 bp
25	CL	Right hand	540	**626 bp**

Table 1 : Sample analyzed by K26-PCR.

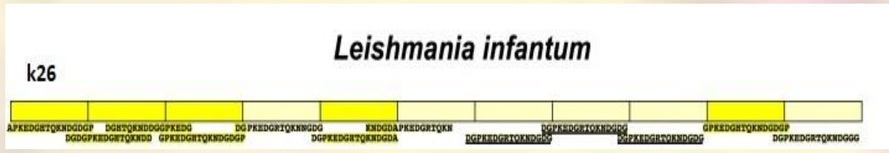

Figure 1: K26 gene structure of highly polymorphic *L. infantum*

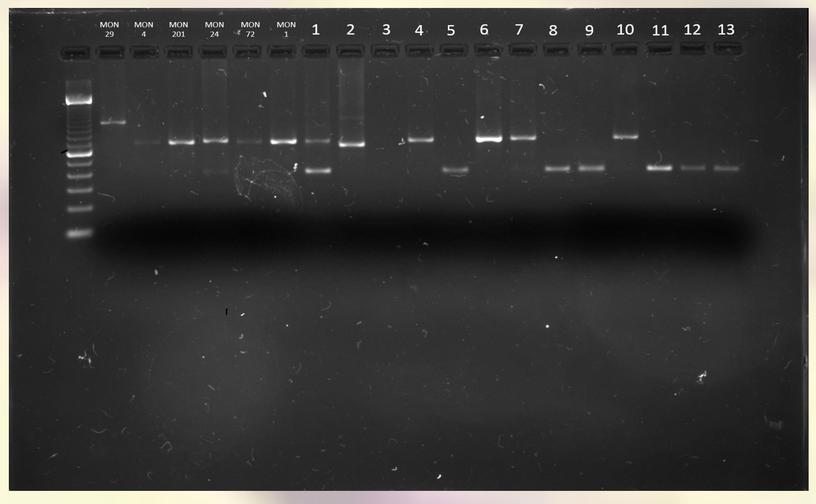

Figure 2: Agarose gel electrophoresis 1,5% of samples human.

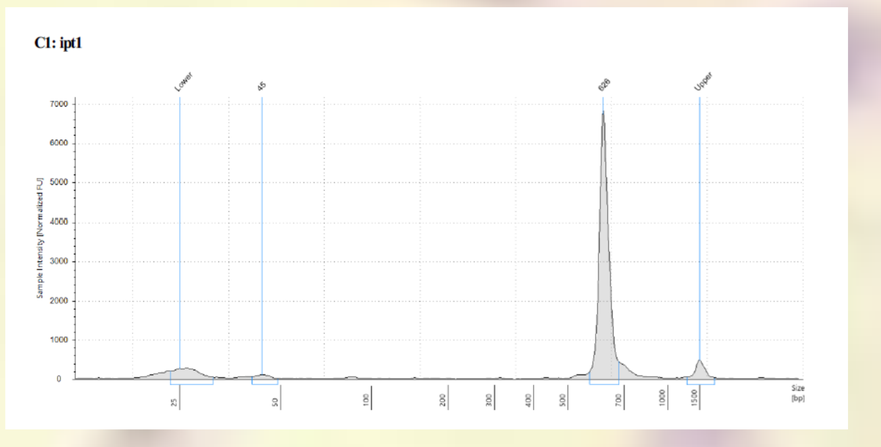

Figure 3: Electropherogram of the IPT1 peak (MON-1) obtained by Capillary electrophoresis

Materials and Methods :

In this study, we explored the genetic polymorphism of Leishmania isolates from twenty-five cases of human Leishmaniasis: two cases of visceral Leishmaniasis (VL) and twenty-three of cutaneous Leishmaniasis (CL) from different areas of the Sicilian territory. DNA extraction from tissue samples (bloods and skin biopsies) was carried out as previously described (2) to evalueted the concentrations of Leishmania infantum DNA . The isolated strains of Leishmania were grown in Tobie agar medium (3) modified by Evans with 15% rabbit blood, 5% fetal bovine serum, 250 µg of gentamycin/ml and 500 µg/ml 5-fluorocytosine. The cultures were incubated at 25 °C for 7 days. The isolates and stabilized strains in culture were subjected to DNA extraction through the special kits (Sigma). The extracted DNA was first quantized through Nanodrop and then subjected to molecular analysis by PCR-K26. The DNA amplifiers obtained from PCR-K26 were loaded into 1.5% agarose gel so as to determine the molecular weights of the amplified fragments of the K26 gene. Subsequently, capillary electrophoresis was performed, which provided validity and confirmation in discriminating the MON-1 strains from non-MON-1 in relation to the bp and the height of the peak identified in the electropherogram.

The *Leishmanial infantum* MON-1 reference strain shows an amplicon of the K26 gene of 626 bp, a quantity that is different in non-MON-1 strains (4, 5) .

Results and conclusions :

The table 1 shows the human samples of CL and VL analyzed by Real Time PCR and positive at DNA of *L .infantum.*
K26 PCR analysis show that all 25 isolates have variable sizes, in particular samples 2, 4, 6, 7, 10, 25 are identified as MON-1 since a band of about 626 bp is detected (Figure 2). The molecular tools applied in this study can be a useful support for the parasites monitoring and for a better understanding of the epidemiological evolution of Leishmaniasis. L. infantum strains, widespread in Europe, are highly polymorphic, in particular have K26 products with a range of sizes ranging from 385 to 1330 bp while the K26 amplicons of MON-1 strains isolated from patients from various areas of Sicily show a unique size of 626 bp.
PCR molecular technique, based on the analysis of a region of K26 gene, allowed in a first approach to discriminate MON-1 strains from non MON-1 strains. The study carried out allowed to identify that 6 out of 25 strains of L.infantum isolated from skin scrapings and of patients affected by CL and VL belong to the MON 1 group, because they showed an amplicon of 626 bp. The development of effective molecular techniques in characterization of the Leishmania strains that are inexpensive, simple and rapid, will allow the identification of the main L. infantum strains present in the endemic areas at the epidemiological level, in order to define the leishmaniosis strains circulating in the Sicilian territory.

References :

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