Microsatellite panel definition to characterize Leishmania strains isolated from human samples in an italian endemic region

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Background

The Leishmaniasis affects people, domestic and wild animals in temperature, subtropical and tropical regions. The natural cycle involves phlebotominae sandfly vectors transmitting the parasite to the vertebrate host. The insects influence the epidemiology of the disease by their geographical distribution in the seasons and the specific vectorial capacity. Human Leishmania infections are increasing every year in Sicily, which represent the region with the highest endemic level of the disease in Italy. Among different approaches employed for the diagnostic the parasites isolation remains the gold standard.

Methods

The samples directly taken from the lesions allowed us to obtain a number of isolated strains that we stored in nitrogen (Table I). We used this strains collection to check the genetic distance trough the parasites isolated in various part of Sicily by applying two different molecular approaches. A multiplex PCR were carried out to amplify 11 independent polymorphic microsatellites to cluster the strains in various groups (Table II). K26 gene targeted PCR-RFLP amplification analysis was conducted to confirm the polymorphisms through the strains too.

Table I: 27 Leishmania strains isolated from human skin lesions.
IPT1 MHOM 1 and MHOM 24 Leishmania reference strains were included in this study.

Patients	Patient from	Genetic clusters
1	Ciminna (PA)	
2	Agrigento (AG)	
3	Caccamo (PA)	
4	Caccamo (PA)	
5	Sciacca (AG)	
6	Racalmuto (AG)	
7	Racalmuto (PA)	
8	Canicattì (CL)	
9	Baucina (PA)	
10	San Carlo-Chiusa Sclafani (PA)	
11	C/da Due Gangi (AG)	
12	Sciacca (AG)	
13	Caltabellotta (AG)	
14	Caltabellotta (AG)	
15	Sciacca (AG)	
16	Riesi (CL)	
17	Caltanissetta (CL)	
18	Collesano (PA)	
19	Bompensiere (CL)	
20	Palermo (PA)	
21	Termini Imerese (PA)	
22	Sciacca (AG)	
23	Montallegro (AG)	
24	Roccapalumba (PA)	
25	Palermo (PA)	
26	Burgio (AG)	
27	Chiusa Sclafani (PA)	
28	IPT1 MHOM1 (reference strains 1)	
29	MHON 24 (ref 2)	
30	ref 3	
31	ref 4	
32	ref 5	
33	ref 6	
34	ref 7	
35	ref 8	

Figure 1: Genetic clustering of the strains: Number 1 (yellow) indicates the strains isolated from patient living in Palermo district ; Number 2 (green) indicates the strains isolated from from patient living in Caltanissetta district;; Number 3 (blue) indicates the Leishmania reference strains; Number 4 (yellow) indicates the strains isolated from from patient living in Agrigento district.

Results

This combined analytical method allowed us to distinguish various genotypes of *L. infantum* strains by isoenzyme independent approach. All the genetic markers with the exception of Li46-67 showed polymorphisms within the zymodeme MON-1, Li46-67 showed difference only in the strain *L. infantum* which corresponds to MON-24 (Table II). The PI, PIC DJ value indicated the discrimination and the informative power linked to the strength of each employed microsatellites. K26 analytical data showed the characterization power of the polymorphism based on SNPS. the method permitted us to discriminate the MHOM1 isolated strains respect the non-MHOM1 (MHOM24). Figure 1 shows the geographical distribution of the isolated strains analyzed by molecular approaches. By using a model-based clustering method, it was possible to delimit a big group of investigated strains of *L. infantum* which strictly correlated to the zymodeme MHOM1. Furthermore, other definite subgroups were observed as highly monomorphic groups. The multilocus microsatellites test could be useful to investigate the diffusion of predominant *L. infantum* zymodeme in endemic areas such as Sicily, and for this purpose, a model-based analysis was carried out. The data comparison shown as strains within the same cluster were isolated in neighboring districts and as genetic distance can reflect a geographic distribution of the strains (Table I).

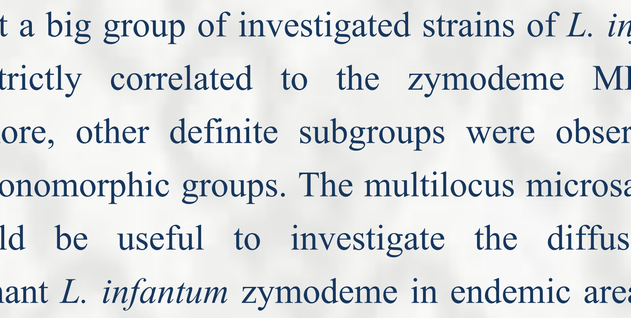

Table II: Genetic parameters at the 11 SSR loci analysed in the grapevine accessions collected

Locus	Na	Allele range (bp)	He	Ho	PI	PIC	Dj
Li41-56	6	80 - 90	0,758	0,600	0,0759	0,7264	0,8130
Li46-67	5	68 - 76	0,681	0,324	0,1001	0,6749	0,7796
Li71-7	4	92 - 98	0,553	0,114	0,2530	0,4197	0,6080
Li71-33	6	104 - 116	0,774	0,257	0,0711	0,7370	0,8197
Li22-35	10	80 - 102	0,853	0,600	0,0259	0,8570	0,9059
Li23-41	6	80 - 92	0,728	0,286	0,1033	0,6687	0,7597
Li21-34	6	72 - 92	0,622	0,286	0,1633	0,5762	0,6508
Li45-24	4	84 - 100	0,685	0,600	0,1229	0,6320	0,7345
Lm2TG	4	108 - 140	0,680	0,514	0,1240	0,6292	0,7382
Lm4TA	8	68 - 80	0,730	0,514	0,0891	0,6972	0,7849
Li715/2	7	100 - 110	0,661	0,400	0,1610	0,5625	0,7008
Mean	6	-	0,702	0,409	0,1172	0,6528	0,7541
All Loci	66	-	-	-	1,30E-11	-	-

Na: Number of Alleles per locus; **He**: Expected heterozygosity; **Ho**: Observed heterozygosity; **PI**: Probability of Identity**; PIC**: Polymorphic Information Content**; Dj**: Discrimination Power

Conclusions

The classical isoenzymatic analysis based on the polymorphisms of the protein structures, is time expensive a can result difficulties for potato amid gel emplying. Microsatellite approach is a semi-automatized analytical method and can use Structure Software. This software uses multi-locus genotype data to investigate population structures by studying hybrid zones and estimating allele frequencies. The results permitted us to found genetic clusters and them geographically distribution. However the parasite infective cases could reflect the movements of the patients in the endemic region. K26 analytical data confirmed the fact that our samples were belonging to the MHOM1 zymodeme.

The methods exhibited a high level of discrimination for closely related strains at epidemiological level and could be used on large-scale investigatio; moreover it permit the unequivocal identification of the same strain in case of re-infection.

Reference:

Haralambous C, Antoniou M, Pratlong F, Dedet JP, Soteriadou K.(2008). ***Development of a molecular assay specific for the Leishmania donovani complex that discriminates L. donovani/Leishmania infantum zymodemes: a useful tool for typing MON-1.*** **Diagn Microbiol Infect Dis. 60(1):33-42.**

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