Acta Tropica 118 (2011) 80–86

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Review

Canine *Leishmania infantum* enzymatic polymorphism: A review including 1023 strains of the Mediterranean area, with special reference to Algeria

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ARTICLE INFO

Article history:
Received 13 December 2010
Received in revised form 2 March 2011
Accepted 5 March 2011
Available online 16 March 2011

Keywords:
Leishmania infantum
Zymodemes
Polymorphism
Canine leishmaniasis
Algeria
Mediterranean basin

ABSTRACT

This bibliographic review reports the isoenzyme polymorphism of 1023 *Leishmania infantum* strains isolated from dogs that have been characterized by multilocus enzyme electrophoresis in the Leishmania Reference Centre of Montpellier, or in other laboratories, to which this typification technique has already been transferred. Between 1981 and 2010, a total of 12 zymodemes were identified around the Mediterranean basin: MON-1, MON-24, MON-34, MON-72, MON-77, MON-80, MON-98, MON-105, MON-108, MON-199, MON-199 var NP1130 and MON-281, of which 6 were present in Algeria. The zymodeme MON-1 was predominant (86.5% of the strains). The dog was confirmed as the main reservoir of *L. infantum* MON-1, while the reservoir of the other zymodemes has not yet been identified. The enzymatic polymorphism is relatively high in Algeria and in Spain in contrast to other Mediterranean countries. The reasons for this polymorphism are discussed.

Contents

1. Introduction

Canine leishmaniasis (CanL) was described by Nicolle and Comte in 1908. It is an endemic disease widespread in the world, and particularly in the Mediterranean basin. The first case in Algeria was reported in 1910 by the Sergent brothers (Sergent and Sergent, 1910). Dogs have long been implicated as the main domestic reservoirs of *Leishmania infantum*, the aetiological agent of both visceral (VL) and cutaneous forms (CL) of human leishmaniasis, and phlebotomine sand flies are the vectors of this protozoal disease.

In 1908, Charles Nicolle named *L. infantum* as the causative agent of infantile kala-azar (Nicolle, 1908). The same year, Nicolle and Comte discovered the same protozoan in dogs in Tunis and developed the NNN medium (Novy-McNeal-Nicolle) for its cultivation (Nicolle and Comte, 1908). Since the seventies, the parasite *Leishmania* has been identified with precision through the isoenzyme

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doi:10.1016/j.actatropica.2011.03.001

analysis, which was widely developed and led to the current classification of the genus (Rioux et al., 1990). This method is considered as the gold standard method for *Leishmania* identification. It is in use for more than 30 years and is the only method which has been applied to several thousands of *Leishmania* isolates obtained around the world from man, reservoir hosts and phlebotomine vectors, leading to a better understanding of the epidemiology of leishmaniasis.

Most of the strains of *L. infantum* in the Mediterranean region belong to zymodeme MON-1, which is also the zymodeme predominantly found in dogs, in the region (Dereure et al., 1999; Pratlong et al., 2004; Campino et al., 2006; Aït Oudhia et al., 2009). However several other *L. infantum* zymodemes have also been identified in dogs, but these data are dispersed in various publications. The present paper aims to review and update the results of isoenzymatic identification of various studies carried out in Algeria and to establish an inventory of the strains identified during epidemiological investigations of all studies carried out around the Mediterranean area.

2. Materials and methods

We carried out a retrospective bibliographic study on the isoenzymatic identifications of *L. infantum* strains obtained from CanL cases between 1981 and 2010.

2.1. The bibliographic study

A total of 45 papers dealing with isoenzymatic identification technique, according to Rioux et al. (1990), of canine strains isolated in Algeria, and in other Mediterranean countries, were included in this review.

2.2. Isoenzymatic identification

The technique used for identification in all cases was multilocus enzyme electrophoresis (MLEE), using starch-gel electrophoresis as described by Rioux et al. (1990), and developed in the Montpellier *Leishmania* reference Center. The following 15 enzyme systems were studied: malate dehydrogenase (EC 1.1.1.37); malic enzyme (EC 1.1.1.40); isocitrate dehydrogenase (EC 1.1.1.42); 6-phosphogluconate dehydrogenase (EC 1.1.1.44); glucose-6-phosphate dehydrogenase (EC 1.1.1.49); glutamate dehydrogenase (EC 1.4.1.3); NADH diaphorase (EC 1.6.2.2); purine nucleoside phosphorylase 1 (EC 2.4.2.1); purine nucleoside phosphorylase 2 (EC 2.4.2.*); glutamate–oxaloacetate transaminases 1 and 2 (EC 2.6.1.1); phosphoglucomutase (EC 5.4.2.2); fumarate hydratase (EC 4.2.1.2); mannose-phosphate isomerise (EC 5.3.1.8); glucose phosphate isomerase (EC 5.3.1.9).

The *Leishmania* parasites have been identified by their enzymatic profiles compared to the standard *L. infantum* reference strain MHOM/FR/78/LEM75 (zymodeme MON-1), in the Montpellier Reference Centre, or in other laboratories in which the technique has been previously transferred. When new zymodemes were found, these identifications were generally confirmed in the Montpellier Reference Centre.

For comparing enzymatic polymorphism by country, we calculated a polymorphic index which corresponds to the number of zymodemes compared to the number of strains studied in the corresponding country (PI = Z/S). This index was calculated only for countries with a sufficient sample of strains (more than 50), resulting from CanL epidemiological surveys.

Table 1
Enzymatic profiles of the 12 zymodemes identified in canine leishmaniasis in the Mediterranean basin, between 1981 and 2010.

Zymodemes MON-	Enzyme profiles													Reference strains
	MDH	ME	ICD	PGD	G6PD	GLUD	DIA	NP_1	NP_2	GOT_1	GOT_2	PGM	FH	MPI	GPI	
1	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	MHOM/FR/78/LEM75
24	**104**	100	100	100	100	100	100	**140**	100	100	100	100	100	100	100	MHOM/DZ/82/LIPA59
34	**104**	100	100	100	100	100	100	100	100	100	100	100	100	100	100	MHOM/FR/84/LEM538
72	100	100	100	100	100	100	100	100	100	100	100	**109**	100	100	100	MHOM/IT/86/ISS218
77	100	100	100	100	**102**	100	100	100	100	100	100	100	100	100	100	MCAN/ES/86/LEM935
80	**104**	100	100	100	100	100	100	**130**	100	100	100	100	100	100	100	MHOM/DZ/83/LEM425
98	100	**90**	100	100	100	100	100	100	100	100	100	100	100	100	100	MHOM/EG/87/RTC2
105	100	100	100	100	**105**	100	100	100	100	100	100	100	100	100	100	MCAN/ES/88/LEM1355
108	100	100	100	100	100	100	**120**	100	100	100	100	100	100	100	100	MCAN/FR/87/RM1
199	**104**	100	100	100	**102**	100	100	**140**	100	100	100	100	100	100	**105**	MHOM/ES/92/LLM-373
199 var. $NP1_{130}$	**104**	100	100	100	**102**	100	100	**130**	100	100	100	100	100	100	**105**	IPER/ES/90/DP169
281	**140**	100	100	100	100	100	100	100	100	100	100	100	100	100	100	MHOM/PS/99/LRC-L773

The profile of enzymes that are different from the reference strain MON-1 are in bold.