Short Report

Visceral leishmaniasis in Sudan: first identifications of *Leishmania* from dogs

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In Sudan, human visceral leishmaniasis (VL) is endemic in various parts of the country. In the southern area VL is caused by various zymodemes of *Leishmania*: MON-18, MON-30 and MON-82 (ASHFORD *et al.*, 1992; OSKAM *et al.*, 1998). The sandfly *Phlebotomus* (*Larroussius*) *orientalis* has been found infected by MON-18 and MON-82 (ASHFORD *et al.*, 1992). In this region of East Africa, VL is commonly considered as anthroponotic although a few wild mammals have been found infected by *L. donovani s.l.*: grass rat (*Arvicanthis niloticus*, Rodentia: Muridae), spiny mouse (*Acomys albigena*, Rodentia: Muridae), serval (*Felis serval*, Carnivora: Felidae) and genet (*Genetta genetta*, Carnivora: Viverridae) (HOOGSTRAAL & HEYNEMAN, 1969), and jackal (*Canis* sp.) (SIXL *et al.*, 1987).

We report here the results of an immunological investigation completed by isolation and identification of strains of *Leishmania* causing canine visceral leishmaniasis (CVL) in a focus of VL in eastern Sudan.

Material and Methods

The area of Gedaref, eastern Sudan, is known as an endemic focus of VL (EL SAFI *et al.*, 1993). In June 1998, during a field investigation carried out in Barbar El Fugarra village, Gedaref State, 51 domestic dogs (*Canis familiaris*) were examined and had blood collected. Serological investigations for anti-*Leishmania* antibodies were made by immunofluorescence antibody test (IFAT), using as antigen a strain of *L. donovani* from VL in the same village (MHOM/SD/98/LEM3566). Nine dogs were selected for lymph-node puncture because they were old or because they had clinical signs compatible with CVL. An aspirate was taken from popliteal lymph nodes: the skin was shaved, then disinfected with ethanol; the lymph-node fluid was aspirated with a syringe containing 2 mL isotonic saline plus 250 000 International Units of sodium benzylpenicillin. The suspension was then distributed into 3 NNN culture tubes. The cultures were incubated for a few days in field conditions in an ice box in order to maintain a temperature of around 25°C (ambient temperature was 45°C) and rapidly forwarded to the laboratory. The isolated strains were mass cultivated and described biochemically by starch gel electrophoresis using the following 15 enzyme systems (RIOUX *et al.*, 1990): malate dehydrogenase (MDH, EC 1.1.1.37); malic enzyme (ME, EC 1.1.1.40); isocitrate dehydrogenase (ICD, EC 1.1.1.42); phosphogluconate dehydrogenase (PGD, EC 1.1.1.44); glucose 6-phosphate dehydrogenase (G6PD, EC 1.1.1.49); glutamate dehydrogenase (GLUD, EC 1.4.1.3); diaphorase NADH (DIA, EC 1.6.2.2); nucleoside purine phosphorylase (NP1, EC 2.4.2.1 and NP2, EC 2.4.2.*); glutamate–oxaloacetate transaminase (GOT 1 and GOT 2, EC 2.6.1.1); phosphoglucomutase (PGM, EC 5.4.2.2); fumarate hydratase (FH, EC 4.2.1.2); mannose phosphate isomerase (MPI, EC 5.3.1.8); and glucose phosphate isomerase (GPI, EC 5.3.1.9). The isolates were compared to reference strains MHOM/FR/78/LEM75 (*L. infantum*, MON-1), MHOM/ET/72/GEBRE1 (*L. archibaldi*, MON-82), MHOM/ET/67/HU3 (*L. donovani s. s.*, MON-18) and MHOM/SD/82/GILANI (*L. infantum s. s.*, MON-30).

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Results and Comments

Out of 51 dogs, 22 (43%) had a serological titre >1:40. Ten dogs had antibodies at 1:20 and 15 dogs at 1:40. Four cultures from 9 dogs were positive (Table): 3 at the 7th day and the fourth at the 14th day. One culture (dog 25, IFAT titre 1:80) was contaminated by a filamentous fungus. One strain (MCAN/SD/98/LEM3556) was identified as MON-82 (dog 21, IFAT titre 1:160), the second (MCAN/SD/98/LEM3562) as MON-257 (dog 49, IFAT titre 1:40) and the third (MCAN/SD/98/LEM 3555) as MON-267 (dog 2, IFAT titre 1:320).

Zymodemes MON-257 and MON-267 are new zymodemes, previously isolated respectively from 5 patients with VL and from 1 patient with post-kala-azar dermal leishmaniasis (PKDL) in the same focus (F. Pratlong, personal communication). They are closely related to MON-82 which was found in humans and sandflies in southern Sudan (ASHFORD *et al.*, 1992). These 3 zymodemes are differentiated by GOT and MPI enzymes: zymodeme MON-257 has the same enzyme GOT^{110} as MON-82 which is intermediate between GOT^{100} which is common to all zymodemes of *L. infantum s. s.* and GOT^{113} which is specific to the zymodemes of *L. donovani s. s.* (RIOUX *et al.*, 1990). In this paper MON-82 is labelled *L. archibaldi*. The difference between MON-82 and MON-257 is made by enzymes MPI^{100} for MON-82 and MPI^{110} for MON-257. The other new zymodeme MON-267 has the enzyme GOT^{100} specific to *L. infantum s. s.* and the enzyme MPI^{110} of MON-257. Thus, according to RIOUX *et al.* (1990) it could be possible to label MON-82 and MON-257 as *L. archibaldi* and MON-267 as *L. infantum s. s.* However, some authors consider that the visceralizing *Leishmania* isolates in Sudan cannot be reliably separated at this taxonomic level. An extensive study on human and canine isolates of this area is in progress and should contribute to the solution of this problem.

The dog is known to be the reservoir host of *L. infantum* in the Mediterranean area. In Asia and East Africa *L. donovani* is currently considered as anthroponotic. In Sudan, as mentioned above, a few wild mammals have been found infected by *L. donovani s. l.* (HOOGSTRAAL & HEYNEMAN, 1969; SIXL *et al.*, 1987). In other countries of East Africa or Central Asia some authors have found *Leishmania* in dogs: 2 strains were isolated in Kenya (MUTINGA *et al.*, 1980) and 1 of them (MCAN/KY/00/D2) was related to *L. donovani s. s.* MON-36; another strain (MCAN/IQ/81/SUKKAR-2), isolated in Iraq (SUKKAR *et al.*, 1981), was also identified as *L. donovani s.s.* MON-3 (MORENO *et al.*, 1986). The high canine seroprevalence (43%) found by us, and the demonstration that 4 of 9 dogs had positive culture and 3 had the same zymodemes of *Leishmania* as occurring in humans (in VL and PKDL), has led us to assume that the dog acts as a reservoir host of kala-azar in this focus of VL in Sudan. Further investigations are needed for a better understanding of the *Leishmania* species and their epidemiological status in Sudan, and of the precise status of the dog, and particularly to determine if man is infected

Table. Positive dogs for NNN medium culture: parasite identification, clinical aspects, results of serological tests, and isoenzymatic characterization of isolated strains (Barbar El Fugarra village, Gedaref State, Sudan; 1998)

Dog number	Age (years)	Clinical signs	IFAT titre	WHO code strains	Characterization	
2	4	No	1:320	MCAN/SD/98/LEM3555	*Leishmania infantum*	MON-267
21	2	Yes	1:160	MCAN/SD/98/LEM3556	*Leishmania archibaldi*	MON-82
25	10	Yes	1:80	Contaminated		
49	5	Yes	1:40	MCAN/SD/98/LEM3562	*Leishmania archibaldi*	MON-257

All 4 dogs were male. IFAT, immunofluorescence antibody test.

from dogs by the sandflies or whether infection is by the contrary route.

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