LEISHMANIOSIS DUE TO *LEISHMANIA INFANTUM* IN A FIV AND FELV POSITIVE CAT WITH A SQUAMOUS CELL CARCINOMA DIAGNOSED WITH HISTOLOGICAL, SEROLOGICAL AND ISOENZYMATIC METHODS

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Summary:

Leishmaniosis caused by *Leishmania infantum* is an endemic zoonosis present in the Mediterranean area. Canidae (dog and fox) constitute the main reservoir hosts for the parasite, whilst wild rodents or the cat can be carriers of the protozoan and are considered as secondary potential reservoirs. This paper describes a case of disseminated feline leishmaniosis with cutaneous (ulcerative), visceral (spleen and lymph nodes) and blood involvement in a FIV-FelV positive cat. The microscopic identification of the *Leishmania* infection was initially made on a skin biopsy of the temporal area, where a squamous cell carcinoma was diagnosed. The diagnosis of the disease was achieved by several serological techniques (ELISA, IFAT and Western-blot). The strain was obtained by blood culture, characterized by electrophoresis of isoenzymes and identified as *Leishmania infantum* zymodeme MON-1. Since the infection due to *L. infantum* is a zoonosis, the potential feline reservoir should be more investigated. Serological analysis by Western blot on domestic cats provides a useful tool. In veterinary practice, feline leishmaniosis should be systematically included in the differential diagnosis when compatible cutaneous lesions are present, especially in the endemic areas of canine leishmaniosis.

KEY WORDS : cat, *Leishmania infantum*, leishmaniosis, squamous cell carcinoma, France.

Résumé : MISE EN ÉVIDENCE DE *LEISHMANIA INFANTUM* CHEZ UN CHAT FIV ET FELV POSITIF AVEC UN CARCINOME ÉPIDERMOÏDE À L'AIDE DE MÉTHODES HISTOLOGIQUE, SÉROLOGIQUES ET ISOENZYMATIQUE

La leishmaniose due à Leishmania infantum *est une zoonose endémique présente dans le Bassin Méditerranéen, où les canidés (chien et renard) constituent les hôtes-réservoir principaux du parasite, tandis que les rongeurs sauvages et le chat peuvent être eux aussi porteurs du protozoaire, donc considérés comme réservoirs secondaires potentiels. Cet article décrit un cas de leishmaniose féline disséminée, avec une atteinte cutanée (ulcérative), viscérale (rate et ganglions lymphatiques) et sanguine chez un chat présentant une sérologie FIV-FelV positive. L'identification microscopique de l'infection leishmanienne a été initialement réalisée à partir d'une biopsie de peau de la région temporale, siège d'un carcinome épidermoïde. Le diagnostic de leishmaniose a été suspecté par la positivité des résultats obtenus par plusieurs analyses sérologiques (ELISA, IFAT et Western-blot) et confirmé par obtention du parasite en culture à partir du sang de l'animal. L'étude isoenzymatique de la souche a permis de caractériser* Leishmania infantum *zymodème MON-1. Compte tenu du caractère zoonosique de l'infection à* L. infantum*, la possible existence d'un réservoir félin pour le parasite doit être recherchée. Les enquêtes sérologiques chez le chat domestique constituent un outil intéressant. En pratique vétérinaire courante, la leishmaniose féline devrait être intégrée de façon systématique au diagnostic différentiel lors de lésions cutanées évocatrices, en particulier dans les zones endémiques de leismaniose canine.*

MOTS CLÉS : *chat,* Leishmania infantum, *leishmaniose, carcinome épidermoïde, France.*

INTRODUCTION

Leishmaniosis by *Leishmania infantum* is a zoonotic infection distributed in the Old World and in some American countries. In France, the disease has spread from the Mediterranean area where it is endemic. In this part of the world, the main reservoir host are canids, principally the domestic dog, with the fox constituting a wild reservoir (Bettini & Gradoni, 1986; Rioux & Golvan, 1969). Rodents (*Rattus rattus*) and the cat are more occasionally infected by *Leishmania infantum* (Morillas-Marquez *et al.*, 1985; del Giudice & Marty, 2003; Morsy *et al.*, 1980). These species should be considered as potential host reservoirs for the parasite. The disease can be of both visceral and cutaneous forms. Parasites multiply in macrophages and they can be easily seen by microscopical examination of cytological Giemsa stained smears in addition to histological examination of the skin and reticuloendothelial organs such as lymph nodes or the spleen.

MATERIAL AND METHODS

A 13-year old domestic neutered female cat, which had always lived in the Grasse city area, (Alpes-Maritimes, Southern France), was presented at

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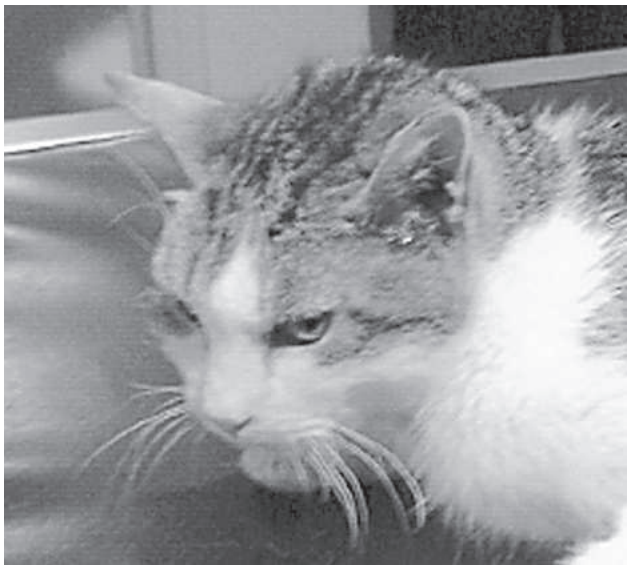

Fig. 1. – Ulcerative lesion on the left temporal area of a good body condition appearing cat.

a veterinary consultation with an ulcerative lesion in the left temporal region (Fig. 1). The owner reported that this cutaneous lesion had appeared two months ago, and at that time had looked like discrete crusts. The cat was sharing the living area with dogs. The clinical examination revealed an animal in general good condition. Considering the ulcerative aspect of the cutaneous lesion, the epidemiologic context and the duration of the process, several hypotheses were suggested; an auto-immune disorder (pemphigus), an infectious process (chronically itched wound with bacterial surinfection) or a neoplastic process (carcinoma). Since the final diagnosis required further investigations, two cutaneous biopsies from the edge of the ulcerative lesion were performed for histological examination. At the owner's discretion, the serological status of the cat towards the feline immunodeficiency virus (FIV) and feline leukemia virus (FelV) were investigated by diagnostic test (Duospeed®, Bio Véto Test, La Seyne-sur-mer, F-83500, France).

Several techniques were performed on serum samples to investigate the diagnosis of leishmaniosis, including the Immuno Fluorescence Antibody Test (IFAT, Resfiz), an Elisa Test (Bordier Affinity Products, Crissier, Switzerland) and a Western-blot test (home made). Culture of parasite was attempted from peripheral blood on NNN and Schneider media in order to characterize the strain of *Leishmania* by isoenzymes (Centre National de Référence des Leishmanioses, Montpellier, France).

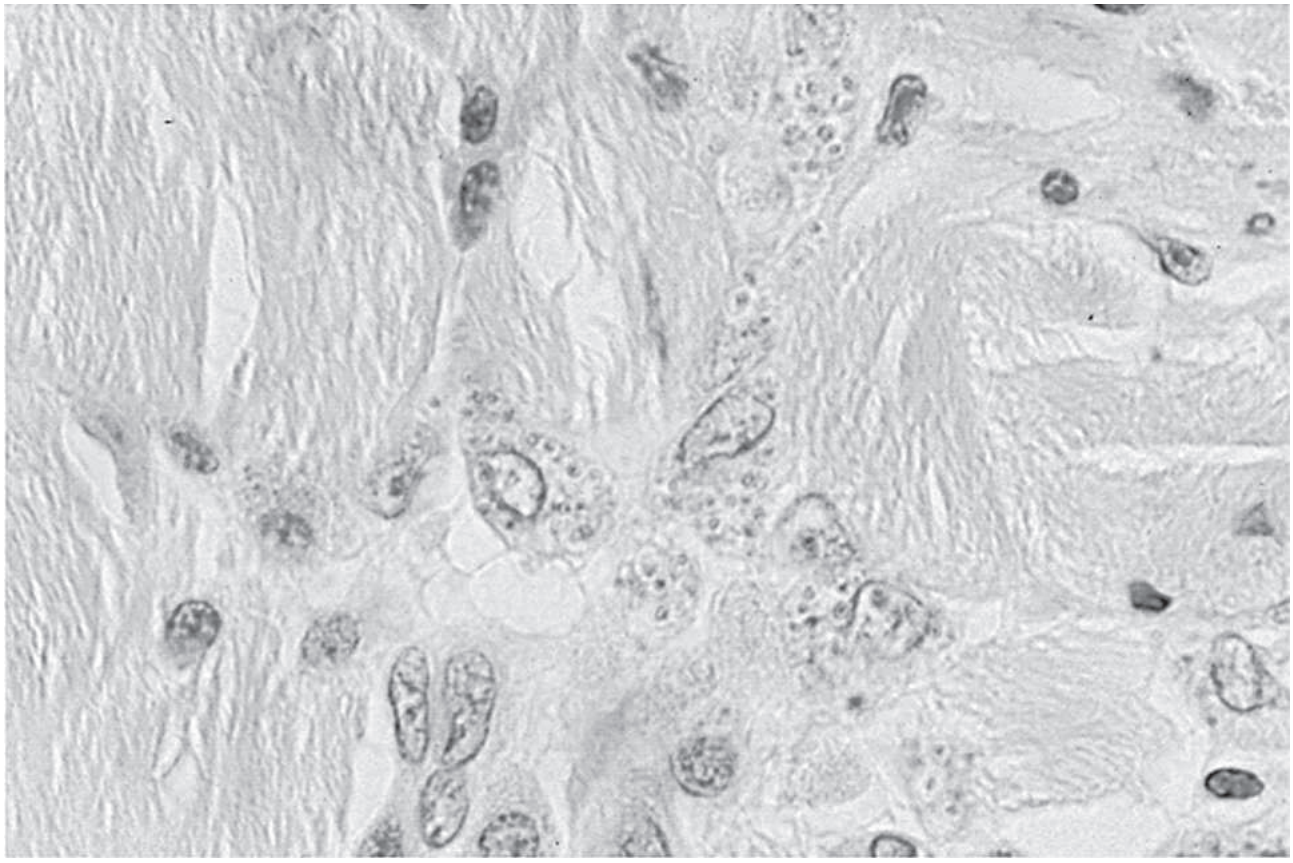

Fig. 2. – Macrophages in the deep dermis. In the clear cytoplasm, many small (2 µm) unicellular basophilic micro-organisms compatible with *Leishmania infantum* amastigotes are visible. Hematoxylin and Eosin. × 400 magnification.

RESULTS

Both serological tests for FeLV and FIV were positive. Considering the reliable value of these tests, it was concluded that the cat was very probably an asymptomatic carrier of both viruses.

At histology, the cutaneous lesion showed a tumor that had developed infiltrative cords of cells invading the dermis and subcutis. The proliferation of large and polyhedral cells was unencapsulated and seemed continuous with the overlying ulcerated epidermis. In a few foci, the neoplastic cells showed a trend to a squamous differentiation; they formed concentric laminations of keratin (keratin pearls) scattered in the tumor parenchyma. A transepidermal inflammatory infiltrate composed of neutrophils, mast cells, lymphocytes and macrophages was observed on both sections. Macrophages predominated in the superficial and deep dermis; they contained many small (2 µm) unicellular basophilic micro-organisms compatible with *Leishmania* amastigotes (Fig. 2). A diagnosis of squamous cell carcinoma associated with *Leishmania* infection was made. IFAT and ELISA tests were positive with 800 Resfiz Units and 10 Arbitrary Units (cut off 4 Arbitrary Units) respectively. The specific bands for patent leishmaniosis were obtained by western blot: bands of 14, 18, 21, 23 and 31 kDa (Marty *et al.*, 1994; Marty *et al.*, 1995). Blood culture was positive and the isoenzymatic characterization allowed the identification of *Leishmania infantum*, zymodeme MON-1.

Considering the poor prognosis of the squamous cell carcinoma, which was worsened by the serological findings, the owner requested euthanasia of the cat and a necropsy was performed.

Macroscopic examination showed only a moderately enlarged spleen. Histological findings showed inflammatory lesions of several organs. Firstly, a diffuse hypertrophy of the splenic white pulp was noted. Some of the macrophages contained numerous intracellular amastigotes with round basophilic nuclei and rod shaped kinetoplasm. A strong haematopoietic activity with many megakaryocytes was also observed in the splenic parenchyma. A subacute inflammatory infiltration of the lymph nodes was seen, with macrophages disseminated in the cortex. Some protozoan organisms could be observed in a clear cytoplasm. A discrete fibrosis of the liver was found associated with hyalinization around the centrolobular veins. A slight mononuclear infiltration and the presence in the cytoplasm of lipid vacuolated hepatocytes was noted. Small lipogranulomas were disseminated in the hepatic parenchyma. The portal zones showed a mild fibrosis with lymphoplasmacytic infiltration. Some haematopoietic cells were disseminated in the hepatic sinusoids. A discrete plurifocal mononuclear infiltration of the salivary gland parenchyma and a lymphocytic infiltration associated with plurifocal intestinal erosions of the villi were also found. In the small intestine, the lamina propria of the villi and the interglandular zones showed lymphoplasmacytic infiltration. Plurifocal clusters of lymphocytes were observed in the sub-mucosa. The microscopic examination of the kidneys revealed a generalized thickening of the glomerular basal membrane associated with interstitial lymphoplasmacytic focal infiltration (membranous glomerulonephritis).

The final diagnosis of this case identified an infection due to *Leishmania infantum* with cutaneous and visceral involvement, associated with a localized squamous cell carcinoma with no macroscopic metastasis and a seropositivity toward the two feline retroviral agents.

DISCUSSION

Leishmaniosis due to *leishmania infantum* is an arthropod-transmitted zoonotic disease, which is endemic in the Mediterranean area, where sporadic cases of human leishmaniosis also occur. In the case of feline infection, the vector is unknown, but as for the canine and human leishmaniosis in France, the sandflies *Phebotomus perniciosus* and/or *Phlebotomus ariasi* are probably involved (Shaw *et al.*, 2001). Some research programs have been developed to better define the importance of the feline reservoir, and the infectivity of cats harbouring *Leishmania*. Among them, PCR assays have provided evidence for the existence of an asymptomatic infection by *L. infantum* (Shaw *et al.*, 2003). In order to investigate the disease in France, a serologic survey was performed on asymptomatic cats living in the surroundings of Nice by Ozon *et al.* (1999); the serum samples were assayed for *L. infantum* by western Blot, and the issue was 12 % of positivity. The animal described in this paper remained its lifetime in Grasse, which is localized in this endemic area (50 km West from Nice).

Since feline leishmaniosis is rarely diagnosed by histopathologists, the suspected infection by *Leishmania* on tissue sample remains usually to be confirmed by another analysis, like serology. Therefore, when serological analysis is not available, immunohistochemical techniques may help in order to confirm the diagnosis. These methods are simple to perform, require no sophisticated equipment and allow unequivocal histopathological diagnosis of leishmaniosis (Ferrer *et al.*, 1998; Bourdoiseau *et al.*, 1997).

In this case the pathogenesis of the skin lesion remains unclear. Cutaneous ulcerative lesion can be considered of classical occurrence in squamous cell carcinoma (Goldschmidt & Hendrick, 2002). However, cutaneous

ulcerative lesions due to *Leishmania* is a frequent finding in canine leishmaniosis as well as in human leishmaniosis (Hepburn, 2003). Moreover, in the few cases of feline leishmaniosis that have been reported since the first case described by Sergent in Alger, involvement of the skin is often described as well (de Souza *et al.*, 2005; Craig *et al.*, 1986; Dunan *et al.*, 1989; Barnes *et al.*, 1993; Bonfante-Garrido *et al.*, 1991; Laruelle-Magalon & Toga, 1996; Pennisi, 2002; Schubach *et al.*, 2004; San Martin Mouriz Savani *et al.*, 2004). In this context, it could be questioned whether the ulceration might have been induced firstly by the carcinoma or the parasite or both. It can also be suggested that both the tumor growth and the presence of the parasite could have worked in a synergistic way. It could thus be questioned whether the carcinoma had improved the protozoan infection or the parasite had led to cutaneous modifications (change in local immune status for example) which would have induced the neoplastic transformation.

To the best of our knowledge, only three cases of feline cutaneous leishmaniosis associated with a visceral infection have been described in the literature (Poli *et al.*, 2002; Costa Durao *et al.*, 1994; Ozon *et al.*, 1998, Hervas *et al.*, 1999). The present case constitutes an original example because it includes a necropsy and an histological examination. In addition, it remains difficult to interpret due to the inflammatory lesions found in most of the observed organs. Indeed, some of them could be age-related (cat of 13 years), but we cannot exclude a general response of the organism toward the widespread presence of the protozoan in the cat organism (host immune response). For the most part, the non-specificity of the symptoms and the rareness of the infection make the diagnosis of feline leishmaniosis difficult because of the confusion with other diseases or with symptoms of aging (Hervas *et al.*, 1999; Hervas *et al.*, 2001).

So far, we are not able to assess the occurrence of an immunodeficiency that could have led to infection by *Leishmania infantum*. In considering the general good body condition of the animal, it is unlikely that the cat was immunodepressed. Both *Leishmania* infection and FelV and FIV could have been a recent occurrence. The serological positivity for immunosuppressive retroviral agents FIV/FelV has been previously described in a feline case of leishmaniosis in Italy (Poli *et al.*, 2002). In our case, the viruses may have impaired the cellular immune response allowing active multiplication of the parasite and the visceral dissemination. In this context, the host cellular immune response would have been weakened by the virus, allowing survival of the parasite in the macrophages by escaping the NK and lymphocytes cytotoxicity. This is not yet elucidated, but one can assume that it could involve the same pathogenic pattern as in the relationship between human leishmaniosis and HIV infection (Desjeux & Alvar, 2003). Additionally, the culture of the parasite has been succeeded from a blood sample even though *Leishmania* are commonly found in macrophages from tissues. Since reports in humans have shown that it is much more frequent to find the protozoan in the blood of HIV positive than of HIV negative patients, we could suggest that infection of the cat by FIV can thus be correlated to the presence of the parasite in the bloodstream (Alvar *et al.*, 1997; Deniau *et al.*, 2003). As a conclusion, it is worth noting that in the French mediterranean area *L. infantum* MON-1 is mainly found in canine and human infections. Though reports on cats infected by this strain are still very few, it can be suggested that cats may be asymptomatic carriers of *Leishmania infantum* MON-1 in this area, and therefore constitute a notable reservoir of the disease.

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