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

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 *Leishmania* were detected in the saliva of the cat (121 kDa and 273 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 hyperplasia of the splenic white pulp was noted. Some of the macrophages contained numerous intracellular amastigotes with round basophilic nuclei and rod shaped kinetoplast. 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 dissemi- nated 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 *Phebotomus 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, 2005). 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 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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**REFERENCES**


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