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Cytauxzoon sp. infection in the first endemic focus described in domestic cats in Europe

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ABSTRACT

Information about epidemiological and clinicopathological aspects of domestic cat infection by species of *Cytauxzoon* other than *Cytauxzoon felis* is limited and it has rarely been reported. Following the detection of clinical cytauxzoonosis in three cats from Trieste (Italy), an epidemiological study was carried out in colony ($n = 63$) and owned ($n = 52$) cats from the same city to investigate the presence of *Cytauxzoon* sp. infection and to assess clinicopathological findings and variables associated with this infection. *Cytauxzoon* sp. infection was detected by 18S rRNA gene PCR in 23% (27/118) and by blood smear examination in 15% (18/118) of domestic cats. The 18S rRNA gene sequences obtained were 99% identical to the *Cytauxzoon* sp. sequences deposited in GenBank® from Spanish, French and Mongolian wild and domestic cats. Erythroparasitemia was observed mainly in apparently healthy cats. *Cytauxzoon* sp. infection was statistically associated with the colony group and the outdoor life style. No statistical association was found between positivity by PCR and breed, gender, age, presence of ticks and/or fleas, clinical status, laboratory findings such as anemia, FIV and/or FeLV status and mortality rate. Persistence of the infection was monitored and documented in four clinical cases. We reported the first clinicopathological description of naturally occurring *Cytauxzoon* sp. infection in domestic cats living in Italy. The predominance of subclinical erythroparasitemia and the evidence of persistent infection support the hypothesis that the domestic cat might serve as a reservoir host for this infection.

1. Introduction

Cytauxzoonosis is a tick-transmitted protozoal disease caused by *Cytauxzoon felis* affecting wild and domestic felids (Meinkoth and Kocan, 2005). It was described in several south central, south eastern (Meinkoth and Kocan, 2005), and mid-Atlantic states of the USA (Birkenheuer et al., 2006). The presumed main reservoir host of this infection appears to be the wild felid bobcat (*Lynx rufus*) (Kocan et al., 1985). Ticks are considered the vector and transmission of *C. felis* by *Dermacentor variabilis* (Blouin et al., 1984) and *Amblyomma americanum* (Reichard et al., 2010) has been demonstrated experimentally. *C. felis* has

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an initial tissue phase where schizonts are present within macrophages lining blood vessels followed by an erythrocytic phase (Nietfeld and Pollock, 2002). Development of the schizogonous form is responsible for the severe and fatal disease (Nietfeld and Pollock, 2002).

In domestic cats, natural and experimental infections have led to a rapid course of illness and death, usually in fewer than five days (Greene et al., 2006). The most common clinicopathological findings are anemia, depression, anorexia, vomiting, icterus, splenomegaly, hepatomegaly and high fever (Birkenheuer et al., 2006). Hypothermia typically develops just prior to the death (Greene et al., 2006). Historically, it was thought that the disease was always fatal in domestic cats. However, survival after infection (Walker and Cowell, 1995) and persistent blood parasitemia without clinical illness (Brown et al., 2008, 2010) have been documented in a few cases. In contrast, wild felids rarely manifest clinical illness and generally develop a subclinical erythroparasitemia (Meinkoth and Kocan, 2005). Nevertheless, occasional cases of fatal cytauxzoonosis, with clinical signs and large schizont-filled macrophages within blood vessels, have been reported also in wild felids (Garner et al., 1996; Nietfeld and Pollock, 2002; Peixoto et al., 2007).

C. felis has been suspected in countries other than United States. Microscopic identification of piroplasms were reported in a captive Bengal tiger from Germany infected presumably from three bobcats that had been imported from the USA about a year earlier (Jakob and Wesemeier, 1996), and in domestic cats in Brazil (Mendes-de-Almeida et al., 2007).

Interestingly, in the last years, other species of *Cytauxzoon* infecting wild and domestic felids have sporadically been described. A new species of *Cytauxzoon* named *Cytauxzoon manul* was molecularly characterized from a Pallas' cat imported into Oklahoma from Mongolia (Ketz-Riley et al., 2003; Reichard et al., 2005). Moreover, molecular recognition of a *Cytauxzoon*-like parasite was documented in a domestic cat (Criado-Fornelio et al., 2004) and Iberian Lynx from Spain (Luaces et al., 2005; Millán et al., 2007, 2009). Recently, *Hepatozoon canis* and *Cytauxzoon* sp. co-infection has been described in a cat from France (Criado-Fornelio et al., 2009). Unfortunately, there is paucity of information about epidemiological and clinicopathological aspects of infection by species of *Cytauxzoon* other than *C. felis*. The present manuscript describes a series of clinical infections with *Cytauxzoon* sp. in cats from Trieste, northeastern Italy. A cross-sectional study was carried out to investigate the presence of *Cytauxzoon* sp. infection in a population of cats in Trieste and to assess clinicopathological findings and variables associated with this infection.

2. Materials and methods

2.1. Study area

The study was carried out in Trieste (45°38′N, 13°48′E) a seaport city (211 km^2) in northeastern Italy. It is situated towards the end of a narrow strip of land lying between the Adriatic Sea and Italy's border with Slovenia, which lies almost immediately south, east and north of the city (http://en.wikipedia.org/wiki/Trieste). The wild animals present in the Trieste's area include roe deer, foxes, wild boars, hedgehogs (Zucca et al., 2003), and Eurasian Lynx (*Lynx lynx*) (Molinari et al., 2006).

2.2. Cats

Diagnosis of *Cytauxzoon* sp. infection was initially made by blood smear examination, PCR and sequencing in three cats (Cats nos. 1, 2, 3). Subsequently, *Cytauxzoon* sp. infection was investigated in a convenience cat population ($n = 115$) divided into group 1 (colony cats, $n = 63$) and group 2 (owned cats, $n = 52$).

2.2.1. Clinical cases

Diagnosis of *Cytauxzoon* sp. infection by microscopic evaluation of blood smear, 18S rRNA PCR analysis (Carret et al., 1999) and sequencing was made between February and June 2008. Cat nos. 1 and 3 were from Trieste, while cat no. 2 lived in Udine (near Trieste) but was adopted from a Trieste's colony 1 year before the diagnosis. Signalment, clinical history, physical examination, serial laboratory test including complete blood count (CBC), biochemical profile, serum protein electrophoresis (SPE), hemostatic profile and urinalysis, medical treatment and follow-up including outcome (survival *versus* nonsurvival) were recorded and evaluated. Other diagnostic tests such as detection of feline leukaemia virus (FeLV) antigen and feline immunodeficiency virus (FIV) antibodies were also assessed. PCR analysis from blood samples for detection of *Mycoplasma haemofelis*, *Candidatus* Mycoplasma haemominutum, *Candidatus* Mycoplasma turicensis (Willi et al., 2005, 2006), *Hepatozoon* sp. (Inokuma et al., 2002), *Bartonella henselae* (Anderson et al., 1994), *Ehrlichia canis* and *Anaplasma phagocytophilum* (Solano-Gallego et al., 2006) were also performed as previously described. When tissue samples were available, PCR was performed from DNA extracted from paraffin-embedded tissues.

Cats ($n = 11$) cohabitating with cat no. 2 were screened for *Cytauxzoon* sp. by CBC, blood smear evaluation and PCR analysis. However, these cats were not included in group 2 (owned cats) because they lived in the town of Udine (near Trieste).

2.2.2. Group 1 (colony cats)

Sixty-three free-roaming apparently healthy cats anesthetized for spay or neuter in a feline population control effort between June 2008 and April 2009 were enrolled. The cats were born and have lived their entire life in cat colonies. A total of 15 cat colonies were studied and 12 colonies were located within Trieste and three colonies near the town (Fig. 1).

The colony from which cat no. 2 was adopted was also included in the study. Signalment, limited clinical history and physical examination were recorded. K_3EDTA blood samples were taken. Blood smears were performed for all the cats, while a CBC was available for only 55 cats. The *Piroplasmidae* 18S rRNA gene PCR analysis (Carret et al., 1999) was carried out on DNA extracted from all blood samples. The detection of FIV antibody and FeLV p27 antigen were done by commercial ELISA tests ($n = 48$) (ViraCHEK®/FIV