Cytauxzoon sp. Infection in Two Free Ranging Young Cats: Clinicopathological Findings, Therapy and Follow Up

Serbest Dolaşan İki Genç Kedide *Cytauxzoon* sp. Enfeksiyonu: Klinikopatolojik Bulgular, Tedavi ve Takip

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ABSTRACT

Two young brother male free-ranging domestic shorthair cats were evaluated for diarrhea. They presented with intraerythrocytic piroplasms on blood smear evaluation. Only the first cat was anemic (mild non-regenerative anemia). A partial segment of the 18S rRNA was amplified and sequenced, revealing a homology of 99% with *Cytauxzoon* sp. and of 93% with *Cytauxzoon felis*. The first cat was treated with doxycycline and imidocarb dipropionate and monitored by serial laboratory exams, resulting negative for *Cytauxzoon* sp. infection after the end of the therapy (follow-up period of 175 days). The second cat received the same therapy, but doxycycline was discontinued by the owner after 1 week. He was monitored for 130 days, remaining erythroparasitemic and asymptomatic. We described cases of *Cytauxzoon* sp. infection in domestic cats with detailed clinical data, description of two therapeutic protocols, and follow-up after treatment with opposite parasitological responses (parasitological cure *versus* persistence of infection). *(Turkiye Parazitol Derg 2014; 38: 185-9)*

Key Words: *Cytauxzoon* sp., cat, clinicopathological findings, therapy

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ÖZET

Serbest dolaşan evcil iki genç erkek kardeş kısa tüylü kedi diyare açısından değerlendirildi. Kan yayması değerlendirmesinde intraeritrositik piroplazmaları mevcuttu. Sadece ilk kedi anemikti (hafif, rejeneratif olmayan anemi).18S rRNA'nın kısmi bir segmenti amplifiye edildi ve sekanslandı, *Cytauxzoon* sp. ile %99 ve *Cytauxzoon felis* ile %93'lük bir homoloji gösterdi. Birinci kedi doksisiklin ve imidokarb dipropionat ile tedavi edildi ve tedavi bitiminden sonra seri laboratuvar muayeneleri ile izlendi, *Cytauxzoon* sp. enfeksiyonu için negatif sonuç verdi (175 günlük takip süresi). İkinci kedi aynı tedaviyi aldı ancak doksisiklin bir hafta sonra sahibi tarafından kesildi. Eritroparazitemik ve asemptomatik kalarak 130 gün boyunca izlendi. Biz ayrıntılı klinik veriler, iki tedavi protokolünün tanımlanması ve zıt parazitolojik cevapla (kalıcı enfeksiyona karşın parazitolojik kür) tedavi sonrası takip ile birlikte evcil kedilerde *Cytauxzoon* sp. enfeksiyonu vakalarını tanımladık. *(Turkiye Parazitol Derg 2014; 38: 185-9)*

Anahtar Sözcükler: *Cytauxzoon* sp., kedi, klinikopatolojik bulgular, tedavi

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INTRODUCTION

Cytauxzoonosis is a tick-transmitted protozoal disease affecting wild and domestic felids (1) caused by *Cytauxzoon felis* (*C. felis*) and reported mainly in the United States (1). A severe, often fatal disease was described in domestic felids (1), but cats surviving natural infection or infected and apparently healthy occur (2). To date, a specific treatment is not available.

Cytauxzoon sp. genetically similar to *C. felis* was identified in Pallas's cats from Mongolia, in a cat and in Iberian lynx in Spain, in a cat in France, and in a feline population from a focus in the northeastern of Italy (3-7). The majority of the *Cytauxzoon* sp. infected cats was apparently healthy, low-erythroparasitemic, and sporadically anemic (7). Some cases with clinical illness and fatal development were described (7). To the author's knowledge, there is limited clinical and epidemiological information about this infection in Europe.

The present case report describes clinicopathological findings, diagnosis, therapy, and follow-up in two free-ranging young cats naturally infected with *Cytauxzoon* sp. with opposite parasitological responses (parasitological cure *versus* persistence of infection).

CASE REPORT

Cat no. 1 was a 6-month-old, male, domestic shorthair that was referred to a veterinarian for diarrhea in October 2009. The cat was adopted from a colony living in Acquapendente (central Italy) (Figure 1). A massive infestation by ticks occurred in the area where he was born during the summer before his adoption. The cat had no history of vaccination and ectoparasiticide treatment. Only a corneal lesion was observed at the physical examination. No ticks or fleas were found. *Toxocara* sp. eggs were detected on fecal microscopic examination. Complete blood count (CBC), biochemical profile, and serum protein electrophoresis were assessed as previously described (7) at the San Marco Laboratory, Padua (Italy) within 24 h after the collection. CBC was performed by an automatic cell counter (ADVIA® 2120, Siemens Healthcare Diagnostics, Erlangen, Germany) in conjunction with blood smear evaluation. Serial laboratory findings of cat no.1 are summarized in Table 1. Mild non-regenerative anemia, mild leukocytosis, monocytosis, and thrombocytosis were present. Intraerythrocytic piroplasms suggestive of *Cytauxzoon* sp., *Theileria* sp., or small form *Babesia* sp. were noted on blood smear evaluation (Figure 2). The parasitemia was classified as low-grade (7).

Deoxyribonucleic acid (DNA) extraction was performed from Ethylendiaminetetracetic acid blood by the High Pure Polymerase Chain Reaction (PCR) Template Preparation Kit (Roche Applied Science, Mannheim, Germany) in accordance with the manufacturer's protocol with some modifications (7). A fragment of the 18S rRNA gene of *Piroplasmidae* species of approximately 412 base pair (bp) was amplified by conventional PCR (7). Then, positive PCR samples were directly sequenced. The sequencing was performed by an Applied Biosystem 3730xl DNA Analyzer (Applied Biosystem, Carlsbad, California) on both strands by BMR Genomics srl (Padua, Italy) by using the dideoxy chain-termination method (8). The consensus sequence was compared to the sequences deposited in GenBank® using the basin local alignment search tool (BLAST) (http://www.nbci.nlm.nih.gov/BLAST/). A nucleotide-nucleotide BLAST search (blastn) was performed using the default settings. The DNA sequences obtained were 99% identical to the *Cytauxzoon* sp. sequences

Figure 1. Geographical location of Acquapendente (42°44'41"N, 11°51'54"E, Viterbo) (blue spot), the little town located in the north part of the Lazio, on the border with Umbria and Tuscany (central Italy) where cats no. 1 and 2 were born and lived until the adoption (http://i.wikipedia.org/wiki/Acquapendente). Geographical location of Trieste (red spot), the town where an endemic focus of *Cytauxzoon* sp. infection was described.

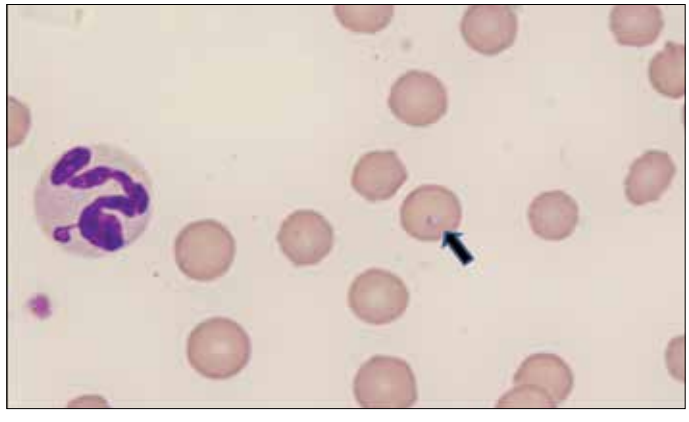

Figure 2. *Cytauxzoon* sp parasite inside red blood cell (arrow) in cat no. 1. in blood smear stained by the modified Wright technique (Aerospray slide stainer 7120 Delcon®, 1000x). *Cytauxzoon* sp appears as individual, small round to oval signet ring intraerythrocytic organisms of 0.5-0.8 µm of diameter with an eccentric basophilic nucleus and a lightly basophilic cytoplasm.

Table 1. Serial laboratory findings for cat no. 1 during the follow up period

	Day 1	Day 43	Day 57	Day 63	Day 87	Day 175
Laboratory Parameters (Reference interval)						
RBC (6.35-9.50 x 10^6/µL)	5.9	9.01	8.67	7.21	8.28	8.93
Hemoglobin (9.6-14.3 g/dL)	8.6	12.8	11.9	9.8	12	12.4
Hct (28-42.5%)	30.1	39.7	35.9	32.3	42.1	43.3
WBC (5-11 x 103/µL)	11.67	8.91	0.64	2.47	8.02	10.72
Segmented neutrophils (2500-7000/µL)	6864	4591	96	649	4452	4601
Monocytes (65-250/µL)	396	249	15	165	104	246
PLT (130-430 x 10^3/µL)	456	458	256	22	441	402
CPK (90-320 IU/L)	130			697		
ALP (19-70 IU/L)	109			23		
Total protein (6.3-7.8 g/dL)	6.5			6.5		
Albumin (3-4 g/dL)	3.1			2.5		
Globulin (3-4.5 g/dL)	3.4			4		
Total bilirubin (0.14-0.26 g/dL)	0.21			0.17		
BUN (32-64 mg/dL)	41			72		47
Creatinine (0.95-1.85 mg/dL)	0.91			1.09		1.35
Glucose (86-116 mg/dL)	99			91		
Iron (50-118 µg/dL)	38			209		
UIBC (130-225 µg/dL)	232			22		
SAA (0.1-0.5 µg/mL)	1.3			77.8		0.1
α-globulin (17.8-27.6%)	16.7			28.2		
β-globulin (6.4-9.4%)	15.2			14.7		
PCR results	POS	NEG	NEG	NEG	NEG	NEG
Presence of piroplasms in blood smear	POS (+)	NEG	NEG	NEG	NEG	NEG

RBC: red blood cells; Hct: hematocrit; WBC: white blood cells; PLT: platelets; CPK: creatinine phosphokinase; ALP: alkaline Phosphatase; BUN: blood urea nitrogen; UIBC: unsaturated iron binding capacity; SAA: serum amyloid a protein; PCR: polymerase chain reaction

present in GenBank®. The highest identity was obtained with *Cytauxzoon* sp. 18S rDNA partial sequences reported in Italian (7), Spanish (4, 5), French (6), and Mongolian (3) wild and domestic felids. In contrast, the sequences revealed an identity of 93% with *C. felis* deposited in GenBank®. The new *Cytauxzoon* sp. nucleotide sequence was deposited in the GenBank® database with accession number KF031139. PCR analysis from the blood sample for detection of *Mycoplasma haemofelis*, *Candidatus* Mycoplasma haemominutum, *Candidatus* Mycoplasma turicensis (9), *Bartonella* henselae (10) and *Leishmania infantum* (11) and to serological detection of Feline Immunodeficiency Virus antibody and Feline Leukemia Virus p27 antigen performed by commercial ELISA tests (ViraCHEK®/FIV and ViraCHEK®/FeLV, Synbiotics Corporation®, Exton, Pennsylvania) were negative.

Doxycycline therapy (Vibravet, Pfizer, New York, USA, 10 mg/kg/q12h PO for 3 weeks) was instituted when piroplasms were found on blood smear evaluation. Then, imidocarb dipropionate (Carbesia, Intervet Italia, Milan, Italy, 5 mg/kg IM for two times 2 weeks apart) was administered after *Cytauxzoon* sp. infection confirmation by PCR and sequencing (day 14). The cat improved and was monitored for 175 days by serial CBC, biochemical profile, and serum protein electrophoresis (Table 1). Two weeks after the last imidocarb dipropionate administration (day 43), *Cytauxzoon* sp. was not detected by CBC and PCR analysis. On the 57th day, the cat was weak, anorectic, and seriously leukopenic (Table 1). Parvovirus infection was revealed by PCR analysis (12). *Cytauxzoon* sp. blood smear evaluation and PCR analysis were negative. Metronidazole (Deflamon, SPA, Milan, Italy 10 mg/Kg/BID, EV), ranitidine (Ranidil, Menarini, Florence, Italy, 2 mg/kg/BID, EV), and fluids were administered. Clinical and laboratory conditions improved. *Cytauxzoon* sp. piroplasms were not detected by blood smear evaluation and PCR analysis during the follow-up period.

Cat no. 2 belonged to the same litter of cat no. 1. He was a 7-month-old, male, domestic shorthair evaluated for diarrhea on November 2009. Fecal microscopic examination was not per-

formed. CBC, blood smear evaluation, PCR analysis, and sequencing were done by the San Marco laboratory, and *Cytauxzoon* sp. infection was diagnosed. The sequence obtained was deposited in the GenBank database with accession number KF031140. Low parasitemia (7), leukocytosis with mature neutrophilia, monocytosis, and thrombocytosis were present. The instituted therapy with doxycycline (10 mg/kg/q24h PO) was discontinued by the owner after 1 week. Then, the cat was treated with imidocarb dipropionate (5 mg/kg IM for two times 2 weeks apart). On the 130th day, a mild increase of erythrocyte number, normal leukocytic concentration, and mild thrombocytosis were observed. Aspartate aminotransferase, alanine aminotransferase, blood urea nitrogen, and creatinine were within normal limits. Other laboratory parameters were not available. The cat was clinically well.

DISCUSSION

In the present work, we described two cats with natural *Cytauxzoon* sp. infection demonstrated by blood smear evaluation and PCR analysis. Sequence comparison of positive samples revealed high homology with isolates from Mongolia, Spain, France, and Italy (3-7). In the present manuscript, for the first time, infection by *Cytauxzoon* sp. was described in cats from central Italy. In fact, *Cytauxzoon* sp. infection was previously reported in domestic cats only in a focus in Trieste (northeastern Italy) (7).

The cats presented with diarrhea and low parasitemia. Cat no. 1 had mild non-regenerative anemia, and cat no. 2 was not anemic. Differentials for the mild non-regenerative anemia include young age and inflammatory disease-associated anemia (13). These data were in agreement with a previous report where the majority of *Cytauxzoon* sp.-infected cats was apparently healthy and not anemic, showing more often subclinical infection and, rarely, signs of illness (7). In fact, both cat no. 1 and cat no. 2 demonstrated low parasitemia, with diarrhea as the only clinical sign. In cat no. 1, diarrhea could be consequent to the *Toxocara* sp. infestation, as observed by fecal microscopic examination. Though a fecal examination was not performed, this infestation could also be the probable cause of diarrhea in cat no. 2, as it belonged to the same litter of cat no. 1. In addition, in the follow-up period, cat no. 2 remained persistently erythroparasitemic with no evidence of illness. In agreement with this data, persistent parasitemia has been previously reported only in three *Cytauxzoon* sp-infected sick cats that were monitored, respectively, for 25, 288, and 49 days after diagnosis, remaining positive on blood smear and PCR analysis (7). On the contrary, *C. felis* infection is characterized by nonspecific clinical signs of fever, lethargy, anorexia, dehydration, icterus, pallor of mucous membrane, dyspnea, and progressive anemia (1). In contrast, wild felids more often are persistently erythroparasitemic and healthy (1). Rarely, wild felids have acute and fatal disease (14). Consequently, infection by *Cytauxzoon* sp. in European domestic cats seems to be more similar to *C. felis* infection in wild felids than in domestic cats. Apparently healthy and persistently parasitemic cats may serve as a reservoir for *Cytauxzoon* sp. infection. Accordingly, descriptions of subclinical, persistently, and naturally *C. felis*-infected domestic cats sometimes are reported in the United States (2). It could be hypothesized that these cats could serve as additional hosts for this parasite (2). The role of *Cytauxzoon* sp.-infected domestic cats in the life cycle of the parasite remains unclear and has to be investigated further.

Cat no. 1 appeared to clear up the infection after a doxycycline and imidocarb dipropionate therapy, resulting in negative blood smear evaluation and PCR analysis for *Cytauxzoon* sp. detection during follow-up. In contrast, cat no. 2 remained erythroparasitemic, but he was treated only with imidocarb dipropionate, because doxycycline was discontinued by the owner. Moreover, two sick infected cats previously described in northeastern Italy were treated with antiprotozoan drugs without improvement (7). However, sick infected cats were not treated with a combination of doxycycline and imidocarb dipropionate. Various treatments for cytauxzoonosis by *C. felis* have been described, but to date, no antiprotozoal therapy has been demonstrated to modify the course of the disease in acutely infected cats (15). In naturally infected cats, diminazene aceturate administration led to survival in acute infection and was unable to eliminate or decrease the parasite in healthy chronic infection (16, 17). Imidocarb dipropionate and enrofloxacin were unsuccessfully used in asymptomatic persistently infected domestic cats (2). Interestingly, the association of imidocarb dipropionate and doxycycline seems to eliminate *Cytauxzoon* sp. erythroparasitemia in cat no. 1. We could not exclude spontaneous elimination of the parasite or very low parasitemia undetectable by PCR analysis after therapy. In fact, very low levels of parasite DNA may result in inconsistent PCR results (2). Further studies need to assess if treatment is needed in this infection and the type of adequate treatment protocol for parasite cure.

The two cats reported in the present study were 6 and 7 months old at the time of diagnosis and belonging to same litter. The way of transmission of *Cytauxzoon* sp. is unknown. Tick bite is the most likely way of transmission, as occurs in *C. felis* infection in cats (18). However, vertical transmission of this infection might be considered, based on the young age of both cats of the same litter. Interestingly, *Cytauxzoon* sp infection was previously reported mainly in young adult cats (7). Moreover, fatal cytauxzoonosis by *C. felis* was described in a free-ranging bobcat cub of approximately 2-3 months (14), and a seven years old *C. felis*-infected kitten was identified in a study involving Florida panthers and Texas cougars (19). In contrast, lack of evidence for perinatal transmission of *C. felis* in domestic cats was reported (20). Further studies need to investigate possible ways of transmission for this new emergent infection in cats.

CONCLUSION

In conclusion, infection by *Cytauxzoon* sp. is present in Italy in the northeastern part of the peninsula but also in the central part in domestic cats. One of the two young cats described appeared to clear up the infection after therapy, while the other presented with persistent erythroparasitemia, suggesting a role as a reservoir in domestic cats as an alternative host to wild felids in the life cycle of this parasite.

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