CASE REPORT

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Co-infection of *Cytauxzoon felis*, *Mycoplasma haemofelis*, and the feline immunodeficiency virus in a domestic cat in Uberlândia, Minas Gerais, Brazil

Co-infecção de Cytauxzoon felis, Mycoplasma haemofelis *e vírus da imunodeficiência felina em gato doméstico em Uberlândia, Minas Gerais, Brasil*

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ABSTRACT

Cytauxzoon felis is a hemoparasite capable of infecting domestic and wild cats. Studies suggest that wild cats are the main reservoirs of the protozoan, and transmission occurs through the bite of an infected tick. Hemotropic mycoplasmas are Gram-negative bacteria responsible for severe hemolytic anemia. The feline immunodeficiency virus (FIV) is a retrovirus capable of generating immunosuppression in the host and persistent infection. The present work describes a case of co-infection of *Cytauxzoon felis*, *Mycoplasma haemofelis,* and feline immunodeficiency virus (FIV) in a cat. A feline from the rural area was admitted to the Veterinary Hospital of Uberlândia - MG of the Federal University of Uberlândia. Fresh whole blood samples were collected for blood count, enzymatic analysis, DNA extraction, real-time PCR for the detection of *Cytauxzoon felis* and *Mycoplasma haemofelis*, and the Point of Care ELISA test for Feline Leukemia Virus (FeLV) antigens and antibodies to feline immunodeficiency virus (FIV). Piroplasms compatible with *Cytauxzoon* spp. were observed in the smear, and the tests performed were positive for *Cytauxzoon felis*, *Mycoplasma haemofelis*, and FIV.
Keywords: Cytauxzoonosis. Feline retroviruses. FIV. Hemoplasmosis. Mycoplasmosis.

RESUMO

Cytauxzoon felis é um hemoparasita que possui capacidade de infectar felídeos domésticos e selvagens. Estudos sugerem que os felídeos selvagens sejam os principais reservatórios do protozoário e a transmissão ocorre através da picada de um carrapato infectado. Os micoplasmas hemotrópicos são bactérias Gram negativas responsáveis por anemia hemolítica grave. O vírus da imunodeficiência felina (FIV) é um retrovírus com capacidade de gerar imunossupressão no hospedeiro e infecção persistente. O presente trabalho descreve um caso de co-infecção de *Cytauxzoon felis*, *Mycoplasma haemofelis* e vírus da imunodeficiência felina (FIV) em um gato. Um felino proveniente da zona rural foi internado no Hospital Veterinário da Universidade Federal de Uberlândia, em Uberlândia-MG. Foram coletadas amostras de sangue total fresco para a realização de hemograma, análise enzimática, extração de DNA, PCR em tempo real para detecção de *Cytauxzoon felis* e *Mycoplasma haemofelis* e o teste de ELISA *Point of Care* para Antígenos de vírus da leucemia felina (FeLV) e anticorpos para o vírus da imunodeficiência felina (FIV). Em esfregaço foram observados piroplasmas compatíveis com *Cytauxzoon* spp. e os resultados dos testes realizados foram positivos para *Cytauxzoon felis*, *Mycoplasma haemofelis* e FIV.
Palavras-chave: Cytauxzoonose. Retroviroses felinas. FIV. Hemoplasmose. Micoplasmose.

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Cytauxzoon felis is a hemoparasite that can infect domestic and wild felids. Wild felids are believed to be the main reservoirs of the protozoa (Furtado et al., 2017). However, *C. felis* infection in cats has been reported in some regions of the national territory, in the urban areas of large cities (André et al., 2017). The transmission of the agent in the form of merozoites occurs through the bite of an infected tick. The parasite form enters monocytes and macrophages of the host, where they make the first schizogonic cycle. The remaining schizogonic cycles occur in the red blood cells, disrupting the cell (Tarigo et al., 2013). The most common clinical signs are high-grade fever, dehydration, jaundice, anorexia, dyspnea, and pale mucous membranes (Clarke & Rissi, 2015). Diagnosis is through visualization of the merozoites inside the erythrocytes. However, the morphological similarity with other infectious agents confirms the diagnosis via molecular tests (André et al., 2017).

Hemotropic mycoplasmas are round to elongated erythrocytic gram-negative bacteria responsible for severe hemolytic anemia, and *M. haemofelis* is considered the most pathogenic of this group (Sykes, 2010). Although the transmission route is not fully understood, potential vectors are fleas and ticks, and infection through scratching has been reported (Díaz-Regañón et al., 2018; Woods et al., 2005). Cats with hemotropic mycoplasmosis may present with anemia, apathy, pyrexia, inappetence, and mucosal pallor (Tasker, 2010). Polymerase chain reaction (PCR) is currently considered the most sensitive detection method (Messick, 2004; Sykes, 2010).

Feline immunodeficiency virus (FIV) is a retrovirus that promotes persistent infection. The main transmission route is through bites or injuries from an infected feline. Infection can cause immunodeficiency in cats and, consequently, a propensity for secondary infections, among other complications (Beatty & Sykes, 2021). Rapid tests identifying anti-FIV antibodies diagnose the infection (Little et al., 2020).

Due to the epidemiological importance of recent reports of cytauxzoonosis, this paper describes a case of co-infection of *C. felis*, *M. haemofelis*, and FIV in a domestic cat.

On October 5, 2021, a 10-year-old female castrated feline, 2.600 kg, from the rural area of Uberlândia was referred to the intensive care unit (ICU) of the Veterinary Hospital of the Federal University of Uberlândia. According to the guardian's report, the animal showed no atypical behavior one week before the consultation. Four days prior to the consultation, apathy, ocular hyperemia, and dehydration were noted. On physical examination, the animal was prostrated, 8% dehydrated, anisocoric, and hyperemic with icteric mucous membranes; moreover, an increased volume in the skull region was pertinent. Vital signs showed normoglycemia (176 mg/dL), hypothermia (35.9 °C), and hypotension (86 mmHg).

The complete blood count showed normocytic and normochromic anemia, lymphopenia, and thrombocytopenia (Table 1). A fresh whole blood smear was performed and stained with the Romanowsky panoptic fast method, where piroplasmas were compatible with *Cytauxzoon* spp. (Figure 1) Moreover, eosinophilic structures in cocci, characteristic of hemotropic mycoplasmas inside the red blood cells, were observed. The animal presented hypoalbuminemia, uremia, and hyperbilirubinemia (Table 1). Medications described in Table 2 were prescribed. The animal was referred for overnight hospitalization; however, the guardian opted to care for the patient at home.

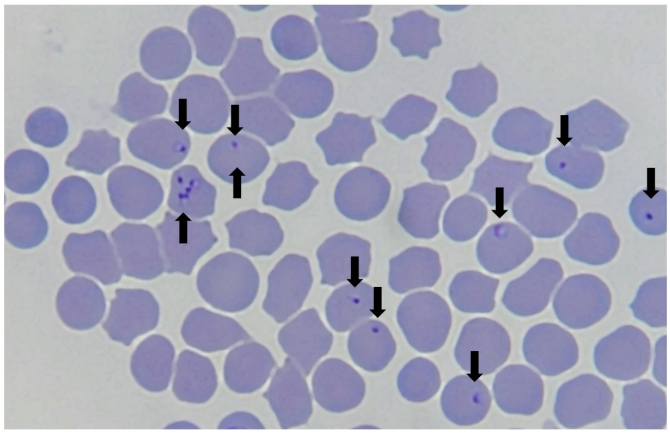

Figure 1 – Blood smear of domestic feline stained by the Romanowsky type method (Fast Panoptic), observing intraerythrocytic structures suggestive of piroplasmids compatible with *Cytauxzoon* spp. (black arrow), 100x magnification in a Nikon Eclipse E200 microscope, Uberlândia – MG.

Table 1 – Hematological and biochemical data of a feline patient from Uberlândia when the diagnosis of infection by *Cytauxzoon felis*, *Mycoplasma haemofelis*, and FIV was obtained

Biochemical and Hematological Parameters	Admission day			Reference values*	
	1st	2nd	4th	Minimum	Maximum
Total red blood cell count (x10⁶µL)	**4.08**	**4.27**	-	5.0	10.0
Hemoglobin (g/dL)	**6.4**	**6.7**	-	8	15
Hematocrit (%)	**17.9**	**19.8**	-	24	45
Mean Corpuscular Volume (fL)	43.9	46.4	-	39	55
Mean Corpuscular Hemoglobin (pg)	15.7	15.7	-	12.5	17.5
Mean Corpuscular Hemoglobin Concentration (g/dL)	35.8	33.8	-	30	36
Plasma Protein (g/dL)	8.4	8.0	-	6.0	8.0
White Blood Cells (mm³)	10,700	8,100		5500	19500
Band Neutrophils (mm³)	0	**405**	-	0	300
Segmented Neutrophils (mm³)	10,272	7,614	-	2500	12500
Eosinophils (mm³)	0	0	-	0	1500
Basophils (mm³)	0	0	-	Rare	Rare
Monocytes (mm³)	214	0	-	0	850
Lymphocytes (mm³)	**214**	**81**	-	1500	7000
Platelets (µL)	**36,000**	**22,000**	-	230,000	680,000
Albumin (g/dL)	**1.90**	-	**1.23**	2.1	3.3
Alanine aminotransferase (U/L)	72	-	40	6	83
Creatinine (mg/dL)	0.61	-	0.92	0.8	1.8
Alkaline Phosphatase (U/L)	69	-	37	25	93
Urea (mg/dL)	**209.6**	-	**122.6**	42.8	64.2
Lactate (mg/dL)	16	-	28	-	-
Total Protein (g/dL)	5.82	-	-	5.4	7.8
Total bilirubin (mg/dL)	**21.52**	-	**24.08**	0.15	0.50
Direct bilirubin (mg/dL)	**11.31**	-	**12.93**		0.20
Indirect bilirubin (mg/dL)	**10.21**	-	**11.15**	-	0.20
Calcium (mg/dL)	-	-	5.71	6.2	10.2
Phosphorus (mg/dL)	-	-	6.64	4.5	8.1

*Kaneko et al., 2008; Feldman et al., 2016.

Table 2 – Information on medication administration to the feline patient from Uberlândia - MG when the diagnosis with infection by *Cytauxzoon felis*, *Mycoplasma haemofelis*, and FIV was obtained

Admission Day	Medication administered	Administered dose (mg/Kg)	Route administered	Administration interval
1st	Omeprazole	10	VO	BID
	Doxycycline	80	VO	SID
	B Complex Vitamins	-	VO	SID
2nd	Omeprazole	10	VO	BID
	Doxycycline	50	VO	BID
	Dipyrone drops	20	VO	SID
	Ondansetron Hydrochloride	4	VO	TID
	Prednisolone	5	VO	BID
	SAME (S-Adenosyl-L-Methionine)	40	VO	SID
	Ursodeoxycholic Acid	30	VO	SID

Caption: VO (oral), SID (once a day), BID (twice a day), TID (three times a day).

On the second hospital day, the animal was returned to the ICU. The guardian reported that the animal did not urinate, defecate, or feed spontaneously during the night. The clinical parameters observed were as follows: icteric mucous membranes, normoglycemia (89 mg/dL), normothermia (38.4 °C), and hypotension (100 mmHg). The animal remained in the ICU and was administered medications described in Table 2.

A new blood count was requested. The results were similar to the first day of hospitalization (Table 1), including the visualization of piroplasmas compatible with *Cytauxzoon* spp. in the ear tip smear and fresh whole blood. The animal was advised for hospitalization for the night. However, the guardian opted for home care, administering the prescribed medications and providing the super-premium adult cat food every 4 h and not

exceeding 25 mL through a nasogastric tube, as prescribed by the manufacturer for a 2.5-kg animal.

On the third hospital day, 300 µL of whole blood sample was collected and submitted for DNA extraction using the commercial Wizard® Genomic DNA Purification Kit (Promega) according to the manufacturer's instructions. After that, the extracted DNA was submitted for real-time PCR for detection of *C. felis (REF1)* and *M. haemofelis*, *Candidatus M. haemominutum*, and *Candidatus M. turicensis* (REF2) using non-specific GoTaq® qPCR Master Mix (Promega) in a Rotor-Gene Q MDx 5 Plex HRM thermal cycler (Qiagen). Feline leukemia virus (FeLV) antigens and FIV antibodies were evaluated by point-of-care enzyme-linked immunosorbent assay test (SNAP® FIV FeLV Combo, IDEXX Laboratories). The test results showed positivity for *C. felis*, *M. haemofelis*, and FIV.

On the fourth hospital day, the animal was again brought with the following clinical parameters: icteric mucous membranes, normoglycemia (123 mg/dL), hypothermia (34.9 °C), and hypotension (120 mmHg). Only biochemical tests and blood transfusion were performed earlier in the day. The biochemical changes included hypoalbuminemia, uremia, and hyperbilirubinemia (Table 1), as per the values available in the reference (Kaneko et al., 2008).

However, due to the significant clinical laboratory alterations and the morbid condition of the animal, euthanasia was offered by the veterinarian, and the guardian authorized the procedure. A necropsy was performed, and the macroscopic alterations found were as follows: icteric skin and mucous membranes, pulmonary and subcutaneous edema, and moderate liver volume increase, with bulging edges and brownish. Multifocal yellowish dots were also observed in the hepatic parenchyma, which deepened on cutting, including a hepatic lobular pattern and renal corticomedullary congestion.

Among the hematological alterations presented by the animal during hospitalization, anemia, lymphopenia, and thrombocytopenia were prominent and congruent with the clinical manifestations Sykes (2010) described in cases of felines infected with hemotropic mycoplasmas. Maia et al. (2013) and Tarigo et al. (2013) reported the presence of lymphopenia, thrombocytopenia, and uremia in *Cytauxzoon* sp.-positive felines, which the animal of the current study manifested. The evolution of hematological alterations that generated an unfavorable prognosis for the animal could be caused by the concomitant infection by different microorganisms.

Among the hemotropic mycoplasmas, *M. haemofelis* is considered the most pathogenic, responsible for severe hemolytic anemia that may culminate in the death of the animal (Messick, 2004). In experimental infections in cats with *M. haemofelis,* Willi et al. (2006) found more severe anemia than in cats infected with other hemoplasma species. Some authors believe that the animal may be an asymptomatic carrier of the agent, and the clinical signs appear when other diseases immunosuppress it (André et al., 2017; Willi et al., 2006). In cats infected with *M. haemofelis*, Firmino et al. (2016) found hematocrit values below the reference value and mild normocytic and normochromic anemia. These data were similar to those presented in this report. The same authors did not find thrombocytopenia in the animals evaluated, contrary to the present study findings.

The hyperbilirubinemia presented by the animal on the first and fourth hospital days was also reported by Maia et al. (2013) study, which was attributed to the infiltration of macrophages with schizonts in the liver and the intense hemolysis caused by *C. felis*. This change can also be found in animals infected with *M. haemofelis,* according to Messick (2004). Contrary to the present findings, Raimundo et al. (2021) found secondary reactive thrombocytosis in an infected feline in Rio de Janeiro.

The presence of icteric mucous membranes and the multifocal yellowish spots in the liver that were reported during necropsy were in line with that reported by Aschenbroich et al. (2012). The same authors further stated that petechial hemorrhages and ecchymoses can be observed throughout the animal's body. However, these changes were not found in the patient. Snider et al. (2010) observed pulmonary edema in cats positive for *Cytauxzoon* sp., also found in the present report. They also noted interstitial pneumonia in cats with cytauxzoonosis, but it was impossible to perform histopathology in this case.

C. *felis* is an agent known and studied since the 1970s in North America and is associated with fever, apathy, lethargy, jaundice, and high lethality in domestic cats (Rizzi et al., 2015). The same authors report the occurrence of chronic carriers of the parasitosis, with 15 months or more of hemoparasitemia. Brazil has had sporadic reports of *Cytauxzoon* spp. since the early 2000s in domestic (Mendes-de-Almeida et al., 2007) and wild cats (André et al., 2009). André et al. (2022) found approximately 26.15% (n=102/390) positivity for *Cytauxzoon* spp. in the states of Minas Gerais and São Paulo in cats with no changes in clinical pathology examinations. Among these, 63.73% (n=65/102) were from the city of Uberlândia and its neighbor Araguari, which reinforces the agent's presence in this study region.

André et al. (2017) identified two felines positive for cytauxzoonosis in a study conducted in cats in Rio Grande do Norte, without hematological and biochemical changes. These studies support the hypothesis that domestic felines may behave as chronic and asymptomatic carriers of *Cytauxzoon* or that Brazilian isolates are less pathogenic to felines than North American isolates. Additionally, its pathogenicity may be potentiated when associated with other pathogens, generating risks to the animal's health and contributing to an unfavorable prognosis.

Moreira et al. (2018) reported the presence of sheep predation by puma (*Puma concolor*) on the same rural property where the cat of the present report lived, alerting to the possibility of contact of vectors (ticks and fleas) with domestic and wild felines in this location and transmission of infectious agents between species. Due to the absence of work proving how transmission occurs in *C. felis* among felines, some authors believe in the possibility that the jaguar is a reservoir of the protozoan (Furtado et al., 2017). André et al. (2011) found a positive sample, among 123 samples analyzed, for *M. haemofelis* in a wildcat (*Leopardus tigrinus*) without clinical signs of the disease from the region of Jundiaí (SP). The cat in the present report may have come in contact with the possible vectors of jaguars or any other wild felid in the area. This could be a source of *C. felis* transmission to the cat. More importantly, since the cat was positive for FIV, wildlife conservation is at risk.

The feline patient in this study presented with clinical signs and laboratory alterations compatible with cytauxzoonosis. However, the co-infection with *M. haemofelis* may have contributed to the hematological and biochemical alterations. The presence of FIV may have contributed to the immunocompromised status of the animal, and these two pathogens may have aggravated the infection. Thus, it is impossible to conclude whether *Cytauxzoon* infection would have a benign outcome without co-infection.

This case report demonstrated the presence of *C. felis* in Uberlândia, Minas Gerais, alerting professionals to the possibility of this infectious agent in animals co-infected with other frequent pathogens, such as FIV and *M. haemofelis*. Thus, we encourage the inclusion of cytauxzoonosis in the request for complementary screening tests in the care of cats, preventing the disease from being diagnosed late or completely undiagnosed, as this has an unfavorable prognosis.

Conflict of Interest

There is no conflict of interest.

Ethics Statement

Not applicable.

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