

Haemobartonellosis in Cats in Ankara, Turkey

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*Received December 30, 2003**Accepted October 26, 2004***Abstract**Kurtde A., K. Ural: *Haemobartonellosis in Cats in Ankara, Turkey*. Acta Vet. Brno 2004, 73: 507-512.

The paper describes four cases of feline haemobartonellosis (FH) in which anaemia and high fever had been the predominant presenting symptoms. In addition coughing was detected in two cats. FH was diagnosed on the basis of blood smear with Romanowski type stain. Blood samples were withdrawn before treatment (day 0) and after treatment (day 29). Mean WBC, RBC, Hb, PCV, MCV and MCHC values on day 0 were found as $16.2150 \cdot 10^9 \cdot L^{-1}$, $4.7425 \cdot 10^{12} \cdot L^{-1}$, $7.8500 \text{ g} \cdot dL^{-1}$, 26.2750% , 50.3575 fL and $32.5125 \text{ g} \cdot dl^{-1}$ respectively. Mean WBC, RBC, Hb and PCV values on day 29 were found as $9.2500 \cdot 10^9 \cdot L^{-1}$, $7.2925 \cdot 10^{12} \cdot L^{-1}$, $11.4000 \text{ g} \cdot dL^{-1}$ and 33.75% , respectively. The abnormalities found on routine haematological examination were a mild normocytic-normochromic regenerative anaemia (cat 1 and cat 2), normocytic-hypochromic anaemia (cat 3) and macrocytic-normochromic anaemia (cat 4), mild eosinophilia (13% in cat 1 and 11% in cat 4) and monocytosis (6% in cat 1 and 12.5% in cat 3). All of the present cats were treated with oxytetracycline hydrochloride at a dose of $25 \text{ mg} \cdot \text{kg}^{-1}$. Clinical recovery and disappearance of *H. felis* organisms was observed on day 29. The objective of this study was to evaluate the clinical cases of haemobartonellosis diagnosed in Ankara, with a view to establishing the clinical signs and response to treatment. To the present author's knowledge FH has previously not been reported in Ankara, in Turkey.

Feline haemobartonellosis, Turkey, clinical cases.

Haemobartonella felis, the causative agent of feline infectious anaemia, is a Gram negative epicellular parasite of feline erythrocytes (Grindem et al.1990; Jensen et al. 2001; AlIeman et al.1999). Recent studies with phylogenetic analysis of 16S rRNA gene sequences from *H. felis* isolates demonstrated that *Haemobartonella* and related organisms have been reclassified, in the genus *Mycoplasma*, as *Mollicutes* (Tasker and Lappin 2002; Neimark et al. 2001). The parasite attaches itself epicellularly to the erythrocyte membrane, causes haemolysis, and an episode of anaemia results from intermittent parasitaemia (Zulty and Kociba 1990; Shaw and Ihle 1997). The disease may be acute or chronic (Carney and England 1993). Only one case, to the authors' knowledge, has previously been reported in Turkey (Tuzer et al. 1993). This report, based on four cases, shows the naturally infected cats in Turkey with haemobartonellosis.

Materials and Methods**Criteria for selection of cases**

Medical records from November 2001 to July 2002 of cats with anaemia identified on clinical examination and haematologic abnormalities at the University of Ankara, Faculty of Veterinary were reviewed. Four (of 20) selected cats were included in this study on the basis of presence of *Haemobartonella felis* on blood smear with Romanowski type stain. Signalment, history, clinical examination findings and associated disorders, radiography (in 2 cats) and available ultrasonography findings were retrieved from records. Age, sex and householding conditions were recorded.

Owners were asked to be interviewed and to complete a questionnaire consisting of the risk factors leading to the clinical suspicion of FH, as follows; vaccination status, previous illness history, catbite abscesses and/or anaemia, flea infestation, age and sex.

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A blood sample was withdrawn from each cat into a tube containing potassium EDTA and also into a tube without anticoagulant, and blood smears were prepared at the same time using fresh blood from venipuncture. Samples were collected at least once a week and forwarded to the Department of Protozoology. Thin blood smears on glass slides were allowed to dry, and were methanol-fixed for 10 min. Then the quick Romanowski stain was used for the blood smears, which then were microscopically evaluated for the visualization of the *H. felis* organisms.

Cell count and erythrocyte indices were evaluated in fresh EDTA-anticoagulated blood by use of an electronic cell counter and packed cell volume, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were detected. Additionally differential white blood cell counting was done manually.

Results

Case histories-clinical examination

Information about the animals and data provided by their owners in a questionnaire are given in Table 1 and follow-up haematological results in pre-(day 0) and post-treatment (day 29) period is given in Table 2.

Table 1
Information on the animals and their owners (based on a questionnaire)

	Age	Gender	Vaccination status	Previous illness history	Catbite abscesses and/or anaemia	Flea infestation	Roaming
Cat 1	4.5	Male	F	-	-	+	Outdoor/Indoor
Cat 2	5	Male	U	F.L.U.T.D	+	+	Outdoor/Indoor
Cat 3	2	Male	F	-	-	+	Outdoor
Cat 4	5	Male	F	-	+	+	Outdoor

F: frequently (with normal feline vaccination protocol including FeLV vaccine, U: unvaccinated, F.L.U.T.D.: feline lower urinary tract disease

Table 2
Haematology data from cats with haemobartonellosis (time interval reflects days from pre-(0) and post-treatment (29))

	Cat 1		Cat 2		Cat 3		Cat 4		Mean	Std. error	Mean	Std. error
	Day 0	Day 29	Day 0	Day 29	Day 0	Day 29	Day 0	Day 29				
WBC ($\cdot 10^9 L^{-1}$)	21.70	9.0	9.0	10.4	18.46	8.2	15.7	9.4	16.2150	2.6995	9.2500	4.573
RBC ($\cdot 10^{12} L^{-1}$)	5.20	8.42	3.96	4.35	5.23	10.8	4.58	5.6	4.7425	3.008	7.2925	1.4462
Haemoglobin (g.dL ⁻¹)	10.70	14	5.8	7.6	7.1	16	7.8	8	7.8500	1.0364	11.4000	2.1197
PCV (%)	34.80	43	17.5	29	23.2	33	29.6	30	26.2750	3.7659	33.7500	3.1983
MCV (fL)	48.33	NT	44.1	NT	44.5	NT	64.5	NT	50.3575	4.8096	.0000	.0000
MCHC (g.dL ⁻¹)	30.75	NT	32.9	NT	30.4	NT	36	NT	32.5125	1.2872	.0000	.0000
Haemobartonella	+++	none	+++	none	+++	none	+++	none	++++		none	

WBC: white blood cell

RBC: red blood cell

PCV: packed cell volume

MCV: mean corpuscular volume

MCHC: mean corpuscular haemoglobin concentration

NT: not tested

Mean: Mean values

Std.Error: Standard error

Cat 1 had a 6-month history of coughing, worsening dyspnea, intermittent fever, depression and weight loss. Throughout the 6 month period the signs had appeared to wax and wane, and the cat was treated with various antibiotics by the referring veterinarian with no significant response. Two months later, the cat was referred to our

clinic for further investigation. On referral, at clinical examination, dyspnoea, respiratory distress, tachypnoea, 'wheezes' on the auscultation of the lung fields, off feed and mildly pale mucous membranes were diagnosed. Rectal temperature was 39.7 °C. Routine haematology revealed normocytic-normochromic regenerative anaemia (red blood cell count $7.20 \cdot 10^{12} \cdot L^{-1}$ [normal range $5.5-10.0 \cdot 10^{12} \cdot L^{-1}$], packed cell volume 34.80% [normal 24-45%], MCV 48.33 fL [normal 39-50 fL], MCHC 32.75 g·dL⁻¹ [normal 32-36 g·dL⁻¹], mild eosinophilia (13%) and monocytosis (6%). A significant *Haemobartonella felis* parasitaemia was detected on peripheral blood smear with Romanowski type stain.

Cat 2 was originally referred to our clinic with a 6-day history of cat bite wound and subcutaneous haemorrhagia in the pectoral area. According to the owner the cat was unvaccinated and often roaming outdoor and stayed outside for 2 days duration during the previous 6 days. Severe icteric and pale mucous membranes, lethargy, fever (40.2 °C) were obtained on clinical examination. Haematological and serum biochemical analysis demonstrated normocytic-normochromic anaemia (red blood cell count $3.96 \cdot 10^{12} \cdot L^{-1}$, packed cell volume 17.5%, MCV 44.1 fL, MCH 14.6 g·dL⁻¹, MCHC 32.9 g·dL⁻¹), increased serum alanine aminotransferase (ALT) (1053 IU·L⁻¹) and increased serum aspartate aminotransferase (AST) (220 IU·L⁻¹) with increased total bilirubin (1.42 mg·dL⁻¹), direct bilirubin (0.87 mg·dL⁻¹), and elevated fasting serum bile acid concentration (23.6 mg·dL⁻¹). *H. felis* parasitaemia was detected on Romanowski type stained blood smears. These findings were consistent with prehepatic icterus, caused by FH and acute hepatic necrosis.

Cat 3 had anorexia for 3 months. It was frequently vaccinated with a normal protocol, including FeLV vaccine, and roaming outdoor. At clinical examination pale mucous membranes and fever (40.6 °C) were obtained. Haematological analysis demonstrated normocytic-hypochromic regenerative anaemia (red blood cell count $5.23 \cdot 10^{12} \cdot L^{-1}$, packed cell volume 23.2%, MCV 44.5 fL, MCHC 30.4 g·dL⁻¹), mild leucocytosis ($18.46 \cdot 10^9 \cdot L^{-1}$) with marked monocytosis (12.5%). *H. felis* parasitaemia was detected on Romanowski type stained blood smears.

Cat 4 was originally presented to the referring veterinary surgeon with a history of an abscess on the neck, fever, regional lymphadenopathy and anorexia. The cat was frequently vaccinated against upper respiratory tract viruses but also roaming outside as other cats included in this study. According to the owner the abscess had occurred as a result of a cat bite 6 days prior to referral. The referring veterinary surgeon had resected and drained the abscess and treated the cat with clavulanic acid-potentiated amoxicillin (Synulox; Pfizer), (dose and duration were unknown). Due to deteriorating conditions and waxing anorexia, after 10 days of this treatment, the cat was referred to our clinic for further investigation. On referral, the cat was in bad body condition, with marked dyspnoea and severe pale mucous membranes. The rectal temperature was 41.2 °C. Blood analysis revealed macrocytic-normochromic anaemia (red blood cell count $4.58 \cdot 10^{12} \cdot L^{-1}$ [normal range $5.5-10.0 \cdot 10^{12} \cdot L^{-1}$], packed cell volume 29.6% [normal 24-45%], MCV 64.5 fL [normal 39-50 fL], MCH 23.2 pg, MCHC 36g·dL⁻¹ [normal 32-36 g·dL⁻¹], mild eosinophilia (11%). *H. felis* parasitaemia was detected on Romanowski type stain blood smears.

Treatment

All cats with FH included in the present study were treated with oxytetracycline hydrochloride (Geosol; Vetas) as an initial therapy at 25 mg·kg⁻¹ intramuscularly three times daily for 7 days and subcutaneously for the next 14 days. Treatment procedure was observed by repeated clinical and haematological examinations in pre- and post-treatment period (Table 2). Oxytetracycline treatment was discontinued at this time, except cat 4.

Cat 2, additionally, had fluid therapy with 0.45% saline and 2.5% dextrose i.v. for 4 days, also metronidazole and lactulose, as an enema.

Discussion

Haemobartonella felis is the most common cause of haemolytic anaemia in cats, with a peak incidence between 4-6 years of age and male cats are affected more often (Shaw and Ihle 1997; Carney and England 1993). The 4 cats included in the present study were also male and ≥ 4 years of age, except cat 3 (2-year-old).

Haemobartonellosis comprises a variety of clinical symptoms, including severe weakness, depression, anorexia, weight loss, fever and pale mucous membranes and anaemia (Grindem et al. 1990; Jensen et al. 2001; Shaw and Ihle 1997). The four cases exhibited all of these previously described historical findings and, in addition coughing was the primary presenting sign noticed by the owners for two of the cats (Cat 1 and Cat 3) but to the authors' knowledge this has not been reported previously for cats. Intermittent fever was also present in all cases and in that reported by Jensen et al. (2001). Hill and Odesnik (2000) reported a case of a cat with haemobartonellosis, presented with behavioural disturbances. However, the four cases reported here showed no behavioural abnormalities.

Cat 1 had a 6-month history of coughing, with no significant response to treatment with various antibiotics. In order to attempt to identify the primary clinical cause for coughing, additional laboratory tests and imaging studies were performed. Bronchial patterns, and elevation of the trachea over the cranial aspect of heart were seen on the thoracic radiography. There was no evidence of tracheal or upper respiratory tract infection and tests for heartworm disease were negative with an unremarkable faecal examination. To the present authors' knowledge there is some concern that whether these changes, although non-specific, are means of primary disease condition or secondary to haemobartonellosis. However previous unresponsive antibiotic treatment and the unremarkable differential diagnosis for coughing of the cat 1 were attributable to the link between coughing and the haemobartonella infection. Therefore coughing was also evident in cat 3.

The abnormalities found on routine haematological examination were a mild normocytic-normochromic regenerative anaemia (cat 1 and cat 2), normocytic-hypochromic anaemia (cat 3) and macrocytic-normochromic anaemia (cat 4), mild eosinophilia (13 % in cat 1 and 11% in cat 4) and monocytosis (6% in cat 1 and 12.5% in cat 3). In haemobartonella cases, the magnitude of the regenerative response expected to correspond to the severity of the anaemia with a macrocytic, normochromic response (Carney and England 1993), but if chronic inflammatory conditions exist in conjunction with haemobartonellosis, the expected response is normocytic, normochromic (Bobade and Nash 1987). The anaemia becomes macrocytic-hypochromic with pre-existing FeLV infection or toxoplasmosis (Bobade and Nash 1987; Hoskins and Barta 1984). In accordance with the normocytic-normochromic response described by Bobade and Nash (1987), the present two cases (cat 1 and 2) red blood cell indices were consistent with underlying chronic inflammatory conditions. Previous authors describe neutrophilia during acute infection (Bobade et al. 1988; Flint et al. 1958), however, monocytosis was present in cat 1 and cat 3 of the present study and in that reported by Carney and England (1993). The haematological variables in the present cases were unstable as reported by Bobade et al. (1988) and Harvey and Gaskin (1978).

Hill and Odesnik (2000) reported a case of a cat with mild azotemia however, serum biochemistry profile was unremarkable, except for cat 4, in the present 4 cases and in that reported by Carney and England (1993).

Previous studies identified potential risk factors such as age, breed, sex, stress, pregnancy, intercurrent infection, anaemia, neoplasia, vaccination, fight wound abscesses, flea infestation, increased roaming and FeLV infection status (Grindem et al. 1990; Foley et al. 1998; Shaw and Ihle 1997; Carney and England 1993). In the present study, all of the cats had risk factors such as roaming outside and illness history as described previously.

H. felis and FeLV viraemia have been reported concurrently in two previous studies (Grindem et al. 1990). FeLV viraemia was unlikely to have been involved in all of the present cats, (except Cat 2), as the cats were routinely vaccinated against FeLV.

Definitive diagnosis of FH is made by direct visualization of the organisms on Wright-Giemsa or acridine orange (Small and Ristic 1971) and mostly on Romanowski (Carney and England 1993; Grindem et al. 1990) stained red blood cells (RBC), although diagnosis by cytological examination of the blood smears has been told to be problematic because of the sporadic visuality of the organism (Tasker and Lappin 2002; Harvey and Gaskin 1977; Flint and Moss 1953). Antibodies can be assayed by indirect fluorescent antibody testing or by ELISA, however, neither procedure is commonly used. Recent molecular studies have been subjected to polymerase chain reaction assays for diagnosis (Tasker and Lappin 2002; Jensen et al. 2001). In the present study, diagnosis is made by direct visualization of the organisms on the surface of the erythrocytes as described previously by Foley et al. (1998) and Carney and England (1993).

It has been reported that, oxytetracycline given at 20 mg·kg⁻¹ twice daily with supportive therapy failed to respond (Stevenson 1997). Doxycycline (McGrotty and Knottenbelt 2002) and enrofloxacin, which has anti-*H. felis* effects, (Dowers et al. 2002) has been told to be effective in *H. felis* infections. In the present study we used oxytetracycline at 25 mg·kg⁻¹ q 8 hours for 21 days, as recommended previously by Carney and England (1993). The authors' experience in the present study was the rapid response to the treatment in 3 of the cats (75%). This may be due to the fact that these cats (cat1, cat 3 and cat 4) were only mildly affected initially and the early diagnosis.

Previous authors described clinical state associated with disappearance and reappearance of *H. felis* organisms on RBC (Splitter et al. 1956; Harvey and Gaskin 1977). Dowers et al. (2002) reported complete clearance of the *H. felis* organism in two cats receiving enrofloxacin treatment. In the present study, the disappearance of the organism with oxytetracycline therapy was observed (Table 2), after clinical recovery for all of the cats.

The 2 of cats (Cat 1 and Cat 3) documented in this study are unusual because of their initial complaint of coughing and response to treatment with oxytetracycline. Primary care clinicians must take care of cats with presenting sign of anaemia and coughing, because haemobartonellosis may be a primary disorder. However, investigation of more cases is needed to improve the prognosis and treatment of this disease in the cats in Turkey.

Felinní hemobartonelóza u koček v Ankaře, Turecko

V práci jsou popsány 4 případy felinní hemobartonelózy (FH) s hlavními symptomy anémie a vysokou teplotou. Kromě toho bylo u 2 koček zaznamenáno kašláni. FH byla diagnostikována z krevního nátěru obarveného dle Romanovského. Vzorky krve byly odebrány před léčbou (den 0) a po léčbě (den 29). V den 0 byly zjištěny tyto průměrné hodnoty bílého a červeného krevního obrazu, Hb, hematokritu, středního objemu erytrocytů a střední korpuskulární koncentrace hemoglobinu: 16,2150·10⁹·L⁻¹, 4,7425·10¹²·L⁻¹, 7,8500 g·dL⁻¹, 26,2750%, 50,3575 fL and 32,5125 g·dl⁻¹ a 29. den byly zjištěny průměrné hodnoty výše uvedených ukazatelů, 2500·10⁹·L⁻¹, 7,2925·10¹²·L⁻¹, 11,4000 g·dL⁻¹ a 33,7500%. Abnormality zjištěné při rutinním hematologickém vyšetření byly: mírná normocytární-normochromatická regenerativní anémie (kočka 1 a 2), normocytární-hypochromatická anémie (kočka 3) a makrocytární-normochromatická anémie (kočka 4), mírná eosinofilie (13% u kočky 1, 11% u kočky 4) a monocytóza (6% u kočky 1, 12,5% u kočky 3). Všechny prezentované kočky byly léčeny dávkou 25 mg·kg⁻¹ oxytetracyklin hydrochloridu. Klinické uzdravení a nepřítomnost *H. felis* bylo zaznamenáno 29. den. Cílem

studie bylo vyhodnotit klinické případy haemobartonellózy diagnostikované v Ankaře, s přehledem klinických projevů a odpovědí na léčbu. Autorům není známo, že by FH byla v Ankaře zaregistrována.

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