Granulocytic *Ehrlichia*-like bodies in a cat with chronic oral disease: case report

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**SUMMARY**

A cat affected by chronic gingivitis/periodontitis and dermatophytosis unresponsive to previous treatments was found to harbour *Ehrlichia*-like inclusions bodies in circulating neutrophils and was diagnosed with Feline Granulocytic *Ehrlichiosis* (FGE). Neutrophilic leukocytosis and hypergammaglobulinemia were the most relevant laboratory findings. The cat was treated with doxycycline at anti-rickettsial dosage for a period of 42 days, leading to clinical cure of both gingivitis and dermatophytosis and to a sharply decreased number of granulocytic inclusions.


**RÉSUMÉ**

Présence de corps d’inclusion ressemblant à *Ehrlichia* dans le cytoplasme des granulocytes neutrophiles d’un chat atteint d’une maladie buccale chronique. Par W. TARELLO.

Un chat atteint d’une gingivite/périodontite et d’une dermatophytie chroniques, qui n’avaient pas répondu aux traitements précédents, a été trouvé porteur de corps d’inclusion semblables à *Ehrlichia* spp. dans le cytoplasme des neutrophiles. Un diagnostic d’Ehrlichiose Granulocytaire Féline (EGF) a été porté. Une hypergammaglobulinémie et une leucocytose neutrophile étaient les anomalies de laboratoire les plus importantes. Un traitement à la doxycycline pendant 42 jours a eu comme résultat la guérison de la gingivite et de la dermatophytie et la nette diminution des inclusions dans les neutrophiles.


**Introduction**

The genus *Ehrlichia* contains obligate intracellular bacteria belonging to the family *Rickettsiaceae* characterized by their unique tropism for circulating leukocytes and platelets [26]. Several *Ehrlichia* spp. can be transmitted to a variety of hosts in nature, including dogs, horses and human beings, usually with ticks as vectors [6]. Recent reports indicate that ehrlichiosis in cats is recognized more and more frequently worldwide [3, 23]. Since the first naturally occurring case of feline ehrlichiosis was documented in France in 1986 [9], *Ehrlichia* morulae (i.e., intracytoplasmatic inclusions formed...
by clusters of rickettsiae) infecting mostly the mononuclear cells [1-3, 7, 9] and rarely the neutrophils [4, 7], have been observed cytologically in 23 feline cases in several countries, in association with varied clinical syndromes [1-4, 23]. Morulae are difficult to find, so a presumptive diagnosis of ehrlichiosis in other clinically ill cats was based on the combination of serum *Ehrlichia* (*E. canis, E. risticii*) antibody detection, an appropriate clinical picture, exclusion of other causes and response to anti-rickettsial drugs [20, 22]. Anorexia, fever, lethargy, weight loss, hyperesthesia, joint pain, pale mucous membranes, splenomegaly, dyspnea and lymphadenomegaly were the most common historical and physical examination abnormalities [3, 23]. Concurrent diseases are rarely reported and include *Haemobartonella felis*, FIV, FeLV and lymphosarcoma [3]. Anemia is a common laboratory abnormality and is usually non-regenerative [23]. Neutrophilia, lymphocytosis, monocytosis, and intermittent thrombocytopenia were reported in some cats [2, 3, 23]. Hyperglobulinemia is also documented with a high frequency [3, 23].

An epidemiological link has been made between the presence of *Ehrlichia* spp. antibodies in serum and monoclonal gammapathy [23]. If the 23 identified morulae cases are combined with 20 cases in USA [20, 22] and 9 in France [3] with a presumptive diagnosis based on serology and response to therapy, 52 cases of clinical ehrlichiosis are reported in cats worldwide. Recently, the first clinical description and characterisation of a granulocytic *Ehrlichia* species in a cat was reported in Sweden [4]. The diagnosis was confirmed by serology, polymerase chain reaction and DNA sequencing. The granulocytotropic *Ehrlichia phagocytophila* strain found in the cat appeared to be 100% identical to DNA sequences found previously in Swedish canine, equine and human granulocytotropic *Ehrlichia* strains [4]. The reader should be aware that after the present article was written, nomenclature of *Ehrlichia* spp. has been reorganized: the current name for *E. phagocytophila* is *Anaplasma phagocytophila* and *Ehrlichia equi* and the agent of Human Granulocytic Ehrlichiosis are considered subjective synonyms of *Anaplasma phagocytophila* [12]. Even though many cats are seropositive for *Ehrlichia* antibodies [5, 18, 22], minimal cross-reactivity exists between antibodies against *Ehrlichia* [26] and other infectious agents, such as *Rickettsia rickettsii* [23]. Consequently, a definitive diagnosis of feline ehrlichiosis is most accurately made by demonstrating intracytoplasmatic inclusion bodies typical of *Ehrlichia* within blood leucocytes or by detection through polymerase chain reaction. It was thus worthy to report a retrospective analysis of a feline case in which granulocytotropic *Ehrlichia*-like bodies, hyperglobulinemia and leukocytosis with neutrophilia were associated with chronic gingivitis-periodontitis responsive to long-term doxycycline treatment.

### Clinical report

A 2-year-old male European outdoor cat, living in a rural area near Castiglione del Lago (Perugia, Central Italy) was presented for a consultation in December 1997, with a history of anorexia, pain and difficulty in mastication. Physical examination revealed lethargy, poor hair coat condition, swallowing difficulties, lumbar pain, dermatophytosis on the head (Fig. 1), enlarged and painful head lymph nodes, reluctance to eat hard food and severe gingivitis/periodontitis (Fig. 2) along with halitosis. The oral disease was qualified at stages 3 to 4 (out of 5) following the WIGGS and LOBPRISE classification [27]. The cat had relapsed after previous unsuccessful treatments with several drugs, including topical and systemic anti-mycotic medications, corticosteroids, non-steroidal anti-inflammatory drugs and amoxicillin + clavulanic acid. No tick/flea exposure was reported by the owner, although the cat was allowed access to indoor and outdoor environments.

FIV and FeLV tests proved negative and all haematological and biochemical results were within normal ranges, with the exclusion of a marked leucocytosis with neutrophilia (white blood cells count = 26.700/mm³; neutrophils = 81 %) and elevated total protein value (TP = 8.78 g/dl). Protein electrophoresis results were normal (albumin = 3.22 g/dl, cd-globulin = 0.24 g/dl, α2-globulin = 0.33 g/dl, β-globulin = 0.85 g/dl) with the only exception of total γ-globulin level (4.13 g/dl) above normal ranges in cats [1.5-3.5 g/dl] [8].

Two smears were prepared from a freshly drawn sample of blood, stained with the Wright technique and analysed microscopically at x 100, leading to the observation of coccus-shaped *Ehrlichia*-like bodies, single or in groups, in the cytoplasm of some neutrophiles (Fig. 3). In 53 neutrophiles out of 1,000 randomly examined, these rickettsia-like inclusions were present, i.e. 5.3 % of neutrophiles appeared affected.

These intracytoplasmatic neutrophilic *Ehrlichia*-like inclusions were similar in shape, size and colour, to the microorganisms previously seen in the neutrophils of dogs from the same area diagnosed with canine granulocytic ehrlichiosis (CGE) in association with *Babesia* spp. and *Dirofilaria repens* infections [24]. Microscopic search for *Haemobartonella felis* and micrococci CFS-associated [25] proved negative and the only additional blood anomaly observed was the presence of a remarkable number of «baskett cells» (Fig. 4), surrounded by disseminated *Ehrlichia*-like bodies.

Treatment with doxycycline, 10 mg/Kg/day was performed for 21 days. On the 10th day of treatment, the dermatophytosis on the head was completely healed (Fig. 5) without using any specific topic or systemic aid, and the owner admitted that the gingivitis/periodontitis was regressing and that mastication was easier. The cat was showing increased appetite and reduced fatigue.
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**FIG. 1.** — Dermatophytosis on the head of the cat.

**FIG. 2.** — Gingivitis/periodontitis stage 3.5 following the WIGSS & LOBPRIS classification (1997), before treatment with doxycycline.

**FIG. 3.** — Granulocytic *Ehrlichia*-like inclusion body in a neutrophil of the diseased cat (x100, Wright stain).

**FIG. 4.** — «Basket-cells» and disseminated *Ehrlichia*-like bodies were easy to observe in the blood smear of the reported case (x100, Wright stain).

**FIG. 5.** — Complete healing of the dermatophytosis on the head after 10 days of doxycycline treatment at anti-rickettsial dosage.

**FIG. 6.** — Marked clinical improvement of the gingivitis/periodontitis (stage 1 of the WIGGS & LOBPRIS classification, 1997) observed 10 days after the end of therapy.
One month after the end of therapy a clinical and haematological check was performed, showing a profound improvement in the oral health status, testifed by the absence of halitosis and pain when gnawing, along with a marked reduction of inflammatory signs around the teeth.

Blood films revealed the presence of Ehrlichia-like bodies in 2.2 % of the neutrophils (out of 1,000 counted neutrophils) and consequently a second 21-day course of doxycycline was programmed.

Ten days after the end of the second course the cat was revisited: the mandibular lymph nodes were significantly decreased in size, appetite and liveliness were excellent and the cat was feeding again on hard food during the last three days, with absence of pain and dysphagia. Gingivitis was reduced to a 2 mi. in width inflammatory line close to the neck of teeth (Fig. 6), and therefore classified as stage 1 of WIGGS and LOBPRISE scale [27] with following clinical improvement.

A second haematological check showed that Ehrlichia-like bodies were reduced but still present in 1 % of neutrophils. Given the slow but on going progress of the health status, the cat was discharged with a prescription for a third doxycycline course with the purpose of eradicating the remaining bacteria. No subsequent blood check was performed. A phone call control made in December 2001, confirmed that the animal had presented no relapses during the last 4 years. The owner reported that no further treatment had been given.

## Discussion

A cat showing Ehrlichia-like bodies in the cytoplasm of 5.3 % of neutrophils and affected by chronic gingivitis and dermatrophytosis found complete clinical remission after three courses of doxycycline at anti-rickettsial dosage. In two controls made after the first and the second course, the number of neutrophils affected was significantly reduced (5.3 % > 2.2 % > 1 %) and the cat was apparently healthy, with no signs of relapses. The response to doxycycline and the significantly reduced number of inclusion bodies observed after 42 days of oral therapy, seemed to confirm the diagnosis of feline granulocytic ehrlichiosis (FGE). The therapeutic results here reported are not in contrast with recent observations that granulocytic ehrlichiosis is difficult to eradicate in both dogs [6, 13] and cats [4] although members of the Ehrlichia phagocytophila group appear to always respond in vitro to doxycycline at anti-rickettsial dosages [14]. The Ehrlichia phagocytophila group cause disease manifestations in cats associated with morulae in the granulocytes [4], E. equi, E. phagocytophila, and the agent of human granulocytic ehrlichiosis (HGE) previously included in the group [26] are today considered synonyms of Anaplasma phagocytophila due to the lack of sufficient differences between them [12]. Human Granulocytic Ehrlichiosis (HGE) is an emerging disease in Europe [19, 21], showing possible zoonotic implications [4].

In the Tuscan region of Italy, which is close to the working area of this study, 5.8 % of 69 dogs tested positive for the agent of human granulocytic ehrlichiosis (HGE) by indirect fluorescent-antibody assay (IFA) and western blotting (WB) [17] and Ixodes ricinus ticks were found to harbour E. phagocytophila in Central Italy [11]. Cases of canine granulocytic ehrlichiosis (CGE) diagnosed cytologically, have already been reported by this author in the same working area [24]. The Ehrlichia-like neutrophilic inclusions visualized in blood smears obtained from local dogs were identical to those of the feline reported case (Fig. 3 and 4). Although the route of transmission of Ehrlichia infection in cats is unknown, it has been suggested that these could be a reservoir for erlichial agents infecting other animals, including humans [23]. It seemed thus important to report a feline case in which granulocytic Ehrlichia-like inclusions bodies were associated with chronic oral disease and dermatrophytosis unresponsive to prior treatments, and which subsequently responded to long-term doxycycline treatment at anti-rickettsial dosage. In this case, features in common with previous feline ehrlichiosis described in literature [23] were the following : 1) presence of Ehrlichia-like inclusion bodies in leucocytes, 2) neutrophilic leucocytosis [4], 3) hyperglobulinemia, 4) response to antirickettsial drugs [5, 22].

Gingivitis has only once been reported in a case of feline ehrlichiosis associated with morulae in lymphocytes observed in France [1] which also tested FIV and FeLV positive and was subsequently submitted to euthanasia. Inflammation of tongue and palate was recently described in two cats showing morulae in lymphocytes [3], but unfortunately one case did not receive treatment and remained stationary, whilst the other was submitted to euthanasia. Differently from other feline ehrlichiosis recently described [3], no concurrent etiologic agent (FIV, FeLV, Haemobartonella felis, Hepatozoon) [1-3, 23] to which could be attributed the general malaise and gingivitis observed in this cat was detected. As in this one, hyperglobulinemia was the most consistent feature of feline ehrlichiosis in all cases observed in the past [3, 23]. Unfortunately, in the case here reported post-therapy controls were limited to the observation of clinical improvement and progressively decreased number of granulocytes affected by Ehrlichia-like bodies.

Nonetheless, these results appear to be compatible with the suggested criteria for the diagnosis of feline ehrlichiosis [23] and with recent observation that percentages of neutrophils with inclusions in diseased dogs varied between 3 and 34 % [13].

It is acknowledged that definitive diagnosis of feline ehrlichiosis is made by demonstration of morulae in the cytoplasm of blood leukocytes or by PCR and that the condition is a differential diagnosis for varied clinical syndromes [23], due to no specific symptoms and lack of fully known pathogenicity.

The case described here shares features in common with most of the chronic oral diseases cases in cats, including: 1) FIV and FeLV negative test results, 2) hyperglobulinemia, 3) response to doxycycline therapy [10].

Oral diseases are the most common abnormality in cats of all ages [15]. Differential diagnostic in feline gingivitis/stomatitis includes primary and secondary immune deficiencies, diabetes, kidney failure, hypothyroidism, senescence, stress, corticosteroid abuse, hyper-sensitivity, auto-immunity, humid food, hyper-vitamin A and hypo-protein diets [10, 15].

The importance of immune suppression in chronic gingivitis/periodontitis/stomatitis is stressed by many authors [15, 29], although a high percentage of cats with chronic oral diseases are FIV and FeLV negative [10]. Nonetheless, this is not in contrast with the case herewith, in which a chronic oral disease is FIV and FeLV negative [10].

Present and previous observations of gingivitis/stomatitis associated with feline ehrlichiosis [1, 3] seem to suggest that the condition may be added to the differential diagnosis of acquired feline oral cavity diseases [15] and that, inversely, the symptom gingivitis should be listed along with those recognised as Ehrlichia-associated in cats.

**References**


