

Probable ehrlichiosis in cheetahs (*Acinonyx jubatus*)

W. TARELLO*, N. RICCIERI

¹ Dacre House Veterinary Clinic, 91, Powder Mill Lane, Southborough, Tunbridge Wells, Kent, TN4 9ED. UNITED KINGDOM.

*Corresponding author: E-mail: wtarello@yahoo.it

SUMMARY

A group of seven cheetahs (*Acinonyx jubatus*) exhibited *Ehrlichia*-like inclusion bodies in the circulating lymphocytes in association with compatible clinical signs responsive to enrofloxacin and imidocarb dipropionate therapy. This study suggests that ehrlichiosis may affect wild felines.

Keywords: Ehrlichiosis, lymphocyte, cheetah, *Acinonyx jubatus*, enrofloxacin, imidocarb dipropionate.

RÉSUMÉ

Probable ehrlichiose chez des guépards (*Acinonyx jubatus*)

Un groupe de sept guépards (*Acinonyx jubatus*) montrait des corps d'inclusions de type *Ehrlichia* dans leurs lymphocytes, en association avec des symptômes évocateurs, et une réponse thérapeutique positive à l'enrofloxacin et au dipropionate d'imidocarb. Cette étude suggère que l'ehrlichiose peut affecter les félins sauvages.

Mots-clés : Ehrlichiose, lymphocyte, guépard, *Acinonyx jubatus*, enrofloxacin, dipropionate d'imidocarb.

Introduction

Intra-monocytic inclusion bodies possibly due to an *Ehrlichia* species were first described in 1986 in a French cat showing non specific signs of disease [10]. Since then, many more cases have been recognized worldwide [21]. Bacteria of the genus *Ehrlichia* are obligate intracellular organisms, belonging to the family Rickettsiaceae [4]. They multiply in the cytoplasm of mononuclear cells or neutrophils as 'initial' or 'elementary' inclusion bodies that over time gradually aggregate within a vacuolar membrane containing up to 40 elements to form mature structures looking like berries, named morulae [4, 11]. Definitive diagnosis is made by cytological demonstration of inclusion bodies in leukocytes [17, 18, 21] or by PCR [6, 13], in association with clinical signs consistent with *Ehrlichia* infection [4, 18] and response to anti-rickettsial drugs administered in the appropriate doses [11, 13, 16, 17]. Treatment based on doxycycline or enrofloxacin is the recommended therapy [4], but imidocarb dipropionate has been reported to be curative as well [15, 18].

Feline mononuclear ehrlichiosis (FME) [2, 7, 16] is far more frequently reported than feline granulocytic ehrlichiosis (FGE) [9, 6, 20] and the two conditions appear to be caused by different, although closely related, *Ehrlichia* species [21].

Bacteria affecting feline mononuclear cells are morphologically [4], serologically [15, 18] and molecularly [3, 8]

undistinguishable from *Ehrlichia canis*, the agent of canine monocytic ehrlichiosis [11]. No feline other than the domestic cat is currently recognized as host species for FME.

Clinic cases

Twelve young adult cheetahs (*Acinonyx jubatus*) imported from Tanzania in 2003 lived in a large fenced area with acacias trees and palm groves providing appropriate shadow and shield, in a desert resort near Dubai (UAE). All animals were regularly vaccinated against the most common infectious diseases (feline herpesvirus, calicivirus, parvovirus, leukaemia and rabies viruses) and daily fed with fresh meat and water. However, during the last six months of year 2005, five cheetahs progressively deteriorated, decreased their food intake, lost weight and died, despite prompt administration of antibiotic therapy (amoxicillin) and supportive medication (vitamins, corticosteroids). Common clinical signs were poor appetite, lethargy, fever, wasting, vomiting, diarrhoea, polydipsia and dehydration. Haematology and biochemistry results were unremarkable apart from a uniform 1-3 fold increase of Creatinine and BUN above the normal average level. Necropsies did not show any particular abnormality and kidney failure due to renal insufficiency was the released common diagnosis.

In December 2005, 7 surviving cheetahs, 5 females and 2 males, 4 to 6 years old, were presented with a 7-day history

of clinical and biochemical picture overlapping that previously noticed in animals succumbed to the fatal syndrome. Two cheetahs appeared so weak that they could be captured without anaesthesia (Fig. 1) whereas the remaining 5 animals were darted with a blowpipe using half of the recommended dose of tiletamine/zolazepam (Zoletil®, Virbac) in wild carnivores. Blood samples were drawn from each and every cheetah (Fig. 2) for biochemical and haematological analysis and thin blood smears for the search of haematozoa were prepared on site. Microscopic examination of Wright-stained smears showed the presence of *Ehrlichia*-like inclusion bodies in lymphocytes.

Five hundred lymphocytes were examined in each slide and the presence of single (Fig. 3) and multiple *Ehrlichia*-like inclusions (Fig. 4), assumed to be initial or elementary bodies, was detected in the cytoplasm of 0.4-2% of lymphocytes. Diagnosis of monocytic ehrlichiosis was based on the same criteria used for cats [21]. Infestation with unspecified tick species was occasionally recorded in some cheetahs. Due to the critical conditions of the animals, initial therapy

was preferentially injected (subcutaneously), based on imidocarb dipropionate (Imizol®, Schering-Plough Animal Health) administered at the same doses used in dogs (i.e. 7.13 mg/kg, thus 1 ml/17 kg) and enrofloxacin (Baytril®, Bayer), given at the anti-rickettial dose of 10 mg/kg. Such medicaments are reported to be efficacious against canine [11] and feline monocytic ehrlichiosis [4, 15, 18] as well.

Diseased animals were isolated in roofed pens and submitted to maintenance therapy with an oral solution of enrofloxacin administered at doses of 10 mg/kg/day for 21 days in the drinking water. Treatment was successful and the clinical signs gradually receded during the following days. None of the animals died and a complete clinical remission was evident 3 weeks later.

Microscopic re-examination of blood smears performed 6 months later showed the disappearance of ehrlichial inclusions in 3 cheetahs and a sharply decreased (0.1%) number of mononuclear inclusions in 4 cheetahs. Eighteen months of follow-up confirmed lasting clinical recovery, with no further sign of disease or death.

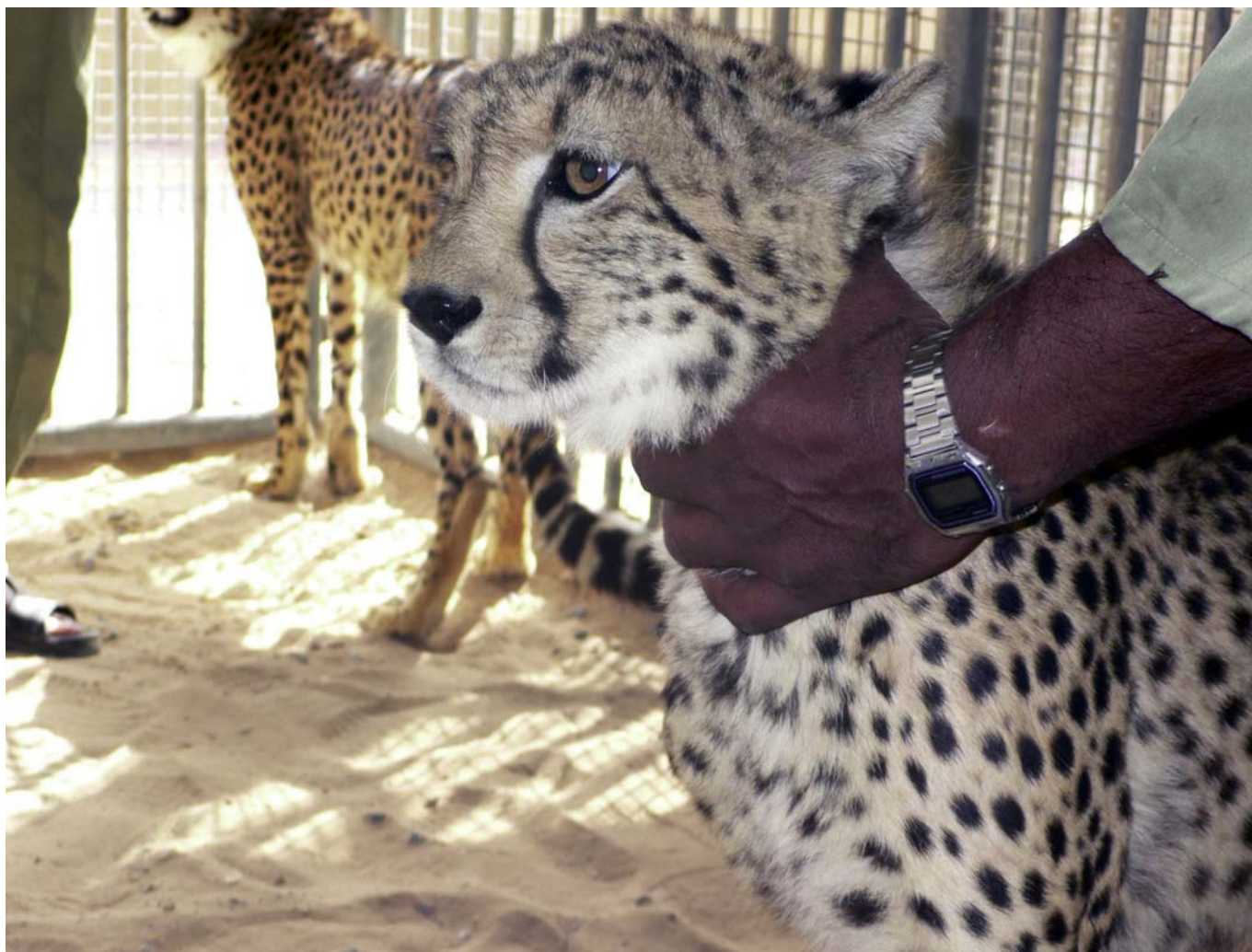


FIGURE 1: Two very weak cheetahs with ehrlichiosis are captured manually.

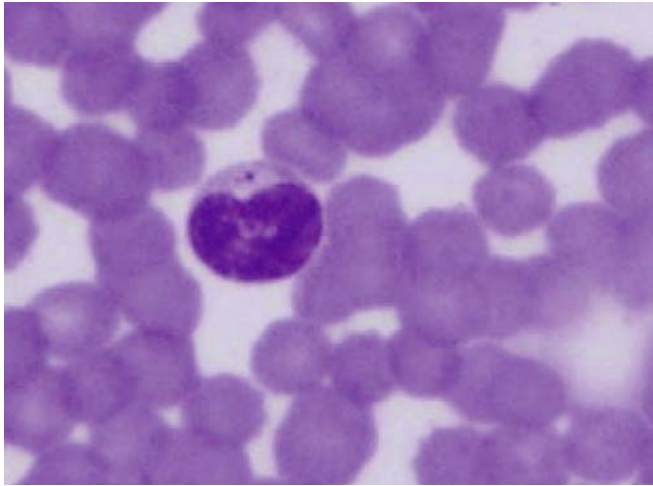


FIGURE 3: *Ehrlichia*-like inclusion body in a lymphocyte from a reported cheetah (x100, Wright stain, microscope Leica DMLS).

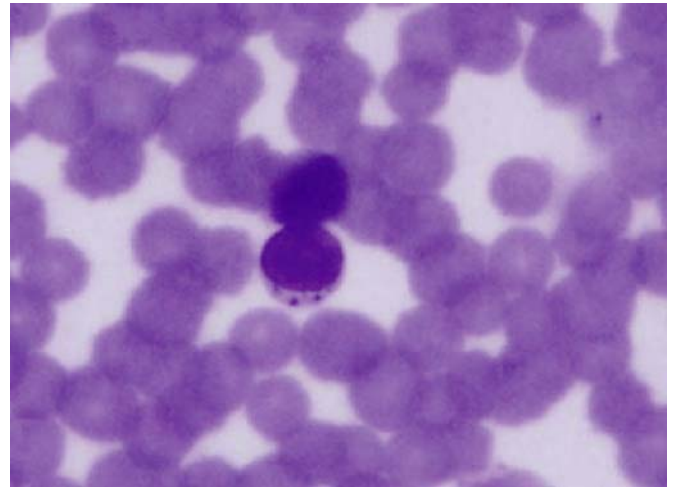


FIGURE 4: Elementary bodies in a lymphocyte from a cheetah diagnosed with ehrlichiosis (x100, Wright stain).



FIGURE 2: Blood samples are drawn from each diseased cheetah.

Discussion

A presumptive diagnosis of monocytic ehrlichiosis in cheetahs, based on evocative clinical signs, detection of unusual lymphocyte inclusions and successful response to specific treatments [4], was apparently confirmed by the long-term symptom-free remission time, the lack of new post-therapy fatal cases and the disappearance or sharp diminution of mononuclear inclusions in blood smears checked 6 months later.

When ehrlichiosis is not associated with a concurrent disease, then prognosis and outcomes of therapy with specific drugs are good, thus confirming the diagnosis [4]. In fact, a positive response to anti-rickettsial treatment, either with doxycycline, enrofloxacin or imidocarb dipropionate, is consistent with the cytological diagnosis of ehrlichiosis [4, 11] and compatible with the suggested criteria for its diagnosis [18, 21].

Most of previous feline reports were linked to the observation of inclusion bodies in mononuclear cells [2-4, 7, 16, 18]. Mononuclear intra-cytoplasmic inclusion bodies (Fig. 3-4) microscopically detectable in cheetahs diagnosed with ehrlichiosis in Dubai were identical to those previously photographed in lymphocytes from cats diagnosed with ehrlichiosis in France [2, 4].

Recognized signs of feline monocytic ehrlichiosis are anorexia (92%), lethargy (85%), wasting (46%), fever (42%), gastrointestinal disorders (vomiting, diarrhoea) (38%), pain (31%), respiratory symptoms (polypnea, wheezing, sneezing) (31%), polydipsia (23%) and de-hydration (15%) [4].

The overlapping clinical and microscopic picture, as well as the satisfactory response to a reference therapy (imidocarb dipropionate and enrofloxacin) seems to indicate a probable monocytic ehrlichiosis in the reported cheetahs. The use of imidocarb dipropionate was initially justified by the reported efficacy of this drug against ehrlichiosis [11, 18]. It became subsequently evident that such medicament was essential in order to eliminate a possible cryptic concurrent babesiosis.

The route of infection in felines is not known [18] but most *Ehrlichia* species are transmitted by ticks [21]. For instance, monocytic ehrlichiosis in dogs is caused by the bite of infective *Rhipicephalus sanguineus* ticks, a species widely distributed in Africa and the Middle East, responsible for the transmission of babesiosis and hepatozoonosis, as well [11].

Surprisingly, 100% of free-ranging cheetahs examined in Tanzania, were found infected with *Hepatozoon* sp. and a *Theileria* sp.-like piroplasm that was morphologically indistinguishable from *Theileria felis* [1].

Whether the infection in cheetahs was acquired locally or imported from the African country is not known. Previous tick infestation was reported in some animals. Living constantly outdoor enhances the risk of tick exposure and of acquiring ehrlichiosis [6]. In cats the disease can develop chronically over the years [21]. Therefore, the chance of an imported condition it is not completely excluded.

Recently, antibodies to *Ehrlichia canis* or a closely related agent were detected in a wild feline, a free-ranging puma (*Puma concolor*), for the first time [14].

In clinically ill cats a presumptive diagnosis of ehrlichiosis has been based on the detection of antibodies against *Ehrlichia canis* or *Ehrlichia risticii*, evocative clinical picture, exclusion of other causes and response to doxycycline at anti-rickettsial dosages [15, 18]. However, positive serologic test results occur in healthy cats as well as in clinically ill cats [18]. Serology testing can result in cross-reaction with other *Ehrlichia* spp. in both healthy and diseased animals [18, 23]. Consequently a diagnosis of feline ehrlichiosis should not be based on serologic test results alone [18]. It seems that false *Ehrlichia* sero-positivity is common in cats.

In fact, 20 (4.3%) out of 460 feline blood samples from sites throughout the USA resulted positive for antibodies to *Anaplasma phagocytophilum*, agent of the granulocytic ehrlichiosis (FGE), using an indirect immunofluorescence assay (IFA). However, these results could not be confirmed by PCR and PCR analysis for other cross-reacting *Ehrlichia/Anaplasma* spp. was also negative [5]. On the other hand, studies also indicated that some seronegative animals can be PCR positive [12, 19].

The diagnostic technique used in this study provides a presumptive diagnosis of ehrlichiosis. Normal mammalian lymphocytes do not show inclusions in their cytoplasm [10]. Occasionally, intracellular granules have been detected in animal lymphocytes and defined as 'toxic granulations' associated with underlying uncharacterized systemic illness [22]. This definition is compatible with the microscopic findings of ehrlichiosis. The evidences presented seem to indicate that ehrlichiosis actually can affect cheetahs.

Acknowledgements

The authors gratefully thank Mr. Mohammad Gul, general supervisor of the zoologic collection located in the Nahklee Resort (Dubai), belonging to Sheik Mohammed bin Rashid al Maktoom, for his continuous help, support and technical advise.

References

1. - AVERBECK G. A., BJORK K.E., PACKER C., HERBST L.: Prevalence of haematozoans in lions (*Panthera leo*) and cheetah (*Acinonyx jubatus*) in Serengeti National Park and Ngorongoro Crater, Tanzania. *Journal of Wildlife Diseases*, 1990, **26**, 392-394.
2. - BEAUFILS J-P., MARTIN-GRANEL J., JUMELLE P., BARBAULT-JUMELLE M.: Ehrlichiose probable chez le chat: étude rétrospective sur 21 cas. *Pratique Médicale et Chirurgicale des Animaux de Compagnie*, 1999, **34**, 587-596.
3. - BEAUFILS J.P., BREITSCHWERDT E.B., HANCOCK S.I., HEGARTY B.C., MARTIN-GRANEL J., JUMELLE P., BARBAULT-JUMELLE M., BLAVIER A.: Ehrlichiose feline: identification génétique de l'agent chez deux chats. *Pratique Médicale et Chirurgicale des Animaux de Compagnie*, 2002, **37**, 235-238.
4. - BEAUFILS J.P.: Feline ehrlichiosis. In: F. Beugnet (ed.): Guide to major vector-borne diseases of pets. Merial S.a.s., Lyon, 2002, 129-134.
5. - BILLETER S.A., SPENCER J.A., GRIFFIN B., DYKSTRA C.C., BLAGBURN B.L.: Prevalence of *Anaplasma phagocytophilum* in domestic felines in the United States. *Veterinary Parasitology*, 2007, **147**, 194-198.

6. - BJOERDORFF A., SVENDENIUS L., OWENS J.H., MASSUNG R.F.: Feline granulocytic ehrlichiosis – a report of a new clinical entity and characterization of the infectious agent. *Journal of Small Animal Practice*, 1999, **40**, 20-24.
7. - BOULOY R.P., LAPPIN M.R., HOLLAND C.H., THRALL M.A., BAKER D., O'NEIL S.: Clinical ehrlichiosis in a cat. *Journal of the American Veterinary Medical Association*, 1994, **204**, 1475-1478.
8. - BREITSCHWERDT E.B., ABRAMS-OGG A.C., LAPPIN M.R., BIENZLE D., HANCOCK S.I., COWAN S.M., CLOOTEN J.K., HEGARTY B.C., HAWKINS E.C.: Molecular evidence supporting *Ehrlichia canis*-like infection in cats. *Journal of Veterinary Internal Medicine*, 2002, **16**, 642-9.
9. - BUORO I.B.J., ATWELL R.B., KIPTON J.C., IHIGA M.A.: Feline anaemia associated with *Ehrlichia*-like bodies in three domestic short-haired cats. *Veterinary Record*, 1989, **125**, 434-436.
10. - CHARPENTIER F., GROULADE P.: Un cas d'ehrlichiose probable chez le chat. *Bulletin de l'Académie Vétérinaire de France*, 1986, **159**, 287-290.
11. - DAVOUST B.: Canine monocytic ehrlichiosis due to *Ehrlichia canis*. In: F. Beugnet (ed.): Guide to major vector-borne diseases of pets. Merial S.a.s., Lyon, France, 2002, 107-113.
12. - DE LA FUENTE J., NARANJO V., RUIZ-FONS F., HOFLE U., FERNANDEZ DE MERA I.G., VILLANUA D., ALMAZAN C., TORINA A., CARAPPA S., KOCAN K.M., GORTAZAR C.: Potential vertebrate reservoir hosts and invertebrate vectors of *Anaplasma marginale* and *A. phagocytophilum* in central Spain. *Vector Borne Zoonotic Diseases*, 2005, **5**, 390-401.
13. - EGENVALL A., LILLIEHOOK I., BJOERSDORFF A., ENGVALL E., KARLMSTAM E., ARTURSSON K., HELDTANDER A., GUNNARSSON A.: Detection of granulocytic *Ehrlichia* species DNA by PCR in persistently infected dogs. *Veterinary Record*, 2000, **146**, 186-190.
14. - FILONI C., CATAO-DIAS J.L., BAY G., DURIGON E.L., JORGE R.S., LUTZ H., HOFMANN-LEHMANN R.: First evidence of feline herpesvirus, calicivirus, parvovirus and *Ehrlichia* exposure in Brazilian free-ranging felids. *Journal of Wildlife Diseases*, 2006, **42**, 470-477.
15. - MATTHEWMAN L.A., KELLY P.J., WRAY K., BRYSON N.R., RYCROFT A.N., RAOULT D., MAHAN S.M.: Antibodies in cat sera from southern Africa react with antigens of *Ehrlichia canis*. *Veterinary Record*, 1996, **138**, 364-365.
16. - PEAVY G.M., HOLLAND C.J., DUTTA S.K., SMITH G., MOORE A., RICH L.J., LAPPIN M.R., RICHTER K.: Suspected ehrlichial infection in five cats from a household. *Journal of the American Veterinary Medical Association*, 1997, **210**, 231-234.
17. - STUBBS C.J., LAPPIN M.R., HOLLAND C.J.: Feline ehrlichiosis. *Journal of Veterinary Internal Medicine*, 1998, **12**, 230.
18. - STUBBS C.J., HOLLAND C.J., REIF J.S., BRUNS C., WHEELER S., LAPPIN M.R.: Feline Ehrlichiosis. *Compendium of Continuing Education for the Practicing Veterinarian*, 2000, **22**, 307-318.
19. - STUEN S., VAN DE POL I., BERGSTROM K., SCHOULS L.M.: Identification of *Anaplasma phagocytophila* (Formerly *Ehrlichia phagocytophila*) variants in blood from sheep in Norway. *Journal of Clinical Microbiology*, 2002, **40**, 3192-97.
20. - TARELLO W.: Granulocytic *Ehrlichia*-like bodies in a cat with chronic oral disease: case report. *Revue de Médecine Vétérinaire*, 2002, **153**, 401-406.
21. - TARELLO W.: Microscopic and clinical evidence for *Anaplasma (Ehrlichia) phagocytophilum* infection in Italian cats. *Veterinary Record*, 2005, **156**, 772-774.
22. - TARELLO W.: Microscopic and clinical evidence for *Ehrlichia* spp. infection in Saker falcons (*Falco cherrug*). *Revue de Médecine Vétérinaire*, 2006, **157**, 203-204.
23. - WANER T., HARRUS S., JONGEJAN F., BARK H., KEYSARY A., CORNELISSEN A.: Significance of serological testing for ehrlichial diseases in dogs with special emphasis on the diagnosis of canine monocytic ehrlichiosis caused by *Ehrlichia canis*. *Veterinary Parasitology*, 2001, **95**, 1-15.