

Baumgartner, K. and Gauckler, A. 1998. Haemobartonellosis (Feline infectious anemia) in a cheetah (*Acinonyx jubatus*) group. European Association of Zoo - and Wildlife Veterinarians, Second scientific meeting, May 21-24, 1998. 1-5. Chester, United Kingdom, European Association of Zoo - and Wildlife Veterinarians.

Keywords: *Acinonyx jubatus*/cheetah/disease/*Haemobartonella felis*/veterinary/zoo

Abstract: In 1997 in the Nürnberg Zoo all female cheetahs fell ill with *Haemobartonella felis* after the contact with two male cheetahs coming from another European facility. One animal died, the other two females could be saved, after diagnosis of *Haemobartonella felis* on stained blood film, by prolonged treatment with Doxycycline.

HAEMOBARTONELLOSIS (FELINE INFECTIOUS ANEMIA) IN A CHEETAH (*ACINONYX JUBATUS*) GROUP

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Abstract

In 1997 in the Nürnberg Zoo all female cheetahs fell ill with *Haemobartonella felis* after the contact with two male cheetahs coming from another European facility. One animal died, the other two females could be saved, after diagnosis of *Haemobartonella felis* on stained blood film, by prolonged treatment with Doxycycline.

Zusammenfassung:

Im Tiergarten Nürnberg erkrankten 1997 alle weiblichen Geparden an *Haemobartonella felis*, nachdem sie mit 2 männlichen Geparden aus einem anderen europäischen Zoo zusammengeführt worden waren. Ein Tier starb, die anderen beiden Weibchen konnten, nachdem anhand gefärbter Blutaussstriche *Haemobartonella felis* diagnostiziert worden war, durch eine Langzeitbehandlung mit Doxycyclin gerettet werden.

Résumé

Dans l'année 1997 dans le jardin zoologique de Nürnberg, tous les guépards féminine contracté une maladie causé *Haemobartonella felis*. Cette maladie se manifestait après des contacts avec deux guépards males, importé d'une autre jardin zoologique Européenne. Une femelle mourut, deux autres peuvent être sauvé par un traitement prolongé avec Doxycycline, après le diagnose „infection par *Haemobartonella felis*“ était établi avec l'aide de frottis de sang colorées.

Key words

Haemobartonella felis - Feline Infectious Anemia - Cheetah

Introduction

Haemobartonella felis is a blood parasite, which lives in the red cells causing haemolysis and haemoglobinuria. This organism is usually found in varying numbers on the surface of the red blood cells (RBC), but is occasionally seen free in plasma. The affected red cells are eliminated totally by the activity of the S.R.E. (3).

Haemobartonella felis is described as cocci or beaded bacilli on the surface of the erythrocyte. They are responsible for the disease called „Feline Infectious Anemia“ and were first reported in Colorado (U.S.) in 1953 by Flint and Moss (5).

Haemobartonella canis is a non-pathogenic parasite for the normal intact dog. It differs from *H. felis* in morphology in that it forms more commonly chains extending across the surface of affected erythrocytes.

Haemobartonella bovis produces anaemia in splenectomized calves.(5)

The mode of transmission of the parasite among cats under natural conditions is not known, but intrauterine infection, vector transmission and bite wounds are suspected (2); although it can be demonstrated to be an infectious disease by inoculation of intact young cats with blood from an infected cat. Infections can also be transmitted iatrogenically during transfusion of blood (4).

Many apparently normal cats carry the parasite for infinite periods of time with no apparent ill effects. Sometimes stress, concurrent infection with „feline leukaemia virus“ or treatment with corticosteroids will cause the disease to flair up. *Haemobartonella felis* causes a haemolytic anaemia. The cats are lethargic and anorectic for two or more days. Frequently the medical history includes some kind of stress factor The animal presents hyperthermia: 39-40 °C, icterus, anaemia (packed cell volumes -PCV - between 6.0 and 23.0% can be observed), weakness and splenomegaly occur within eight days (5).

An increase of *H. felis* in the peripheric blood cells can be observed within the third day post infection. In chronically or slowly developing cases, there may be normal or subnormal temperature, weakness, depression, and emaciation, but no jaundice or splenomegaly. Dyspnea varies with the degree of anaemia (4).

Specific diagnosis of Feline Infectious Anemia presents a problem since it depends on identifying the parasite microscopically on specially prepared and stained blood films. In addition the parasite is present in peripheral blood in large numbers only in the acute stage. When the disease is suspected, but the parasite has not been observed in the stained blood film, it is advisable to examine the blood at least on four separated occasions, generally on consecutive days (5).

The parasite can be found either in the blood or in the bone marrow. The best method is to stain the erythrocytes with Giemsa-solution. The parasite is typically a small ringlike structure that may be seen in large numbers on the surface of the erythrocytes at the height of the parasitaemia. In the blood films other changes of the blood cells can also be seen: diffuse basophilic granules in the larger cells, nucleated RBC, polychromasia, anisocytosis, increased number of reticulocytes. The number of the erythrocytes can decrease to 1×10^{12} /l (3). Another diagnostic possibility is the dry staining of the unfixed blood films with new methylene blue, called NMB: *Haemobartonella* can then be demonstrated, but this technique requires considerable experience, since the parasite is very small.

A differential diagnosis should always be made with Cytauxzoonosis (4), produced by *Cytauxzoon felis*, which has a life cycle that ends in the host cats. *C. felis* appears as a intracellular ring, rod or coccoid-shaped protozoon. Also the Piroplasmosis, caused by *Babesia felis* should be considered in the differential diagnosis. This infection in cats has been described in the famous lioness „Elsa“, the animal died shortly after release in the wild from Piroplasmosis (2). Dogs with Piroplasmosis show the same symptoms as cats with Haemobartonellosis.

Reports of *H. felis* in wild Felidae are uncommon (2), but positive findings occurred in a pallas cat (*Otocolobus manul*), a margay (*Leopardus wiedi*) and a steppe wild cat (*Felis silvestris ornata*). These were diagnosed by Erwin Small, D.V.M., M.S. at the University of Illinois using an acridine orange stain technique. Also in the Cincinnati Zoo an outbreak occurred in two litters of several kittens; offspring of a single pair. All members of the both litters died from the infection or related disorders (2). The geographical distribution of F.I.A. is widespread, with many clinically normal individuals being carriers.

In animals which suffer an acute stadium of Haemobartonellosis a fluid therapy is required, or if available, a blood transfusion. The therapy can be done with Oxytetracycline (20 mg/ kg BW p.o.) or with Tetracycline HCl (33-110 mg/kg BW p.o.), but in this case reduced appetite and vomiting can be observed. Therefore it is the best to use Doxycycline as there are no such side-effects and is sufficient to administer it once a day (1). It is necessary to continue the treatment for at least 10-20 days to prevent relapses. Also Caparsolate, a tarsenamide can be used in a dose of 0,1 mg/kg BW two times (3).

Case report

In the Nürnberg Zoo we had 3 female cheetahs (*Acinonyx jubatus*) at the middle of 1996, when 2 males arrived from another Zoo in Germany. For the first months the two males were kept in a separated enclosure and in January 1997 we first tried to keep them together in the bigger enclosure. In the first hours they had some physical contact and than showed no more interest in each other. The females continued to stay near each other and so did the males.

Two weeks later our hand reared female „MIMA“, born on 10.04.92 in the Pretoria Zoo, showed the first symptoms. She was not feeding well, showed extreme salivation and the protrusion of the third eyelid. We gave her 5 ml of StaglobanSHP® (Immunoglobulin of dog against Distemper, Canine Viral Hepatitis and Parvovirus Infection), 1 g/5 ml of Chloromycetin succinate® (Chloramphenicol) i.m. and Vibramycine® (Doxycycline) orally supposing, that there was an infection going on. The next day she stopped feeding and we gave her again 5 ml of Stagloban® and 1 g/5 ml of Chloramphenicol (Chloromycetin®). It is important to mention, that the animal was vaccinated against Rhinotracheitis, Calicivirus, Panleukopenia, FIP and Rabies.

As she was doing even worse the following day, which was the third day of obvious symptoms, we decided for an anaesthesia. The anaesthesia was induced with Xylazin and Ketamine (Hellabrunner Mischung), we had a pulsoxymeter attached to the ear to control the heart rate (mean 71 BPM) and the oxygen saturation (mean 93% SpO₂). The X-rays did not show anything peculiar, but by palpation a hepatomegaly could be discovered. The mucous membranes were yellow-coloured and the animal seemed to be dehydrated; the body weight was 33,95 kg. We treated her with 2 g/10 ml of Chloromycetine succinate®, 2 ml of Baypamu® (para-immunity inducer), 15 ml of Stagloban® and 5 ml of Catosal® (metabolism- stimulant). In this occasion we took blood samples and did some analysis: in one of the with Giemsa stained blood films we saw cocci on the surface of the erythrocytes, but we could not identify them as *Haemobartonella*. We supposed a *Babesia* to be the agent. The remaining samples were sent to the laboratory. In faecal samples no parasites could be discovered and they were also sent to the laboratory for further investigations.

The following day her condition was even worse, she showed dyspnea, was neither feeding nor drinking and had a protrusion of the third eyelid. We could not handle her, so we decided for another anaesthesia to give her a fluid therapy, which consisted of 1000 ml of RingerLactate, 500 ml of Glykofusal® i.v., furthermore we gave her 3 ml of Acutol® (Flumetason) i.v., 1 ml of Baypamun® (para-immunity inducer) and 2 g/10 ml of Chloromycetine succinate® i.m..

The following day the animal did not move at all, and there was no necessity to use the blowpipe for injections, we gave her 2 ml of Benadryl® (Diphenhydraminhydrochlorid) and 1 g/5 ml Chloromycetine succinate®, in the afternoon she drank a little bit of water and we gave her a fluid therapy (without anaesthesia) i.v. with 500 ml of Ringer Lactate® and 200 ml of Glykofusal®. The next day the animal died in the morning and we performed the post mortem examination the same day at the University of Munich. The pathological findings were: icterus, hepatomegaly, hyperaemia of the lung, hyperplasia of the spleen, haemolysis with final haemolytic crisis and they found *Haemobartonella felis* in Giemsa stained blood films.

The blood picture showed a blood urea of 216 mg/dl in the first and of 364 mg/dl in the second sample, the PCV was 39%, the leucocytes 14.40/nl, the thrombocytes 24 G/l, the BILG (total

bilirubin) 12.0 mg/dl and the BILD (direct bilirubin) 7.7 mg/dl. The laboratory provided us with the following results.

Blood samples:

- Coronavirus: negative
- Leptospira bratislava: titer 1:200, which is considered doubtful
- Panleucopenia: negative
- Babesiosis: negative
- Toxoplasmosis: immunity-titer
- Haemobartonella: the first results were negative; only the second sample was positive.

Faeces samples:

- Parvovirus: negative
- Salmonellosis: negative

(Three days after the death of the first female cheetah, the second female „DUMA“, born on 15.05.92 in the Pretoria Zoo, did not feed well and showed abnormal behaviour and dyspnea. This animal was also vaccinated against Rhinotracheitis, Calicivirus, Panleucopenia, FIP and Rabies. The following day, as the laboratory analyses of the first animal were still going on and we did not have the pathological results yet, we decided for anaesthesia: the body weight was 38,15 kg, the mucous membranes were yellow and the kidneys seemed enlarged, the temperature was 41°C. We gave her 500 ml of Ringer-lactate®, 1 ml of Baypamun®, 1 g/3 ml of Streptomycin Grünwald® (as we supposed Leptospirosis) and 2 ml of Cytobion® (Vit. B12, 1000 microgram/ml). The next day, we still had no results from the tests and we were still supposing Babesia to be the agent, we treated the cheetah with Berenil® (Diminazendiazeturat), which is the best therapy for Babesiosis.

Also in this animal we did different laboratory analysis from the blood samples and we got the following results:

Blood samples:

- Parvovirus (Panleucopenia): negative
- Coronavirus (FIP): negative
- Leptospirosis: negative
- Toxoplasmosis: negative
- Piroplasmosis: negative
- Leishmaniasis: negative
- Filariasis: negative
- Borreliosis: negative
- Aspergillosis: negative

The blood picture showed 12.30/nl leucocytes, a PCV of 45%, BILG (total bilirubin) 4.1 mg/dl and BILD (direct bilirubin) 3.1 mg/dl. The following day we got the result: *Haemobartonella felis*. From this day on we treated the animal with Doxycycline, first i.m. with the blowpipe (6 ml of Vibravenös® = 120 mg of Doxycycline) and after the second day, as she was doing better, breathing normally and feeding again, we gave it orally (4 tablets of Vibramycine® = 400 mg of Doxycycline). After three days she was doing fine, drank normal and fed well. We continued to give 200 mg of Doxycycline daily for two weeks.

In this period we treated all our cheetahs, which included the two males (as prophylaxis) and the two females orally with Doxycycline.

Five weeks later our third female „TONGA“, born on 29.03.90 in the Pretoria Zoo, showed the same symptoms. We decided for anaesthesia: the body weight was 34,50 kg and the mucous membranes were yellow. We immediately treated her with 200 mg of Doxycycline and Berenil® (as we had not any laboratory results from this animal and she already had been treated against *H. felis* a month before). The blood picture showed 10.60/nl leucocytes, a PCV of 42%, BILG (total bilirubin) 4.6 mg/dl and BILD (direct bilirubin) 3.4 mg/dl. From the other laboratory analyses we got the following results:

Blood samples:

- Babesiosis: negative
- Leishmaniasis: negative
- Toxoplasmosis: negative
- Haemobartonellosis: negative

The bacteriological investigation of the faeces sample showed some *Proteus mirabilis* and *Escherichia coli*, but no *Salmonella*.

Two days later she was feeding well and took the Doxycycline orally (300 mg daily), we continued the therapy for 18 days and the animal did not show the symptoms any more.

Discussion

Two of our females fell ill with *Haemobartonella felis*, one female showed the same symptoms one month later, but *H. felis* could not be discovered, the two males never showed any clinical signs. The greatest difficulty was the diagnosis of the blood parasite, as soon as the parasite was discovered the treatment with Doxycycline gave almost immediate results. Until now we do not know how *H. felis* could have affected our animals; maybe the outbreak was caused by the stress situation created by the entering of the two males or maybe it was introduced by these two cheetahs. All our inquiries in other countries, such as U.S. and South Africa did not give any results; in the International Cheetah Studbook (1994-1995), where all the different causes of death are listed: FIP, Panleucopenia, Coronavirus, Feline Distemper, Salmonellosis, Babesiosis (n° 1042-1043), Parvovirus, TBC, only one case of icterus (n°1465) and one case of acute haemolytic anaemia (n° 2579) are mentioned, but in both cases the agent had not been discovered.

After the third case and the prolonged treatment with Doxycycline, none of the animals showed symptoms any more. The animals were kept together until December 1997, when the two females were separated from the males for mating reasons.

Acknowledgements

We would like to thank Dr.T.Hänichen from the Institute of Veterinary Pathology at the University of Munich for the necropsy and Dr.Göbel from the Institute of Tropical Medicine and Parasitology in Munich for the diagnosis of *Haemobartonella felis*.

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