Acta vet. scand. 1972, 13, 323-331.

### From the Department of Pathology, College of Veterinary Medicine, Helsinki, Finland.

# HAEMOBARTONELLOSIS IN THE DOMESTIC CAT

### By

### M. Hatakka

HATAKKA, M.: Haemobartonellosis in the domestic cat. Acta vet. scand. 1972, 13, 323—331. — Post-mortem examination of two cases of natural Haemobartonella felis infection in the cat is reported. In both cases haemobartonellosis was confirmed on blood smears from the diseased animals. The most noticeable macroscopic finding was a pronounced general anemia.

The microscopic examination revealed hyperplasia of the bone marrow and a moderate extramedullary hematopoiesis in case 1 and in case 2 a reduction of the M/E index in the bone marrow.

haemobartonellosis; Haemobartonella felis; Eperythrozoon felis; feline infectious anemia; blood diseases; anemia.

Haemobartonella felis (feline infectious anemia) appears in the domestic cat as an acute or chronic disease characterized by haemolytic anemia. The disease is caused by a blood parasite of the genus Haemobartonella, for which the designation Haemobartonella felis has been suggested.

The parasite occurs on the surface of the red blood cell in a coccoid, rod or annular form. Only the coccoid and the rod forms have previously been found in cats in Finland (Tuomi 1970).

Although extensive clinical studies have been carried out, few reports deal with the pathology of the disease (*Splitter et al.* 1956, *Flint et al.* 1959, *Flagstad & Larsen* 1969, *Wilkinson* 1969). The only more comprehensive report on the pathology of feline haemobartonellosis has been presented by *Flint et al.* Noticeable post-mortem findings were general anemia, icterus and in many cases bone marrow hyperplasia. Extramedullary hematopoiesis was demonstrated in the liver and the spleen. Different degrees of splenomegaly, as well as centrolobular necroses and interlobular infiltration with lymphocytic cells in the liver were occasionally found. Besides, the liver and the spleen often showed a considerable amount of hemosiderin. Feline infectious anemia was first reported in Finland by *Taylor et al.* (1967). These authors assumed that many cases of haemobartonellosis might be diagnosed as panleukopenia. It would therefore be essential to find a more relevant basis for the post-mortem diagnostics of the disease.

This paper deals with preliminary studies on the pathology of the disease in two cats, representing the first post-mortem investigated cases of feline haemobartonellosis in this country.

### MATERIAL AND METHODS

### Microscopic methods

Tissue samples were collected from different organs, fixed in 10 % neutral formalin, processed in the usual manner and stained with hematoxylin and eosin (H&E). Selected sections were stained according to the following methods stated by *Roulet* (1948): Giemsa, May-Grünwald-Giemsa, periodic acid-Schiff (PAS) with and without diastase digestion, Best's carmine staining, Ladewig-Mallory (L-M), Hueck's and Perl's stainings for iron and Kossa's staining for calcium. Amidoblack staining for hemoglobin was employed according to *Puchtler & Sweat* (1962). Frozen sections were stained for neutral fats with Sudan III and blood smears with Giemsa.

## Case history, clinical features and microbiological findings Case 1

Abessinian female cat, 3 years old. The cat was received for clinical treatment 6 months before death. Haemobartonella was found on the surface of the red blood corpuscles stained with Giemsa. Symptoms were vomitus, diarrhae and abortion. The cat was succesfully treated with tetracyclin, but died without showing clinical symptoms several months after recovery. On blood smears prepared after death no organisms could be established due to postmortal disintegration of the erythrocytes.

### Case 2

Finnish race, 4 months old male cat. When received for clinical treatment the cat was in a very poor condition. The most important clinical features were pronounced anemia and low hemoglobin (1.5 g/100 ml). Blood smears were prepared and the presence of Haemobartonella was established. The annular form was predominant (Fig. 1). However, the organisms were quite infrequent and occurred usually singly on the surface of the erythrocytes. The preparations showed an abundance of normoblasts and Howell-Jolly bodies. The animal was euthanatized in consequence of poor condition.



F i g u r e 1. Haemobartonella felis organisms on erythrocytes (case 2). Annular forms of the parasite (long arrows). Howell-Jolly body in an erythrocyte (short arrows). Giemsa stain  $\times$  1825.

### RESULTS

### Macroscopic findings

### Case 1

The animal showed moderate nutritional condition, pronounced anemia and small amounts of transudate in the thoracic cavity and in the pericardial sac. The spleen was moderately swollen and the bone marrow of the long bones solidly red in colour.

### Case 2

The animal showed moderate nutritional condition and pronounced anemia. In the subcutaneous tissues of the perianal area



and on the base of the tail extensive irregular, probably traumatic hemorrhages were found. In areas adjacent to the bladder, two subperitoneal hematomas  $(3 \times 2 \text{ cm})$  were observed. The abdominal cavity showed about 10 ml blood-mixed transudate. Slight hydrothorax was noted. Approx. 30 roundworms (Toxocara cati) were found in the alimentary tract. The mesenterial lymphatic nodes were moderately swollen. No splenomegaly was noted, but the bone marrow was diffusely red.

### Microscopic appearance\*

### Case 1

About 30 % of the cells of the *bone marrow* were identified as erythropoietic cells, thus M/E was about 2.3. Due to slight postmortal changes no detailed examination could be made.

The red pulp of the *spleen* was increased and infiltrated with numerous, mainly erythropoietic cells in different degrees of maturation. Late normoblasts appeared abundantly, but some erythroblasts and proerythroblasts (Fig. 3) were also present. Megacaryocytes were numerous and the hemosiderin content in the RE-cells was pronounced. The lymphatic tissue in the malpighian bodies appeared slightly atrophic, but no alterations were found in the cell picture.

A small amount of hematopoietic cells comprising proerythroblasts, erythroblasts and normoblasts, as well as a few solitary megacaryocytes were identified in the sinusoids of the *liver* 

| Figure     | 9   | Done memory (core 2) Activation of the set the              |
|------------|-----|---|
| rigure     | 2.  | Bone marrow (case 2). Activation of the erythropolesis.     |
|            |     | Early forms of erythrocytes (arrows). Giemsa $\times$ 2425. |
| Figure     | 3   | Red nuln of the spleen (case 1) Prograthroblast (D)         |
| inguit     | υ.  | the pulp of the spicen (case 1). Hoerythrobiast (F).        |
|            |     | Erythroblast (E). Giemsa $\times$ 840.                      |
| Figure     | 4.  | Liver (case 1). Degeneration of liver cells Hematopoie-     |
| 8          |     |   |
|            |     | tic cells in the sinusoids (arrows). HE $\times$ 870.       |
| Figure     | 5.  | Liver (case 1), Kunffer cell containing hemosiderin (A)     |
| 8          | ••• |   |
|            |     | Hemosideringigmentation of the cytoplasm in liver cells     |
|            |     | (B). HE $\times$ 840.                                       |
| <b>D</b> • | 0   |   |
| rigure     | υ.  | Myocardium (case 2). Degeneration of myofibrils. In-        |
|            |     | filtration with granulocytes and reproduction of fibro-     |
|            |     | blastic colla HE X 710                                      |
|            |     | Diastic cens, $\Pi_{12} \propto 110$ .                      |

<sup>\*</sup> The analysis of the histological picture of the bone marrow was confirmed by professor Raimo Tenhunen, University of Helsinki, Department of Clinical Chemistry, Meilahti, Helsinki.

(Fig. 4). Some solitary megacaryocytes also occurred in the interlobular tissue.

Scattered foci of inflammatory cells appeared in the periportal area. The connective tissue was slightly increased. Macrophages displaying a profuse accumulation of a brownish, PAS positive substance were frequent in the interlobular tissue and in the sinusoids (Fig. 5).

Some irregular, mainly spheroid acidofilic bodies of different sizes were found scattered in the sinusoids. These bodies were similar to, but somewhat bigger than the erythrocytes. However, they showed no reaction for hemoglobin stained with amidoblack and displayed a bluish color stained with L-M, thus distinctly diverging from the yellowish stained erythrocytes.

The parenchyma showed a focal, mostly centrolobular degeneration of liver cells (Fig. 4). The affected cells were swollen, inducing a compression of sinusoids and displaying vacuoles of different sizes. The vacuoles revealed no periodate-reactive substances and did not contain neutral fat. The nucleus of the degenerated liver cell mostly had a pycnotic appearance, but vesicular and other types of abnormal nuclei were also found. A brown fine-granular Hueck-positive pigment was noted in the liver cells in all sections of the liver (Fig. 5). No glycogen was observed.

Solitary inflammatory cells, mainly eosinophilic granulocytes were found between the muscle fibres of the *heart*. The myocardium showed a few regression foci. The myofibres were swollen, lacking striation, but displayed no fat infiltration. A few scattered foci of inflammatory cells, mainly lymphocytes, were observed in the cortex of the *kidney*. Most tubules showed extensive degeneration of epithelial cells. No hemosiderin was demonstrated.

### Case 2

The dominating cells of the *bone marrow* belonged to the erythropoietic group (Fig. 2). Thus the M/E index was determined a little below 1. These erythropoietic cells often accumulated, forming small islets. Early forms of erythrocytes, especially erythroblasts, but also proerythroblasts were more frequent than in the normal bone marrow. A distinct shift to the left was noted in the myelopoiesis, and rod shaped nuclei were dominant.

No crythropoietic cells were observed in the *spleen* and only a few megacaryocytes were found. Isolated macrophages displayed an accumulation of a brownish pigment, but no positive reaction for iron could be demonstrated.

No cells of the hematopoietic group were found in the *liver*. Inflammatory infiltration processes were scanty and only a few fagocytizing macrophages were observed. Degeneration processes in the liver cells were slight and no pigmentation granules were noted in the cytoplasm.

The myocardium displayed a focal and in some areas a diffuse infiltration with granulocytes and immature cells of mesenchymal origin (Fig. 6). Eosinophilic granulocytes were infrequent. The muscle fibres showed slight regression processes and solitary foci of fatty degeneration.

The *kidneys* were the site of slight degeneration processes in the tubular epithelial cells and the *adrenals* displayed several focal calcified areas in zona fasciculata.

### DISCUSSION

In the two cases of feline infectious anemia presented in this paper haemobartonella-organisms were demonstrated on the surface of the erythrocytes. In case 1 the organism displayed a coccoid or rod form and in case 2 a predominantly annular form.

The only significant macroscopic observation was a pronounced general anemia.

The microscopic examination revealed a marked activation of the erythropoiesis. In case 1 the liver and the spleen were the site of a distinct extramedullary hematopoiesis and in case 2 the M/E index indicated an activation of the erythropoiesis in the bone marrow. Thus the M/E index value determined by the author was lower than the value (varying from 1.46 to 3.5) observed in the normal cat (Sawilsky & Meyer 1940, Schryver 1963, Gilmore et al. 1964, Penny et al. 1970).

Case 2 displayed only isolated hematopoietic cells in the spleen. In the liver no hematopoietic activity could be established, probably consequent to the remarkably acute course of the disease. A pronounced hemosiderin content in the liver cells and in the macrophages of the spleen indicated the occurrence of hemolytic processes in case 1. However, no hemosiderin could be found in the epithelial cells of the ascending Henle's loop, which is characteristic for an infection with Eperythrozoon ovis, a closely related agent causing hemolytic disease in sheep ( $\emptyset$ verås 1969).

The origin of the acidophilic bodies found in the sinusoids of the liver could not be defined. Possibly they constitute the result of degeneration processes in the red blood corpuscles, socalled "blood shadows" or in accordance with earlier references (*Popper & Schaffner* 1957) disintegration products of degenerated liver cells.

The inflammatory processes in the myocardium revealed by the microscopic examination were somewhat confusing. A direct connection with the infection mechanism could not be established. The traumatic hemorrhages in the subperitoneal and the perianal area and on the tail could possibly have been responsible for a reduced resistance to infection with haemobartonella. Besides it should be considered that the infection with Toxocara cati may have caused a reduction of the resistive ability of the cat.

### REFERENCES

- Flagstad, A. & S. A. Larsen: The occurrence of feline infectious anemia in Denmark. Nord. Vet.-Med. 1969, 21, 129-141.
- Flint, J. C., H. M. Roepke & R. Jensen: Feline infectious anemia. II. Experimental cases. Amer. J. vet. Res. 1959, 20, 33—40.
- Gilmore, C. E., V. H. Gilmore & T. C. Jones: Bone marrow and peripheral blood of cats: Technique and normal values. Path. vet. 1964, 1, 18-40.
- Penny, R. H. C., C. H. Carlisle & H. A. Davidson: The blood and bone marrow picture of the cat. Brit. vet. J. 1970, 126, 459-463.
- Popper, H. & F. Schaffner: Liver: Structure and Function. Mc Graw-Hill Book Company, New York 1957, 204.
- Puchtler, H. & F. Sweat: Amidoblack as a stain for hemoglobin. Arch. Path. 1962, 73, 81-85.
- Roulet, F.: Methoden der pathologischen Histologie. (Methods of histopathology). Springer Verlag, Wien 1948.
- Sawitsky, A. & L. M. Meyer: The bone marrow of normal cats. J. Lab. clin. Med. 1940, 32, 70-75.
- Schryver, H. F.: The bone marrow of the cat. Amer. J. Vet. Res. 1963, 24, 1012-1017.
- Splitter, J. E., R. E. Castro & W. Kanawyer: Feline infectious anemia. Vet. Med. 1956, 51, 17-22.
- Taylor, D., M. Sandholm, M. Valtonen & J. Tuomi: Feline infectious anemia recognized in Finland. Nord. Vet.-Med. 1967, 19, 277— 281.

330

Tuomi, J.: Personal communication 1970.

- Wilkinson, G. T.: Feline infectious anemia. Vet. Rec. 1969, 84, 331-333.
- Øverås, J.: Studies on Eperythrozoon ovis-infection in sheep. Acta vet. scand. 1969, 10, Suppl. 28.

### SAMMANFATTNING

#### Haemobartonellos hos tamkatt.

Författaren refererar obduktionsfynden hos 2 kattor med naturlig Haemobartonella felis infektion. Hos båda fallen observerades haemobartonella-organismer i blodutstryk från de insjuknade djuren. Det väsentligaste makroskopiska fyndet var en markerad allmän anemi.

Den mikroskopiska undersökningen visade hyperplasier i den hematopoetiska vävnaden i benmärgen och måttlig extramedullär hematopoes hos fall 1 och hos fall 2 en reduktion av M/E indexet i benmärgen.

### (Received June 26, 1971).

Reprints may be requested from: M. Hatakka, Department of Pathology, College of Veterinary Medicine, Hämeentie 57, 00550 Helsinki 55, Finland.