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ANAEMIA IN THE CAT WITH PARTICULAR REFERENCE TO INFECTION WITH *HAEMOBARTONELLA FELIS*

by

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Thesis submitted for the degree of
Master of Veterinary Medicine
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October, 1980.

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ACKNOWLEDGEMENTS

First, my thanks to God for His mercies. To Him be the glory for this work.

I wish to thank Professor W.I.M. McIntyre for providing me with the opportunity to carry out this work in the Department of Veterinary Medicine. I am greatly indebted to my supervisor, Mr. A.S. Nash, who initiated this project and has been an invaluable and constant source of ideas, information, constructive criticisms and encouragement. He has been very understanding and patient. I also wish to thank Professors E.W. Fisher and I.E. Selman for their constructive criticisms and encouragement.

I am grateful to Miss P. Rogerson for performing the post mortem examinations. Also I wish to thank Mrs. Joanne Maisey (née Groves) who carried out the haematological analyses. But for her conscientiousness and competence it would have been very difficult to carry out this investigation My thanks also go to the other technicians in the Department of Veterinary Medicine for their co-operation and assistance, the staff of the Photography Department for the plates and figures, Miss Alison Kean and other nurses in the Department of Veterinary Medicine for their assistance in handling, housing and feeding the cats, and all the staff of the Department of Veterinary Medicine for their friendliness and assistance in various ways.

Thanks are also due to Miss Cathie Love for her patience in typing the draft and the final text.

I am also indebted to the Ministry of Overseas Development of the British Government for sponsoring my studies and to the University of Ibadan, Nigeria, for additional financial support during my stay in Britain.

I wish to express my deep gratitude to the Head of the Department of Veterinary Medicine, University of Ibadan, Nigeria, Professor O.O. Oduye, whose kindness, sacrifice and encouragement made it possible for me to have the opportunity to do this work.

I am greatly indebted to my wife, Olusola, for her great courage, patience, endurance, love, understanding and support during the course of this study, in spite of her predicament during most of the year.

Finally, I wish to thank my friends, relatives and all who have made my stay in Britain worthwhile.

May God bless you all.

Akin Bobade

24th October, 1980

DECLARATION

I declare that the work presented in this thesis has been carried out by me. The clinical aspects were carried out in conjunction with Mr. A.S. Nash, Department of Veterinary Medicine and the pathology in conjunction with Miss P. Rogerson, Department of Veterinary Pathology.

P.A. Bobade

SUMMARY

This study was designed to investigate the prevalence and aetiology of anaemia in cats referred to Glasgow University Veterinary Hospital and to relate the prevalence to that of feline haemobartonellosis in these cats. A total of 84 male and 71 female cats, mostly domestic short haired, were examined. Their ages ranged from one month to 16 years.

Anaemia was found in 41 (25.45%) of the 155 cats. It was slightly more prevalent in males than females and it increased with age reaching a peak at four to four and a half years. Marked anaemia (PCV less than 25%) occurred in 28 of the cases and the others were cases of mild anaemia (PCV 25-29%). The final diagnoses in the cases of anaemia were as follows. Concurrent *Haemobartonella felis* (*H. felis*) and feline leukaemia virus (FeLV) infections (12 cases), FeLV infection (7 cases), *H. felis* infection (3), concurrent *H. felis* and acute myeloid leukaemia (1), renal diseases (2), Heinz body anaemia due to intestinal intussusception(1) and feline infectious peritonitis (1). The diagnoses in the cases of mild anaemia were *H. felis* infection (5 cases), concurrent *H.felis* and FeLV infections (2), FeLV infection (1), renal diseases (2) severe flea infestation (1) and non-effusive peritonitis (1). No diagnosis was made in one case each of marked anaemia and mild anaemia.

H. felis infection was detected in the blood of 36 (23.2%) of the cats examined and the prevalence of infection was slightly higher in males than females.

The prevalence of infection increased with age reaching a peak at seven to seven and a half years. However the prevalence of the clinical disease as manifested by anaemia reached a peak at six to six and a half years. The prevalence of H. felis infection was significantly much higher ($P < 0.001$) in cats with external parasitism than other cats in the population sample, as well as in cats with FeLV infection compared with FeLV-free cats.

Marked anaemia occurred in 16 of the cats infected with H. felis, mild anaemia in seven and the others were non-anaemic.

Seventeen of the H. felis infected cats had concurrent FeLV infections. A much larger proportion of these cats were anaemic than the proportion of those with only H. felis infection. The anaemia due to concurrent H. felis and FeLV infection was generally more severe than that observed in cats with only H. felis infection and the mortality was much higher in the former group than in the latter.

This study showed that H. felis infection on its own causes little or no clinical problem. However, when it is complicated by FeLV infection, a severe anaemia occurs in most cases. This suggests that FeLV infection may be more important in the pathogenesis of anaemia in clinical feline haemobartonellosis than the parasite H. felis itself.

The anaemia due to concurrent H. felis and FeLV infections was usually so severe that compensatory regenerative responses were not adequate to produce

remissions. Therefore the prognosis for anaemic cats with concurrent H. felis and FeLV infections should be regarded as poor.

This study showed that acridine orange stain is more efficient in demonstrating H. felis organisms than the Romanowsky stains and that the May-Grunwald-Giemsa staining method is superior to the other Romanowsky stains in demonstrating H. felis organisms. However it also highlighted the need for developing a serological or immunological technique for the diagnosis of H. felis infection as all the aforementioned staining procedures at times failed to demonstrate the organism in the peripheral blood of infected cats.

The H. felis organisms found in this study were mostly coccoid forms, with a few rod forms in two cases. The diameter of the coccoid forms ranged from 0.63μ to 1.73μ, while the length of the rod forms ranged from 0.79μ to 1.1μ and the diameter 0.17μ to 0.24μ.

This study confirmed that H. felis organisms are chelated from the erythrocytes in blood samples stored in E.D.T.A. for some hours.

INTRODUCTION

Anaemia has been defined in a variety of ways. Laboratory definitions describe anaemia in terms of haemoglobin concentration in peripheral blood or of an equivalent such as the haematocrit or erythrocyte count when the values obtained are compared to the accepted normal values for the appropriate species, taking into account the age and sex. Further subdivision is made by calculations of mean cell volumes (MCV), mean cell haemoglobin concentration (MCHC) and mean cell haemoglobin (MCH).

The clinical diagnosis and definition of anaemia are dependent upon the recognition of a number of clinical signs forming together a syndrome.

In the domestic cat (*Felis domesticus*) anaemia is a commonly encountered clinical syndrome (Cramer 1974; Hathaway 1976; and Mackey 1977) and it has been said to occur more frequently in this domestic species than in any other (Wright 1973). Moreover, anaemia has been described as an important cause of mortality in cats (Holzworth 1956; Mackey 1977).

Feline infectious anaemia (Haemobartonellosis) caused by *Haemobartonella felis* (*Eperythrozoon felis*) is one of the causes of anaemia in cats and is the most prevalent cause of haemolytic anaemia in cats (Wright 1973; Loeb 1975; and Hathaway 1976). The infection was first reported in the United Kingdom in 1959 by Seamer and Douglas (Seamer and Douglas 1959) and since that time there have been other reports from England and Wales but none from Scotland.

No survey has been conducted in Scotland to determine the prevalence of this infection or its relationship to the prevalence of anaemia in cats.

Cases of feline anaemia have been referred to the University of Glasgow Veterinary Hospital for many years. At least 20 percent of the 509 cats presented in the Medicine Department of the hospital between 1977 and mid-1979 were anaemic. Therefore it was decided to investigate the following:

1. The occurrence and aetiology of anaemia in cats presently referred.

2. The prevalence of H. felis in these cats.

3. The efficiency of various techniques for the demonstration of H. felis.

4. The assessment of therapy in clinical cases of haemobartonellosis.

CHAPTER I

REVIEW OF LITERATURE

SECTION I

REVIEW OF LITERATURE ON NORMAL FELINE HAEMATOLOGY

In order to define anaemias that have been described in the domestic cat, it is necessary first to review what is known of the normal haematological parameters.

Several studies have been made of the blood of cats, in haematological values. As a result of these differences it has not been possible to state the true limits of normal values. Therefore, some of the results of the studies by various authors are presented in Table 1.1.

The differences in the erythrocytic parameters, viz. the packed cell volume (PCV) or haematocrit, the red blood cell (RBC) or erythrocyte count, and haemoglobin concentration (Hb) values, make it difficult to state how low these values should be before a cat can be regarded as being anaemic. The lower limits of the normal values obtained by Gilmore, Gilmore and Jones (1964); Schalm, Jain and Carroll (1975); and Osbaldiston (1978) would be considered anaemic according to the results of the others, (Archer 1965; Penny, Carlisle and Davidson 1970; Anderson, Wilson and Hay 1971). In the same way, the upper limits for white blood cell (WBC) or leucocyte count obtained by Gilmore and others (1964); Anderson and others (1971) and Osbaldiston (1978) would be regarded as cases of leucocytosis when compared with results of the others, (Schryver 1963; Archer 1965; Penny and others 1970b; Schalm and others, 1975).

TABLE 1.1

Haematological values in normal cats as reported by various authors

REFERENCE	PCV(%)	RBC ($x10^6$/ul)	Hb (g/dl)	MCV (fl)	MCHC (g/dl)	Reticulocyte (%)	H-J Bodies (%)	Total WBC ($x10^9$/l)
Schryver (1963)	29.0 ± 0.48	-	10.8 ± 0.19	-	-	-	-	18.72 ± 1.22
Gilmore and others (1964)	24 - 46 (37.0)	-	-	-	-	-	-	4.80 - 29.00 (15.25)
Archer (1965)	30 - 50 (40.0)	6 - 10 (8.0)	8 - 14 (11.0)	-	-	-	-	9 - 23 (16.0)
Penny and others (1970b)	36.15 ± 4.94	6.45 ± 0.87	12.48 ± 1.75	56.16 ± 6.22	34.53 ± 3.26	0.05 ± 0.09	0.13 ± 0.28	13.86 ± 5.18
Anderson and others (1971)	36.6 ± 3.6	7.7 ± 0.8	13.3 ± 1.8	47 ± 3.9	36 ± 3.1	-	-	24.0 ± 12.5
Schalm and others (1975)	24 - 45 (37.0)	5 - 10 (7.5)	8 - 15 (12.0)	39 - 55 (45.0)	30 - 36 (33.2)	0.2 - 1.6 (0.6)	-	5.5 - 19.5 (12.5)
Osbaldiston (1978)	25 - 49 (36)	4.8 - 10.9 (8.3)	7.4 - 16.2 (11.9)	35.1 - 55.4 (42.8)	33 - 34			8.0 - 28.0 (18.0)

In considering these data, it is pertinent to mention the circumstances under which they were obtained and factors that might be responsible for such wide variations.

The authors referred to in Table 1.1, with the exception of Anderson and others (1971) and Osbaldiston (1978), obtained their blood samples from randomly selected apparently healthy household cats, rather than from cats raised specifically for research purposes. It is therefore possible, as pointed out by Mackey (1977), that minor subclinical infections could have been present in these cats. This could not even be ruled out in the cats sampled by Anderson and others (1971). Also it was not stated if the cats used by Anderson and others (1971) and Osbaldiston (1978) were free from helminth infections. Both hookworm and ascarid infections are known to cause anaemia in cats (Todd 1975).

Feline leukaemia virus (FeLV) infection is an important cause of anaemia in cats (Mackey, 1975). Hoover, Perryman and Kociba (1973) observed that a decrease occurred in the erythrocytic parameters before the onset of clinical disease in cats experimentally infected with FeLV. Only Osbaldiston (1978) obtained blood samples from cats that were definitely free of FeLV. However the similarity between the values obtained for the FeLV-free cats and the healthy survivors in a second group of cats any of which could have been infected with FeLV, suggests that the FeLV-free cats could have had other conditions or subclinical

infections that might have affected the haematological values. Also none of the authors screened the cats for Haemobartonella felis infections which can also produce mild anaemia in cats before acute anaemia appears (Coles 1980).

The blood vessels from which blood samples were obtained from the cats were probably another cause of discrepancy in the haematological values obtained by the different workers. Schryver (1963) and Penny (1970) obtained their samples from the cephalic vein, Gilmore and others (1964) and Anderson and others (1971) from the ear vessels, and Osbaldiston (1978) from the jugular vein. Schalm and others (1975) pointed out that if the blood does not flow freely from the ear vessels but is forced out by squeezing the tissues, the RBC and WBC counts will not reflect their true peripheral blood levels. Even where larger vessels are used for obtaining blood, if some difficulty is experienced, withdrawal of the sample is slow and clumping of platelets may result. This may lead to the formation of little clots in the sample thereby giving low PCV and RBC values.

It is interesting to note that the values for the lower normal limits for erythrocytic parameters obtained by the American authors Schryver (1963), Gilmore and others (1964), Schalm and others (1975) and Osbaldiston (1978) were lower than those reported by the others. This raises the question of probable location differences in these values. From Mexico, Velasco, Landaverde, Lifshitz and Parra (1971) reported elevated values for cat's blood at an altitude of 7,200 feet above sea level.

In view of the foregoing, it is apparent that more studies need to be made, in the light of new scientific developments and discoveries in the field of feline medicine, to establish acceptable definitive normal ranges for the haematological parameters in the domestic cat. For example, it appears that the data published by Schalm in 1965 were merely transferred into the new edition of the book (Schalm and others 1975), without any reference to the effects that new information, for example, from the field of FeLV research, may have had on the data obtained more than ten years previously.

Schalm and others (1975) have reviewed the changes which occur in feline haematological values with age. The newborn kitten has larger but fewer erythrocytes than the adult. The haemoglobin concentration is reduced during the nursing period and this has been attributed to the low concentration of iron in milk. The RBC count gradually increases from the 3rd week of life onward and reaches the adult level by the third to fourth month of life.

The adult level of haemoglobin concentration is not attained until the 5th or 6th month of life.

The kitten's PCV is high at birth (45%) but it decreases progressively reaching a value of about 30 per cent at four weeks of age. This is followed by a progressive increase to the adult level of about 37 per cent at about five months of age.

Anderson and others (1971), working with cats in a range of age groups from four weeks to one year, obtained similar results.

There are slight sex differences in feline haematological values. All the four reports cited by Schalm and others (1975) indicated slightly higher RBC and Hb values for males. However, two of the reports (Lewis 1941; Hauser 1963) indicated higher WBC in females while the other two (Landberg 1940; Windle, Sweet and Whitehead 1940) gave higher WBC values for males. Berman (1974) has reported a decrease in the erythrocytic parameters of the female during the last trimester of pregnancy. No reports of breed differences in feline haematological values have been found.

Physical and emotional stress have been observed to produce increases in the values for the erythrocytic parameters and the WBC in domestic cats (Schalm and others 1975; Kleinsorgen, Brandenburg and Brummer 1976).

Apart from the haematological values discussed above, other parameters which may be of value in assessing anaemia in the cat include the number of reticulocytes in

circulating blood, the presence of nucleated erythrocytes (normoblasts) and the presence of erythrocytic inclusion bodies such as Howell-Jolly bodies and Heinz bodies.

The peripheral blood reticulocyte count is the simplest means of measuring bone marrow activity (Fan, Dorner and Hoffman, 1978). In most studies, reticulocyte counts in normal cats have been below 1.0 percent of the RBC count (Table 1.1). Schalm and others (1975) classified reticulocytes into three types: erythrocytes of uniform size, staining light green with new methylene blue and having a faint blue stipling represented Type I; those varying somewhat in size and having large dark granules represented Type II while those with blue-green cytoplasm and a heavy dark-blue reticular network represented Type III. Cramer and Lewis (1972), on the other hand, grouped types I and II together as "punctate" reticulocytes and referred to type III as "aggregate" reticulocytes. When both types were counted they found a normal mean of 4.6 percent and a range of 1.4 - 10.8 percent while the range for the aggregate form alone was 0 - 0.4 percent. However only eight cats were used and this casts some doubts on the validity of these results. The authors mentioned that in their experience, approximately 50 percent of feline erythrocytes are reticulocytes at two to three months of age. They also cited Krafka (1931) as claiming that all erythrocytes of new-born kittens are reticulocytes.

Fan and others (1978) have suggested that excitement or physical exertion can elevate the reticulocyte count in peripheral blood.

Howell-Jolly bodies are small spherical bodies which are frequently encountered in feline erythrocytes. They are nuclear remnants and there are rarely more than one per erythrocyte in normal feline blood. Though Penny and others (1970b) reported a mean occurrence of 0.13 ± 0.28 percent, they may occur in up to one percent of the erythrocytes (Schalm and others 1975; Harvey 1977).

Nucleated erythrocytes rarely occur in the blood of healthy cats.

Heinz bodies or erythrocyte refractile bodies have been reported in normal feline blood (Schalm and others 1975). These are very small crystalline inclusions of denatured haemoglobin. They are easily differentiated from Howell-Jolly bodies in that they are not stained by the routine Romanowsky stains. However, they can be stained with vital stains such as methyl violet or new methylene blue in saline. They have a refractile appearance when stained supravitally with new methylene blue. Penny and others (1970b) reported the occurrence of Heinz bodies in 0.06 ± 0.24 percent of normal feline erythrocytes.

SECTION II

REVIEW OF LITERATURE ON ANAEMIA IN CATS

Anaemia is one of the most common clinical signs encountered in the domestic cat, probably occurring more frequently in this species than in any other species of domestic animals (Wright 1973; Cramer 1974). In fact it is regarded as an important cause of mortality in the species (Holzworth 1956; Mackey 1977). Though these facts have been stated by several workers, it seems that there is no record of any planned survey to determine the incidence or prevalence of this clinical entity in any cat population. In 1978, the "Modern Veterinary Practice" (Anon.) published a panel report on anaemia in cats. The panel consisted of nine veterinary practices in eight states of America; cases of anaemia were encountered frequently in six of the practices, with the figures ranging between 15 percent and 25 percent of all cats presented.

General clinical signs of feline anaemia

The clinical signs of anaemia in the domestic cat are variable depending on the rapidity of onset, degree of physical activity, severity of the anaemia and the underlying aetiology. The domestic cat by nature seems able to adjust readily its level of activity so that most cases of anaemia are not recognised in the early stages. The PCV may fall below 10 percent without obvious respiratory distress (Hathaway 1976). In fact, some cats have been presented to veterinarians in such a state of profound anaemia that any further delay would have been fatal (Holzworth 1956).

The general signs of anaemia in the cat are attributable to tissue hypoxia or anoxia. These include pallor of the mucous membranes of the rhinarium, tongue, gums and conjunctivae, as well as the skin. There is fast, shallow respiration; and severe respiratory distress may be noticed after exertion. Lethergy, depression and weakness are common and occasionally episodes of collapse may occur in profoundly anaemic cats (Hathaway 1976). Anaemic cats have diminished appetite and may exhibit degrees of pica including licking of stones, concrete or iron work, although this habit is commonly seen in many apparently normal cats (Wright 1973). Increased thirst may also be present.

On examination, tachycardia, with the heart rate exceeding 200 beats per minute, may be noted and in cases with PCV below 15 percent, a systolic, soft, blowing cardiac murmur may be auscultated (Hathaway 1976). There may be a persistent low fever (Loeb 1975) or no rise in the body temperature at all; and usually the ears and other extremities are cold to the touch (Wright 1973).

Other signs which may be specific for a particular type of anaemia are discussed in the next section.

Classification of feline anaemias

As in other species of domestic animals, there are several systems for classifying anaemia in the cat. The various classifications of anaemia in the domestic cat have been dealt with by many authors (Wright 1973; Cramer 1974; Loeb 1975; Hathaway 1976; Perman 1977). However the most

commonly used ones are discussed briefly:

1. Morphologic classification: this involves using the erythrocyte indices, namely the mean corpuscular volume (MCV), the mean corpuscular haemoglobin concentrations (MCHC), and the mean corpuscular haemoglobin (MCH). When this classification is used, anaemias with normal MCV are termed normocytic while those with MCV values below or above normal ranges are termed microcytic or macrocytic respectively. Anaemias in which the MCH and MCHC are normal are termed normochromic and those with MCH and MCHC below the normal ranges are termed hypochromic. True hyperchromia (absolute increase in haemoglobin within the erythrocyte) does not occur, but in acute haemolytic anaemia, a false hyperchromia may be seen due to excess free haemoglcbin in the plasma (Penny 1978). The MCHC is of more practical value in assessing anaemia than the MCH (Jain 1979).

With this classification, anaemias are described using both the MCV and MCHC or MCH. Thus anaemias are described for example as being normocytic normochromic; normocytic hypochromic, microcytic hypochromic, etc.

2. Classification according to bone marrow responses: In this system, anaemias are classified either as regenerative (responsive) or non-regenerative (non-responsive) (Perman 1977). Regenerative anaemia is seen when the bone marrow responds to anaemia by actively producing erythrocytes. This indicates that the primary cause of anaemia has no pathologically depressive effects on the bone marrow.

The characteristic haematologic findings are reticulocytosis (an increase in the number of reticulocytes in peripheral blood) and polychromasia (variation in colour among erythrocytes resulting from the admixture of normal staining reticulocytes with the more basophilic ones).

Non-regenerative anaemia is seen when the bone marrow cannot respond adequately to the anaemia. There is decreased erythrocyte response because the primary cause of the anaemia has a pathologic or suppressant effect on the bone marrow. The usual findings in these cases are an absence of reticulocytes and no polychromasia.

3. Classification according to pathophysiologic mechanism or aetiology: This is based on the pathophysiologic mechanism(s) that produce the anaemia. According to this classification, anaemia can be put into three groups, viz:

a. Blood loss or haemorrhagic anaemia

b. Haemolytic anaemias due to increased erythrocyte destruction.

c. Anaemia caused by impaired erythrocyte production (aplastic/hypoplastic anaemia).

For the purpose of this review, a combination of these classifications is used. While the pathophysiologic mechanisms are employed for grouping the types of anaemia encountered in the domestic cat, the other classifications are used for descriptive purposes. This combination gives a clearer picture of each type of anaemia than would be obtained by employing just one type of classification.

Types of anaemia in the domestic cat

1. Haemorrhagic or blood loss anaemia

This is uncommon in the cat (Hathaway 1976). However, when it occurs, it may be acute or chronic (Wright 1973).

Acute blood loss is usually caused by internal or external haemorrhage resulting from trauma such as in accidents, post-surgical procedures or dystocia (Hathaway 1976). Also rupture of friable primary or metastatic neoplasms of the liver and spleen, particularly haemangiosarcoma, may cause acute blood loss anaemia (Mackey 1977). Acute blood loss may be accompanied by signs of hypovolaemic shock. It is usually characterised by a sudden parallel fall in RBC, PCV and Hb levels (Wright 1973). This is followed three to five days later by evidence of active regenerative response.

Chronic blood loss anaemia may be caused by bleeding in the gastrointestinal tract from such causes as haemorrhagic gastroenteritis, an ulcer or neoplasm, hookworm infection, coccidiosis (in kittens) and foreign objects or hairballs (Hathaway 1976; Mackey 1977). Heavy infestation with blood sucking lice and fleas have also produced chronic blood loss anaemia (Cramer 1974).

Other less common causes of blood loss anaemia in the cat include coagulation defects and warfarin poisoning (Hathaway 1976; Penny 1978).

Haemorrhagic or blood loss anaemias are macrocytic due to the regenerative response. There is reticulocytosis

and polychromasia. Initially, the anaemia is normochromic but if iron is continually lost from the body, it may become hypochromic (Mackey 1977).

2. Haemolytic anaemia

This is made up of a group of anaemias which are produced as a result of decreased erythrocyte survival rate. There is an increased rate of erythrocyte destruction and this may occur both intravascularly and extravascularly. In the cat, the usual mechanism of erythrocyte destruction is by extravascular haemolysis or erythrophagocytosis by cells of the reticuloendothelial system (Hathaway 1976).

Haemolytic anaemias in the cat are often sudden in onset and evoke a regenerative response. There is reticulocytosis and the erythropoietic response is often accompanied by leucocytosis (Wright 1973). There may be excessive amounts of haemoglobin degradation products in the plasma, urine or faeces. Icterus, fever and splenomegaly may be present. The splenomegaly results from increased sequestration of erythrocytes and extramedullary haemopoiesis.

The causes of haemolytic anaemia in the cat are reviewed below.

a. Haemobartonellosis

This is regarded as the most prevalent cause of haemolytic anaemia in domestic cats (Wright 1973; Hathaway 1976). Since feline haemobartonellosis is the central focus of this investigation the literature on it is reviewed in the next section of this chapter.

b. Heinz body anaemia

This is a disease in which haemolysis is associated with the presence of Heinz bodies in a large proportion of the erythrocytes. The bodies tend to be larger than those seen in normal cat blood. Altman (1974) described the condition as a spontaneous disease of cats. It has also occurred following administration of drugs containing methylene blue (Schechter, Schalm and Kaneko 1973; Boon and Rich 1974; Schalm 1977), and in association with the administration of acetaminophen (Finco, Duncan Schall and Prasse 1975; Schalm 1977) and phenazopyridine (Harvey and Kornick 1976). Schalm (1977) has also associated the disease with autointoxication following intestinal obstruction.

Like other haemolytic anaemias, Heinz body anaemia is regenerative and it is characterised by reticulocytosis (Schalm 1977).

c. Immune mediated haemolytic anaemia

Only a few cases of this type of anaemia have been described in the literature.

Autoimmune haemolytic anaemia (AIHA) was first described in the cat by Sodikoff and Custer (1965) and later documented by Scott, Schultz, Post, Bolton and Baldwin 1973). This disease results from the presence of immunoglobulin (Ig) on the erythrocyte membrane (Mackey 1977). An immune reaction involving complement may cause lysis of the cells intravascularly, or the affected cells may be removed from the blood by phagocytosis in the spleen where

they are destroyed. Based on the study of seven affected cats and four other cases from previous literature, Scott and others (1973) concluded that the disease was characterised by haemolytic crises during which the cats showed fever, anorexia, pallor, lethargy and splenomegaly. Jaundice occurred rarely. The typical features of haemolytic anaemia were present. Most cases were macrocytic and normochromic with reticulocytosis and extramedullary haemopoiesis being evident. Autoagglutination was evident in blood samples from the cats and six of the seven cats studied gave positive results with the direct Coombs' test. a majority of the cases occurred in male cats.

While Scott and others (1973) seem to suggest that this is a primary disease, Hathaway (1976) believes that immune mediated haemolytic anaemia in the cat is generally a secondary rather than a primary autoimmune phenomenon. He suggested that viral infections and drug induced changes in the erythrocytic membrane may lead to immunologically induced erythrocytic destruction. Actually four of the seven cats studied by Scott and others (1973) were infected with FeLV and two of them developed lymphosarcoma. More cases will need to be studied before this condition can be fully understood.

A fatal case of autoimmune haemolytic anaemia associated with evidence of systemic lupus erythematosus (SLE) has been described in the domestic cat by Heise and Smith (1973) There was hepatosplenomegaly and lymphadenopathy. At necropsy, there was evidence of erythrophagocytosis in the spleen, liver and lymph nodes.

The nodes were hyperplastic. Glomerulonephritis, which occurs frequently in SLE, had developed and the bone marrow showed erythroid hyperplasia.

d. Feline Porphyria

Porphyrin compounds are intermediates in the synthesis of haem which in turn is an intermediate compound in haemoglobin synthesis. Porphyria is a rare hereditary defect of haemoglobin formation in which abnormal porphyrin, which cannot be utilised in haem synthesis,accumulates and becomes deposited in the tissues. Porphyria may be of the erythrocytic type in which there is a defect in the biosynthetic pathway, or of the hepatic type when the syndrome results from disordered porphyrin metabolism. Though only the congenital erythropoietic type has been described in the cat (Loeb 1975; Mackay 1977), Hathaway (1976) stated that the features of both erythrocytic and hepatic porphyria exist together in this species.

The main features of the syndrome are anaemia and a pink or brown discolouration of the teeth, bones and other tissues resulting from porphyrin deposition. The pigment fluoresces a bright pink or red colour in ultra-violet light.

The urine also may show brown discolouration as a result of porphyrin excretion. The accumulation of abnormal porphyrin in the erythrocytes leads to their removal and destruction in the spleen and this results in a haemolytic anaemia. The anaemia is macrocytic and hypochromic, the latter being due to failure of normal

haemoglobin synthesis. The degree of anaemia varies from moderate to severe (Mackey 1977). There may be aniscoytosis, poikilocytosis, target cells, normoblasts and an increased number of Howell-Jolly bodies depending on the severity of the anaemia. There is also splenomegaly due to erythrophagocytosis, and extramedullary haemopoiesis. The anaemia may be fatal in some cases.

Other causes of haemolytic anaemia in the cat.

Those which have been reported include haemolytic bacterial infections and transfusion reactions (Perman 1977); and isoimmune disease of kittens (Schall and Perman 1975). Lead poisoning, though uncommon in the cat can also cause haemolytic anaemia (Hathaway 1976).

Infection with the subgroup A feline leukaemia virus (FeLV) has produced anaemia whose characteristics suggest haemolysis as the underlying mechanism (Mackey, Jarrett, Jarrett and Laird (1975). However, it has been suggested that an immunohaemolytic mechanism may be involved (Madewell and Feldman 1980). Experimental studies have shown that FeLV infection with the subgroup A virus isolate may induce both haemolytic and aplastic forms of anaemia (Mackey and others 1975). The haemolytic form is often transient and of moderate severity.

3. Anaemias due to decreased erythrocyte production

These are non-regenerative anaemias and they account for the majority of anaemias in the domestic cat (Holzworth 1956; Wright 1973; Hathaway 1976). The anaemia is generally normochromic and normocytic though minor changes in shape or size may occur. Nucleated

erythrocytes may be present in the peripheral blood in the absence of polychromasia, and the reticulocyte counts are low.

These anaemias have a very insidious onset and the severity depends on the underlying aetiology. The various causes of these non-regenerative anaemias are reviewed briefly.

a. Nutritional anaemia

Nutritional anaemia is not common in cats unless the cat is grossly malnourished.

Iron deficiency anaemia occurs mostly in kittens. It is transient, prior to weaning and it has been attributed to low dietary intake of iron (Loeb 1975; Mackey 1977). In adult cats, iron deficiency anaemia is uncommon and is usually related to chronic blood loss (Hathaway 1976; Mackey 1977) or a totally inadequate diet (Loeb 1975). Iron deficiency anaemia is characterised by hypochromia, poiklocytosis and microcytosis.

Anaemia associated with folic acid and vitamin B12 deficiency has been reported in a cat following a three week treatment with tetracycline for haemobartonellosis (Loeb 1975). Also experimental macrocytic anaemia has been produced in cats by feeding a diet deficient in folic acid (Loeb 1975). Hathaway (1976) has suggested that folic acid deficiency may result from inadequate intestinal absorption of folate or an increased need for the vitamin. Drugs such as diphenylhydantoin and methotrexate inhibit folate absorption.

b. Feline leukaemia virus (FeLV) associated anaemia

Non-regenerative anaemia has been reported in the absence of leukaemia in cats naturally infected with FeLV (Hardy, Old, Hess, Essex and Cotter 1973; Mackey 1975; Cotter 1979). The anaemia is normochromic and normocytic in most cases and there is no reticulocytosis. Nucleated erythrocytes were present in the blood of 49 percent of the cats studied by Cotter (1979) but she stated that there was no correlation between the number of these cells and the magnitude of reticulocyte count.

The anaemia has been attributed to erythroid hypoplasia (Mackey 1975; Cotter 1979). Cotter (1979) found the degree of overall cellularity in the 40 cats whose bone marrow was examined to be normal or hypocellular in most cases although a small number were hypercellular.

The anaemia seems to occur more in males than females and most of the anaemic cats were young adults (Cotter 1979). The clinical signs were vague.

Aplastic (or non-regenerative) anaemia has been produced by experimental infection of neonatal cats with FeLV (Hoover, Kociba, Hardy and Yohn 1974; Mackey and others 1975). In these cases, the cats were inoculated either with the subgroup C virus (Mackey and others 1975) or a combination of A, B and C (Hoover and others 1974).

The aplastic anaemia in these studies was characterised by a short latency and very high incidence in the infected cats. The disease progessed rapidly

producing profound reductions in erythrocytic parameters and death occurred between one and five months of age. There was no evidence of erythrocytic response as reticulocytes and normoblasts were absent from the blood. At necropsy, the bone marrow was severely depleted of erythroid tissue. There was no sign of extramedullary haemopoiesis though erythrophagocytosis was evident. Death was due to cardiac failure resulting directly from the anaemia.

c. Anaemia associated with myeloproliferative disorders

A myeloproliferative disorder is a primary neoplasm of the bone marrow which may involve one or more haemopoietic lines and is characterised by abnormal proliferation of bone marrow cells. This disease complex includes granulocytic leukaemias, myelofibrosis, erythremic myelosis or erythroleukaemia. The incidence of this condition may be high in the cat. (Searcy 1976). One of the features of this disorder in the cat is a progressive, pronounced, non-regenerative anaemia due to damaged bone marrow (Schalm 1973; Gibbs, Buhles and Montgomery 1974; Sutton, McKellow and Bottrill 1978; Falconer, Irving, Watson and Ludwig 1980). The typical signs are pyrexia, progressive weight loss, hepatosplenomegaly and occasional lymphadenopathy (Hathaway 1976). Examination of the blood smears may reveal abnormal leucocytes.

Some myeloproliferative disorders have been found in association with FeLV infection (Schalm and Theilen 1970; Madewell, Jain and Weller 1979).

d. Aplastic/hypoplastic anaemia

This is a rather vague term which encompasses drug and chemically induced marrow damage, radiation and idiopathic damage and perhaps damage caused by certain infectious agents (Hathaway 1976). Most of these agents affect all haemopoietic lines thereby producing pancytopaenia.

Anticancer drugs frequently cause myelosuppression and can result in irreparable marrow damage. Chloramphenicol and acetylsalicylic acid (aspirin) have been incriminated as causes of aplastic anaemia in cats (Penny 1978) and so also has phenylbutazone (Carlisle, Penny, Prestcott and Davidson 1968).

Aplastic anaemia has been associated with both bacterial and viral diseases of cats. These include abscessation caused by Staphylococci sp, Streptococci and Pasteurella species as well as infectious peritonitis, rhinotracheitis and pneumonitis (Wright 1973; Mahaffey and Smith 1978). This anaemia may be due to inflammatory processes as has been pointed out by Perman (1977), and Mahaffey and Smith (1978).

Other substances that have been incriminated as causes of aplstic anaemia in cats include radio-active and radiomimetic materials; benzole and its derivatives, especially coal tar derivatives; arsenates; sulphonamides; salts of heavy metals especially lead and mercury; streptomycin, tridione and insecticides (Loeb 1975).

Aplastic anaemias are generally normocytic and normochromic and show absence of regeneration.

e. Secondary anaemias

Moderate anaemia is very common in any chronic illness in the cat (Hathaway 1976; Searcy 1976). Chronic renal disease results in some degree of anaemia due to either decreased secretion of erythropoietin (a hormone produced by the kidney which regulates erythropoiesis), or toxic damage to bone marrow (Hathaway 1976; Perman 1977). Neoplasms and thyroid deficiency have also been associated with some degree of anaemia in the cat (Loeb 1975).

SECTION III

REVIEW OF LITERATURE ON FELINE HAEMOBARTONELLOSIS

Feline haemobartonellosis (Syn. Feline infectious anaemia) is a haemolytic anaemia of cats which results from infection of the erythrocyte with the rickettsial parasite *Haemobartonella felis* (Syn. *Eperythrozoon felis*).

The disease was first reported from South Africa by Clark (1942). He observed small eperythrocytic bodies attached to the erythrocytes in blood and spleen smears obtained from an anaemic cat at necropsy. He proposed the name *Eperythrozoon felis* for the organism. Eleven years later, Flint and Moss (1953) in America described an infectious anaemia of cats in which the causative organisms were identified as small, round bodies attached to erythrocytes. They were considered to be either *Eperythrozoon* or *Haemobartonella*, or possibly a mixture of both. However, in 1955, Flint and McKelvie proposed the name *Haemobartonella felis* (*H. felis*) for the organism.

Since then there has been little agreement among authors regarding differentiation between *H. felis* and *E. felis*. While British, Australian and some European authors (Seamer and Douglas 1959; Harbutt 1963; Wilkinson 1969; Bedford 1970 etc.) designated the organism as *E. felis*, American, Canadian, Japanese and other authors preferred *H. felis* (Holzworth 1956; Splitter, Castro and Kanawyer 1956; Schwartzman and Besch 1958; Flagstad and Larsen 1969; Hatakka 1972, Maede 1975). However the organism is now most commonly termed *Haemobartonella felis* (*H. felis*) (Mackey 1977).

THE AETIOLOGICAL AGENT OF FELINE INFECTIOUS ANAEMIA

Classification

The taxonomy of this parasite and the classification of the group to which it belongs has presented some difficulty. While some authorities favour its inclusion in the rickettsial group, others consider it to be more akin to the protozoa (Mackey 1977).

Small and Ristic (1967), after studying the organism using electron microscopy agreed that there was a great degree of resemblance in the ultrastructure of *H. felis*, *Eperythrozoon ovis* and *Eperythrozoon wenyoni*. This led them to support the classification of *H. felis* in the order Rickettsiales. Thus Kreier and Ristic (1974) in Bergey's Manual of Determinative Bacteriology also classified the organism in the order Rickettsiales. Splitter and others (1956) suggested that all structural forms of *H. felis* derived from the *Haemobartonella*-like rod form. It was thus concluded that feline infectious anaemia was a haemobartonellosis rather than an eperythrozoon infection.

Seamer (1964) in a review of feline infectious anaemia noted that although *Eperythrozoon* and *Haemobartonella* have been differentiated on morphologic grounds, they so closely resembled one another that the need for two separate genera had been questioned. He then suggested that both *E. felis* and *H. felis* be regarded as the same organism and the disease in the cat as one entity.

Kreier and Ristic (1968) reviewed the *Haemobartonellae* and noted that no comparative studies on *E. felis* and *H. felis* had been published. They then suggested that the two organisms might be the same parasite. This line of thought was also supported by Mackey (1977) on the basis that the organisms recognised in different countries were morphologically alike and induced the same type of disease in the cat.

Demaree and Nessmith (1972) studied the organism from a natural case of feline infectious anaemia with electron microscopy. They noticed the similarity between the *H. felis* they studied and *Haemobartonella muris*, *Haemobartonella canis* and *Haemobartonella bovis*. It was then concluded that the proper taxonomic designation of the organism was *H. felis*.

In Bergey's Manual of Determinative Bacteriology, Kreier and Ristic (1974) grouped both *H. felis* and *E. felis* in the genus Haemobartonella. They described the haemobartonellae as obligate parasites on or within erythrocytes of many vertebrate species. "The organisms are coccoid or rod-shaped as seen by light microscopy; the rods appearing to be chains of coccoids. The organisms occur singly, in pairs or in groups in shallow or deep indentations on the erythrocyte surface, but rarely in plasma. The organisms stain well with Romanowsky type stains. They have a single or double limiting membrane and have neither cell wall nor distinct

nuclear structures. They are Gram-negative, not acid fast and have not been cultivated outside the host".

For the purpose of this investigation, E. felis and H. felis are regarded as being synonymous and the name H. felis will be used.

Morphology of H. felis

The morphology of the parasite that causes feline infectious anaemia (F.I.A.) as described by the earlier workers has been extensively reviewed by Kreier and Ristic (1968). The descriptions of H. felis by later authors, Flagstad and Larsen (1969), Demaree and Nessmith (1972), Manuel and Abalos (1975), Harvey and Gaskin (1977) and Simpson, Gaskin and Harvey (1978) did not differ in any essential way from those of the earlier workers.

The aetiological agents of F.I.A. described were similar whether the parasite was called H. felis or E. felis. Most reports portrayed the organism as staining intensely with Romanowsky stains and exhibiting pleomorphism when examined by light microscopy. The organism occurred frequently as coccoid, ring or rod forms. The coccoid form occurred singly or in pairs, though chains of cocci consisting of three to eight organisms have been observed (Splitter and others 1956; Flint, Roepke and Jensen 1958). The cocci could be irregularly arranged on the erythrocyte or were sometimes arranged in rosette form (Wilkinson 1963). The diameter of the cocci ranged from 0.1 μ to 0.8 μ (Flint and McKelvie 1955), though some were as large as 1.5 - 2.0 μ in diameter (Splitter and others 1956).

Rod forms occurred singly, in pairs or chains arranged end to end. They also occurred as beaded bacillary forms (Seamer and Douglas 1959) or as short rods at the periphery of an erythrocyte ringing it in part. The length varied from 0.7 µ (Flint and others 1958) to 3.3 µ (Manuel and Abalos 1975) while the diameter varied from 0.2 µ (Flint and McKelvie 1955) to 1.2 µ (Manual and Abalos 1975).

Discoid forms of the organism have been observed (Maede and Sonoda 1975) as also have those shaped like commas and tennis racquets (Clark 1942; Flint and Moss 1953).

Harvey and Gaskin (1977) have suggested that the relative frequency of the different forms observed by light microscopy may be influenced by the nature of the blood film itself. They found that the ring forms were more prominent in thin areas of blood films than in thicker areas where the coccoid form seemed more abundant. Also rod forms were infrequently observed in the centre of films but were evident in the feathered edge. The parasites were noted free between cells in areas where mechanical forces were greatest in blood film preparation.

<u>H. felis</u> has been stained with toluidine blue using a method which had earlier been described for <u>Anaplasma</u> by Roger and Wallace in 1966 (Wilkinson 1969). The organism stained deep purple while the erythrocytes stained blue.

Small and Ristic (1967) stained _H. felis_ with acridine orange and illuminated the preparation with ultra-violet light. The organism fluoresced a bright orange with an undertone of yellow green. It was thus concluded that _H. felis_ contained both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA); the reddish orange staining of the RNA masking the diffuse yellowish green of DNA. The authors also utilized a fluorescent-labelled antibody procedure to stain the parasite. Stained in this way, the parasite appeared as intensely fluorescent yellow-white coccoidal bodies meauring 0.3 - 0.4 µ in diameter and attached to the erythrocyte in groups, chains or as individual bodies.

The ultrastructure of _H. felis_ has been studied with both transmission and scanning electron microscopy.

Kreier and Ristic (1968), citing the work of Small and Ristic (1967), who utilized transmission electron microscopy, described the parasite as coccoid, occurring singly, in pairs and in groups of three or four or more. They appeared to be partially embedded in the erythrocyte membrane. The diameter ranged from 0.2 µ to 0.4 µ and they had neither flagella nor cilia and appeared to lack a rigid cell wall. In thin section, the parasites also appeared coccoid; they were surrounded by two membranes and contained a granular dense material embedded in an electron lucent ground substance. No true nucleus, organelles or cell wall were observed. The parasites were also attached to the erythrocyte membrane and partially embedded in it.

There was some erosion of the erythrocytic membrane at the point of contact between it and the parasite. The diameter in ultrathin section was between 0.3 µ and 0.4 µ.

There is very little similarity between the description of the ultrastructure of H. felis by Small and Ristic (1967) and that of Demaree and Nessmith (1972) who also employed transmission electron microscopy. The latter described the organism as being pleomorphic, occurring as rods, coccoid, rings and intermediate shapes of these. Most of the parasites were rod shaped. All the forms were surrounded by a single membrane and it was suggested that the outer membrane described by Small and Ristic (1967) could be the plasmalemma of the erythrocyte. Demaree and Nessmith (1972) also observed that the parasites usually indented the host erythrocytes and that though some of them were completely surrounded by erythrocytes, they were all on the surface of the erythrocytes, none being intracellular.

The description of H. felis by Simpson and others (1978) who studied the organism with transmission electron microscopy is very similar to that of Demaree and Nessmith (1972). They observed pleomorphism, including rods, ring and coccoid forms. The parasites appeared granular and were surrounded by a single membrane. They usually adhered to the smudged plasmalemma of erythrocytes at narrow areas of intermittent contact or occasionally they were separated from the erythrocytes by a gap of varying depth.

Jain and Keeton (1973) studied H. felis with scanning electron microscopy and observed that marked pleomorphism occurred. In addition to coccoid and rod forms, a conical form was observed. There was no ring form. The conical forms appeared to have their ends tapered to a variable length. The rod forms were rare and adhered to the erythrocytes either along their entire length or only at one end. The parasites were present on the surface of the erythrocytes either singly, clustered in small groups or arranged as small beaded chains. They appeared to be partially embedded in the erythrocytes with the cell surface in the immediate vicinity being slightly depressed.

Maede and Sonoda (1975) also employed scanning electron microscopy in studying the ultra-structure of H. felis using blood in which the erythrocytes were "highly parasitised". They observed two forms, a discoid form and a coccoid form. The discoid form was predominant, measured 0.5 - 0.6 µ in diameter and had a shallow concavity at the centre. It was suggested that the discoid form might be the vegetative form of H. felis.

The coccoid or lemon shaped ones had diameters of 0.2 - 0.3 µ. No rod forms were seen. The parasites were attached to the erythrocyte either singly, in pairs in a row or in a cluster, with the surface areas of the erythrocyte on which they were attached appearing to be indented.

These descriptions of the ultrastructure confirm the pleomorphic and eperythrocytic nature of H. felis. The parasite has no nucleus but both RNA and DNA have been demonstrated in it. The exact nature of the limiting membrane is still uncertain as evidences have been advanced for the presence of single as well as double membrane structures.

Replication and development of H. felis

The mode of replication of H. felis is poorly understood. However the available reports seem to indicate some form of budding or binary fission.

The first indication of how the parasite replicates was observed by Splitter and others (1956) who noted that some rod forms of the parasite were divided into two to six segments. This division was followed by swelling and growth of the segments into coccoid and ring forms identical to those seen in the blood preparation. It was then suggested that all the forms of H. felis were derived from the rod form.

There was no other report until 1972 when Demaree and Nessmith, using transmission electron microscopy, observed indications of two types of possible division, budding and binary fission. They observed situations in which two parasites attached to erythrocytes were just separating and stated that this suggested binary fission. They also described buds attached to the parent organism by a cytoplasmic stalk as an evidence of budding.

Jain and Keeton (1973) studying the parasite with scanning electron microscopy proposed a form of budding. According to them, round or coccoid bodies first appeared to become conical, with subsequent tapering of their free ends. These tapered forms, it was suggested, might be the progenitors of the rod-shaped forms.

Màede and Sonoda (1975) also employed scanning electronmicroscopy and observed discoid forms which appeared to be attached to the erythrocyte in pairs. This, they stated, seemed to be the result of binary fission. Since the blood sample used was collected at a period when almost all the erythrocytes were parasitised by H. felis, it was presumed that there was an explosive proliferation of the parasite just before sampling. It was then suggested that the discoid shaped organism might be the vegetative form of H. felis.

Cultivation and Preservation of H. felis

Attempts to cultivate H. felis in ordinary laboratory media have been unsuccessful (Flint and Moss 1953; Splitter and others 1956). Balazs, Robinson, Grey and Grice (1961) tried to cultivate the parasite using aseptically collected heparinised blood. The blood was inoculated into the surface of an agar slant and into liquid Gieman's medium. Both cultures were incubated at 28°C for four days. A thin film of growth developed on the surface of the agar culture, while a fine granular sub-surface growth was evident along wall of the tube containing Gieman's medium. Smears made from the growth

contained small Gram-negative rods and cocci both of which appeared singly, in pairs or in short chains. Transfers made from the initial culture to freshly prepared media consistently failed to develop. According to the authors, the morphological appearance of the cells present in the initial culture and its pattern of development very closely resembled those described by other workers for Haemobartonella muris. A cat which was inoculated intraperitoneally with 2 ml of the culture grown on the liquid medium developed a slight anaemia 10 days post inoculation. Blood smears from the cat revealed organisms on the erythrocyte that resembled Haemobartonellae. However, the blood was negative when examined a month later and the cat showed no clinical illness.

There seems to be no report on the preservation of H. felis. However Flint and Moss (1953) reported that citrated blood from infected cats which was frozen for two weeks was still infective for susceptible cats. This suggests that the parasite may be preserved in this way for this period of time.

The presence of H. felis in peripheral blood

The organism is usually attached to the surface of the erythrocyte (Demaree and Nessmith (1972) and is rarely seen free in plasma (Small and Ristic 1971). Despite this, the parasite is not always detectable on the erythrocyte of infected cats even during the most acute stages of the disease (Splitter and others (1956). It

appears and disappears from the peripheral blood from time to time (Flint and McKelvie 1955; Harvey and Gaskin 1977). Harvey and Gaskin (1977) reported a case in which parasites disappeared from the circulation of a cat, in which over 90 percent of the erythrocytes were parasitised, within a period of one and a half hours.

In an attempt to explain this phenomenon Maede and Murata (1978) showed that H. felis was detached from parasitised erythrocyte in the spleen. Simpson and others (1978) found free H. felis organisms in macrophages in the spleen and lungs and stated that this could be the explanation for the fate of the parasite following removal from the circulation. The authors also suggested that in addition to this mechanism of removal, because of the close association of erythrocyte borne organisms to boundaries between endothelial cells of small capillaries, the parasite could slide between endothelial cells and become located extravascularly.

Maede (1979) explained how the parasite is removed in the spleen without the erythrocyte being destroyed in most cases. The principal means is by phagocytosis of the parasite located on erythrocytes by a cordal macrophage. This is preceded by the adhesion of extended processes of the marcophage to the parasite. The second and less frequent means of removal is by a process called pitting. The parasite is pitted from the parasitised erythrocyte when the parasite passes between reticular cells or when the

parasitised erythrocyte passes among cytoplasmic processes of the reticular cells in the splenic cord.

However the synchronised disappearance of the parasite has not been explained. Also it is not known how the parasite returns into circulation. The parasite has been observed in the immature erythrocytes of the bone marrow (Holzworth, 1956).

EPIZOOTIOLOGY OF FELINE HAEMOBARTONELLOSIS

Distribution:

Feline haemobartonellosis is probably world wide in distribution. Following the initial reports from South Africa (Clark 1942) and America (Flint and Moss 1953) there have been reports from other parts of the world. These included those from Britain (Seamer and Douglas 1959; Wilkinson 1963) and from other parts of Europe, France (Florio, Lescure, Dorchies, Guelfi and Franc 1977); Germany (Prieur 1960); Denmark (Flagstad and Larsen 1969); Finland (Hataka 1972) and Sweden (Lundborg, Karlborm and Christensson 1978). Reports from Africa included those from Niger (Gretillat 1977) and Nigeria (Bobade and Akinyemi 1980); from South America (Ojeda and Skewes 1978); and from Australia (Manusu 1961; Harbutt 1963). There have also been reports from Japan (Maede, Hata, Shibata, Niiyama, Too and Sonoda 1974) and the Philippines (Manuel and Abalos 1975).

Occurrence

Clinical feline haemobartonellosis does not seem to be a common disease. Holzworth (1956) found 28 cases

in a group of 120 cats examined over a period of five years. Also Flint and others (1958) encountered 30 cases over a similar period of time. Harbutt (1963) working in Australia reported a low incidence of the disease in the state of Victoria.

In Britain, Seamer and Douglas (1959) found H. felis in the blood of only six out of 105 cats examined in a survey and none of these had the clinical disease. Bedford (1970) found the parasite in the blood of 16 cats in the London area and nine of these showed signs of clinical haemobartonellosis. Hayes and Priester (1973) reported that only about 0.9 percent (374 out of 43,514) of the cats seen in 11 Veterinary College hospitals/clinics in the U.S.A. were diagnosed as suffering from haemobartonellosis

Most reports of clinical haemobartonellosis concerned individual cases. There have been very few outbreaks of the disease (Harbutt 1963; Douglas 1970; Ojeda and Skewes 1978).

Seasonal Incidence

Little is known about the seasonal incidence of the disease. Results of studies made to determine this are conflicting. Holzworth (1956) reported that three-quarters of the cases seen occurred between September and January. Flint and others (1958) reported that though there was no marked seasonal variation, most cases occurred in autumn, winter and spring months. On the other hand Hayes and Priester (1973) found a slightly higher frequency of the disease in spring and summer months, with a peak in May.

Host Range

The infectivity of H. felis for species other than the cat has been studied experimentally. The parasite was not infective for rats, mice, swine, cattle, sheep or dogs (Splitter and others 1956, Flint, Roepke and Jensen 1959). However an anaemia resembling feline haemobartonellosis and associated with Eperythrozoon (Haemobartonella) has been diagnosed in three exotic felidae, a tiger-cat (Leopardus pardinoides), an ocelot (Leopardus pardalis) and a fishing cat (Prinonailurus viverrinus) (Kraft 1974). Thus it seems that the parasite is specific for cats and other felidae.

All breeds of domestic cats are susceptible to the infection. However a slight but significant increased risk to the infection has been reported in cats of mixed breed origin when compared with all breeds combined (Hayes and Priester 1973).

Age and Sex incidence

Naturally occurring feline haemobartonellosis is usually seen in young cats, one to three years old (Prier 1975) and it seems to be more common in males.

Holzworth (1956) noted that 75 percent of the 28 clinical cases of feline haemobartonellosis seen occurred in male cats and that half of the infected cats were under three years of age. But she pointed out that the distribution was closely parallel to that of sick cats in the hospital. Flint and others (1958) also reported that 28 of the 30 clinical cases encountered occurred in

male cats and that 22 of the cases were in cats between one and three years of age.

In the survey conducted by Seamer and Douglas (1959), five of the six cats whose blood had E. felis (H. felis) were males and five also were under three years of age. However it must be noted that 86 males and 19 females were examined and over half of the examined cats (58) were under three years of age.

From the foregoing, it can be concluded that the reports are most likely a reflection of the sex and age distribution of the animals presented or examined, as noted by Holzworth (1956). However Hayes and Priester (1973) using a controlled reference population reported a similar sex distribution. They found that the risk of haemobartonellosis for males was two and a half times that for females and that the risk of infection for all cats increased with age, reaching a maximum at four to six years and then declining.

Manuel and Abalos (1975) suggested that both sexes are equally susceptible but their results did not support this. They found that three of the 175 male cats examined (1.54%) and two of the 317 females (0.63%) had H. felis. These results suggest a higher risk in the male.

The higher incidence of infection in the male cat has been suggested to be due to the frequency of combat wounds which play a major role in the onset of the disease (Switzer 1971).

Transmission

The natural mode of transmission of feline haemobartonellosis has not been established. The disease has been transmitted experimentally by intraperitoneal inoculation of infected blood (Flint and Moss 1953; Splitter and others 1956; Flint and others 1959; Balazs and others 1961; Flagstad and Larsen 1969) as well as by the intravenous and oral routes using infected blood (Flint and others 1959).

Splitter and others (1956) reported that intraperitoneal inoculation of filtered serum obtained from acute cases and carriers of *H. felis* failed to produce infection in kittens. Injection of urine from an infected cat into a susceptible cat also failed to produce infection. Also it was not possible to produce infection by keeping susceptible cats in the same cage as carrier cats for 170 days.

It has been suggested that the natural infection may be spread by biting during cat fights (Flint and others 1958). Intra-uterine transmission has also been suggested (Harbutt 1963; Harvey and Gaskin 1977) and so has transmission by milk (Harvey and Gaskin 1977). The possibility of transmission by arthropod vectors such as sucking lice, ticks and fleas has been suggested (Holzworth 1956; Splitter and others 1956; Thomsett 1960; Prier 1975). None of these suggested modes of natural transmission of *H. felis* has been proved or established conclusively.

PATHOGENESIS OF FELINE HAEMOBARTONELLOSIS

Incubation period

Nothing is known about the incubation period of the clinical disease since *H. felis* has been detected in blood of apparently healthy cats (Kreier and Ristic 1968). Therefore data on incubation periods are only available for experimental infections. The results of earlier experiments have been reviewed by Kreier and Ristic (1968). The review showed a wide variation in the incubation periods obtained by different authors irrespective of the route of infection and quantity of inoculum used. The recorded periods ranged from two to 69 days. It was also found that splenectomy had little effect on the incubation period.

Other authors (Flagstad and Larsen 1969; Maede and Hata 1975; Harvey and Gaskin 1977) also obtained varying results in experimental infections. The periods ranged from two to 18 days.

From these reports, it is apparent that there is not enough information to relate the length of incubation period to the level of parasitaemia in and the dose of the inoculated blood. Harvey and Gaskin (1977) gave an indication of the level of parasitaemia in the blood they used as inoculum. However their results are not sufficient for any meaningful conclusion to be reached since only one level of parasitaemia was used for infecting the cats.

Effects of stress and coincidental disease

It is generally considered that clinically normal cats can be carriers of *H. felis* (Kreier and Ristic 1968).

Flint and Moss (1953) reported the development of haemobartonellosis in kittens given blood transfusions from a donor cat while Splitter and others (1956) and Flint and others (1959) produced the disease in cats by inoculation of pooled blood from clinically normal cats. Cats that have recovered from the disease may also become carriers (Loeb 1975; Harvey and Gaskin 1977).

It has been stated that carrier cats can develop active haemobartonellosis following periods of stress (Small and Ristic 1971; Schalm and others 1975; Mackey 1977). This stress can be produced by another disease or surgery (Fishler and Birzele 1974), poor nutrition, worms or coincidental infection (Anon 1972), fight wound abscesses, parturition, severe respiratory disease or other ill-defined illnesses (Switzer 1971). Flint and others (1958) put it thus "It is believed that many mature cats are carriers of the disease as a result of earlier exposure. The infection presumably lies dormant until some stress situation such as pregnancy, abscesses, neoplasia and other debilitating conditions, lowers host vitality and permits the disease to recur in its original virulent form as a severe parasitaemia and anaemia".

This statement apparently remained unproved but also unrefuted until 1978 when Harvey and Gaskin attempted to induce relapses in chronically infected cats (Harvey and Gaskin 1978a). Several methods were used to induce relapses in carrier cats that had recovered from the clinical disease. Parasitaemias were not observed in cats

following the administration of the immunosuppressive drugs cyclophosphamide and 6-mercaptopurine despite the fact that one of the cats that received the latter drug died as a result of drug administration. Parasitaemia was also not observed in two cats in which abscessation was experimentally produced, though one of them died of purluent pneumonia. Moderate parasitaemias were observed in splenectomised cats while parasitaemias were irregularly observed in cats given glucocorticoid steroids. However none of the cats developed clinical haemobartonellosis.

Another report has shown that prednisolone therapy also has no effect on the parasitaemia and the PCV in experimentally infected cats (Watson, Farrow and Hoskins 1978).

In view of the foregoing, though circumstantial evidences have been advanced to show that periods of stress may activate a latent infection, this is yet to be proved experimentally. It can therefore be assumed that stress conditions have little or no effect in activating *H. felis* infections. It is however possible that severely stressed cats may be more susceptible to *H. felis* infection than healthy cats.

Mechanism(s) by which anaemia develops in *H. felis* infection.

This has not been fully elucidated though it has been stated that erythrophagocytosis, and less importantly, intravascular haemolysis are responsible for the destruction of erythrocytes in *H. felis* infection (Harvey and Gaskin 1977).

In a study of the ultrastructure of H. felis with scanning electron microscopy, Jain and Keeton (1973) noticed small shallow pock-marks on some erythrocytes that were parasitised with H. felis. These marks were thought to represent lesions created by adherence of the parasite, which became apparent after its dislodgement. It was then conjectured that haemolysis would follow the development of these lesions at the site of parasitic attachment. In support of this theory, the authors argued that if the area of erosion on the erythrocytic membrane was large enough, haemoglobin would escape. On the other hand, while smaller erosions would not result in direct haemolysis, such injuries might create ionic imbalances which would lead to osmotic swelling of the erythrocytes and haemolysis might ensue.

Maede and Hata (1975) tried to elucidate the mechanism of anaemia development in H. felis infection by using experimentally infected cats. They found a decrease in erythrocyte counts, an increase of erythrocyte osmotic fragility, a positive direct Coomb's reaction in all the cats, and appearance of erythrophages in peripheral blood. These findings, according to the authors, were similar to those observed in naturally infected cats earlier on. In this study, the erythrocyte count decreased rapidly following the appearance of H. felis in the peripheral blood but it tended to return gradually to the previous level after the disappearance of the parasite from the erythrocytes.

The osmotic fragility on the other hand increased markedly after the first appearance of the parasite and continued to increase even when the parasite had disappeared. It was then conjectured that parasitised erythrocytes might be sequestrated in the spleen or some other organs of the reticulo-endothelial system. This was confirmed by later studies (Maede and Murata 1978; Simpson and others 1978, Maede 1979).

Maede and Hata (1975) further stated that some of the erythrocytes might return to the peripheral circulation after being freed of the parasite. Hence the increase in erythrocyte count following disappearance of the parasite. However, the cell may have been damaged by the parasite, the authors argued, and this might result in increased osmotic fragility.

Maede and Hata (1975) also found a further increase in osmotic fragility with frequent re-appearance of H. felis on the erythrocytes. This, the authors stated, seemed to be a result of increased numbers of damaged erythrocytes in the blood and it was felt that this might have led to the production of anti-erythrocyte antibody and the occurrence of phagocytosis by macrophages.

Later studies (Maede 1975) showed that erythrocytes showing increased osmotic fragility following H. felis infection had a short life span.

Simpson and others (1978) also attempted to explain the pathogenesis of the anaemia in H. felis infection in the light of their findings. Using electron

microscopy they found that *H. felis* caused smudging of erythrocytes and not erosion. They also found that often there was attachment of adjacent erythrocytes by an intervening series of *H. felis* organisms. Further, intra-erythrocytic crystalloid inclusions which were interpreted as being crystalloid haemoglobin were found in erythrocytes infected with *H. felis*. The number of these inclusions in each erythrocyte was directly proportional to the number of parasites present. It was then postulated that the attachment of erythrocytes together due to binding by intervening organisms would enhance erythrocyte sequestration in narrow vascular spaces and potentiate their phagocytosis by macrophages in the reticulo-endothelial system. It was also presumed that the intra-erythrocytic haemoglobin crystals would decrease the deformability of erythrocytes and thereby increase the likelihood of their phagocytosis.

It is apparent from these that *H. felis* damages the erythrocyte in one way or the other. This alters the structure of the erythrocyte and enhances its eventual phagocytosis or lysis.

CLINICAL FELINE HAEMOBARTONELLOSIS

Clinical signs

Clinical reports on cats with naturally occurring haemobartonellosis have been published by various authors (Flint and Moss 1953; Holzworth 1956, Flint and others 1958, Balazs and others 1961, Harbutt 1963, Wilkinson 1963 and 1965, Flagstad and Larsen 1969). Also clinical reports

on experimentally produced haemobartonellosis have been published (Splitter and others 1956, Flint and others 1959, Harvey and Gaskin 1977). The clinical signs of both the naturally occurring and the experimentally produced disease were similar.

The disease could be acute or chronic. The commonly observed symptoms in affected cats included listlessness, anorexia or inappetence for a variable period of time, lethargy, weakness and loss of weight. In some cases, the medical history included the presence of other conditions such as abscesses and bite wounds (Flint and others 1958, Flagstad and Larsen 1969): infections, internal parasitism, pneumonitis, nephritis and liver disease (Holzworth 1956); as well as stress factors (Schalm and Switzer 1972).

The most consistent finding was anaemia which was manifested by pallor of the visible mucous membranes. It has been stated that the onset of the anaemia was usually rapid (Switzer 1971). There was increased heart rate and the pulse was fast and feeble (Balazs and others 1961). The anaemia could be profound enough to produce anoxia with hyperpnoea, the cat tending to lie on its side and pant even after moderate exertion (Wilkinson 1969). Dyspnoea and cyanosis have been reported in a cat following exertion (Flagstad and Larsen 1969).

Mild to marked icterus has been reported in some cases (Holzworth 1963; Flagstad and Larsen 1969; Maede and others 1974) and the urine could be normal in colour,

or brown or contain traces of blood (Holzworth 1956).

Fever has been reported in some cases (Splitter and others 1956) but was not a consistent finding. Moribund cases usually had subnormal body temperatures (Flint and others 1958).

A palpably enlarged spleen (Holzworth 1956, Flagstad and Larsen 1969), and enlargement of the submaxillary lymph nodes with resultant dysphagia (Wilkinson 1963 and 1965) have been reported.

Fischer (1970) found *H. felis* in the blood of some anaemic cats that had retinal haemorrhages. However the role of *H. felis* as a primary aetiological agent of the severe anaemia in these cases was questioned. Prolapse of the third eyelid has been observed in some cases of feline haemobartonellosis (Harbutt 1963). Dehydration has also been reported in many cases (Harbutt 1963, Flagstad and Larsen 1969, Ojeda and Skewes 1978).

Without treatment, the disease could run a cyclic course with remission alternating with relapses (Loeb 1975).

Haematological findings

The degree of anaemia depended on the stage of the disease at which the cat was seen. The blood of affected cats could be light pink and watery (Flint and McKelvie 1955), and the PCV varied from eight percent to 20 percent (Schalm and Switzer 1972). There was a reduction in erythrocyte count and Hb value, and values as low as 1.2 million/cubic millimetre (Wilkinson 1965) and 1.5 gm per

100 ml of blood (Holzworth 1956) respectively have been reported.

As noted in the section on the aetiological agent, the parasite appeared and disappeared from circulation in a synchronised manner. In experimentally induced haemobartonellosis, it has been found that the PCV declined as the number of parasites increased, and in many cases increased dramatically following disappearance of parasites from the circulation (Harvey and Gaskin 1977). Splitter and others (1956) also found that blood values decreased most rapidly during, and immediately following the appearance of parasites in the blood, and sometimes increased slightly after their disappearance until the next parasitic invasion.

The anaemia in feline haemobartonellosis has been described as haemolytic (Wilkinson 1969, Mackey 1977), characterised by regenerative responses (Clark 1942, Perman 1977); and usually macrocytic and normochromic (Kreier and Ristic 1968; Small and Ristic 1971; Mackey 1977). However Flagstad and Larsen (1969) found a macrocytic but hypochromic anaemia in naturally and experimentally infected cats. According to Schalm and Switzer (1972), if the erythrocyte replacement was minimal, the anaemia would be normocytic, with the MCV remaining within the normal range.

Increased erythrocytic sedimentation rates (Flint and others 1959) and increased osmotic fragility of erythrocytes (Jain 1973, Maede and Hata 1975) have been reported.

In stained blood smears, anisocytosis, polychromasia and less frequently, poikilocytosis have been observed (Holzworth 1956, Flint and others 1958, Flagstad and Larsen 1969). Macrocytosis and an increase in the number of Howell-Jolly bodies also occurred (Wilkinson 1965, Mackey 1977). There was also reticulocytosis with the reticulocyte count as high as 85 percent (Watson and others 1978) and often approaching 100 percent (Mackey 1977). This was accompanied by extramedullary haemopoiesis, with numerous normoblasts (Flagstad and Larsen 1969, Mackey 1977) and occasional erythroblasts being present in circulation (Clark 1942, Mackey 1977).

A variety of leucocytic manifestations have been reported in clinical feline haemobartonellosis. Both leucopaenia and leucocytosis have been reported (Kreier and Ristic 1968, Harvey and Gaskin 1977). Wilkinson (1969) in a review of feline infectious anaemia stated that there was usually a leucocytosis with neutrophilia and lymphopaenia. Small and Ristic (1971) on the other hand stated in their review that leucocytosis and a normal leucocyte count could be encountered in two thirds of the cases during the acute phase of the disease but peucopenia might develop in the terminal phase. According to Mackey (1977), there may be neutrophilia early in the disease but this is usually followed by neutropaenia. In her experience, neutropaenia was a typical finding in acutely ill cats. In the cases reported by Holzworth (1956) normal leucocyte distribution and neutrophilia

occurred with about equal frequency. Lymphocytosis and occasionally monocytosis were observed in a few cases.

Schalm and Switzer (1972) believed that the total and differential leucocyte counts in feline haemobartonellosis were not sufficiently characteristic to be of value in arriving at a diagnosis. Both leucopaenia and leucocytosis might be encountered, but more commonly, the total leucocyte count remained within the normal range.

An increase in the platelet count has also been reported (Holzworth 1956).

Very little is known about the changes that occur in the plasma compostion in feline haemobartonellosis.

In experimental infections, Harvey and Gaskin (1977) found that the plasma protein content tended to be moderately elevated during the acute phase of the disease compared to pre-inoculation values and it often seemed to increase following increased parasitaemia. Also moderate elevations in the plasma icterus indices were observed in some of the cats during the acute phase of the disease. The authors also found normal plasma glucose levels in some of the cats tested though one other cat had a low plasma glucose level when it was moribund.

Ojeda and Skewes (1978) have reported normal calcium and phosphorus levels in one clinical case.

Morbidity in feline haemobartonellosis

Reports regarding the morbidity of the disease and its importance as a cause of anaemia are scanty.

Holzworth (1956) stated that about 25 percent of the anaemic cats studied were infected with H. felis. Cramer (1974) reported that only 10 out of the 140 cases of feline anaemia he studied were due to haemobartonellosis.

In an outbreak in a cattery of about 30 cats, Harbutt (1963) found 100 percent infection over a period of about 10 months while Ojeda and Skewes (1978) reported infection in seven out of the 11 cats in another outbreak in a cat colony.

Experimentally, 100 percent infection rates have been reported following inoculation of infective material into susceptible cats (Splitter and others 1956, Seamer and Douglas 1959, Harvey and Gaskin 1977). On the other hand only 56 of the 76 cats developed infection. Flint and Moss (1953) also reported an unsuccessful attempt to infect a cat.

Mortality in feline haemobartonellosis

Many of the reported cases of natural feline haemobartonellosis have been fatal (Clark 1942, Holzworth 1956, Flint and others 1961, Wilkinson 1965, Bedford 1969, Flagstad and Larsen 1969, Hataka 1972).

According to Holzworth (1956) about 33 percent of cats that suffered from the uncomplicated disease died, while about 75 percent of those in which the disease was complicated by other conditions died. Flint and others (1958) also reported about 75 percent mortality among the 30 clinical cases seen, 15 of them having been moribund when presented. Manusu (1962) stated that mortality from

feline haemobartonellosis in Australia was under 50 percent. Harbutt (1963) also working in Australia reported only two deaths in a cattery of 30 cats.

On the other hand, there are reports of cases in which all infected cats showing clinical signs of the disease recovered (Bedford 1970, Ojeda and Skewes 1978).

In the experimentally induced disease, Splitter and others (1956) reported 35 percent mortality among infected cats while Harvey and Gaskin (1977) recorded only one death among the eight cats that were inoculated. Flint and others (1959) also recorded some deaths among experimentally infected cats.

Necropsy findings

The necropsy findings in feline haemobartonellosis as reported by earlier workers have been reviewed by Kreier and Ristic (1968). More recent reports have been published by Flagstad and Larsen (1969), Bedford (1970), Hataka (1972), Tury, Papp and Horvath (1977), Leeflang, Kleyn and Mieog (1970).

The most common gross findings were emaciation, and anaemia as manifested by pallor of the tissues (Bedford 1970), the presence of transudate in the pericardial sac (Wilkinson 1965, Hataka 1972), and cardiac dilation and hypertrophy (Balazs and others 1961). While various degrees of splenomegaly have been described by most workers, Clark (1942) and Bedford (1969) found no evidence of such in their affected cats. Enlargement of the lymph nodes particularly the mesenteric was observed in some cases

(Holzworth 1956, Leeflang and others 1970, Hataka 1972, Tury and others 1977). The liver was pale and swollen in some cases (Balazs 1961, Wilkinson 1965).

The bone marrow was solidly red throughout the length of the diaphyses in the few cases where it was examined (Wilkinson 1965, Hataka 1972). Icterus was seen only occasionally (Holzworth 1956, Flagstad and Larsen 1969).

Histologically, the enlarged spleens showed a marked degree of haemopoiesis, follicular hyperplasia, erythrophagocytosis and haemosiderosis (Holzworth 1956, Flagstad and Larsen 1969, Hataka 1972). The liver parenchyma showed centrilobular degeneration and necrosis (Wilkinson 1965, Hataka 1972). Wilkinson (1965) regarded the hepatic necrosis as a feature of most anaemias irrespective of aetiology, and ascribed it to anoxia. Haemopoiesis in the hepatic parenchyma (Balazs and others 1961, Hataka 1972) as well as lymphocytic infiltration of the portal canal (Splitter and others 1956) have been reported.

The bone marrow showed increased erythropoietic activity (Holzworth 1956, Flint and others 1958, Flagstad and Larsen 1969) except in prolonged and refractory cases where the erythropoietic response became exhausted and there was evidence of bone marrow depletion (Holzworth 1956).

The enlarged lymph nodes showed hyperplasia and lymphadenitis (Holzworth 1956) and erythrophagia (Leeflang and others 1970).

Renal lesions described included fatty changes in the cortex (Clark 1942), foci of lymphocytic infiltration in the cortex and slight to extensive degeneration of tubular epithelial cells (Hataka 1972). The significance of these was not stated.

IMMUNOLOGICAL RESPONSES TO *H. felis* INFECTION

Little is known about immunological responses to *H. felis* infection. Splitter and others (1956) were unable to demonstrate antibody in the sera of cats experimentally infected with *H. felis* by a complement fixation test using infected blood as antigen, nor with *Anaplasma marginale*, *Eperythrozoon suis* or *Haemobartonella muris* antigens. On the other hand Small and Ristic (1967) stained *H. felis* parasites with fluorescent labelled antibody derived from cats that were experimentally infected with *H. felis*. However despite this demonstration of antibody production, infected cats could remain chronic carriers for many months. Since it is not known whether clinically recovered cats completely eliminate the parasite, it is also not known whether such cats are resistant to re-infection (Mackey 1977).

LABORATORY DIAGNOSIS OF *H. felis* INFECTION

Laboratory assistance in confirming the diagnosis of feline haemobartonellosis has depended mainly on the demonstration of the parasite, *H. felis*, in stained smears. This has been achieved by employing Romanowsky-type stains. While most workers have used Giemsa stain (Clark 1942,

Flint and McKelvie 1955, Splitter and others 1956 and other workers), Flint and Moss (1953) and Balazs and others (1961) used Wright's stain, Wilkinson (1965) Leishman's stain and Flagstad and Larsen (1969) the May-Grunwald-Giemsa staining method.

With the exception of Clark (1942) who described the parasite as staining a pale violet, most other workers portrayed H. felis as staining intensely basophilic with Romanowsky-type stains. Usually a shade of deep purple colour was obtained. Maede and Sonoda (1975) described the parasite as staining purple or faint violet with Giemsa stain.

Small and Ristic (1967) reported staining the parasite with acridine orange. With ultraviolet illumination, the parasites fluoresced a bright orange with an undertone of yellow-green. The same authors also ultilized the fluorescent-labelled antibody procedure to stain the parasite. When stained by the acridine orange or fluorescent antibody techniques the parasite appeared morphologically the same as in Giemsa stained preparations made by the authors except that rod forms were not observed. The authors believed that the fluorescent antibody and acridine orange techniques demonstrated the parasites in blood films in which Giemsa staining failed to reveal them. They found a high degree of correlation between the results obtained with acridine orange staining and fluorescent antibody technique and therefore stated that both methods were equally accurate for diagnostic purposes, though the former is non-specific.

As noted in the section on the aetiological agent, H. felis was not always present in the peripheral blood. Also it has been stated that the parasites could be removed from erythrocytes by chelating agents such as ethylene diamine tetra acetic acid (EDTA) (Harvey and Gaskin 1977, Hathaway 1976). It has therefore been suggested that cats may need to be examined daily for a week or more before the parasite can be observed (Harvey and Gaskin 1977) and that smears should be made from fresh blood samples (Penny 1978). These factors have limited the usefulness of the acridine orange and fluorescent antibody techniques. Furthermore the latter requires a source of labelled antibody, while small Howell-Jolly bodies will stain with acridine orange and must be differentiated from H. felis (Harvey and Gaskin 1977).

Sheriff (1974) reported positivity to Coombs' test in six cats that showed signs of feline haemobartonellosis. Two of the cats had detectable parasitaemias. Maede and others (1974) also reported that four of five cats in which H. felis was found showed positive results with the direct Coombs' test. All of these were natural infections.

In experimental infections, Maede and Hata (1975) obtained Coombs' positive results over a period of seven to 25 days following the appearance of the parasite in the blood. In another study, Maede (1978) obtained positive results to the direct Coombs' test in all cats tested during the acute phase of the disease (30-35 days

post-inoculation) while all the cats in another group which were tested in the early phase of infection (10-15 days post-inoculation) were negative.

The value of Coombs' test in the laboratory diagnosis of feline haemobartonellosis still has to be assessed by further studies since autoimmune haemolytic conditions, though rare in the cat, would also give positive results with Coombs' test.

TREATMENT OF FELINE HAEMOBARTONELLOSIS

A review of the treatments adopted for the early cases of feline haemobartonellosis has been made by Kreier and Ristic (1968). Other reports of treatment regimes and the success or otherwise of these have been published (Flagstad and Larsen 1969, Bedford 1969 and 1970, Ojeda and Skewes 1978, Watson and others 1978).

Various antimicrobial agents have been used with variable results. In most cases the treatment also included supportive therapy with blood transfusions, fluid electrolytes, vitamins and other haematinics.

Penicillin, liver extract, blood transfusions and vitamins were used in the successful treatment of a clinical case of feline haemobartonellosis (Flint and Moss 1953). Holzworth (1956) reported using penicillin, or combined penicillin and streptomycin, together with supportive therapy and obtained varying results. Flint and others (1958) considered penicillin and sulphonamides ineffective in the treatment of feline haemobartonellosis.

Arsenical compounds - neoarsphenamine (Flint and McKelvie 1955) oxyphenarsine hydrochloride (Flint and others 1958), oxyphenylarsen (Balazs and others 1961), thiacetarsamide sodium (Fishler and Birzele 1972, Watson and others 1978) have also been used with varying results. Fishler and Birzele (1972) claimed that thiacetarsamide sodium at a dosage rate of 0.5 ml/10 pounds body weight on two alternate days was effective in clearing the blood of H. felis. However Watson and others (1978) reported failure of this drug to clear the blood of H. felis even one month after treatment. The authors cited Harvey and Gaskin (1978b) as also reporting thiacetarsamide to be ineffective in removing H. felis from infected cats. Harbutt (1963) stated that arsenical therapy seemed to have little effect on H. felis. Besides, these drugs are said to be toxic in the cat (Flint and others 1959, Small and Ristic 1971).

Chloramphenicol and tetracyclines have been recommended for the treatment of feline haemobartonellosis (Switzer 1971, Loeb 1975, Prier 1975). Chloramphenicol given in doses of up to 100 mg twice daily for prolonged periods of up to 21 days was effective in the treatment of some clinical cases (Flint and McKelvie 1955, Wilkinson 1963, Flagstad and Larsen 1969). However this drug was found to be ineffective in other cases (Wilkinson 1965, Watson and others 1978). Seamer (1964) questioned the effectiveness of the drug in the treatment of feline haemobartonellosis. Also the drug is known to produce

toxicity in cats (Penny, Carlisle, Prestcott and Davidson 1970a) and may cause further anaemia.

Some good results have been obtained with tetracylines (Holzworth 1956, Wilkinson 1965, Bedford 1970). However in other cases, initial improvement was followed by relapses (Splitter and others 1956, Balazs and others 1961). Watson and others (1978) and Harvey and Gaskin (1978b) have reported that tetracycline was ineffective in eliminating H. felis infection.

Harbutt (1963) stated that antibiotics and cortisone had little effect on H. felis. Watson and others (1978) also reported the inability to raise the PCV in experimentally infected cats with prednisolone therapy.

Successful treatment of natural cases of feline haemobartonellosis with lincomycin at a dosage rate of 50 mg/kg body weight intramuscularly has been reported (Ojeda and Skewes 1978).

Flint and McKelvie (1955) highlighted the importance of blood transfusion in the successful therapy of feline haemobartonellosis. According to them, blood transfusions alone would save many cats, but frequently the animals would develop a chronic state of infection if no other treatment was given and would continue in a poor state of health. Some cats would eventually recover but others would relapse and die. Flint and others (1959) found that a number of cats with both clinical and experimental infection made uneventful recoveries after two or more blood transfusions without the aid of antibiotics.

They suggested that transfusions should always be the first therapeutic measure.

Harbutt (1963) further stressed the importance of supportive therapy in the treatment of feline haemobartonellosis. According to her, glucose, liver extract and Vitamin B appeared to give best results. Though this therapy did not reduce the number of parasites in the blood, the anaemia abated.

In view of the foregoing, it is apparent that more controlled evaluations of treatments for haemobartonellosis are required to establish the therapeutic value of the arsenical drugs and antibiotics. No records of treatments with antipiroplasm drugs like phenamidine isethionate, imidocarb dipropionate and diminazine aceturate were found.

RELATIONSHIP BETWEEN FELINE HAEMOBARTONELLOSIS AND FELINE LEUKAEMIA VIRUS (FeLV)

A higher than expected frequency of feline leukaemia has been found in cats following feline haemobartonellosis (Priester and Hayes 1973). Essex and others (1974) found that cats with haemobartonellosis have a higher frequency of FeLV infection than other cats in the same population. Also one case of haemobartonellosis with simultaneous FeLV infection has been reported (Bradley 1976).

The close relationship between feline haemobartonellosis and FeLV has been suggested to result from the activation of one disease by the other (Priester and Hayes 1973) and Mackey (1977) has suggested that the

immunosuppressive effects of FeLV may possibly predispose to H. felis infection.

FELINE HAEMOBARTONELLOSIS IN BRITAIN

The aetiological agent of feline haemobartonellosis was first reported in Britain in 1959 (Seamer and Douglas). In a survey carried out in Cambridge in 1958, the authors detected the parasite in the blood of six out of 105 cats examined giving an incidence of 5.7 percent. Five of the cats were males and the other one a neutered female. However this distribution parallels the sex distribution of the cats examined (86 males and 19 females). The parasite was provisionally designated Eperythrozoon felis (E. felis) "until such a time as the taxonomy and nomenclature of the parasites occurring in South Africa, America and Great Britain can be classified further". The parasites seen were mostly coccoid and annular. The authors succeeded in transmitting the parasites to four cats by intraperitoneal injection of infected blood and all the cats developed a mild anaemia. However no clinical case of haemobartonellosis was seen either in the naturally or experimentally infected cats. Five of the clinical cases showed evidence of ectoparasites though similar evidence was also found in about half of the cats examined in the survey.

Thomsett (1960) published results of a similar survey carried out in the London area. One hundred and twenty-six cats were examined but none of them was found to be infected with E. felis despite the fact that 76 of them

had ectoparasites - fleas, otodectes and lice. The author suggested that the parasites (E. felis) found in the earlier report by Seamer and Douglas (1959) might have been introduced into Britain through an infected cat imported from abroad.

However in 1963, the first clinical case of feline haemobartonellosis in Britain was reported from Norwich. (Wilkinson 1963). It occurred in a two year old domestic short haired neutered male cat. No evidence of ectoparasitism was found on the cat, and it was successfully treated with chloramphenicol at a dosage rate of 100 mg daily for 11 days. The author suggested that a centre of infection might exist in East Anglia since the only other report of the parasite (E. felis), came from the Cambridge area. Two more cases were reported by the same author two years later (Wilkinson 1965). Two male cats were involved and one of them died before any treatment could be given. In the other, initial treatment with chloramphenicol was not effective.and was replaced with chlortetracycline which proved effective.

The first clinical case of feline haemobartonellosis inthe London area was reported in 1969 (Bedford 1969). It occurred in a female cat. Chloramphenicol and Vitamin B12 therapy were ineffective and the cat died. The same author (Bedford 1970) detected the parasite (E. felis) in the blood of 16 cats inthe London area. In nine of the cats, the presence of the parasite was accompanied by signs of haemobartonellosis. All the nine cats were under 12 months

of age and seven of them were males.

In a review of the feline infectious anaemia, Wilkinson (1969) stated that the condition had been reported in Bristol, Cambridge, Glasgow, Liverpool and Norwich. However, the sources of the reports from Bristol, Glasgow and Liverpool were not stated nor found.

More recently in South Wales in a survey of the incidence of feline infectious anaemia, two out of the 15 cats examined showed evidence of *E. felis* (Jessop 1976). It was concluded that there was a low incidence of the disease in the area. The report stated that the acute form of the disease usually responded to a prolonged course (five weeks) of oxytetracycline coupled with supportive therapy.

CHAPTER II

SECTION I

MATERIALS AND METHODS

Animals

The animals used for this investigation were cats referred to the Medicine Department of Glasgow Universtiy Veterinary School between November 1979 and July 1980 inclusive. Records of age, sex, breed and the previous and immediate medical history of each cat were kept. Cat owners were closely questioned about any previous evidence of ectoparasite infestation. Each cat was examined and records kept of the clinical findings.

Following clinical examination, a blood sample was collected from each cat by jugular puncture as described by Hovell, O'Reilly and Povey (1970) using a 21 gauge one inch needle into a 5ml disposable syringe. Instead of the sleeve described by Hovell and others (1970) the cat was wrapped in a laboratory coat of strong cloth with only the head and neck exposed. Most of the cats were allowed to become accustomed to the surroundings before blood was collected and in some cases this was not done until about 24 hours after the cat had been admitted. Samples that contained clots were discarded and the procedure repeated.

Cats that appeared ill or were referred for observation were admitted to the hospital. They were examined daily and records of the rectal temperature, heart rate, respiratory rate, defaecation, urination, appetite and other clinical findings were kept. Blood

sampling was repeated in these cats at intervals of up to one week.

Out-patient cases referred for FeLV tests or minor conditions were re-examined subsequently and blood samples collected on each occasion.

Handling of blood samples

After collection of the blood sample into the syringe, smears were made on glass slides that had been previously cleaned as described below. The remainder of the sample was transferred into two plastic sample tubes (Brunswick, Essex) containing ethylene diamine tetra-acetic acid (EDTA), and lithium heparin as anticoagulants, for haematological analysis and FeLV testing respectively.

Haematology

Each sample was anlaysed as soon as possible on the day of collection. Where this was not possible, the sample was stored in a refrigerator and analysed the following day.

The packed cell volumes (PCV) or haematocrit, erythrocyte (RBC) and leucocyte (WBC) counts, and haemoglobin (Hb) determinations were carried out in an electronic counter (Coulter Counter, Model ZF6 (Coulter Electronics Ltd., Luton, Bedfordshire)). The WBC count was not corrected for nucleated erythrocytes.

The reticulocyte count was done as described by Cramer and Lewis (1972) using new methylene blue. A minimum of 500 red blood cells were counted and the reticulocyte count was the sum of the number of punctate

and aggregate forms. The reticulocyte count was not corrected for the PCV.

Platelet counting was done by the direct method as described by Schalm and others (1975) but using one percent ammonium oxalate as the diluting fluid and allowing 20 minutes for the platelets to settle.

Blood from anaemic cats was examined for Heinz bodies as described by Dacie and Lewis (1970) using methyl violet.

The mean corpuscular volume (MCV) and the mean corpuscular haemoglobin concentration (MCHC) were calculated from the PCV, RBC and Hb using the formulae below:

$$MCV = \frac{PCV\ (\%) \times 10}{RBC\ (10^6/ul)}$$

$$MCHC = \frac{Hb\ (g/dl) \times 100}{PCV\ (\%)}$$

The MCV was expressed in femtolitres (fl) and the MCHC in grams per decilitre.

Staining Procedures

Glass slides for making blood smears were cleaned and rendered grease-free by immersion in a two percent solution of a laboratory cleaning agent (Decon 90 - Decon Laboratories Ltd., Sussex) for at least 24 hours. They were then rinsed in tap water and wiped with soft laboratory tissue paper. The dry slides were immersed in absolute methanol until needed, when they were removed and dried with clean tissue before use.

Blood smears were made from fresh samples as mentioned earlier and more smears were made in the laboratory with the EDTA sample.

Routinely, the smears were stained with Leishman and Giemsa stains, and by the May-Grunwald-Giemsa and acridine orange methods. Fresh stain solutions were prepared each day and the stock solutions were filtered with ashless filter paper (Whatman 44 Ashless - Whatmans Ltd., England) before dilution.

Giemsa staining was done as described by Schalm and others (1975) using a ten percent solution of the stock stain solution (BDH Chemicals Ltd., Poole) in distilled water and staining for 30 minutes. Leishman staining was done as described by Archer (1977). The smear was fixed in the undiluted stock solution (BDH Chemicals) for two minutes and then stained in the double diluted solution for 10 minutes.

The May-Grunwald-Giemsa procedure was also followed as described by Archer (1977) using stock solutions made by BDH Chemicals. The smear was fixed in absolute methanol for 10 minutes and the staining in Giemsa was done in two stages. Two Coplin jars of 10 percent Giemsa solution were used. The smear was stained in each for 15 minutes with a brief rinsing in phosphate buffer (pH 7.2) for a minute in between.

Acridine orange staining was done as described by Small (1971).

Examination of Smears

Stained smears were allowed to drain dry before being examined. They were examined with a Leitz Orthoplan research microscope (Leitz Instruments Ltd., Luton).

Those stained with the Romanowsky stains (Leishman, Giemsa and May-Grunwald-Giemsa) were examined in bright field with an oil-immersion objective (x100). About 100 fields, extending across the length and the width of each smear were examined for parasites and cellular morphology studies.

Differential leucocyte counts were carried out on the May-Grunwald-Giemsa preparations. A total of 200 nucleated cells were counted.

For examining the acridine orange preparations, the nose piece of the microscope was replaced with a fluorescence vertical illuminator (Leitz Instruments Ltd.). The smears were illuminated with ultraviolet light (blue-light excitation) and examined in incident light. The smears were examined for the presence of H. felis in the erythrocytes, care being taken to differentiate these from small Howell-Jolly bodies.

FeLV testing

The heparinised blood samples were submitted to the virology laboratory of Glasgow University Veterinary School for FeLV testing. The samples were first tested with a commercially prepared testing kit (Leukassay F, C-Vet, Suffolk). All those that gave positive or doubtful results were subjected to further testing by virus isolation using techniques which have been developed in the laboratory.

Statistical analysis

The results were analysed using the standard Chi-squared test (2 x 2 contingency table) with $P \leq 0.05$ as the acceptable level of significance.

SECTION II

SURVEY OF ANAEMIA IN CATS REFERRED TO THE UNIVERSITY OF GLASGOW VETERINARY HOSPITAL

MATERIALS AND METHODS

The materials and methods used for this investigation were as described in Section 1.

Anaemia was determined by the results of haematological analysis for each cat rather than by physical observations. For the purpose of this investigation, the anaemias were divided into two groups, namely marked anaemia and mild anaemia. A marked anaemia was one in which the PCV was below 25 percent while the PCV in mild anaemia was between 25 percent and 29 percent. The normal haematological values for healthy cats which were used for this investigation were as follows:

PCV (%): 30 - 50

MCV (fl): 39 - 60

MCHC (g/dl): 30 - 36

Reticulocyte Count (%): 0 - 10.8 (Cramer & Lewis 1972)

Normoblast Counts (%): Rare

WBC ($x10^3/\mu l$): 8 - 20

Absolute Neutrophils /µl: 2,500 - 12,500

Absolute Lymphocytes /µl: 2,000 - 7,000

Absolute Monocytes /µl: 0 - 600

Absolute Eosinophils /µl: 0 - 750

Leucocyte precursor cells: Rare

In this investigation, the absolute leucocyte counts were calculated by using the differential and the total

leucocyte counts as shown below. The absolute neutrophil count for example, was obtained by the following formula:

$$\text{Absolute neutrophils} = \frac{\text{Differential neutrophil count}}{100} \times \text{WBC}$$

The clinical diagnoses in this investigation were confirmed by other relevant investigative techniques and necropsy findings whenever possible.

RESULTS

A total of 155 cats were examined and this number included 114 domestic short haired (DSH), three domestic long haired, 27 Siamese, four Persian, five Burmese, one Chinchilla and one Havana. There were 84 males and 71 females of which 23 and 38 respectively had not been neutered. The ages of the cats ranged from one month to 16 years. Table 2:1 shows the age and sex distributions of the cats examined.

Prevalence of anaemia

Anaemia was detected in 41 of the cats examined. This represented 26.45 percent of the total. Marked anaemia occurred in 28 cats (18.07%) while the remaining 13 cases (8.38%) were mild anaemias. Figure 2.1 shows the percentage distribution of anaemia within the population of cats examined in each age group.

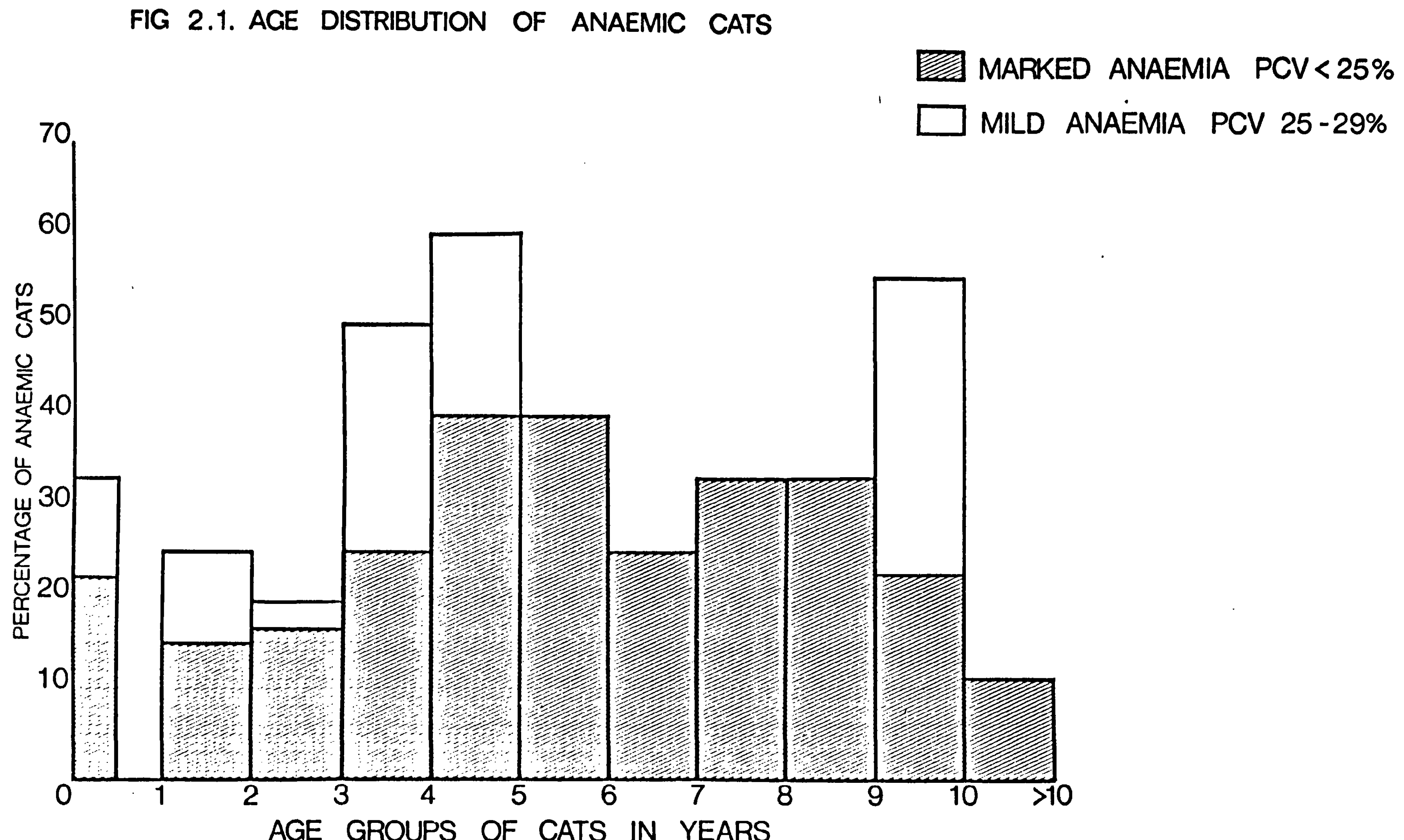
Anaemia was observed in 26 (30.95%) of the males and 15 (21.13%) of the females giving a male to female percentage ratio of approximately 3:2. Five males and seven females in the anaemic group had not been neutered. Marked anaemia was present in 20 of the males and eight

TABLE 2.1

Age and Sex distribution of cats examined

AGE (in Years)	MALE	FEMALE	TOTAL
<0.5	2	7	9
0.5 - 0.9	7	14	21
1.0 - 1.9	14	10	24
2.0 - 2.9	21	15	36
3.0 - 3.9	7	5	12
4.0 - 4.9	1	4	5
5.0 - 5.9	5	5	10
6.0 - 6.9	5	3	8
7.0 - 7.9	5	1	6
8.0 - 8.9	5	1	6
9.0 - 10.0	5	4	9
Over 10.0	7	2	9

FIG 2.1. AGE DISTRIBUTION OF ANAEMIC CATS



of the females giving a male to female percentage ratio of approximately 2:1. Figure 2.2a shows the male to female percentage ratio among the anaemic cats in each age group.

Anaemia was not observed in any of the 21 cats aged between six and 11 months or in any of the female cats aged six to seven and a half years, or those over 10 years old. Marked anaemia was more prevalent in males than females in the cats under six months of age and those between one and seven and a half years old, while the reverse was the case in cats eight to 10 years old (Figure 2.2b). On the other hand mild anaemia was more prevalent in females than males in all the age groups in which it occurred except in cats nine to 10 years old. Mild anaemia did not occur in any of the cats between the ages of five and eight and a half years and those over 10 years old (Figure 2.2c).

There were 31 cases of anaemia among the domestic (common) cats, seven among the Siamese and three among the other breeds put together, thus giving breed prevalence percentages of 26.5, 25.9 and 27.3 respectively. Marked anaemia was present in 22 of the cases among the domestic (common) cats, four among the Siamese and one among the other breeds.

FIG 2.2a. MALE TO FEMALE RATIOS AMONG ALL THE ANAEMIC CATS

FIG 2.2b. MALE TO FEMALE RATIOS AMONG CATS WITH MARKED ANAEMIA

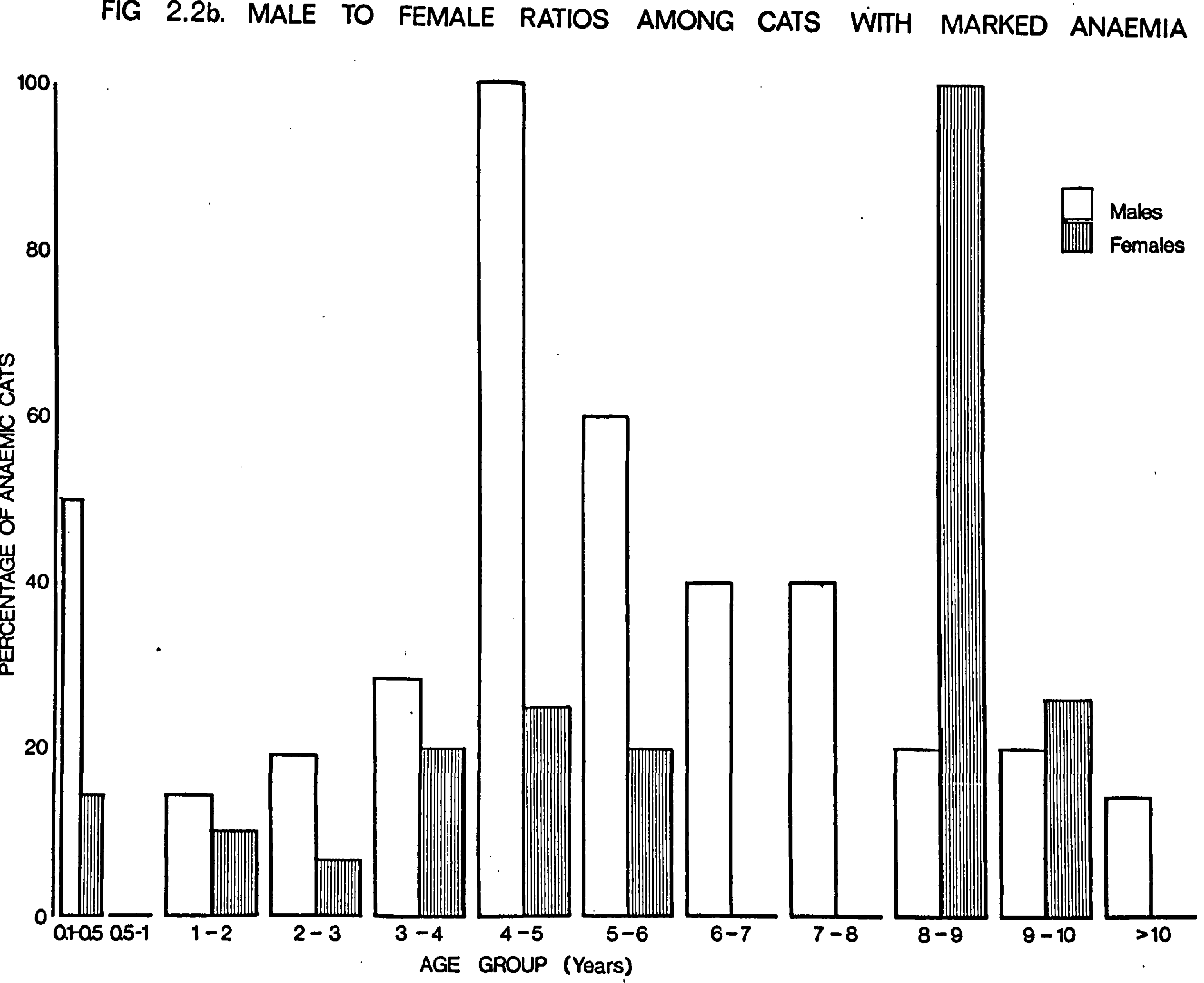


FIG 2.2c. MALE TO FEMALE RATIOS AMONG CATS WITH MILD ANAEMIA

Possible aetiology of the anaemias

The final diagnoses in the 41 cases of anaemia observed are listed in Table 2.2

Concurrent H. felis and FeLV infections were responsible for the largest number of marked anaemias (42.9%). Next to this were the anaemias caused by FeLV infection on its own (25%) while H. felis on its own accounted for 14.2 percent of the marked anaemias.

Among the mild anaemias, H. felis infection was responsible for the largest number (38.5%), while concurrent H. felis and FeLV infections, FeLV on its own, and renal diseases together accounted for the same number as H. felis infection.

No diagnosis could be made in one case each of marked anaemia and mild anaemia.

The various possible causes of the anaemias were well distributed within the age groups between one and five years while H. felis associated anaemias accounted for the majority of cases in cats over five years old. (Table 2.3).

The FeLV associated anaemias

This section deals with the cases of anaemia in which FeLV infection, probably complicated by other conditions but excluding H. felis infection, was diagnosed. There were eight such cases, only one of which was a mild anaemia. All the seven cats with marked anaemia were males (four were neutered). Six of them were domestic short haired cats and the other a Siamese cat. The case of mild anaemia was observed in a female domestic short haired cat.

TABLE 2.2

Final Diagnosis in the 41 cases of Anaemia

	Diagnosis	No. with Marked Anaemia	No. with Mild Anaemia
1.	*H. felis* infection	3	5
2.	*H. felis* and myeloid leukaemia	1	-
3.	*H. felis* and FeLV infections	12	2
4.	FeLV infection	7	1
5.	Renal diseases	2	2
6.	Heinz body anaemia	1	-
7.	F.I.P. + hepatic and pancreatic carcinoma	1	-
8.	Flea infestation and polyarthritis	-	1
9.	Peritonitis	-	1
10.	Undetermined	1	1

TABLE 2.3

Age Distribution of the 41 cases of Anaemia

AGE (In Years)	*H. felis* Infection		*H. felis* + FeLV Infections		FeLV Infection		Other Causes	
0.1 - 0.5	-		-		1	(1)	1	
0.5 - 0.9	-		-		-		-	
1.0 - 1.9	-	(2)	-		-		1	(1)
2.0 - 2.9	-		3	(1)	2		-	(1)
3.0 - 3.9	-	(1)	1	(1)	1		1	(1)
4.0 - 4.9	-	(1)	1		1		-	
5.0 - 5.9	-		3		1		-	
6.0 - 6.9	-		1		-		1	
7.0 - 7.9	1		1		-		-	
8.0 - 8.9	-		1		-		1	
9.0 - 10.0	1	(1)	1		-		-	(2)
>10.0	1		-		-		-	

NOTE: The figures in brackets are the numbers of cats with mild anaemia while the other figures represent marked anaemia.

The FeLV associated anaemias occurred in cats less than six months old and in those between one and five and a half years of age. None of the cats over five and a half years old had FeLV associated anaemias (Table 2.3).

The clinical signs observed in the cats were mostly attributable to anaemia. These were dullness, pallor of the visible mucous membranes, dehydration, anorexia of between one and five days, weight loss, recurrent pyrexia and increased heart rates. The heart rates were mostly between 200 and 230 beats per minute. A distinct systolic murmur and hyperpnoea were observed in one of the cats (No. 11). The other sign observed in these cases was enlargement of lymph nodes. In three cats (Nos. 24, 133 and 143), the submaxillary lymph nodes were enlarged, and one of the cats (No. 24) also had firm enlarged kidneys. Palpably enlarged mesenteric lymph nodes were observed in cat 11.

Haematological analyses on admission showed a macrocytic and slightly hypochromic anaemia in cat 11, normochromic and hypochromic anaemias in cats 78 and 99, while the other five cats had normocytic and normochromic anaemias with no evidence of bone marrow regeneration (Table 2.4a).

The reticulocyte and normoblast counts in all the cats were within the adopted normal ranges for healthy cats. The total leucocyte counts were within the normal ranges in six of the cats. The other two cats (Nos. 133 and 148) had marked and mild leucocytosis respectively.

TABLE 2.4a

Some Initial Haematological Values in Anaemic Cats Infected with FeLV

CAT No.	PCV (%)	MCV (fl)	MCHC (g/dl)	Reticulocyte Count (%)	Normoblast Count (%)	WBC ($x10^3/\mu l$)	Neutrophils (No./µl)	Lymphocytes (No./µl)	Monocytes (No./µl)	Eosinophils (No./µl)	Granulocytic percursors (%)	Lymphocytic percursors (%)
11	14.6	65	28	6	-	9.9	7,326	1,881	198	99	-	-
24	14.2	43	33	1	-	20.0	3,600	3,000	40	-	-	62
74	22.5	49	30	1	-	12.4	11,656	496	24	-	-	-
78	6.9	54	19	2	-	18.9	U N D I F F E R E N T I A T E D				80	-
99	7.9	48	25	1	-	6.4	4,800	1,408	13	6	-	-
133	19.6	51	30	4	<1	52.6	41,554	7,890	53	53	3	-
148	10.6	56	37	1	-	21.7	20,181	651	22	0	3	-
143	27.6	57	30	1	-	8.7	87	5,829	2,001	0	-	-

TABLE 2.4b

Concurrent clinical diagnoses and outcome in eight cases of FeLV associated anaemias

CAT No.	CONCURRENT DISEASES	No. OF TRANSFUSIONS	OUTCOME
11	Alimentary lympho-sarcoma	-	Died
24	Glomerulonephritis and lymphoid leukaemia	1	Euthanasia
74	Feline infectious peritonitis	-	Died
78	Leukaemia (undifferentiated)	1	Died
99	Aplastic anaemia with ossification of bone marrow	2	Euthanasia
133	Lymphademopathy	-	Alive
148	Chronic myeloid leukaemia	1	Euthanasia
143	Lymphadenopathy	-	Alive

The leucocytosis in cat 133 was accompanied by a marked neutrophilia, moderate lymphocytosis, and the presence of myelocytes and metamyelocytes in the blood smears; while that in cat 148 was accompanied by a neutrophilia and lymphopenia. Cat 99 had a leucopaenia but the differential leucocyte counts were normal. Promeylocytes, myelocytes and metameylocytes were observed in the blood smears from cats 78 and 148 while lymphoblasts and prolymphocytes were present in the smears from cat 24 (Table 2.4a).

The anaemic cats received whole blood transfusions (50ml intravenously) as part of the supportive therapy. Cat 99 showed a transient improvement after the first transfusion but relapsed and a second had to be given a week later.

Six (75%) of the cats with FeLV associated anaemias eventually died or were destroyed in extremis (Table 2.4b). Only two of the cats, one of which had mild anaemia, were still alive at the conclusion of this investigation.

The clinical entities diagnosed in these cases were alimentary lymphosarcoma, lymphoid, myeloid and undifferentiated leukaemias, and an aplastic anaemia with ossification of the bone marrow (Table 2.4b). The infection in cat 24 was complicated by glomerulonephritis and that in cat 74 by feline infectious peritonitis (F.I.P.).

In this investigation FeLV infection was found in eight other cats but they were not anaemic. The ages of the cats ranged from six months to eight years, with a gap from three to seven years. The clinical signs observed in these cats were swollen and reddened conjunctivae, protrusion of the 3rd eyelid, iridocyclitis, sudden onset of blindness, respiratory distress and enlargement of the submaxillary lymph nodes (one case). Apart from FeLV infection one of the cats had F.I.P. and glomerulonephritis and another had thymic lymphosarcoma. The former died and the latter as euthanised.

The other six cats were still alive at the conclusion of this investigation.

Anaemias associated with renal diseases

Anaemic cats with renal diseases were diagnosed as having chronic nephritis (three cases) and membranous nephropathy (one case). The affected cats (Nos. 75, 106, 149 and 38) were three, six, eight and 10 years old respectively and were all neutered males. Marked anaemia was observed in two of the cats with chronic nephritis (Nos. 106 and 149) while the other two had mild anaemias (Table 2.5). The condition in cat 106 was complicated by an acute gastritis and that in cat 38 by intestinal adenocarcinoma. The anaemias in all the cases were normocytic and normochromic with no evidence of bone marrow regeneration (Table 2.5). Cat 38 had a moderate leucocytosis and accompanying neutrophilia while cat 75 had a leucopaenia.

TABLE 2.5

Initial Haematological Values in Some Anaemic Cats

CAT No.	DIAGNOSIS(ES)	PCV (%)	MCV (fl)	MCHC (g/dl)	Reticulocyte Count (%)	WBC ($x10^3/\mu l$)	Neutrophils (no./μl)	Lymphocytes (No./μl)	Monocytes (No./μl)	Eosinophils (No./μl)	Outcome
106	Chronic nephritis + Acute gastritis	20.9	48	33	-	19.9	12,139	7,363	398	-	Died
149	Chronic nephritis	22.9	45	35	-	13.0	12,090	780	130	-	Died
75	Membraneous nephropathy	27.6	52	31	-	6.7	4,02o	2,278	201	201	Euthanasia
38	Chronic nephritis + Intestinal adenocarcinoma	27.4	50	35	<1	28.0	24,920	1,960	280	840	Euthanasia
139	Heinz body anaemia and old instestinal intususception	23.4	48	32	<1	15.3	9,639	4,437	306	612	Died
48	Chronic FIP and Carcinoma	18.5	70	36	6	41.8	32,604	1,672	1,254	-	Euthanasia
30	Heavy flea infestation and Polyarthritis	29.0	47	29	<1	7.7	5,775	1,540	308	77	Alive
141	Peritonitis	24.5	51	31	<1	81.6	75,092	4,896	816	-	Died
134	No diagnosis	29.0	43	38	<1	11.4	8,208	1,938	456	798	Alive
135	No diagnosis	6.8	38	32	-	16.6	11,620	4,316	332	166	Died

The two cats with marked anaemia died and the other two were euthanised.

Heinz body anaemia

This occurred in an 18 month old neutered female domestic short haired cat (No. 139). The cat was bright but very thin and was losing weight despite a voracious appetite. The visible mucous membranes were pale and a firm mass was palpable in the abdomen. Exploratory laparotomy revealed a thick, fibrosed intussusception in the distal jejunum. This was removed and the intestine anastomosed. The cat died the day following the laparotomy.

Haematological analysis on admission showed a normocytic and normochromic anaemia (Table 2.5). The total and differential leucocyte counts were normal. Smears from the blood showed anisocytosis, poikilocytosis and polychromasia. Large Heinz bodies were present in over 50 percent of the erythrocytes when the blood was supravitally stained with methyl violet.

At necropsy, the marrow of the long bones was red but there was no evidence of extramedullary haemopoiesis in the spleen or liver.

Other anaemias

A marked anaemia was observed in a three year old female domestic short haired cat (No. 48) that had F.I.P. as well as hepatic and pancreatic carcinoma. The anaemia was macrocytic and normochromic. There was marked leucocytosis and an accompanying neutrophilia with a left shift (band neutrophils = 2,508/μl) as well as monocytosis.

The cat was euthanised.

Heavy flea infestation was associated with a transient mild anaemia that was found in a nine year old female cat (No. 30) which also had polyarthritis. After treatment with an insecticidal aerosol spray (Nuvan Top - Ciba Geigy, Cambridge) the haematological values returned to normal within a week and were normal for three subsequent samplings. The anaemia was normocytic and slightly hypochromic.

A normocytic and normochromic anaemia was observed in cat 141 (Table 2.5). The cat had a non-effusive peritonitis following previous ovariohysterectomy. There was marked leucocytosis and an accompanying neutrophilia.

The anaemias associated with H. felis and concurrent H. felis and FeLV infections are dealt with in the next chapter.

DISCUSSION

In this investigation, both mild and marked anaemias were noted. This is in contrast to other studies in which only marked anaemias were noted (Cramer 1974, Anon 1978, Cotter 1979). Therefore the results for marked and mild anaemias were presented separately where necessary.

The prevalence percentage for the marked anaemia found in this study was 18.1 percent and this falls within the approximate ranges of 15-25 percent that have been reported in America (Anon 1978).

The male to female ratio of three to two among all the anaemic cats and two to one among the cats with marked anaemia suggest a higher though insignificant ($P > 0.05$) prevalence of anaemia in males than female cats. No reports of sex distribution for all types of anaemia together in the cat has been found.

Anaemia did not occur in cats aged six to 11 months which constituted 13.5 percent of the total number examined, neither in the female cats six to seven and a half years old nor those over 10 years. The reason for this is unknown. More studies are required to determine if this is the actual field situation or just a peculiarity of the cases available for this investigation.

The domestic (common) cats had a slightly higher prevalence of anaemia than the Siamese breed. More studies are required to establish the breed prevalence of incidence of anaemia in the cat.

Over 70 percent of all anaemia cases and almost 70 percent of the marked anaemias occurred in cats less than six years old. The different possible causes of anaemia diagnosed in this study were well distributed in this group of cats whereas most of the anaemias in cats over six years (8/12) were associated with H. felis infection.

In this study, marked anaemia occurred twice as often as mild anaemia probably because mild anaemia occurred only in cats less than five years old and in those nine to 10 years of age. After an initial drop from the

level in cats less than six months old to zero in cats six to 11 months old, marked anaemia increased with age reaching a peak at four to six years. It appears that the six to six and a half year old cats in this study had a lower than expected prevalence of marked anaemia and the seven to eight and a half year olds had a higher than expected prevalence. More studies with larger populations of cats are required to establish the true age distribution of anaemia in domestic cats.

The causes of anaemia diagnosed in this study have been reported in the literature (Holzworth 1956, Wright 1973, Cramer 1974, Mackey 1975, Hathaway 1976, Schalm 1977). Cramer (1974) reported that haemobartonellosis, nephritis and F.I.P. were diagnosed in 7.1 percent, 5.7 percent and 3.6 percent respectively of the 140 cases of feline anaemia he studied. Holzworth (1956) on the other hand reported that haemobartonellosis was diagnosed in about 25 percent of the 120 cases of feline anaemia studied. While the percentage of marked anaemia found in association with F.I.P. in this study was similar to that reported by Cramer (1974), renal diseases accounted for a slightly higher percentage than he reported. The percentage of marked anaemia in which H. felis was diagnosed was much higher than those reported by the two authors (Holzworth 1956, Cramer 1974). FeLV infection was diagnosed in approximately 68 percent of the marked anaemia cases in this study. This is similar to that reported by Cotter (1976). She found that approximately 70 percent of cats with anaemia in a large series of clinical cases were positive for FeLV.

The clinical manifestations of FeLV observed in this study were similar to those that have been reported (Jarrett, Anderson, Jarrett, Laird and Stewart 1971, Hoover and Kociba 1974, Mackey and others 1975, Mackey 1975). Glomerulonephritis and F.I.P. have been found in field cases of FeLV infection (Cotter, Hardy and Essex 1975) while glomerulonephritis has been reported in experimental infections (Mackey 1975). Both conditions were found in association with FeLV infection in this study.

While most FeLV associated anaemias have been normocytic and normochromic (Mackey 1975, Cotter 1979), macrocytic and normochromic anaemia has been reported (Mackey 1975) and bone marrow hyperplasia has been found in a few cases of FeLV associated anaemias (Cotter 1979). In this study, most of the FeLV associated anaemias were normocytic and normochromic. However, a macrocytic and hypochromic anaemia was found in a cat with alimentary lymphosarcoma, while normocytic and hypochromic anaemias were found in two cats, one of which had undifferentiated leukaemia; and in the other the bone marrow had been replaced by bone tissue. The leucopaenia seen in the last cat (No. 99) was a reflection of the general pancytopaenia. The leucocytosis found in cats numbers 148 and 133 as well as the presence of leucocyte percursors in the peripheral blood of these cats, and cats numbers 24 and 78 were manifestations of leukaemia.

Anaemia had been reported in cats with renal diseases (Holzworth 1956, Cramer 1974, Nash, Wright, Spencer, Thompson and Fisher 1979). As was confirmed in this study,

anaemia, due to chronic renal disease is non-regenerative (Perman 1977).

The one case of Heinz body anaemia in this investigation was probably due to auto-intoxication as a result of the intestinal intussusception. A similar case due to intestinal obstruction caused by a piece of plastic has been reported (Schalm 1977). Though Heinz body anaemia is classified as haemolytic (Schalm 1977) the haematological analyses in field cases have usually shown a normocytic and normochromic anaemia (Schechter and others 1973, Boon and Rich 1974). In the cases reported by Schechter and others (1973) reticulocytosis became evident only about a week after the onset of anaemia despite the presence of normoblasts in the peripheral blood of one of the cats from the onset. In the case found in this study, the anaemia was normocytic and normochromic and the reticulocyte counts were very low.

Severe flea infestation has been reported as a cause of blood loss anaemia (Cramer 1974). Heavy flea infestation was associated with a transient mild anaemia found in this study. However, it must be pointed out that only 18 of the 36 flea infested cats found in this study were anaemic. The anaemia in the other 17 cases was associated with H. felis or FeLV infections. The anaemia in these cases was normocytic and hypochromic. Though blood loss anaemia is normocytic and normochromic, it has been pointed out (Mackey 1977) that if iron is continually lost from the body the anaemia may become hypochromic. This may be the situation in the case observed.

Anaemia commonly occurs secondarily to chronic illness in cats (Wright 1973, Hathaway 1976). Thus the anaemias found in association with peritonitis and F.I.P. were probably secondary to these diseases. The leucocytosis and accompanying neutrophilia found in both cases suggested this.

The mortality in the cases of anaemia considered in this chapter was very high. Seventy-five percent of the FeLV associated anaemias and 80 percent of the other cases of anaemia died or had to be euthanised. However the contribution of anaemia to the mortality rates is not really known as some of the cats had concurrent diseases that might have been responsible for their death. Holzworth (1956) reported a mortality rate of about 80 percent among the 120 anaemic cats studied.

CONCLUSIONS

This study has shown that anaemia may be responsible for up to 25 percent of clinical cases seen in cats. Anaemia tends to be more prevalent in male cats than females and it increases in prevalence with age reaching a peak at four to six years. It has a slightly higher frequency of occurrence in domestic (common) cats than in Siamese cats.

The largest number of anaemias occurred in association with either FeLV or concurrent H. felis and FeLV infections. However some of the cases were due to H. felis infection, renal disease and intestinal obstruction. These should be considered in the differential diagnosis of anaemia in cats. Other conditions such as F.I.P. should also not be overlooked as causes of secondary anaemia.

Though mortality rates in the anaemias considered in this chapter were high, the actual number of deaths due to anaemia alone is unknown.

CHAPTER III

STUDIES ON *HAEMOBARTONELLA FELIS* (*H. felis*) INFECTION

STUDIES ON HAEMOBARTONELLA FELIS (H. felis) INFECTION

MATERIALS AND METHODS

These were as described in the previous chapter.

In addition, where a cat from a multi-cat household had H. felis infection, the other cats in the household were examined and sampled for infection.

A star system was used for denoting the level of parasitaemia as shown below:

1* Less than 10 percent erythrocytes infected

2* Up to 25 percent erythrocytes infected

3* Up to 50 percent erythrocytes infected

4* Up to 75 percent erythrocytes infected

5* Over 75 percent erythrocytes infected

RESULTS

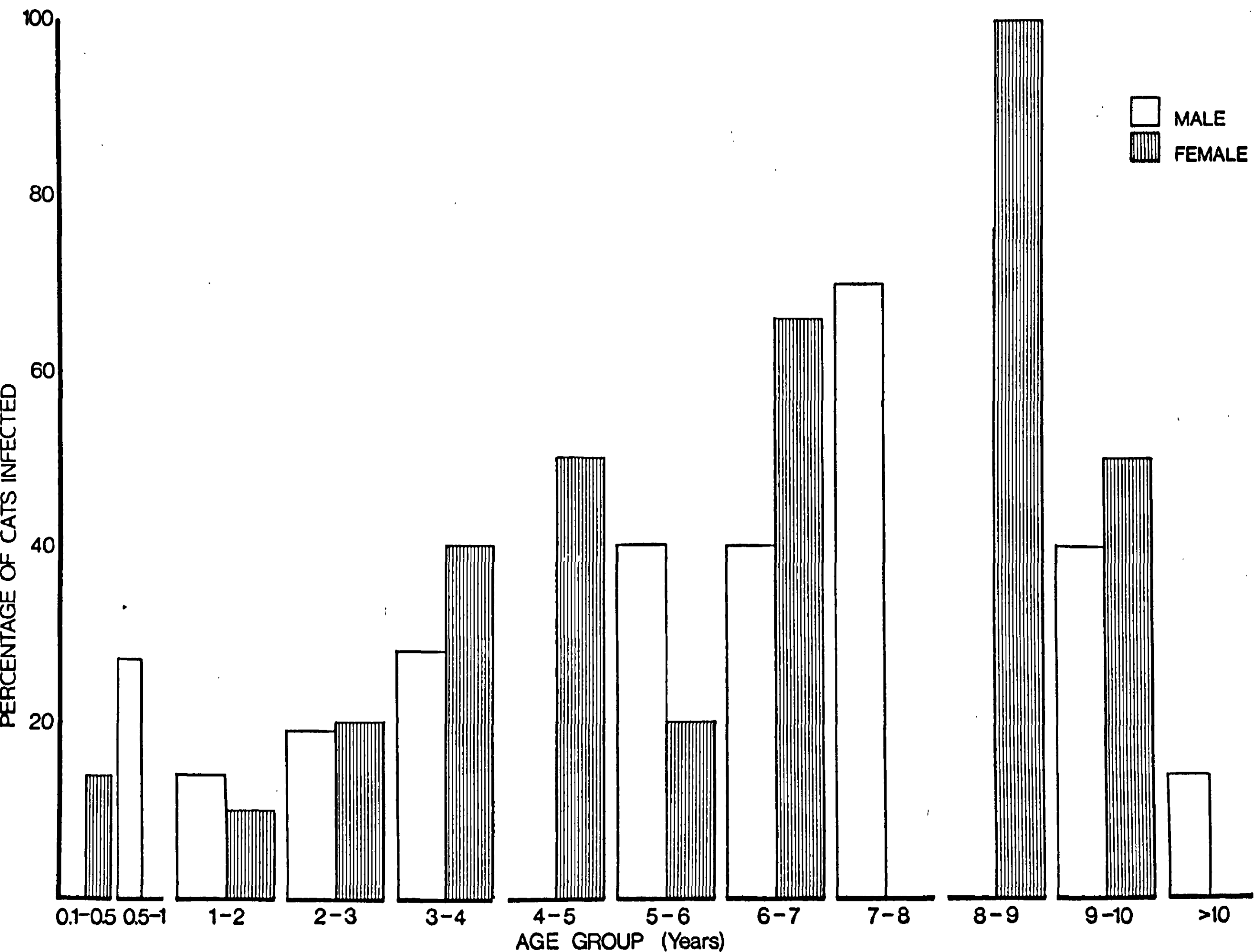
Epidemiological aspects of H. felis infection

H. felis was detected in the blood of 36 (23.25%) of the 155 cats examined. The infection occurred in all age groups and the rate of infection generally increased with age reaching a peak at seven to seven and a half years and then declining (Figure 3.1).

Twenty-one of the infected cats were males and 15 were females. Thus 25 percent of the males and 21.1 percent of the females examined had H. felis infection, giving a male to female percentage ratio of approximately 1.2 to 1.0. Figure 3.2 shows the male to female ratio among infected cats in each age group. Of the 26 infected cats, 30 were domestic (common) cats, five were Siamese and one a Persian cat.

FIG 3.1 AGE DISTRIBUTION OF *H. felis* INFECTION

FIG 3.2. MALE TO FEMALE RATIOS AMONG *H. felis* INFECTED CATS



Fleas were found on 15 of the infected cats and Otodectes sp.on another, while seven others had a history of flea infestation. Thus approximately 64 percent of the H. felis infected cats had external parasites either at the time of examination or previously. However 21 of the uninfected cats had fleas when examined, two had a history of flea infestation and five were infested with Otodectes. Thus 23 (approximately 45%) of the 51 cats that had external parasitism were infected with H. felis. Using the four-fold analysis table and the standard chi-squared test, it was found that the prevalence of infection with H. felis was significantly much higher ($P < 0.001$) in cats with external parasitism than in the other cats in the sample population.

Anaemia occurred in 23 of the 36 cats infected with H. felis. Sixteen of the anaemic cats had marked anaemia while the other seven had mild anaemia. Eleven of the former and four of the latter were males. The percentage of anaemic cats among the infected cats in each age group is shown in Figure 3.3 None of the infected cats less than a year old was anaemic. From one year of age, the percentage of cats with anaemia generally increased with age reaching a peak at six to six and a half years, at which age all the infected cats had marked anaemia.

Seventeen of the cats infected with H. felis had concurrent FeLV infection and one cat which was FeLV negative had acute myeloid leukaemia. The other 18 cats with H. felis infection were FeLV negative. The percentage

FIG 3.3. DISTRIBUTION OF ANAEMIA IN CATS INFECTED WITH <u>H. felis</u>

of infected cats with concurrent FeLV infection in each age group is shown in Figure 3.1.

The chi-squared test showed that the prevalence of infection with *H. felis* was significantly higher ($P < 0.001$) in cats infected with FeLV than in the other cats in the sample population.

H. felis infection in multi-cat households

In this study, five multi-cat households designated Household one to Household five were investigated.

Household 1

There were four cats (Nos. 39, 45, 46 and 47) in this household. Cats 39 and 45 were neutered males aged three and five years respectively while cats 46 and 47 were spayed females both aged two and a half years. Cats 39, 45 and 46 were domestic short haired and cat 47 a Burmese cat. All the cats had close contact and they had a history of flea infestation.

Cat 39 was hospitalised because of anaemia caused by concurrent *H. felis* and FeLV infections. The anaemia did not respond to treatment and the cat had to be euthanised. When the other three cats were examined, they were all healthy and none was anaemic. However cat 46 was infected with *H. felis* but not FeLV while the other two were uninfected.

Household 2

This household also had four cats - two females Nos. 54 and 56, aged five and three and a half years respectively and two males, Nos. 55 and 57, aged eight and

two years respectively. All the cats were domestic short haired and had a history of flea infestation.

Cat 54 had a severe anaemia caused by concurrent H. felis and FeLV infections and she was eventaully euthanised. On examination, none of the other three cats were anaemic. However cat 56 had concurrent H. felis and FeLV infections and cat 55, FeLV infection. Cat 57 was free of both infections.

Household 3

There were two female Siamese cats (Nos. 101 and 102) aged eight and two years respectively in this household. Both cats had fleas when examined.

Cat 101 had concurrent H. felis and FeLV infections which resulted in a severe anaemia and eventual euthanasia. Cat 102 was not anaemic when examined but it also had both H. felis and FeLV infections.

Household 4

This household had three domestic short haired cats (Nos. 9, 115 and 123) in succession. Cat 115 lived with cat 9 for about two months before the latter died. Cat 123 was brought into the household three months after the death of cat 9. Cats 9 and 115 were males aged one and a half years and six months respectively and cat 123 was a neutered female aged eight months. Cats 9 and 115 had a history of flea infestation while cat 123 was free of fleas.

Cat 9 had concurrent H. felis and FeLV infections which caused a severe anaemia and his eventual death. When examined cat 115 also had both infections but was not anaemic. Cat 123 was free of both infections.

Household 5

This household also had three domestic short haired cats (Nos. 105, 121 and 122). They were all neutered males aged two and a half, six and two years respectively. All three cats had fleas when examined.

Cat 105 died of anaemia resulting from concurrent *H. felis* and FeLV infections. When examined, the other two cats were not anaemic though cat 121 had *H. felis* infection.

Clinical aspects of *H. felis* infection

For comparison purposes, the cases of *H. felis* infection with concurrent FeLV infection are dealt with separately from the cases without FeLV infection.

H. felis infection without FeLV

The 18 cases of *H. felis* infection on FeLV-free cats occurred in nine males and nine females giving a male to female percentage ratio of the total number examined in each sex of 1 to 1.2. Sixteen of the cats were domestic short haired, one a Siamese and the other a Persian. The age distribution of the cats is shown in Figure 3.4

Marked anaemia occurred in three of the cats (two males and a female) while five others (two males and three females) had mild anaemia. Thus approximately 56 percent of the FeLV-free cats infected with *H. felis* were not anaemic.

FIG 3.4. AGE DISTRIBUTION OF *H. felis* INFECTION IN FeLV-FREE CATS

The marked anaemia occurred in cats over seven years old. The percentages of anaemia among the infected cats are shown in Figure 3.4.

While all the anaemic cats showed various degrees of ill-health, three of the non-anaemic cats (Nos. 22, 46 and 121) were healthy.

The clinical symptoms observed in the anaemic cats were complicated by those attributable to co-existing conditions (Table 3.3). The clinical disease had a gradual onset and in most cases the cats had shown signs of ill-health for periods ranging from two to four weeks before veterinary attention was sought.

All the eight anaemic cats were dull, and those with marked anaemia were weak. One of the cats (No. 104) slept more than was usual. Anorexia or inappetence for varying lengths of time were observed in all eight cats. In addition vomiting was observed in two cats (Nos. 112 and 119) while two others (Nos. 81 and 150) had diarrhoea.

Weight loss occurred in the three cats with marked anaemia and in two with mild anaemia (Nos. 17 and 150).

In most of the cats the rectal temperature was within the normal range. However one cat (No. 36) had a sudden onset of pyrexia during the course of the disease and two others (Nos. 15 and 81) had bouts of pyrexia (104-104.5^{o}F) which alternated with periods during which the body temperature was normal.

TABLE 3.1

Some Initial Haematological Data of Anaemic *H. felis* infected Cats

Cat Number	PCV (%)	MCV (fl)	MCHC (g/dl)	Reticulocyte Count (%)	Normoblast Count (%)	Total WBC ($x10^3/\mu l$)	Neutrophils (No./µl)	Lymphocytes (No./µl)	Monocytes (No./µl)	Eosinophils (No./µl)	Granulocytic percursors (%)	Lymphocytic percursors (%)
81	15.4	64	31	48	85	9.4	752	658	-	-	-	-
112	18.2	45	25	1	-	9.3	5,952	2,976	186	186	-	-
119	21.4	50	35	1	-	10.8	9,936	432	324	108	-	-
15	25.9	45	31	2	-	3.8	1,482	2,014	114	190	-	-
36	27.0	44	31	3	2	9.1	7,462	1,183	273	-	-	-
117	26.5	46	31	1	-	37.6	32,336	3,760	376	1,128	-	-
150	26.9	44	33	6	-	9.1	4,732	3,458	546	364	-	-
104	29.0	48	32	2	-	20.3	15,225	3,857	609	812	-	-

TABLE 3.2

Some Initial Haematological Data of non-Anaemic H. felis infected cats

Cat Number	PCV (%)	MCV (fl)	MCHC (g/dl)	Reticulocyte Count (%)	Normoblast Count (%)	Total WBC ($x10^3/\mu l$)	Neutrophils (No./µl)	Lymphocytes (No./µl	Monocytes (No./µl)	Eosinophils (No./µl)	Granulocytic percursors (%)	Lymphocytic percursors (%)
16	30.0	53	32	-	1	25.7	22,873	2,313	257	-	-	-
22	44.0	58	31	<1	-	18.6	7,812	9,300	186	1,302	-	-
40	36.0	54	30	6	1	25.9	15,022	7,511	1,036	1,036	3	-
42	33.7	51	32	8	9	5.5	3,685	935	330	55	-	-
46	43.0	55	32	<1	-	7.4	5,254	1,628	222	296	-	-
83	47.1	53	32	5	-	7.2	3,672	2,952	216	360	-	-
98	40.7	51	25	3	1	28.7	25,830	1,722	287	574	-	-
114	35.7	48	35	1	-	9.7	5,917	1,455	388	1,940	-	-
121	36.9	47	33	-	-	19.0	14,060	3,610	760	570	-	-
140	30.5	49	32	-	-	13.8	9,522	3,036	552	690	-	-

TABLE 3.3

Parasitaemia, Concurrent Diseases and outcome in *H. felis* infected cats.

Cat Number	PCV (%)	Highest Level of Parasitaemia	Co-existing Disease	No. of Blood Transfusions	Outcome
81	15.4	2*	Diarrhoea	1	Alive
112	18.2	1*	Mast cell tumour of skin	-	Died
119	21.4	1*	Polycyctic kidneys and Chronic nephritis	-	Died
15	25.9	2*	Polyarthritis	-	Alive
36	27.0	2*	Myeloproliferative disease and Glomerulonephritis	-	Died
117	26.5	1*	Membranous nephropathy	-	Alive
150	26.9	1*	Head injury in traffic accident and tooth abscess	-	Alive
104	29.0	1*	Membranous nephropathy	-	Alive
16	30.0	1*	Membranous nephropathy	-	Died
22	44.0	1*	-	-	Alive
40	36.0	1*	Intestinal tumour	-	Died
42	33.7	1*	Colonic impaction	-	Died
46	43.0	1*	-		Alive
83	47.1	1*	Lymphadenopathy	-	Alive
98	40.7	1*	Pancreatitis and Hepatitis	-	Euthanasia
114	35.7	1*	Cough	-	Alive
121	36.9	1*	-	-	Alive
140	30.5	1*	Ringworm	-	Alive

The anaemia was manifested by pallor of the visible mucous membranes and increased heart rates in most of the cats,as well as a distinct systolic haemic murmur in one cat (No. 81).

The other symptoms observed were halitosis (cats 36 and 112), scaly skin with harsh, staring coat (cats 81 and 112) dark coloured urine (cat 81) and subcutaneous haemorrhages (cat 36).

Oedema of the limbs and ascites were observed in two cats (Nos. 117 and 104) both of which also had the nephrotic syndrome.

The haematological data for the initial samples obtained from the infected cats are presented in Tables 3.1 and 3.2. In the cats with marked anaemia, the anaemia was macrocytic and hypochromic in one (No. 81), macrocytic and normochromic in another (No. 112), and normocytic and normochromic in the third (No. 119). The mild anaemias were all normocytic and normochromic. Reticulocytosis and normoblastosis occurred in only one cat (No. 81).

The total and differential leucocyte counts in most of the cats were within normal ranges. However cat 15 showed a leucopaenia with an accompanying neutrophilia while cat 117 had a leucocytosis and an accompanying neutrophilia. Though the total leucocyte count was normal in cat 81, there was neutropaenia and lymphopaenia.

Examination of the stained blood smears from the anaemic cats showed anisocytosis in all the cats,

polychromasia in seven and poikilocytosis in three (Nos. 81, 36 and 117). The anisocytosis in cat 117 was very severe. Also there were more numerous Howell-Jolly bodies in the smear from cat 81.

Among the non-anaemic cats, the blood of one cat (No. 98) was hypochromic and that of cat 42 showed evidence of erythrocytic regeneration.

The H. felis infection in the anaemic cats was complicated by renal diseases in three cases (Nos. 119, 117 and 104), myeloproliferative disorder and renal disease in one case (No. 36) and mast cell tumour in another (No. 112). The other complications are listed in Table 3.3.

Two of the cats with marked anaemia (Nos. 112 and 119) and one of those with mild anaemia (No. 36) died before the completion of this study. Also three of the non-anaemic cats died and one was euthanised. Thus 11 of the 18 infected cats were still alive at the completion of this investigation (Table 3.3).

Five of the dead cats (Nos. 112, 119, 36, 42 and 98) were available for necropsy. Most of the necropsy findings were manifestations of co-existing diseases rathern than anaemia. The only findings attributable to anaemia were seen in three cats (Nos. 36, 98 and 119).

The carcase of one of the cats (No. 98) was pale and slightly icteric. The liver was pale in the three cats (Nos. 36, 98 and 119) and in addition the liver of cat 119 was mottled while that of cat 98 was enlarged and soft but without any evidence of biliary obstruction.

The submandibular and retropharyngeal lymph nodes were enlarged in cat 36. The same cat also had haemorrhages in the myocardium, kidneys and submucosa of the bladder, and pink bone marrow.

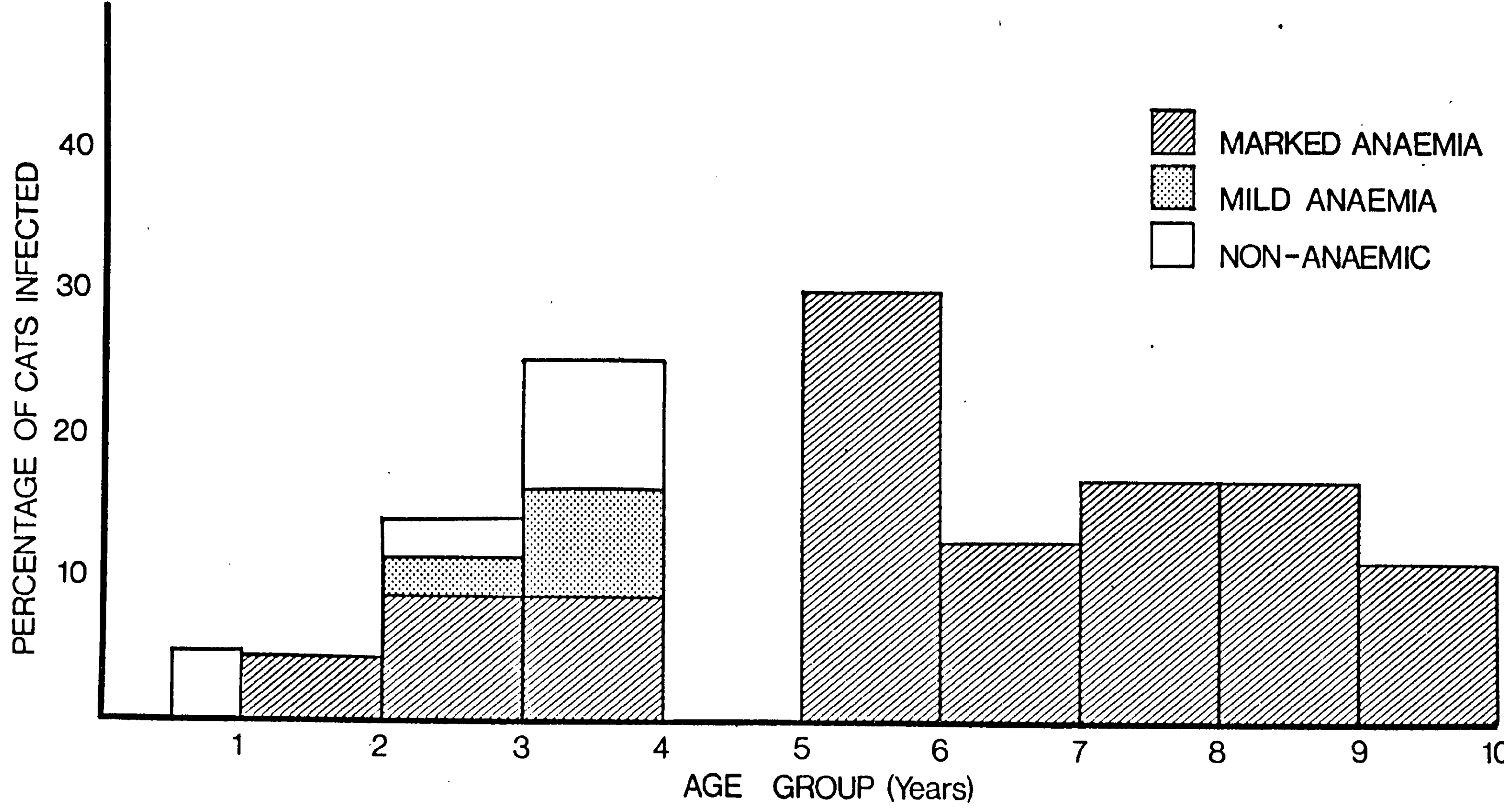
Histologically, there was hepatocellular degeneration in the livers of cats 36 and 98 and this was centrilobular in the former. In addition there was deposition of bile pigment and low grade focal hepatitis in the liver of cat 98. On the other hand the liver of cat 119 contained fatty cysts.

The bone marrow of cat 36 which had a myeloproliferative disease, was expanded, with cells of the myeloid series predominating. Myeloid precursors were also present in the spleen and mesenteric lymph nodes while a section of the retropharyngeal lymph node was filled with plasma cells.

H. felis with concurrent FeLV infection

Concurrent H. felis and FeLV infections occurred in 17 cats, 12 of which were males, giving a male to female percentage ratio of approximately two to one. Thirteen of the cats were domestic short haired and the others Siamese. The concurrent infections occurred in cats between six months and 10 years of age with none in the four to four and a half year age group. The rate of the infections increased with age reaching a peak between five and five and a half years and then declined. Figure 3.5 shows the age distribution of the concurrent infection.

FIG 3.5. AGE DISTRIBUTION OF CONCURRENT *H. felis* AND FeLV INFECTIONS



Marked anaemia occured in 12 (nine males and three females) of the cats with the concurrent infections while mild anaemia occurred in two, Nos. 19 and 39 (both males). All the non-anaemic cats (Nos. 56, 102, 115) were from multi-cat households (Households 2, 3 and 4) in which other cats had had concurrent *H. felis* and FeLV infections. The three non-anaemic cats and the two with mild anaemia were less than four years old. All infected cats more than five years old had marked anaemia (Figure 3.5).

All the anaemic cats showed signs of ill-health while all the non-anaemic ones were healthy. While in almost all the ill cats the clinical disease had a gradual onset, in one cat (No. 9) it was marked by a sudden collapse and prostration.

All the anaemic cats were dull, weak and lethargic. There were various degrees of inappetence over periods ranging from three days to four weeks; complete anorexia being observed in six of the cats. This was accompanied by weight loss in all the cats except two (Nos. 9 and 39).

Pyrexia (104-106°F) was observed in five of the cats (Nos. 9, 32, 105, 128 and 130) during the course of the disease. The pyrexia was intermittent and there were periods of about one week during which the body temperature remained normal.

The anaemia was severe in most of the cats (Table 3.4). The visible mucous membranes were pale, and in some cases (Nos. 9, 21 and 105) almost paper-white at initial examination. There was tachycardia (200-240 beats per minute) and the pulse was feeble in most of the cases.

TABLE 3.4

Some Initial Haematological Data in Cats with Concurrent *H. felis* and FeLV infections

Cat Number	PCV (%)	MCV (fl)	MCHC (g/dl)	Reticulocyte Count (%)	Normoblast Count (%)	Total WBC ($x10^3/\mu l$)	Neutrophils (No./µl)	Lymphocytes (No./µl)	Monocytes (No./µl)	Eosinophils (No./µl)	Granulocytic percursors (%)	Lymphocytic percursors (%)
9	6.3	97	28	91	6	35.0	25,900	3,850	700	350	6	-
21	7.8	57	24	6	-	8.2	7,544	410	82	-	2	-
32	14.2	64	27	3	-	50.1	45,090	2,004	1,002	-	-	-
54	12.9	86	26	18	-	2.9	2,204	580	87	29	-	-
77	15.4	51	33	<1	-	1.8	216	1,566	18	-	-	-
101	11.5	89	28	3	-	81.2	UNDIFFERENTIATED				>80	-
105	7.7	85	31	1	1	8.3	4,648	1,577	83	-	12	-
116	10.0	56	27	2	10	4.1	2,624	533	123	82	8	-
128	10.6	70	28	64	51	18.6	4,836	2,790	-	-	8	-
130	16.8	87	30	50	3	15.8	11,692	316	-	158	20	-
132	8.7	72	29	<1	-	11.8	4,248	5,900	-	1,416	2	-
145	9.4	81	31	1	-	8.0	4,560	2,240	320	80	-	-
19	27.2	60	32	5	-	10.9	8,175	2,180	218	327	-	-
39	25.0	65	29	6	2	19.2	17,856	384	-	384	1	-
56	37.1	51	32	3	-	8.9	4,272	4,183	178	267	-	-
102	38.7	60	32	1	2	7.6	5,016	1,520	76	760	-	-
115	43.9	45	32	3	-	14.7	7,350	5,800	735	735	-	-
*65	3.0	80	120	14	12	6.4	2,688	1,536	128	-	20	-

* FeLV negative Leukaemia

A distinct systolic haemic murmur was heard on cardiac ausculation in two cats (Nos. 32 and 145). The anaemia was accompanied by dehydration in three of the cats (Nos. 21, 54 and 132). Also there was hyperpnoea in three cats (Nos. 9, 128 and 14), and dyspnoea in another (No. 54). Auscultation of the chest in cat 9 revelaed evidence of pulmonary oedema.

Icterus was observed at initial examination in two cats (Nos. 128 and 39) and a slight icterus developed in cat 9 as the disease progressed. This was accompanied by dark coloured urine in the latter.

Palpably enlarged livers were observed in two cats (Nos. 9 and 145) while splenomegaly was observed in only one cat (No. 101). The submandibular lymph nodes were enlarged in four cats (Nos. 105, 128, 132 and 145). In addition the popliteal lymph nodes were enlarged in cat 128.

The other symptoms of the disease observed were halitosis in six cats (Nos. 21, 32, 54, 105, 132 and 145), pica, manifested by licking of stones in one cat (Nos. 32), vomiting (cat 116), polydipsia (cat 105) and dry scaly skin (cats 128 and 145).

One cat (No. 54) suddenly started showing nervous symptoms during the course of the disease. There were convulsive fits with paddling of the feet, circling to the right, and champing of the jaws. Periods of convulsive fits alternated with those of semi-consciousness for 24 hours before the cat was euthanised. The convulsions became more frequent as the disease progressed.

The haematological data for samples obtained from the cats at initial sampling are presented in Table 3.4.

The anaemia was macrocytic and hypochromic in seven of the cats (Nos. 9, 32, 54, 101, 128, 132 and 39), macrocytic and normochromic in four (Nos. 105, 130, 145 and 19) normochromic and hypochromic in two (Nos. 21 and 116) and normocytic and normochromic in one (No. 77). Though reticulocytes were present in the peripheral blood of all the cats, only four cats (Nos. 9, 54, 128 and 130) had abnormal counts. Normoblastosis was observed in six of the anaemic cats (Nos. 9, 105, 116, 128, 130 and 39).

Examination of stained blood smears revealed mild to severe anisocytosis in 12 of the anaemic cats, polychromasia in 10 and poikilocytosis in three (Nos. 9, 21 and 19). Howell-Jolly bodies were more numerous than normal in most of the cats and in two cases (cats 9 and 54) they were observed in more than 50 percent of the erythrocytes.

The leucocyte counts were within normal ranges in many of the cats. However three cats (Nos. 9, 32 and 101) had leucocytosis while three others (Nos. 54, 77 and 116) had leucopaenia. The leucocytosis in cats 9 and 32 was accompanied by neutrophilia while that in cat 101 was due mainly to the presence of circulating myeloid precursors. The neutrophils in cat 32 showed hypersegmentation. The leucopaenia in cats 77 and 116 was accompanied by neutropaenia and lymphopaenia, respectively, while that in cat 54 was accompanied by both.

The granulocytic (myeloid) precursors found in nine of the anaemic cats (Table 3.4) included myeloblasts, promyelocytes, myelocytes and metamyelocytes. Also megakaryocytes and giant platelet clumps were found in blood smears from three cats (Nos. 32, 128, 132).

The concurrent diagnoses made in this group of cats are listed in Table 3.5.

At the completion of this study, 13 of the anaemic cats had either died or had been euthanised. Thus only one of the anaemic cats was alive while all the non-anaemic cats were still alive and healthy (Table 3.5).

Necropsy was performed on eight of the dead cats, the other five (Nos. 101, 105, 116, 19 and 39) being unavailable for necropsy.

At necropsy, the carcase was pale in four cases (Nos. 9, 21, 54 and 132) and icteric in another (No. 128). The viscera were also pale in cat 9. An oily yellow fluid was present in the thoracic and abdominal cavities in cat 128 while a small amount of straw coloured fluid was found in the abdominal cavity of cat 145.

The lungs were pale and oedematous in one cat (No. 9). Splenomegaly was present in six cats (Nos. 9, 21, 32, 116, 132 and 145). In addition the spleens in cats 132 and 145 were pale and had a pulpy consistency. So also was the spleen in cat 128 which also showed evidence of recent infarction.

Most of the lymph nodes in two of the cats (Nos. 21 and 128) were enlarged while in two others (Nos. 9 and 132) only the retropharyngeal lymph nodes were enlarged.

TABLE 3.5

Parasitaemia, concurrent diagnosis and outcome in cats with concurrent *H. felis* and FeLV infections.

Cat Number	PCV (%)	Highest Level of Parasitaemia	Concurrent diagnosis	No. of Blood Transfusions	Outcome
9	6.3	4*	Lymphosarcoma, hepatitis and mycarditis	2	Died
21	7.8	3*	Mesenteric lymphadenitis	2	Euthanasia
32	14.2	3*	Cholangitis	2	Euthanasia
54	12.9	4*	Mesenteric lymphadenitis	1	Euthanasia
77	15.4	1*	Renal lymphosarcoma and chronic enteritis	-	Euthanasia
101	11.5	2*	Myeloproliferative disease	-	Euthanasia
105	7.7	3*	-	3	Died
116	10.0	3*	-	-	Died
128	10.6	1*	Myeloproliferative disease	1	Died
130	16.8	1*	-	-	Alive
132	8.7	1*	-	-	Euthanasia
145	9.4	1*	Osteoarthritis Myeloproliferative disease	-	Died
19	27.2	3*	-	-	Died
39	25.0	5*	-	-	Euthanasia
56	37.1	1*	-	-	Alive
102	38.7	2*	-	-	Alive
115	43.9	1*	-	-	Alive
65*	3.0	5*	Acute myeloid Leukaemia	-	Euthanasia

65* - FeLV Negative Leukaemia

Pale liver was observed in four cases (Nos. 32, 54, 116 and 132) and heptomegaly in one (No. 145). None of the livers showed any evidence of biliary obstruction.

The bone marrow was pink in three cases (Nos. 9, 21 and 32) and reddish brown in one (No. 128).

In cat 9, a small discoid mass was found on the dorsal surface of the tongue immediately medial to the right tonsil.

Histologically, the enlarged spleen in five cats (Nos. 9, 21, 32, 132 and 145) showed evidence of extramedullary haemopoiesis. The spleen in cat 128 contained macrophages, megakaryocytes and polymorphonuclear cells (polymorphs). The macrophages contained haemosiderin.

The lymph nodes in cat 21 showed in increase in the number of lymphocytes while the mesenteric lymph nodes in cats 32 and 54 contained polymorphs and macrophages. The lymphs nodes in cat 132 showed diffuse lymphadenitis and necrosis as well as some evidence of extramedullary haemopoiesis. Lymphoid depletion occurred in the mesenteric lymph nodes of one cat (No. 145).

There was centrilobular degeneration in the liver in five cats (Nos. 21, 54, 128, 132 and 145). In addition, small numbers of macrophages laden with haemosiderin were present in the liver of cat 21. Also there was evidence of extramedullary haemopoiesis in the liver of cat 145. In one cat (No. 9) the liver had focal hepatitis, and in cat 32 there was cholangitis with inflammatory cells present around the portal triad.

The bone marrow was fully expanded in four cats. In three of them (Nos. 9, 21 and 145) it contained erythroid

cells mainly, while in the other (No. 32) it contained polymorphs, erythroblasts and megakaryocytes. In another cat (No. 54) there was increased connective tissue (myelofibrosis) and spongy bone (ossification) in the bone marrow. In cat 128, most of the bone marrow had been replaced by sheets of tightly packed macrophages interspersed by megakaryocytes, polymorphs and haemopoietic tissue.

The lingual mass found in cat 9 was a lymphosarcoma and the same tumour was present in the right retropharyngeal lymph node.

In cat 54, there were microscopic changes in the brain which were consistent with anaemic cerebral anoxia. Neurones in the cerebral grey matter, particularly the piriform areas, were ischaemic.

H. felis infection with FeLV-negative leukaemia

This case is being reported separately from the other cases in FeLV-free cats because there was evidence of leukaemia in blood smears at initial examination.

This case occurred in a four year old neutered female domestic short haired cat (No. 65). The cat had been anorexic for about a week and was wasted by the time it was first examined. The mucous membranes were almost paper white, the cat was very dull and weak and remained in lateral recumbency. The temperature was subnormal, the pulse very feeble and the heart rate was 250 beats per minute.

Haematological data from initial sampling are presented in Table 3.4. The anaemia was macrocytic and there was an increase in the MCHC. Reticulocytosis and

normoblastosis were also present. The stained blood smear revealed very few erythrocytes per microscopic field. There was polychromasia and very severe poikilocytosis. Almost all the erythrocytes were infected with H. felis, most of them containing more than one parasite.

The total leucocyte count was below the normal range but the differential count was normal. There were promyelocytes, myelocytes and metamyelocytes in the blood smear.

A diagnosis of haemobartonellosis with acute myeloid leukaemia was made. The cat was euthanised but permission for necropsy was refused.

Treatment of cats with H. felis infection

The first line of treatment for the severely anaemic cats was whole blood transfusions except where euthanasia was requested. One of the H. felis infected FeLV free cats and six of those with the concurrent infections received whole blood transfusions (Table 3.3 and 3.5). The cats in the latter group received an average of two transfusions. Blood was withdrawn via cardiac puncture into a 60ml heparinised syringe from a donor cat anaesthetised with ketamine and this was immediately transfused intravenously into the recipient.

The infected cats were treated with tetracyclines to remove the parasites. Two cats (Nos. 9 and 32) were treated with oxytetracycline hydrochloride (Engemycin 5% - Mycofarm. Essex) at a daily dose of 50 milligrams (mg) (1.0ml) intramuscularly for five days. The intravenous

route was used in another cat (No. 54) at a daily dose of 25mg (½ml) for five days. The other cats were treated with oxytetracycline dihydrate tablets (Occrycetin "100" Tablets - Willows Francis Veterinary, Bolton) at a dose of 100mg twice daily for 10 days.

The blood transfusions sustained the FeLV-free cat (No. 81) and the clinical anaemia abated. However the tetracycline did not clear the blood of the parasites which could still be detected three months after completion of the treatment.

In the cats with concurrent *H. felis* and FeLV infections, the blood transfusions only gave transient improvement and the conditions relapsed. A second transfusion (and even a third in cat 105) had to be given about a week after the previous one in most cases. Despite the transfusions, the cats died or had to be euthanised due to lack of response to treatment.

The tetracyclines also failed to clear the blood of the parasites as they were still present in blood samples taken prior to euthanasia or before death.

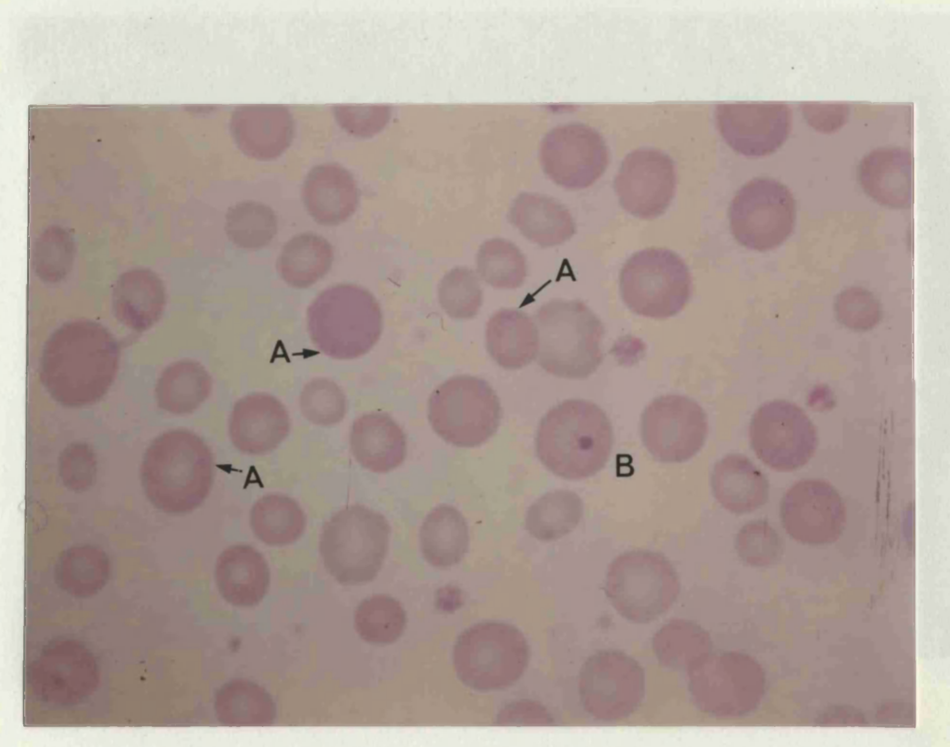


Plate 3.1

Blood film from a cat infected with H. felis. Some of the erythrocytes contain H. felis organisms (A) and the erythrocyte (B) in the centre of the film contains a Howell-Jolly Body. The film also shows anisocytosis.

Leishman's stain X 1200.

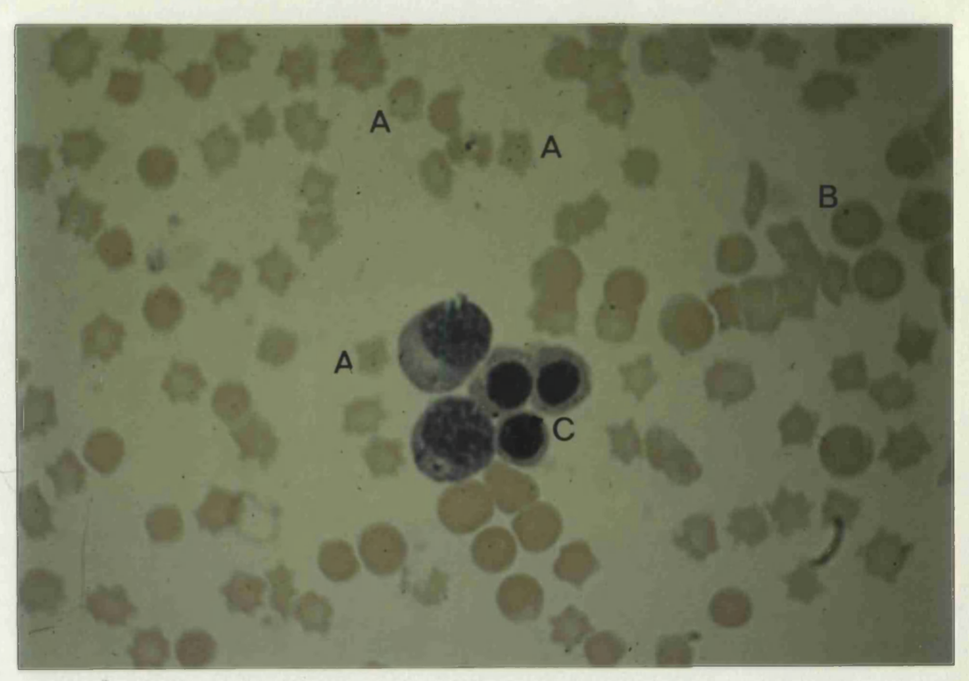


Plate 3.2

Blood from a cat with concurrent H. felis and FeLV infections. Organisms are present in the erythrocytes (A and B). Erythrocyte B contains a pair of H. felis organisms and a Howell-Jolly Body. The film also shows the presence of normoblasts (C) and poikilocytosis.

May-Grunwald-Giemsa stain (photographed with blue filter) X 1200.

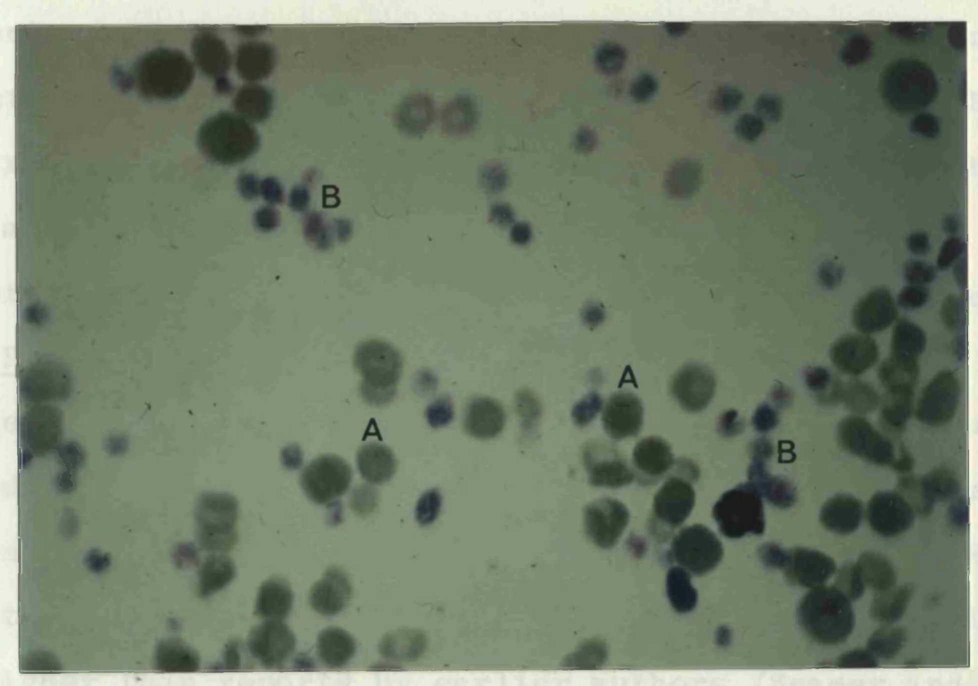


Plate 3.3

Blood film from another cat with concurrent *H. felis* and FeLV infections. *H. felis* organisms are present in the erythrocytes (A). The film also shows the presence of abnormal platelet clumps (B) which occurred in all blood films obtained from this cat throughout the course of the disease.

Giemsa stain (photographed with blue filter) X 1200.

DISCUSSION

The prevalence percentage of 23.2 for H. felis infection obtained in this survey is the highest so far in Britain. Seamer and Douglas (1959) obtained a prevalence percentage of 5.7 while Thomsett (1960) did not find any infected cat among the 126 examined. A search through the case records of Glasgow University Veterinary School showed that at least 7.1 percent of the 509 cats presented in the Medicine Department between 1977 and mid-1979 were infected with H. felis. The high figure obtained in this study may be due partly to the fact that the sample population was biased in favour of anaemic cats because such cases were requested. However, it has been suggested (Seamer, 1964) that the actual prevalence of H. felis infection in Britain was higher than reports by earlier authors (Seamer and Douglas 1959 and Wilkinson 1963) had suggested. In the United States Holzworth (1956) reported that at least 25 percent of the anaemic cats she saw had H. felis.

The prevalence of H. felis infection was slightly higher in males than females in this study. However the difference was not significant ($P > 0.20$) even among infected cats that had marked anaemia. Hayes and Priester (1973) in a retrospective study of 43,514 cats in the United States found that the risk of infection in males was two and a half times that in females among the 374 cats with clinical feline haemobartonellosis. However, the results obtained by these authors (Hayes and Priester, 1973) depended on diagnoses made by many other people and

this casts some doubts on their results as it was not stated how the diagnosis of haemobartonellosis was arrived at in the different hospitals/clinics.

Manuel and Abalos (1975) in a survey in the Philippines also found a higher prevalence in males though they stated that there was no sex difference in the prevalence of the infection. However only five of the 512 cats examined were infected with H. felis.

H. felis infection was found in all age groups in this study but the clinical disease as manifested by anaemia occurred mostly in cats one to 10 years old. The proportion of anaemic cats increased with age reaching a peak at six to six and a half years. Hayes and Priester (1973) also found an increase of the clinical disease with age but obtained a peak at four to six years. If cats with concurrent H. felis and FeLV in this study only are considered, a peak for the clinical disease would be obtained at five to five and a half years.

In FeLV-free cats infected with H. felis marked anaemia was only seen in cats older than seven years. On the other hand, anaemia occurred in cats from one year of age upwards in those with concurrent H. felis and FeLV infections. However while some of the cats less than four years old in the latter group had mild anaemia or were not anaemic, all the infected cats above five years of age had marked anaemia. Thus it seems that H. felis infection is associated with a clinical disease more commonly in older cats.

Though H. felis infection had a higher prevalence in domestic (common) cats than the pedigree cats in this study, the difference was not significant ($P > 0.20$). However, the number of pedigree cats examined in this study (less than 25 percent of the total number) is too small for any meaningful conclusion to be drawn.

The presence of fleas and Otodectes sp. on almost two-thirds of the cats infected with H. felis and the significantly higher prevalence of H. felis infection in cats with ectoparasites than the other cats in the sample population support the earlier suggestions that arthropod parasites might serve as vectors of transmission of the infection (Holzworth 1956, Splitter and others 1956, Thomsett 1960). Furthermore, the detection of H. felis in some of the cats that had fleas in the multi-cat households suggest the possibility of fleas being vectors of transmission for the infection. However, experimental evidence is still required to prove that fleas are actually involved in the transmission of the parasite.

The prevalence of H. felis was significantly much higher ($P < 0.001$) in cats with FeLV infection than the other cats in the population sampled. This suggests a high degree of association between the two infections as has been stated by earlier workers (Priester and Hayes 1973, Essex 1974, Jarrett 1979).

In this study, over 50 percent of the FeLV-free cats with H. felis infection were not anaemic, while five of the eight anaemic cats had only mild anaemia. Also

four of the eight cats had renal diseases which might have contributed to the anaemia. On the other hand, over 80 percent of the cats with concurrent H. felis and FeLV infections were anaemic, the anaemia being severe in most cases. Furthermore, the non-anaemic cats in this group were all cats that had close contact with other cats that had the concurrent infections. Of the cats that had FeLV infection without H. felis (previous chapter) only 50 percent were anaemic. These findings therefore suggest that the presence of H. felis and FeLV infections together has an additive effect which leads to a severe anaemia in most of the infected cats.

The clinical symptoms of the disease observed in this study were similar to those reported in clinical cases of haemobartonellosis by earlier authors (Flint and Moss 1953, Holzworth 1956, Flint and others 1958, Balazs and others 1961, Harbutt 1963, Wilkinson 1963 and 1965, Flagstad and Larsen 1969, Maede and others 1974). The clinical symptoms in cats with concurrent H. felis and FeLV infections were more attributable to anaemia than those in FeLV-free cats with H. felis, in which many of the cases were complicated by other diseases. In the former group, icterus, splenomegaly, heptomegaly and lymph node enlargement were observed in some cases, while these were not present in any of the cats in the latter group. With the exception of lymph node enlargement, these symptoms were not observed in any of the FeLV associated anaemias reported in the previous chapter. This further supports

the hypothesis that *H. felis* and FeLV infections together produce more severe symptoms of anaemia than does either of them on its own.

The anaemia due to the concurrent *H. felis* and FeLV infections was generally more severe than that observed in cats with *H. felis* infection alone. While most of the anaemias in FeLV-free cats with *H. felis* infection were normocytic and normochromic, seven of the 14 cats with concurrent *H. felis* and FeLV infections had macrocytic and hypochromic anaemias and four others had macrocytic and normochromic anaemias. Thus while some of the cases were macrocytic and normochromic as would be expected from the description of Kreier and Ristic (1968), about half of the marked anaemia cases were similar to those described by Flagstad and Larsen (1969). It is therefore likely, as Schalm and Switzer (1973) suggested, that when erythrocyte replacement is minimal, the anaemia is normocytic. Thus the morphology of the erythrocytes in the cases observed probably depended on the rate at which the erythrocytes were being replaced. The reduction in the MCHC below the normal range in many of the anaemic cats with concurrent *H. felis* and FeLV infections in this study might have been due to the fact that the body iron reserve and iron uptake were not sufficient to cope with the increased erythropoiesis. In experimentally infected cats Flint and others (1959) found that while the average MCV for the infected cats increased, the average MCHC decreased, though it was within the normal range.

Only one of the FeLV free cats with *H. felis* infection showed evidence of bone marrow regeneration while many of the cats with concurrent *H. felis* and FeLV infections showed evidence of bone marrow regeneration. However two of the cats with marked anaemia in the former group in which bone marrow regeneration would have been expected had concurrent diseases (mast cell tumour and renal diseases - Table 3.3) which could have affected the bone marrow responses.

In stained smears, anisocytosis and polychromasia occurred more frequently than poikilocytisis in the two groups of *H. felis* infected cats. This supports an earlier statement to this effect (Mackey 1977).

A variety of leucocytic manifestations were seen in the two groups of infected cats, though in most cases the total and differential leucocyte counts were within normal ranges. This observation lends weight to the statement by Schalm and Switzer (1973) that both total and differential leucocyte counts are not sufficiently characteristic to be of value in the diagnosis of *H. felis* infection. Thus *H. felis* associated anaemia may not be differentiated from any other type of anaemia by the leucocytic manifestations. However, there seems to be a difference between the leucocytic manifestations seen in cats with concurrent *H. felis* and FeLV infections and in FeLV-free cats with *H. felis*. Myeloid percursors occurred more frequently in the peripheral blood in the former than in the latter group. The anaemic cats in the former group had myeloid

precursors in the peripheral blood while none of the anaemic ones in the latter group did. In the FeLV infected cats without H. felis (previous chapter) myeloid and lymphocytic precursors were observed in the peripheral blood and these in many cases were manifestations of leukaemia. Thus the precursor cells found in cats with concurrent H. felis and FeLV infections were either manifestations of leukaemia (Table 3.5) or an expression of the FeLV component.

Comparing the clinical symptoms and haematological findings in this study with those reported by some earlier authors (Holzworth 1956, Harbutt 1963, Wilkinson 1965, Flagstad and Larsen 1969, Bedford 1969) it seems likely that some of the reported cases of clinical haemobartonellosis also had concurrent FeLV infections.

Most of the co-existing conditions in the cats with concurrent H. felis and FeLV infections were attributable to FeLV infection (Table 3.5). On the other hand, most of the co-existing conditions found in the FeLV-free cats with H. felis infection were unrelated to the infections.

It is difficult to assess the mortality in the infected cats since many of the FeLV-free cats with H. felis infection died from causes unrelated to the anaemia and many of those with concurrent H. felis and FeLV infections were euthanised. However the mortality in cats in the latter group was much higher than that in the former. This is similar to the report by Holzworth (1956) that about 33 percent mortality occurred among cats with uncomplicated H. felis associated anaemia while about 75 percent mortality

occurred in those with complicated disease. Flint and others (1958) also reported 75 percent mortality among 30 clinical cases.

The necropsy findings in this study were similar to those that have been reported (Kreier and Ristic 1968, Bedford 1970, Leeflang and others 1970, Hataka 1977, Tury and others 1977). The cats with concurrent H. felis and FeLV infections had more pronounced pathological lesions than those with only H. felis infection. However, some of the lesions in the former, such as lymphadenopathy and lymphosarcoma could be attributed to FeLV infection.

The clinical signs and haematological findings in the cat with H. felis and FeLV-negative myeloid leukaemia were similar to those in some of the cats with concurrent H. felis and FeLV infections. This suggests that the development of leukaemia might have played a prominent role in the pathogenesis of the anaemia in some of the cats with concurrent H. felis and FeLV infections.

The failure of tetracyclines to clear the blood of H. felis in this study confirms earlier reports that the antibiotic is ineffective in the treatment of the infection (Watson and others 1978, Harvey and Gaskin 1978b). Also the failure of blood transfusions and other treatments to induce remission of the anaemia in the cats with concurrent H. felis and FeLV infections suggest a poor prognosis in these cases. Flint and others (1959), reported poor response to blood transfusion in four of the 20 transfusions given to their experimentally infected cats.

CONCLUSION

This is probably the first investigation in which *H. felis* and FeLV were studied together as causes of anaemia in the same cat population. This study has shown that while *H. felis* infection may occur in a sizeable number of cats in any population (up to 20 percent or more), the uncomplicated infection causes little or no clinical problem. However, where the infection is complicated by FeLV infection or leukaemia, there is usually a severe anaemia in most cases. This suggests that FeLV infection or feline leukaemias may be more important in the pathogenesis of anaemia in clinical feline haemobartonellosis than the parasite *H. felis* itself.

This study has also shown that the erythrocytic morphology observed in *H. felis* associated anaemias probably depends on the rate at which the erythrocytes are being replaced. Though macrocytosis occurred in most of the cases in this study, the mean cell haemoglobin concentration (MCHC) was normal in some cases and below normal in others.

Though there were evidences of bone marrow regeneration in many of the cats with concurrent *H. felis* and FeLV infections, the anaemias were usually so severe that the compensatory regenerative responses were not adequate to produce remissions. Therefore the prognosis for anaemic cats with concurrent *H. felis* and FeLV infections or *H. felis* and leukaemia should be regarded as poor.

CHAPTER IV

DEMONSTRATION OF *H. felis* IN INFECTED CAT BLOOD

DEMONSTRATION OF *H. felis* IN INFECTED CAT BLOOD

MATERIALS AND METHODS

These were as described in the previous chapters (Chapters 2 and 3). In addition, blood samples from 100 cats whose *H. felis* status were unknown at the time of sampling were used to compare the efficiency of the Romanowský (Leishman, Giemsa and May-Grunwald-Giemsa) staining procedures and the acridine orange staining method in demonstrating the parasite in infected blood. A modified Wright's stain (Haemofast - Centropa Test, distributed by Immuno Ltd. Kent) was also used in staining smears from the samples as follows. Air dried smears prepared as described in chapter 2 were immersed in the Wright's stain solution for 10 seconds after which they were immersed in distilled water for one minute. The smears were then rinsed in distilled water and allowed to drain dry.

Some blood smears were also stained with freshly prepared toluidine blue (Gurr Ltd. London) employing a rapid staining technique for *Anaplasma* which was described by Rogers and Wallace (1966).

A further comparison between the efficiency of acridine orange and May-Grunwald-Giemsa staining methods in demonstrating *H. felis* in infected blood was made using 100 blood samples from cats in whose blood *H. felis* organisms had previously been detected.

H. felis organisms were measured with a screw micrometer eyepiece (Leitz (Instruments) Ltd., Luton) which

was calibrated with a stage micrometer (Leitz Ltd.). One thousand coccoid organisms and 100 rod forms, both in May-Grunwald-Giemsa stained smears were measured. Also 50 coccoid organisms each in May-Grunwald-Giemsa, Giemsa and Leishman stained smears from two cats were measured for comparison purposes.

A limited study to determine the effect of EDTA on H. felis was carried out using blood from eight cats known to be infected with the parasite. After making smears from fresh blood samples, the samples were stored in EDTA tubes as described in Chapter 2. Smears were made from the EDTA samples 30 minutes, one hour, two, three, six and 24 hours after collection. These smears stained with the Romanowsky stains and acridine orange were examined for the presence of the parasite.

RESULTS

Most of the smears stained with the modified Wright's stain had stain deposits despite filtering the stain several times. This made the detection of H. felis difficult.

Toluidine blue stained the smears faintly and no organisms could be detected in the smears. Thus the results obtained were discarded as being unreliable.

The staining procedures compared in this study were Leishman, Giemsa, May-Grunwald-Giemsa and acridine orange. With the first three staining procedures (the Romanowsky stains) H. felis stained a deep purple colour. In addition, the deep purple colour had a reddish-brown tinge in the May-

Grunwald-Giemsa stained smears and a bluish tinge in the Giemsa stained smears. Though Howell-Jolly bodies also stained deep purple with the Romanowsky stains, they stained more intensely and appeared denser than the H. felis organisms. Also they were usually larger than the parasite. In the May-Grunwald-Giemsa smears, cytoplasmic granules such as may be found in reticulocytes were at times found in the erythrocytes, especially the larger ones. These were differentiated from H. felis by the more sharply defined outline and shape of the latter. Also the granules were usually out of focus when the H. felis organisms were in focus.

With acridine orange, H. felis fluoresced a bright yellowish colour with an orange tinge, against a dark-green background. Howell-Jolly bodies on the other hand fluoresced an orange colour which was more intense than that seen with the parasite.

The H. felis organisms seen in this study were mostly coccoid forms. In two cats (Nos. 39 and 105) short rod forms were observed in addition to the more predominant coccoid forms. The coccoid forms occurred singly, in pairs and on a few occasions in short chains of three or four organisms. Also aggregates of coccoid forms were found, so also were those arranged in a circle within the periphery of the erythrocyte.

The rod forms occurred singly and on the periphery of the erythrocytes. No beaded rod forms were seen. No organism was observed free in plasma.

The diameter of the coccoid forms ranged from 0.63µ to 1.73µ with a mean of 0.98µ (± 0.19). The length of the rod forms ranged from 0.79µ to 1.1µ with a mean of 0.82µ (± 0.21) while the diameter was between 0.17µ and 0.24µ with a mean of 0.18µ (± 0.02). The mean values obtained for the diameter of the coccoid forms in May-Grunwald-Giemsa stained smears were slightly higher than those obtained in Leishman and Giemsa stained smears in the two blood samples in which they were compared.

The results of the study carried out to compare the efficiency of the three Romanowsky and acridine orange staining procedures are presented in Table 4.1 The acridine orange method gave the best result, followed by May-Grunwald-Giemsa while Leishman's staining method revealed H. felis organisms in only 12 of the 26 smears in which acridine arange revealed them.

May-Grunwald-Giemsa stained smears usually revealed more organisms per microscopic field than did Giemsa and Leishman stained smears for the same samples. Furthermore fewer stain deposits were found in smears stained with the May-Grunwald-Giemsa method than in the Giemsa and Leishman stained smears. The Leishman stained smears usually had more stain deposits than the others.

In the comparison between acridine orange and May-Grunwald-Giemsa staining procedures using blood from known infected cats, the former revealed H. felis in 95 of the 100 blood samples while the latter revealed them in 80.

TABLE 4.1

Comparison of different staining procedures for the demonstration of *H. felis* in 100 blood samples.

Staining Procedure	No. Positive	% Positive using Acridine orange as 100%
Acridine Orange	26	100
May-Grunwald-Giemsa	23	88.5
Giemsa	14	53.9
Leishman	12	46.2

TABLE 4.2

Initial Parasitaemia and Erythrocytic Parameters in anaemic *H. felis* infected cats.

Cat Number	Parasitaemia	PCV (%)	RBC ($x10^6/\mu l$)	Hb (g/dl)
9	4*	6.3	0.64	1.8
21	3*	7.8	1.38	1.9
32	2*	14.2	2.2	3.8
54	2*	12.9	1.5	3.3
65	5*	3.0	0.37	2.9
77	1*	15.4	3.0	5.2
81	- ve	15.4	2.4	4.8
101	2*	11.5	1.29	3.2
105	2*	7.7	0.9	2.4
112	1*	18.2	4.1	4.5
116	3*	10.0	1.8	2.7
119	1*	21.4	4.3	7.4
128	- ve	10.6	1.5	3.0
130	- ve	16.8	1.9	5.0
132	- ve	8.7	1.2	2.5
145	1*	9.4	1.16	2.9
15	1*	25.9	5.9	8.1
19	3*	27.2	4.47	8.8
36	2*	27.0	6.1	9.0
39	5*	25.0	3.7	7.2
104	- ve	29.0	6.2	9.5
117	1*	26.5	5.9	8.2
150	1*	26.9	6.2	9.0

TABLE 4.3

Variations in the level of H. felis parasitaemia in 11 of the infected cats

Cat Number	Initial sampling	Number of days after initial sampling									
		3	7	10	14	17	21	24	28	31	35
9	4*	3*	1*	1*	NS	2*	NS	1*			
15	1*	-ve	-ve	-ve	2*	1*	-ve	1*			
21	3*	1*	-ve	-ve	1*						
32	2*	1*	NS	3*	4*	-ve	1*	-ve	3*		
36	2*	NS	NS	NS	-ve	-ve	1*	NS	1*		
54	2*	2*	NS	2*	2*	2*	4*				
81	-ve	1*	1*	2*	2*	NS	NS	NS	NS	2*	2*
104	-ve	NS	-ve	1*	NS	1*					
105	2*	2*	1*	3*	3*	3*	NS	2*	Ns	2*	
128	-ve	1*									
132	-ve	1*	1*								

KEY
NS = Not sampled
-ve = Negative

TABLE 4.4

Variations in Parasitaemia in *H. felis* infected blood samples stored in E.D.T.A.

Cat Number	Fresh Sample	Number of hours after sampling					
		0.5	1.0	2.0	3.0	6.0	24.0
9	3*	3*	3*	1*	1*	-ve	-ve
21	3*	3*	3*	3*	1*	1*	1*
32	3*	3*	3*	1*	-ve	-ve	-ve
39	5*	5*	5*	4*	3*	3*	1*
54	4*	4*	4*	4*	4*	4*	1*
65	5*	5*	5*	5*	5*	5*	4*
101	2*	2*	2*	2*	1*	1*	-ve
105	3*	3*	3*	3*	3*	3*	2*

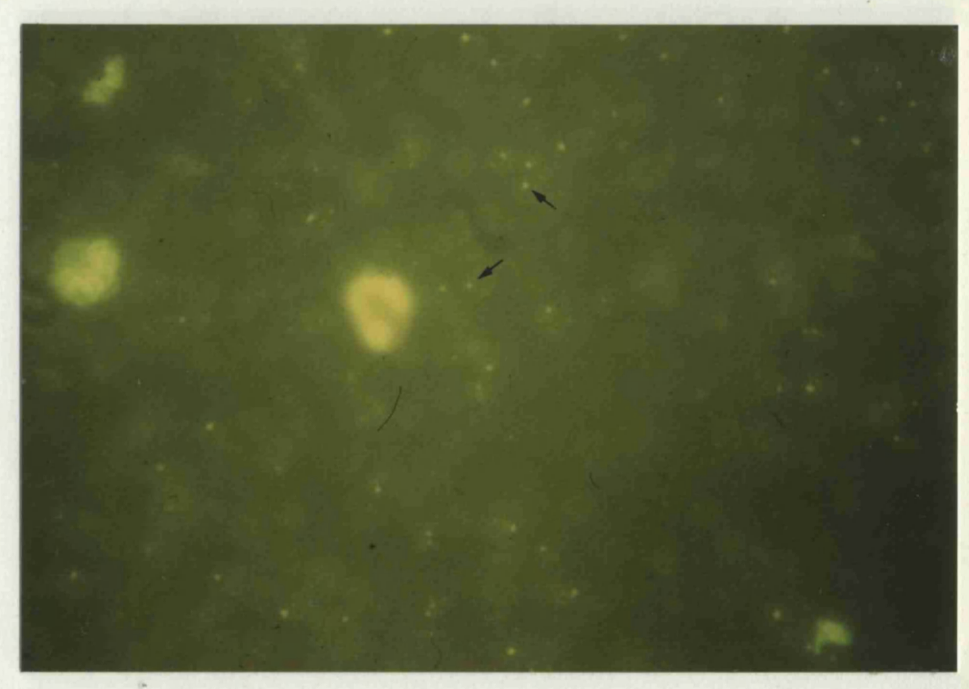


Plate 4.1

Blood film stained with acridine orange showing *H. felis* organisms (arrowed). Fluorescence microscopy with blue excitation. Note the lighter green outline of the erythrocytes against the darker green background. The cells showing orange fluorescence are leucocytes.

Acridine orange stain X 800.

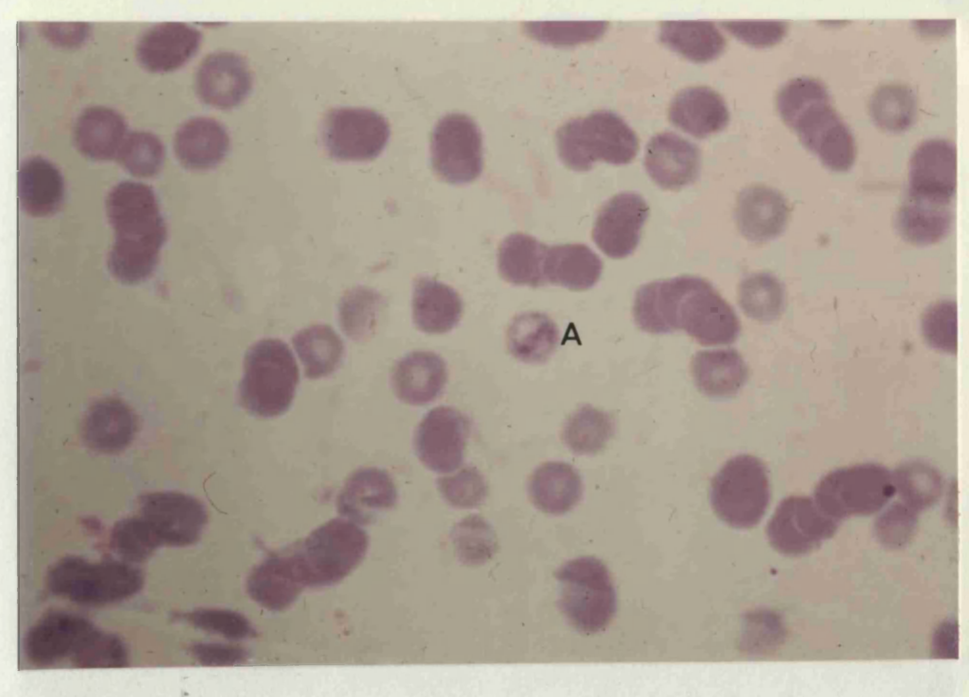


Plate 4.2

Blood film from a cat with H. felis infection. The film was made from freshly sampled blood before it was mixed with E.D.T.A. H. felis organisms are present in many of the erythrocytes. Erythrocyte A contains H. felis organisms arranged in a circle within the periphery of the erythrocyte.

May-Grunwald-Giemsa stain X 1200.

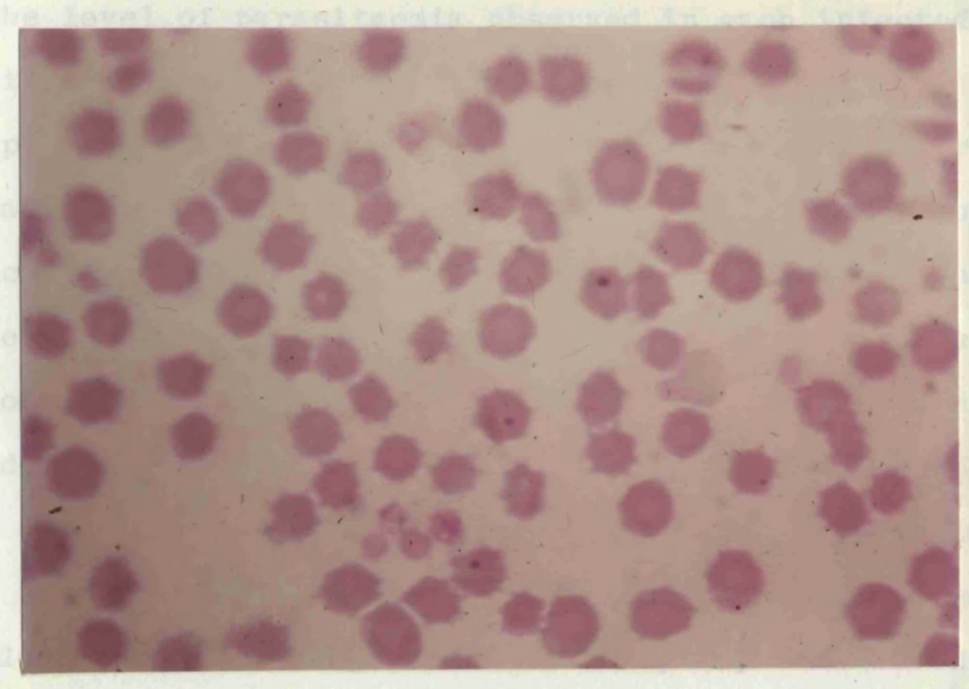


Plate 4.3

Blood film prepared from the same sample used for the film in Plate 4.2, after 24 hours storage in E.D.T.A. Note the absence of H. felis organisms and (crenation) of the erythrocytes.

May-Grunwald-Giemsa stain X 1200.

There was no correlation between the parasitaemia either at initial sampling or at the highest level attained, and the degree of anaemia observed in the infected cats (Tables 4.2, 3.3 and 3.5). Also there were fluctuations in the level of parasitaemia observed in each infected cat. At times the parasites could not be detected in the peripheral blood for some days but were detected later on. In fact in some severely anaemic cats, H. felis was not detected at initial sampling but was detected later on. Table 4.3 shows the fluctuations in the level of parasitaemia in some of the infected cats. In this study H. felis was not detected with any of the staining procedures used in five of the 36 cats infected with H. felis (Nos. 81, 104, 128, 130 and 132) at initial sampling. However subsequent sampling revealed the parasite in the peripheral blood of these cats.

In the study to determine the effect of EDTA on H. felis it was observed that smears from blood stored in EDTA for periods of time contained fewer parasites than smears made from fresh blood samples. Table 4.4 shows the result of the study conducted on eight blood samples from infected cats. In three of the cats, (Nos. 32, 9 and 101) no parasite could be found in the blood samples after they had been stored in EDTA for three, six and 24 hours, respectively.

DISCUSSION

The staining reactions of H. felis organisms found in this study were similar to those that have been described

previously (Kreier and Ristic 1968). Also the two forms observed, coccoid and rod forms, have been described by earlier workers (Kreier and Ristic 1968). In Britain, coccoid and rod forms have been the predominant forms observed (Seamer and Douglas 1959, Wilkinson 1963, Bedford 1969). However, Seamer and Douglas (1959) and Bedford (1969) described an annular form and the former also described a beaded bacillary form. Neither forms were found in this study.

The sizes of the forms observed in this study fall within the ranges described by earlier workers (Flint and McKelvie, 1955, Splitter and others 1956, Flint and others 1958, Manusu 1961, Manuel and Abalos 1975). However some of the rod forms observed had slightly smaller diameters than those that were described by the aforementioned authors. There are no records of the sizes of the organisms found by earlier workers in Britain.

The results of the comparative study of the efficiency of acridine orange and the Romanowsky staining procedures in demonstrating H. felis in infected blood confirm the earlier statement by Small and Ristic (1967) that acridine orange revealed the organism in smears in which Giemsa stain did not. This study has shown that acridine orange is superior to the Romanowsky stains in the demonstration of H. felis in blood smears.

Among the Romanowsky staining procedures, the May-Grunwald-Giemsa has been shown by this study to be more efficient in demonstrating H. felis than Giemsa and Leishman.

Based on the results obtained, the May-Grunwald-Giemsa staining procedure is at least one and a half times and about twice as efficient as Giemsa and Leishman's stains respectively. Also the fact that the organisms are more prominent (larger diameter) and more numerous in May-Grunwald-Giemsa than in Giemsa and Leishman stained smears, as well as the fewer number of stain deposits in the former, make it more suitable for demonstrating H. felis. According to Archer (1977), results obtained when blood smears are stained with the May-Grunwald-Giemsa method are excellent and he advised that it should be used where particularly well stained cytoplasmic granules are required. The author also stated that nuclear details appear inferior with Giemsa staining and that the stain is less satisfactory than Leishman and Wright's stains where leucocyte morphology is important. Thus the results obtained are most likely a reflection of the properties of the stains themselves.

The May-Grunwald-Giemsa procedure suffers from the disadvantage of being generally more complex than Giemsa and Leishman staining procedures and more time consuming than the latter. However, it eliminates the need to stain blood smears in Giemsa stain for 90 minutes as was done by Seamer and Douglas (1959) and Thomsett (1960), or overnight (Watson and others 1978), in order to demonstrate H. felis organisms.

The disappearance of H. felis from the peripheral blood as was observed in this study was also observed by earlier workers (Flint and McKelvie 1955, Splitter and others 1956, Harvey and Gaskin 1977). This study has

emphasised the need for repeat sampling of cats as has been suggested by Harvey and Gaskin (1977) in order to detect the parasite. In this study, the blood of five of the 36 cats infected with H. felis was negative for the parasite when first sampled, and if these cats had not been sampled again, they would have been regarded as being free of the parasite. This shows that while acridine orange and May-Grunwald-Giemsa staining procedures are useful in diagnosing H. felis infection, their usefulness is limited by the disappearance of the organism from the peripheral blood. There is therefore, a need for the development of a new technique which does not require the presence of the organism in the peripheral blood, for the diagnosis of the infection. The fluorescent antibody technique described by Small and Ristic (1967) does not meet this need since it also requires that the organism be present in the blood for a positive diagnosis to be made.

The decrease in the number of H. felis organisms in blood samples stored in EDTA for some hours stresses the importance of making smears from fresh samples as suggested by Penny (1978).

It was stated in the last Chapter, that the high prevalence percentage of 23.2 for H. felis infection obtained in this study might be due partly to the fact that the sample population was biased in favour of anaemic cats. Another reason for this high figure may have been the different staining techniques used in this study to demonstrate H. felis. Earlier workers in Britain used

either Giemsa (Seamer and Douglas, 1959, Thomsett, 1960, and Bedford 1970) or Leishman (Wilkinson 1963, Bedford 1969) staining procedures. Based on the results obtained in this study, Seamer and Douglas (1959) probably detected the parasite in about half of the infected cats. In fact the authors (Seamer and Douglas 1959) recorded "doubtful" infections in three other cats and these were not taken into account in the prevalence percentage of 5.7 they obtained. Furthermore, repeat sampling of the cats used in this study, enabled more infected cats to be detected than would have been detected by single sampling. Five of the 36 H. felis infections found in this study would have been missed if the cats had been sampled only once.

CONCLUSION

This study has shown that the May-Grunwald-Giemsa staining procedure is superior to Giemsa and Leishman's procedures for the demonstration of H. felis. It has also shown that while acridine orange and May-Grunwald-Giemsa staining procedures are very useful in the diagnosis of H. felis infection, their usefulness is limited by the synchronised disappearance of the parasite from peripheral blood. There is therefore the need for the development of a new technique, such as a serological or immunological technique, for the diagnosis of the infection. Until such a technique is developed, it is suggested that cats suspected to be infected with H. felis be sampled several times before being declared free of the infection.

Also because of the effect of EDTA on *H. felis* organisms observed in this study, it is suggested that blood samples submitted to laboratories for diagnosis of *H. felis* infection be accompanied by smears made soon after sampling. The smears should be made on clean grease-free slides to minimise the presence of artifacts that may be confused with the parasite.

CHAPTER V

GENERAL CONCLUSIONS

GENERAL CONCLUSIONS

In this study, the final diagnoses in the cases of feline anaemia were: concurrent *H. felis* and FeLV infections, FeLV infection, concurrent *H. felis* and acute myeloid leukaemia, *H. felis* infection on its own, renal diseases, Heinz body anaemia, feline infectious peritonitis, non-effusive peritonitis and flea infestation. Of these the largest number of cases of anaemia occurred in association with concurrent *H. felis* and FeLV infections and next to this was the anaemia associated with FeLV infection. Most of the anaemias in which FeLV was isolated were severe. *H. felis* infection on its own was diagnosed in the same number of cases of anaemia as FeLV infection on its own. However, more than 50 percent of the anaemias in this group were mild and some of the cases of marked anaemia occurred in cats with renal diseases. This study has shown that FeLV infection is more important as a cause of anaemia in cats than *H. felis* infection and it is probably the most important cause of feline anaemia. This is in contrast to the general tendency among previous authors to associate feline anaemia primarily with haemobartonellosis.

The prevalence percentage of 23.2 obtained for *H. felis* infection in this study is the highest recorded in Britain. Though this high figure is partly due to the fact that the sample population was biased in favour of anaemic cats, the staining techniques used in this study and repeated blood sampling of the cats examined are

important contributing factors. Earlier workers in Britain probably obtained low prevalence figures partly because they used only Giemsa and Leishman's stains and probably because most of the cats examined were sampled for H. felis infection only once. This study has shown that the prevalence of H. felis infection in cats in Britain is higher than earlier reports have indicated.

This study has also shown that though H. felis infection may occur in a sizeable proportion of any cat population, the infection on its own causes little or no clinical problem. However when it is complicated by FeLV infection, there is usually a marked anaemia which in most cases is refractory to treatment. It has therefore been shown for the first time that FeLV infection is probably more important in the pathogenesis of anaemia in clinical feline haemobartonellosis than the parasite H. felis itself. It is probable that most of the cases of clinical haemobartonellosis reported by earlier workers were complicated by FeLV infection. Most of the earlier work on feline haemobartonellosis preceded either the discovery of the existence of FeLV or simple techniques for the demonstration of FeLV infection.

The severity of the anaemia which develops in concurrent H. felis and FeLV infections is such that the compensatory regenerative responses that occur are inadequate to produce remissions. Also the anaemia does not respond to blood transfusions and most of the affected cats die or have to be euthanised. Therefore the

prognosis for anaemic cats with concurrent H. felis and FeLV infections should be regarded as poor.

Apart from FeLV infections, other conditions, such as leukaemias and renal diseases may also lead to the development of anaemia when they complicate H. felis infection. However these complications occur much less frequently than complications with FeLV infection.

The diagnosis of H. felis infection to date depends on the demonstration of the parasite in infected blood. This study has shown that the acridine orange staining procedure is better than the Romanowsky stains for demonstrating the parasite in infected blood. However it has also been shown in this study that the parasite is not always present in the peripheral blood even in severely anaemic cats. In order for greater accuracy in the diagnosis of H. felis infections, a new technique, either serological or immunological should be investigated. Until such a technique is developed, it is suggested that the blood of cats suspected to be infected with H. felis be sampled on several occasions before the animal is declared free of the infection. This is particularly important in cats being screened for use as blood donors as it is known that H. felis infection can be transmitted through blood transfusion from a healthy carrier to another cat.

REFERENCES

REFERENCES

Altman, N.H. (1974). Intraerythrocytic crystalloid bodies in cats and their comparison with haemoglobinopathies of man. Ann. N.Y. Acad. Sci. 241, 589-93.

Anderson, L., Wilson, R. and Hay, D. (1971). Haematological values in normal cats from four weeks to one year of age. Res. Vet. Sci. 12, 579-583.

Anon (1972). Feline infectious anaemia. Feline Pract. 2, 54-55.

Anon (1978). Anaemia in cats: Panel Report. Mod. Vet. Pract. 59 (12) 934-935.

Archer, R.K. (1965). Haematological techniques for use on animals. Blackwell Scientific Publications, Oxford. pp.120-121.

Archer, R.K. (1977). IN: Comparative Clinical Haematology. Eds. R.K. Archer and L.B. Jeffcott. Blackwell Scientific publications, Oxford, London, Edinburgh, Melbourne, pp. 537-610.

Balazs, T., Robinson, J., Grey, D. and Grice, H.C. (1961). Feline haemobartonellosis - A case report. Can. J. Comp. Med. Vet. Sci. 25, 220-222.

Bedford, P.G.C. (1969). Feline infectious anaemia: A clinical case report. Vet. Rec. 84, 601-602.

Bedford, P.G.C. (1970). Feline infectious anaemia in the London area. Vet. Rec. 87, 305-310.

Berman, E. (1974). Hemogram of the cat during pregnancy and lactation and after lactation. Am. J. Vet. Res. 35 (3) 457-460.

Bobade, P.A. and Akinyemi, J.O. (1980). Haemobartonellosis in a cat in Ibadan. J. Nig. Vet. Med. Ass. In Press.

Boon, G.D. and Rich, L.J. (1974). Heinz body anaemia associated with methylene blue administration in a cat. Feline Pract. 4 (6) 49-51.

Bradley, R.L. (1976). A case of feline infectious anaemia (haemobartonellosis) with simultaneous feline leukemia virus. Vet. Med./Small Anim. Clin. 71 (9) 1233-1234.

Carlisle, C.H., Penny, R.H.C., Prestcott, C.W. and Davidson, H.A. (1968). Toxic effects of phenylbutazone on the cat. Br. Vet. J. 124, 560.

Clark, R. (1942) Eperythrozoon felis (Sp. Nov.) in a cat. J. S. Af. V.M.A. XIII (1) 15-16.

Coles, E.H. (1980). Veterinary Clinical Pathology. 3rd Edn. W.B. Saunders Company, Philadelphia, London, Toronto. 72-122, 456-457.

Cotter, S.M. (1976). Feline leukemia virus induced disorders in the cat. Vet. Clin. N. Am. 6 (3), 367-378.

Cotter, S.M. (1979). Anaemia associated with feline leukemia virus infection. J.A.V.M.A. 175 (11), 1191-1194.

Cotter, S.M., Hardy, W.D. and Essex, M. (1975). Association of feline leukemia virus with lymphosarcoma and other disorders in the cat. J.A.V.M.A. 166 (5) 449-454.

Cramer, D.V. (1974). Anaemia in the cat. IN: Current Veterinary Therapy V. Small animal practice. Ed. Kirk, R.W., W.B. Saunders Company, Philadelphia, London, Toronto. 354-357.

Cramer, D.V. and Lewis, R.M. (1972). Reticulocyte response in the cat. J.A.V.M.A. 160 (1) 61-67.

Dacie, J.V. and Lewis, S.M. (1970). Practical Haematology, 4th Edn. J.A. Churchill, London. 104-105.

Demaree, R.S. and Nessmith (1972). Ultrastructure of Haemobartonella felis from a naturally infected cat. Am. J. Vet. Res. 33 (6) 1303-1308.

Falconer, G.J., Irving, A.C., Watson, P.R. and Ludwig, J. (1980). A case of erythremic myelosis in a cat. N.Z. Vet. J. 28 (4) 83-84.

Fan, L.C., Dorner, J.L. and Hoffman, W.E. (1978). Reticulocyte response and maturation in experimental acute blood loss anaemia in the cat. J.A.A.H.A. 14 (2) 219-224.

Finco, D.R., Duncan, J.R., Schall, W.D. and Prasse, K.W. (1975). Acetaminophen toxicosis in the cat. J.A.V.M.A. 166, 469.

Fischer, C.A. (1970). Retinopathy in anaemic cats. J.A.V.M.A. 156 (10) 1415-1427.

Fishler, J.J. and Birzele, F.D. (1972). A practical approach to diagnosis and control of feline infectious anaemia. Feline Pract. 2 (5) 36-37.

Flagstad, A. and Larsen, S.A. (1969). The occurrence of feline infectious anaemia in Denmark. Nord. Vet. Med. 21, 129-141.

Flint, J.C. and Moss, L.C. (1953). Infectious anaemia in cats. J.A.V.M.A. 122 (910) 45-48.

Flint, J.C. and McKelvie, D.H. (1955). Feline infectious anaemia - Diagnosis and treatment. Proc. 92nd meeting Am. Vet. Med. Ass. 240-242.

Flint, J.C., Roepke, M.H. and Jensen, R. (1958). Feline infectious anaemia I. Clinical aspects. Am. J. Vet. Res. 19, 164-168.

Flint, J.C., Roepke, M.H., and Jensen, R. (1959). Feline infectious anaemia II. Experimental cases. Am. J. Vet. Res., 20, 33-40.

Florio, R., Lescure, F., Dorchies, P., Guelfi, J.F. and Franc, M. (1977). L'hemobartonellose du chat. A propos de quelques cas observes dans la region Toulousaine. Revue de Med. Vet. 128 (3) 313-318, 321-322. (English Summary: Vet. Bull. 47 (9) Abst. 5023).

Giles, R.C. Jr., Buhles, W.C., and Montgomery, C.A. Jr., (1974). Myeloproliferative disorder in a cat. J.A.V.M.A. 165 (5) 456-457.

Gilmore, C.E., Gilmore, V.H. and Jones, T.C. (1964). Bone marrow and peripheral blood of cats: Technique and normal values. Path. Vet. I, 18-40.

Gretillat, S. (1977). (An infectious anaemia of dog, cat and horse (due to Haemobartonella) in Niger). Bull de L'Academie Veterinaire de France. 50 (4) 519-525. English Summary: Vet. Bull (1978). 48 (12) Abst. 6999.

Harbutt, P.R. (1963). A clinical appraisal of feline infectious anaemia and its transmission under natural conditions. Aust.Vet. J. 39 (10) 401-404.

Harbutt, P.R. (1969). The incidence of Eperythrozoon (Haemobartonella) felis in Victoria. Aust.Vet. J. 45 (2) 87-88.

Hardy, W.D. Jr., Old, L.J., Hess, P.W., Essex, M. and Cotter, S. (1973). Horizontal transmission of feline leukemia virus. Nature 244 (5415) 266-269.

Harvey, J.W. (1977). Feline haematology. Sc. Proc. 44th Boston Am. Anim. Hosp. Ass. Meeting 75-79.

Harvey, J.W. and Gaskin, J.M. (1977). Experimental feline haemobartonellosis. J.A.A.H.A. 13 (1) 28-38.

Harvey, J.W. and Gaskin, J.M. (1978a). Feline haemobartonellosis: Attempts to induce relapses of clinical disease in chronically infected cats. J.A.A.H.A. 14 (4) 453-456.

Harvey, J.W. and Gaskin, J.M. (1978b). Feline haemobartonellosis. Proc. 45th Ann. meeting A.A.H.A. 117-123.

Harvey, J.W. and Kornick, H.P. (1976). Phenzopyridine toxicosis in the cat. J.A.V.M.A. 169, 327.

Hataka, M. (1972). Haemobartonellosis in the domestic cat. Acta. vet. Scand. 13 (III) 323-331.

Hathaway, J.E. (1976). Feline anaemia. Vet. Clin. N. Am. 6 (3) 495- 510.

Hauser, P. (1963). Quantitatives und Qualitatives blutibild der Gesunden Katze. Schweiz. Arch. Tierheil, 105, 438.

Hayes, H.M. and Priester, W.A. (1973). Feline infectious anaemia. Risk by age, sex and breed; prior disease, seasonal occurrence, mortality. J. Sm. An. Pract. 14, 797-804.

Heise, S.C. and Smith, R.S. (1973). Lupus erythematosus with haemolytic anaemia in a cat. Feline Pract. 3 (3) 14-19.

Holzworth, J. (1956). Anaemia in the cat - J.A.V.M.A. 128 (10) 471-488.

Hoover, E.A. and Kociba, G.J. (1974). Bone lesions in cats with anaemia induced by feline leukemia virus. J. Natl. Cancer Inst. 53 (5) 1277-1284.

Hoover, E.A., Kociba, G.J., Hardy, W.D. Jr. and Yohn, D.S. (1974). Erythroid hypoplasia in cats inoculated with feline leukemia virus. J. Natl. Cancer Inst. 53 (5) 1271-1276.

Hoover, E.A., Perryman, L.E. and Kociba, G.J. (1973). Early lesions in cats inoculated with feline leukemia virus. Cancer Res. 33 (1) 145-152.

Hovell, G.J.R., O'Reilly, K.J. and Povey, R.C. (1970). A method of venipuncture in the cat. Vet. Rec. 87, 184-185.

Jain, N.C. (1973). Osmotic fragility of erythrocytes of dogs and cats in health and in certain haematologic disorders. Cornell Vet. 63, 411-423.

Jain, N.C. (1979). Haematologic characteristics of anaemia. Part II. Interpretive aspects. Calif. Vet. 33 (10) 15-18.

Jain, N.C. and Keeton, K.S. (1973). Scanning electron microscopic features of Haemobartonella felis. Am. J. Vet. Res. 34 (5) 697-700.

Jarrett, O. (1979). Feline leukemia virus. In Practice (Supp. to Vet. Rec.) 1 (2) 15-23.

Jarrett, W.F.H., Anderson, L.J., Jarrett, O., Laird, H.M. and Stewart, M.F. (1971). Myeloid leukemia in a cat produced experimentally by feline leukemia virus. Res. Vet. Sci. 12, 385-387.

Jessop, L. (1976). B.S.A.V.A. Regional Survey report. Feline infectious anaemia. Vet. Pract. 8 (9).

Kleinsorgen, A., Brandenburg, C. and Brummer, H. (1976). (Influence of using force on cats on blood values). Berliner und Munchener Tierarzliche Wochenschrift. 89 (18) 358-360. English Summary: Vet. Bull. 47 Abst. 643.

Krafka, J. Jr. (1931). Reticulocytes in the premature and stillborn. A test of oxygen tension theory of erythropoietic control. Folia. haemat. 43, 318-322.

Kraft, W. (1974). (Eperythrozoonosis in a tiger-cat, an ocelot and a fishing cat). Kleintier-Praxis 19 (8) 267-269. English Summary. Vet. Bull. 45 (8) Abst. 4267.

Kreier, J.P. and Ristic, M. (1968). Haemobartonellosis, Eperythrozoonosis, Grahamellosis and Ehrlichiosis IN: Infectious Blood Diseases of Man and Animals Vol. II. Eds. D. Weinman and M. Ristic. Acad. Press New York and London, 387-472.

Kreier, J.P. and Ristic, M. (1974). Genus IV. Haemobartonella. IN: Bergeys Manual of Determinative Bacteriology. 8th Edn. R.E. Buchanan and N.E. Gibbons (Eds). 910-912.

Landsberg, J.W. (1940). The blood picture of normal cats. Folia haemat. 64, 169.

Leeflang, P., Kleyn, E.F. and Mieog, W.H.W. (1970). (Feline Infectious anaemia II. Occurrence of the disease in the Netherlands). Tijdschr. Diergeneesk. 95, 223-235. English Summary Vet. Bull. 40 Abst. 3335.

Lewis, L.A. (1941). The blood picture of adrenalectomised animals treated with different adrenal fractions. Endocrinology 28, 821.

Loeb, W.F. (1975). Blood and blood forming organs. IN: Feline Medicine and Surgery. Ed. E.J. Catcott. 2nd Edn. Am. Vet. Publ. Inc., Santa Barbara. California. 225-250.

Lundborg, L.E., Karlbom, I. and Christensson, D. (1978). (Infectious anaemia in cats due to Haemobartonella felis). Svensk. Vet. 30 (10), 417-419. English Summary Vet. Bull. 48 (11) Abst. 6591.

Mackey, L. (1975). Feline leukemia virus and its clinical effects in cats. Vet. Rec. 96 (1), 5-11.

Mackey, L. (1977). Haematology of the cat. IN: Comparative Clinical Haematology. Eds. R.K. Archer and L.B. Jeffcott. Blackwell Scientific Publications. Oxford, London 441-482.

Mackey, L., Jarrett, W., Jarrett, O., and Laird, H. (1975). Anaemia associated with feline leukemia virus infection in cats. J. Natl. Cancer Inst. 54 (1), 209-217.

Madewell, B.R. and Feldman, B.F. (1980). Characterization of anaemias associated with neoplasia in small animals. J.A.V.M.A. 176, (5), 418-425.

Madewell, B.R., Jain, N.C. and Weller, R.E. (1979). Haematologic abnormalities preceding myeloid leukemia in three cats. Vet. Pathol. 16 (5), 510-519.

Maede, Y. (1975). Studies on Feline haemobartonellosis IV. Life span of erythrocytes of cats infected with Haemobartonella felis. Jap. J. Vet. Sci. 37, 269-272.

Maede, Y. (1978). Studies on feline haemobartonellosis V. Role of the spleen in cats infected with Haemobartonella felis. Jap. J. Vet. Sci. 40, 141-146.

Maede, Y. (1979). Sequestration and phagocytosis of Haemobartonella felis on the spleen. Am. J. Vet. Res. 40 (5), 691-695.

Maede, Y. and Hata, R. (1975). Studies on feline haemobartonellosis II. The mechanism of anaemia produced by infection with Haemobartonella felis. Jap. J. Vet. Sc. 37, 49-54.

Maede, Y. and Murata, H. (1978). Ultrastructural observation on the removal of Haemobartonella felis from erythrocytes in the spleen of a cat. Jap. J. Vet. Sci. 40, 203-205.

Maede, Y. and Sonoda, M. (1975). Studies on feline haemobartonellosis III. Scanning electron microscopy of Haemobartonella felis. Jap. J. Vet. Sci. 37, 209-211.

Maede, Y., Hata, R., Shibata, H., Niiyama, M., Too, K. and Sonoda, M. (1974). Clinical observations on six cases of feline infectious anaemia. J. Jap. Vet. Med. Ass. 27, 267-272.

Mahaffey, E.A. and Smith, J.E. (1978). Depression anaemia in cats. Feline Pract. 8 (5), 19-20, 22.

Manuel, M.F. and Abalos, H.P. (1975). A preliminary survey on the incidence of Haemobartonella felis in Philippine domestic cats. Phil. J. Vet. Med. 41 (1), 125-135.

Manusu, H.P. (1961). Infectious feline anaemia in Australia. Aust. Vet. J. 37, 405.

Manusu, H.P. (1962). Feline infectious anaemia. Aust. Vet. J. 38, 232-234.

Nash, A.S., Wright, N.G., Spencer, A.J., Thompson, H. and Fisher, E.W. (1979). Membranous nephropathy in the cat: A clinical and pathological study. Vet. Rec. 105, 71-77.

Ojeda, J.D. and Skewes, H.R. (1978). Haemobartonellosis felina: Communicacion de un brote Y su tratamiento. Vet. Mexico. 9 (2), 55-60.

Osbaldiston, G.W. (1978). Haematological values in healthy cats. Br. Vet. J. 134, 524-536.

Penny, R.H.C. (1978). Practical haematology for the small animal clinician. J. Small Anim. Pract. 19, 479-492.

Penny, R.H.C., Carlisle, C.H. and Davidson, H.A. (1970b). The blood and bone marrow picture of the cat. Br. Vet. J. 126 (9), 459-463.

Penny, R.H.C., Carlisle, C.H., Prestcott, C.W. and Davidson, H.A. (1970a). Further observations on the effect of chloramphenicol on the haemopoietic system of the cat. Br. Vet. J. 126, 453-458.

Perman, V. (1977). The anaemic cat. Sc. Proc. 44th Boston Am. Anim. Hosp. Ass. Meeting 51-59.

Prier, J.E. (1975). Bacterial and ricketsial diseases. IN: Feline Medicine and Surgery. Ed. E.J. Catcott. 2nd Edn. Am. Vet. Publ. Inc. Santa Barbara. 63-70.

Priester, W.A. and Hayes, H.M. (1973). Feline leukemia after feline infectious anaemia. J. Natl. Cancer Inst. 51 (1), 289-291.

Prieur, W.D. (1960). Beitrag zur infektiosen aneamie der Katze. Kleintier - Praxis, 5, 87-89.

Rogers, T.E. and Wallace, W.R. (1966). A rapid staining technique for Anaplasma. Am. J. Vet. Res. 27 (119) 1127-1128.

Schall, W.D. and Perman, V. (1975). Diseases of the red blood cells. IN: Textbook of Veterinary Internal Medicine Vol. 2. Ed. S.J. Ettinger. W.B. Saunders Co., Philadelphia, London, Toronto. 1581-1626.

Schalm, O.W. (1965). Veterinary Haematology 2nd Edn. Bailliere, Tindall and Cassell, London.

Schalm, O.W. (1973). Myeloproliferative disorder in a cat with a period of remission followed by a relapse two years later. Calif. Vet. 27 (4) 26-29.

Schalm, O.W. (1977). Heinz body haemolytic anaemia in the cat. Feline Pract. 7 (6), 30-33.

Schalm, O.W. and Switzer, J.A. (1972). Feline infectious anaemia. Feline Pract. 2 (5), 26-30.

Schalm, O.W. and Theilen, G.H. (1970). Myeloproliferative disease in the cat, associated with C-type leukovirus particles in the bone marrow. J.A.V.M.A. 157 (11), 1686-1696.

Schalm, O.W., Jain, N.C. and Carroll, E.J. (1975). Veterinary Haematology. 3rd Edn.Lea and Febiger, Philadelphia.

Schechter, R.D., Schalm, O.W., and Kaneko, J.J. (1973). Heinz body haemolytic anaemia associated with the use of urinary antiseptics containing methylene blue in the cat. J.A.V.M.A. 162 (1), 37-44.

Schryver, H.F. (1963). The bone marrow of the cat. Am. J. Vet. Res. 24, 1012-1017.

Schwartzman, R.W. and Besch, E.D. (1958). Feline infectious anaemia. Vet. Med. 53, 494-497.

Scott, D.W., Schultz, R.D., Post, J.E., Bolton, G.R. and Baldwin, C.A. (1973). Autoimmune haemolytic anaemia in the cat. J.A.A.H.A. 9 (6), 530-539.

Seamer, J. (1964). Symposium on diseases of cats II. Infectious anaemia of cats. J. Small. Anim. Pract. 5, 511-516.

Seamer, J. and Douglas, S.W. (1959). A new blood parasite of British cats. Vet. Rec. 71 (20), 405-408.

Searcy, G.P. (1976). The differential diagnosis of anaemia. Vet. Clin. N. Am. 6, 567-580.

Sheriff, D. (1974). Feline infectious anaemia. Vet. Rec. 94, 385.

Simpson, C.F., Gaskin, J.M. and Harvey, J.W. (1978). Ultrastructure of erythrocytes parasitised by *Haemobartonella felis*. J. Parasitol. 64 (3), 504-511.

Small, E. (1971). Feline infectious anaemia. IN: Current Veterinary Therapy IV. Small Animal Practice. W.B. Saunders Co. Philadelphia. 681.

Small, E. and Ristic, M. (1967). Morphologic features of *Haemobartonella felis*. Am. J. Vet. Res. 28, (124), 845-851.

Small, E. and Ristic, M. (1971). Haemobartonellosis. Vet. clin. N.Am. 1 (2), 225-230.

Sodikoff, C.H. and Custer, M.A. (1965). Autoimmune haemolytic anaemia in a cat. J.A.A.H.A. 1, 261-262.

Splitter, E.J., Castro, E.R., and Kanawyer, W.L. (1956). Feline infectious anaemia. Vet. Med. 51, 17-22.

Sutton, R.H., McKellow, A.M. and Bottrill, M.B. (1978). Myeloproliferative disease in the cat: a granulocytic and megakaryotic disorder. N.Z. Vet. J. 26 (11) 273-274, 279.

Switzer, J.W. (1971). Feline infectious anaemia. IN: Current Veterinary Therapy IV. Small Animal Practice. Ed. R.W. Kirk. W.B. Saunders Co., Philadelphia, 261-262.

Todd, K.S., Jr. (1975). Internal metazoal and protozoal diseases. IN: Feline Medicine and Surgery. Ed. E.J. Catcott. 2nd Edn. Am. Vet. Publ. Inc. Santa Barbara, California, 85-112.

Thomsett, L.R. (1960). *Eperythrozoon felis*: Observations on incidence and relationship to external parasitism in the cat. Vet. Rec. 72 (21) 397-399.

Tury, E., Papp, L., and Horvath, Z. (1977). Occurrence of feline infectious anaemia in Hungary (Preliminary Report) Magyar Allatorvosok Lapja 32 (4), 249-250. English Summary. Vet. Bull. 47 (10) Abst. 5506.

Velasco, M., Landaverde, M., Lifshitz, F. and Parra, A. (1971). Some blood constituents in normal cats. J.A.V.M.A. 158, 763-764.

Watson, A.D.J., Farrow, B.R.H. and Hoskins, L.P. (1978). Some observations on treating cats infected with *Haemobartonella felis*. Aust. Vet. Practitioner 8 (3), 129-132.

Wilkinson, G.T. (1963). Feline infectious anaemia. A case in a cat and its successful treatment. Vet. Rec. 75, 324-325.

Wilkinson, G.T. (1965). Two further clinical cases of feline infectious anaemia. Vet. Rec. 77 (16), 453-454.

Wilkinson, G.T. (1969). Feline infectious anaemia. Vet. Rec. 84, 331-333.

Windle, W.F., Sweet, M., and Whitehead, W.H. (1940). Some aspects of prenatal and postnatal development of the blood of the cat. Anat. Rec. 78, 321.

Wright, A.I. (1973). Anaemia in cats. Bull. Feline Advisory Bureau. 12 (10), 6-8.

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