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HAEMOPARASITES OF FELINES - AN OVERVIEW

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ABSTRACT

Haemoparasitic infections persist as one of the major challenges in companion animal health. Despite the increased incidence, studies on tick-borne apicomplexan haemoparasites infecting domestic cats are comparatively fewer. These vector-borne diseases are emerging problems in cats across the globe. Feline haemoparasites include *Hepatozoon* spp. such as *H. felis*, *H. canis* and *H. silvestris*, Piroplasm-causing organisms such as *Cytauxzoon felis*, *Babesia felis, B. cati*, *B. herpailuri* and *B. pantherae* and haemoflagellates such as *Trypanosoma evansi.* Ticks have the major role in the transmission of hepatozoonosis, cytauxzoonosis and babesiosis. Haematophagous insects including *Tabanus, Stomoxys and Lyperosia* act as vectors for trypanosomosis. Routine diagnosis of infection is based on microscopic identification of parasites in blood smears or tissues. Application of molecular diagnostic techniques like polymerase chain reaction (PCR) for rapid diagnosis and epidemiological studies will enhance the potential of veterinary services. Knowledge on occurrence and clinical manifestations of haemoparasitic infections will aid in improving the health of cats and facilitate the development of an efficient disease control strategy.

Keywords: Felines**,** Hepatozoonosis, Cytauxzoonosis, Babesiosis, Trypanosomosis

INTRODUCTION

Feline vector-borne diseases (VBDs) are being increasingly reported worldwide. The population of domesticated cats has drastically increased during the last decades. With the recently identified rise in cat population, appropriate management strategies are required to maintain proper health and to control infections including VBDs. The knowledge about VBDs in cats is still limited, mainly due to the rapidly changing epidemiology, with wider geographical distribution and increasing

global prevalence. Factors such as climate change, environmental, demographic and human behaviour are involved in this process. Most of the vector-borne agents cause morbidity and mortality in the domestic cats.

There are no significant symptoms to rule out the disease apart from that it also can occur in healthy animals making the diagnosis of these diseases challenging. Co-infection of haemoparasites with retrovirus or haemotropic mycoplasmas has also been reported which may cause immunosuppression and worsen the condition of the animal (Malangmei *et al.,* 2021). Diagnosis is the major challenge considering their occurrence in healthy cats, the presence of non-specific clinical signs in infected animals or the simultaneous occurrence with other infections. Morphological identification of parasites using light microscopy is an important and common diagnostic test. Recent application of molecular diagnostic techniques such as polymerase chain reaction (PCR) and DNA sequence analysis are beneficial for the diagnosis of individual clinical cases as well as for epidemiological studies on tick- and other vector-borne agents of domestic cats (Oliveira *et al.,* 2018).

Information about the presence and distribution of haemoparasites in cats will promote in establishing early diagnostic tools and immediate treatment of cats and kittens that host these parasites. This will aid in improving the health of cats and facilitating in development of an efficient disease control strategic plan. Cats having access to outdoors are more vulnerable to parasitic infections as a result of exposure to variety of ectoparasites that may transmit these diseases.

FELINE HEPATOZOONOSIS

The genus *Hepatozoon* are Apicomplexan parasites of the family *Hepatozoidae*. Currently more than 340 species of *Hepatozoon* affecting mammals, birds, reptiles and amphibians have been identified. The first report of hepatozoonosis in domestic cat was from India in 1908. Later, the infection was reported from domestic cats of several countries including South Africa, Asia, southern Europe and USA. The exact identity of the species which infect cats, their pathogenicity and vectors involved in the transmission have not been explained. Three different *Hepatozoon* species were described to infect domestic cats which include, *H. felis, H. canis* and *H. silvestris*. *Hepatozoon felis* is the most often diagnosed species in cases of feline hepatozoonosis in India (Malagmei *et al*., 2021; Vincy, 2022).

Transmission

Blood-sucking arthropods are the

definitive hosts for *Hepatozoon* spp. but arthropod vectors of feline infections are not identified conclusively. It is assumed that ticks are the vectors of *H. felis* and *H. silvestris*. The sexual development and sporogony take place in these arthropods. The infection is transmitted to the vertebrate host by the ingestion of the arthropod or part of it which contains mature oocysts of the parasite (Morelli *et al.,* 2020). Recently, transplacental transmission and predation were also reported (Hodžić *et al.,* 2017). Merogony and gametogony occurs inside the vertebrate host. Gamonts formed by this are found in leukocytes, usually in neutrophils. The occurrence of small tissue cysts containing single or multiple parasitic stages (cystozoites) have been described in several species of *Hepatozoon* accenting the complexity of their life cycles.

Clinical signs

Feline hepatozoonosis is related with infection of muscle tissues. Meronts of *Hepatozoon* are recognized in the myocardium and skeletal muscles of domestic cats with hepatozoonosis. The infection is mostly subclinical in domestic cats. More pathogenic effects may be expressed in stressed and immunocompromised cats or cats with concomitant infections (Díaz-Regañón *et al.,* 2017). Poor body condition, lethargy, anorexia, icterus, abdominal distension, fever, lymphadenopathy, ruffled hair and tick infestation are the common clinical signs noticed in feline hepatozoonosis (Basso *et al.,* 2019). Elevated muscle enzyme creatinine kinase (CK) is found in majority of cats with hepatozoonosis. Other consistent pathological findings reported were anaemia, neutrophilic leukocytosis, and increased serum total bilirubin and indirect bilirubin concentrations (Tuna *et al*., 2018).

Diagnosis

Microscopical examination of blood smears is not sensitive enough for the detection of *Hepatozoon* gamonts, due to the low level of parasitaemia. Less than one per cent of neutrophils and monocytes only contain gamonts of *Hepatozoon* (Pereira *et al.,* 2019). *Hepatozoon felis* gamonts usually locate in the cytoplasm of neutrophils and monocytes sometimes compress the lobulated host cell nucleus. The gamonts of *H. felis* are relatively shorter with a mean length of 10.5µm when compared with the *H. canis* gamont which is 11µm long.

The *H. felis* meronts are found in muscular tissues especially myocardium and skeletal muscles. The *H. felis* meront does not form the typical wheel spoke shape like *H. canis* meront. *Hepatozoon felis* meronts contain 10–15 micromerozoites which are

larger, rectangular or triangular in shape and oriented perpendicularly to the meront wall (Hodžić *et al.,* 2017). Morphological characteristics of meronts of *H. silvestris* include wheel spoke-shape with thinner capsule which has 20–30 smaller and round to oval shape micromerozoites.

Polymerase chain reaction is more sensitive than blood smear examination for diagnosis of *Hepatozoon* infection. Current technique uses Piroplasmid-F/Piroplasmid R primers targeting a partial sequence of the 18S rRNA gene of *Hepatozoon* spp. for diagnostic purpose (Baneth *et al.,* 2013). Merging of morphologic and genetic findings supported by a broad-based epidemiological study, is required for detailed characterization and description of *Hepatozoon* spp. in domestic cat.

Treatment

The treatment of choice is not known, but single cases have been treated with doxycycline or oxytetracycline and primaquine. Imidocarb dipropionate (two doses of 6 mg/kg body weight, SC, with an interval of 14 days) in combination with doxycycline monohydrate (5 mg/ kg body weight twice a day, orally, for four weeks) was effective to treat hepatozoonosis in domestic cats (Basso *et al.,* 2019). Although the mode of transmission and the type of vector is not proved, preventive treatment against blood-sucking vectors (fleas and ticks) is advised.

FELINE CYTAUXZOONOSIS

Cytauxzoonosis is an emerging, tick-borne disease of domestic and exotic cats. *Cytauxzoon* spp. are apicomplexan haemoparasites belonging to family Theileriidae. Several *Cytauxzoon* spp. have been identified among which *C. felis* being the most important (Díaz-Regañón *et al.,* 2017). *Cytauxzoon felis* was first identified in Missouri in 1973 (Brown *et al.,* 2009). Infection is characterized with an extremely high fatality rate in domestic cats. Bobcats (*Lynx rufus*) are considered to be the primary reservoir of *C. felis* and harbour infection without clinical signs (Pollard *et al.,* 2017).

Transmission

Cytauxzoon felis has a complex lifecycle that includes an asexual stage within a felid host and a sexual reproductive stage within a competent ixodid tick vector. The parasite is transmitted by ticks that acquire the infection by feeding on parasitaemic infected hosts. *Amblyomma americanum* (lone star tick) is the principal vector. *Dermacentor variabilis* (American dog tick) also considered as the vector capable of transmitting the infection. In Bobcats, infections are usually mild or subclinical and they can act as persistently infected

carriers and are the major reservoir hosts. Domestic cats that survived the acute illness can also remain persistently parasitaemic rendering the animal a subclinical chronic carrier. Piroplasms persist in the RBCs of the cats survived the infection throughout the life span. These chronic carriers may serve as reservoirs and increase the risk of *C. felis* exposure to other domestic cats (Pollard *et al.,* 2017).

Clinical signs

Onset of clinical signs occurs one to two weeks after tick transmission. Initial signs are non-specific. There can be sudden onset of marked lethargy and anorexia in healthy cat. Later the disease progresses to severe form. The cat show vocalization, weakness, icterus, respiratory distress, abnormal mentation and sometimes seizures. Fever (103^0-107^0F) is a consistent finding. Dehydration, pale or icteric mucous membranes, tachypnoea, tachycardia and mild to moderate enlargement of lymph nodes, spleen and liver may be found on physical examination. Bilaterally elevated and hyperaemic nictitating membrane is a frequent finding. Due to generalized pain most of the infected cats are reluctant to move. Dyspnoea, hypothermia and comatose are noted in the terminal stages of the disease. Haemolytic anaemia occurs frequently.In some cats neurological signs may occur in late stages. Due to the rapid disease progression most cats die within one week of the onset of clinical signs (Meinkoth and Kocan, 2005).

Cytauxzoonfelis infection frequently cause anaemia in domestic cats. This is due to the schizogonous phase in which the blood vessels of the organs, liver, lungs, lymph nodes and spleen are occluded by schizont-laden mononuclear phagocytes. After maturation the organisms enter into erythrocytic phase and invade erythrocytes as piroplasms. This will result in haemolysis (Pollard *et al.,* 2017). Presence of low haematocrit and haemoglobin values, thrombocytopaenia, increased level of glucose, hepatic enzymes and serum albumin are the haemato-biochemical changes reported (Díaz-Regañón *et al.,* 2017).

Diagnosis

Examination of peripheral blood smears is the most common method of diagnosing Cytauxzoonosis. The organism may appear pleomorphic. The classic form of *C. felis* is a small ring (1–3 μm in diameter) with a thick round nuclear area at one point of the ring (signet ring). Other forms include elongated ‘‘safety-pin’’ form with two nuclear structures on opposite sides and small comma-shaped or linear forms which lack a distinct nuclear thickening. Schizonts of *C. felis* can be observed in

fine needle aspirates of infected organs (spleen, lymph nodes), histopathology, or impression smears (Reichard *et al.,* 2021).

Polymerase chain reaction (PCR) is the molecular method employed for diagnosing *C. felis*. Different genetic targets used for *C. felis* diagnosis include 18S rRNA, internal transcribed spacer 1 (ITS1), cytochrome b (cytb) and cytochrome c oxidase subunit III (cox3). These methods are much more sensitive and specific compared to light microscopy but are more time-consuming and costly (Reichard *et al.,* 2021).

Treatment

The current recommended treatment of combination of atovaquone (15 mg/kg PO q8h) and azithromycin (10 mg/kg PO q24h) along with aggressive supportive and nursing care has great survival rate. Use of diminazene aceturate is not effective and adverse side effects are common (Reichard *et al.,* 2021). But Meier and Moore (2000) reported efficiency of diminazene (2 to 3.5 mg/kg body weight, intramuscularly [IM]) or imidocarb (2 mg/kg body weight, IM) administered twice within one week, together with adjunctive heparin and intravenous maintenance fluid therapy. Use of parvaquone or buparvaquone may be efficacious in the treatment of feline cytauxzoonosis, but these drugs have not shown significant potential. Concurrent supportive treatment and critical care is extremely important to improve the prognosis. Cats that survive the infection may become chronic carriers for life.

FELINE BABESIOSIS

Babesiosis is a tick-borne haemoparasitic disease caused by the apicomplexan parasite *Babesia*. First clinical cases of feline babesiosis were reported from the Cape Town, South Africa, in 1937. Earlier, based on the morphology of the organisms, feline *Babesia* species were classified either as "large" or "small" (Penzhorn and Oosthuizen, 2020). Small babesial organisms included *B. felis* and *B. cati* (1.0–2.5 µm) and large babesial organisms were *B. herpailuri* and *B. pantherae* (2.5–5.0 µm) (Panicker *et al.,* 2020). The most virulent species in felines is *B. felis* and the least one is *B. cati* (Ayoob *et al.,* 2010).

Transmission

The natural transmission of Babesiae to vertebrate hosts occurs through the bite of the vectors. Ticks of the genera *Ixodes, Dermacentor, Rhipicephalus, Amblyomma* and *Haemophysalis* were known to infest cats and were likely vectors for transmission. Mechanical transmission through other biting insects and arthropods may also occur (Ayoob *et al.,* 2010).

Ingestion of erythrocytes lodging merozoites by the ticks from parasitized host results in the transmission of infection to arthropod vector. Within the arthropod salivary gland multiple fission of merozoites results in the production of sporozoites. These sporozoites will be inoculated into the host circulation during the time of blood meal by the vectors. A prepatent period of 3 to 28 days is reported for *B. felis*. Transstadial and transovarial transmission occur in adult female ticks. This has great role in maintaining infection with in vector population.

Clinical signs

Generally, babesiosis in domestic cats is associated with anorexia, lethargy, anaemia and icterus. The acute infection of *B. felis* is characterized by a severe clinical illness. Immunocompetence of the host, chronicity of infection and concurrent disease influence the level of parasitaemia. Macrocytic hypochromic regenerative anaemia, hyperbilirubinemia and elevated alanine aminotransferase (ALT) activity are common. Concurrent infection with feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) can aggravate the condition (Solano-Gallego and Baneth, 2011).

Diagnosis

Examination of stained blood smears has been the standard diagnostic technique and reliable when a moderate to high parasitaemia is present. Smears made from capillary blood (from ear tip or toe nail) may be beneficial and freshly prepared smears are suggested for the accurate diagnosis. Similarly, automated flow cytometric techniques have been employed to detect *Babesia* in reticulocytes and mature erythrocytes (Solano-Gallego and Baneth, 2011).

The piroplasms of *B. felis* are small and most commonly observed as singular or paired annular bodies (signet ring), pear shaped forms and rarely, tetrads (Maltese cross forms). *Babesia cati* appear as single or paired annular bodies within the erythrocyte and results in milder clinical disease. The morphology of large feline babesial species *B. herpailuri* is single or paired annular bodies (Ayoob *et al.,* 2010). Piroplasms of *B. pantherae* are smaller than *B. herpailuri* (Penzhorn and Oosthuizen, 2020).

Molecular characterization is crucial in distinguishing between *Babesia* species and in identifying and describing new taxa (Penzhorn and Oosthuizen, 2020). Recent technique of 18S rRNA gene (rDNA)-based PCR method provides greater sensitivity and specificity in terms of detection and differentiation of *Babesia* species (Simking *et al.,* 2010).

Use of Indirect fluorescent antibody (IFA) testing to detect antibabesial antibodies in the blood of infected or exposed animals is described, but is not recommended for routine diagnosis (Ayoob *et al.,* 2010).

Treatment

It is difficult to treat feline babesial infections (especially small *Babesia* spp.) because of poor initial response to therapy and a high rate of relapse. Current protocol mainly emphasizes on the resolution of clinical signs and anaemia and not the complete elimination of infection. Monitoring through routine haematology evaluation and blood smear analysis is indicated. Primaquine phosphate is the drug of choice for small feline babesia infections at a dose rate of 0.5 to 1.0 mg/kg PO, IV, or IM once, or administered daily for three consecutive days. Rapid resolution of clinical signs and reduction in parasitaemia is seen within 24–72 hours. The drug is proven to be reliably efficacious but doses exceeding 1 mg/ kg should be avoided because of the fatal toxicity. Vomiting after oral administration is very common. Long term therapy should be depending on hematologic variables and clinical signs rather than parasitaemia.

Diminazine aceturate is a potential drug in the treatment of large babesial species in cats. Clinical efficacy can be varied by the use of a single dose of 3.5 mg/kg, IM in the treatment of *B. felis.* Imidocarb dipropionate is not efficient in the treatment of *B. felis* infections in cats. A single dose of 2.5 mg/kg, IM or co-administration with doxycycline are both effective in the resolution of clinical signs in *B. canis* subsp. *presentii* and *B. herpailuri* infections. Adverse effect is due to the anticholinergic nature of drug that elicit signs including salivation, lacrymation, vomiting, diarrhoea, muscle tremors, restlessness, tachycardia and dyspnoea.

Fluid therapy is indicated for maintenance of blood volume and adequate end-organ perfusion, correction of acid-base and electrolyte abnormalities, diuresis, and prevention of RBC sludging in capillaries. Blood transfusion may become necessary if anaemia is significant. As the anaemia results from haemolysis, packed red blood cells are the blood component of choice. Animal can be supported with vitamin and mineral preparations, corticosteroids, anabolic steroids, nonsteroidal anti-inflammatory drugs, antioxidant therapy, nutritional support, and appetite stimulants (Ayoob *et al.,* 2010).

FELINE TRYPANOSOMOSIS

Trypanosoma evansi is a haemoflagellate protozoan parasite. Even though the infections in livestock and dogs are widely documented, reports on natural

infection of trypanosomosis in domestic cats is very rare (Tarello, 2005). Feline trypanosomosis is frequently reported in wild animals from India (Patel and Patel, 2020).

Transmission

Haematophagous insects of the genera *Tabanus, Stomoxys, Atylotus* and *Lyperosia* can mechanically transmit the disease. Infection can also be transmitted by ingestion of infected meat through the abrasion in oral mucosa especially in wild carnivores (Patel and Patel, 2020).

Clinical signs

Partial blindness, anorexia, pyrexia, lameness, extended fore limbs, enlarged lymph nodes, pale mucus membranes, abnormal mentation and elevated heart rate and respiratory rate are the common clinical signs reported in feline trypanosomosis in India (Thirunavukkarasu *et al.,* 2000; Sivajothi and Sudhakara Reddy, 2018).

Diagnosis

Microscopic methods like wet film examination and conventional staining techniques including Giemsa staining, Field's staining and Acridine orange staining can be used for the diagnosis of trypanosomosis (Rani *et al.,* 2022). Serological tests which include card agglutination test (CATT), ELISA, immunoblotting, immunofluorescence test and immunochromatographic test can also be used for the diagnostic purpose (Kumar *et al.,* 2022).

Elevated muscular enzymatic activities (aspartate aminotransferase and creatine kinase), hyperproteinaemia, hyperglobulinemia and hypoalbuminemia were observed in infected cats. No alteration in serum activity of alanine aminotransferase, gamma-glutamyl transferase, creatinine and urea were noted in cats experimentally infected with *T. evansi* (Da Silva *et al.,* 2010).

Treatment

Da Silva *et al.* (2009) reported 85.7 per cent efficacy of diminazene aceturate for the treatment of trypanosomosis in cats at a dose rate of 3.5 mg/ kg BW. Sivajothi and Sudhakara Reddy (2018) reported complete cure in a seventeen-month-old tom cat infected with *T. evansi* with single dose administration of diminazene aceturate at 3.5mg/kg and supportive therapy after six days.

CONCLUSION

Feline vector borne diseases have emerged in recent years, showing a wider geographic distribution and increased global prevalence. Most of the feline haemoparasitic infections are manifested

as subclinical infection in domestic cats. But may develop in association with immunosuppression induced by a concurrent disease that can lead to the intensification of parasitaemia. Preventive and control measures against ectoparasite infestation including regular individual use of ectoparasiticide formulations, seem the most effective tool to prevent infection in cats and other hosts. The recognition of cats as hosts of different vector-borne pathogens is of paramount importance towards a better management of these diseases in animals. Molecular based diagnostic methods applied to vector-borne pathogens are very effective to detect and characterize infecting organisms, for monitoring cure after chemotherapy and to evaluate the role of sub clinically-infected cats in the transmission of infections.

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