GUIDELINE for Haemoplasmosis in Cats

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Key points

- Haemoplasmas are bacteria that attach themselves to the surface of red blood cells and can induce haemolysis, sometimes resulting in anaemia.
- *Mycoplasma haemofelis* is the most pathogenic of the three feline haemoplasma species.
- ‘*Candidatus Mycoplasma haemominutum*’ and ‘*Candidatus Mycoplasma turicensis*’ infections are less pathogenic but can result in disease, especially in immunocompromised cats or cats with concurrent disease.
- Male non-pedigree cats of increasing age with outdoor access are more likely to be haemoplasma-infected.
- The natural mode of transmission of haemoplasma infection is not known; aggressive interactions and vertical transmission are possible routes.
- Transmission by blood transfusion can occur and all blood donors should be screened for haemoplasma infection.
- The evidence for transmission by vectors is poor.
- Polymerase chain reaction (PCR) assays are the preferred diagnostic method for haemoplasma infections.
- Cats chronically infected with haemoplasmas do not usually show clinical signs of infection – subclinical infections can exist for all three feline haemoplasma species.
- Treatment with doxycycline for 2 weeks is usually effective for the treatment of *haemofelis*-associated clinical disease, but doxycycline treatment does not always clear infection completely, even if clinical signs resolve. A protocol comprising 4 weeks of doxycycline, followed by 2 weeks of marbofloxacin for those cats that are still PCR-positive after the doxycycline treatment, has been described to clear chronic *M. haemofelis* infection. This protocol can be considered if clinical disease is severe and/or recurrent and/or when concurrent disease is present.
- We do not recommend the use of corticosteroids for the treatment of haemoplasmosis unless a cat with a positive Coombs’ test fails to respond to appropriate antibiotic treatment alone and the diagnosis of haemoplasmosis is uncertain. In such cases, immune-mediated haemolytic anaemia could be the cause of the cat’s anaemia.
- Treatment of healthy subclinical infections with any haemoplasma species, in which cats do not show any clinical signs, is not recommended.
Little information is currently available on the antibiotic responsiveness of ‘M. haemominutum’ and ‘Ca. M. turicensis’.

Agent properties

The haemoplasmas are haemotropic mycoplasmas, bacteria that parasitize red blood cells and can induce haemolytic anaemia. The haemoplasmas were initially classified as rickettsial organisms within the *Haemobartonella* and *Eperythrozoon* genera, but sequence analysis of the 16S rRNA gene of haemoplasmas resulted in their reclassification within the genus *Mycoplasma* in the *Mycoplasmataceae* family (Neimark et al., 2001; Messick et al., 2002; Neimark et al., 2002). However, research suggests that although the haemoplasmas probably do belong to this family, they might be better placed in their own separate genus (Hicks et al., 2014a). In contrast to many ‘classical’ mycoplasmas, haemoplasmas are uncultivable. Their propagation is possible in living animals only, but not yet in vitro.

The three main haemoplasma species known to infect cats are *Mycoplasma haemofelis*, ‘*Candidatus Mycoplasma haemominutum*’ and ‘*Candidatus Mycoplasma turicensis*’. These mycoplasmas have a worldwide distribution. A canine haemoplasma species-like organism, described as ‘*Candidatus Mycoplasma haematoparvum*-like, has also been reported in a small number of cats in two studies (Sykes et al., 2007a; Martinez-Diaz et al., 2013). The clinical importance of this haemoplasma species in cats remains unclear.

Epidemiology

Prevalence

In general, studies that evaluated domestic cats for the presence of all three of the feline haemoplasma species by PCR, found that ‘*Ca. M. haemominutum*’ is more prevalent (4.4 – 46.7% of cats are infected) than *M. haemofelis* (0.4 – 27.0% of cats) and ‘*Ca. M. turicensis*’ (0 – 26.0% of cats). Reported prevalences vary with geographical variation and also differ quite widely, likely because the cats sampled in different studies are very variable, i.e. some studies test only ill anaemic cats, whereas others sample only healthy cats, some test stray feral cats whereas others focus on owned cats registered with veterinary clinics. Feline haemoplasma infections have been identified in prevalence studies performed worldwide, including in and around Europe: Cyprus (Attipa et al., 2017), Denmark (Rosenqvist et al., 2016), Germany (Bauer et al., 2008; Bergmann et al., 2017), Iran (Ghazisaeedi et al., 2014), Ireland (Juvet et al., 2010), Italy (Gentilini et al., 2009; Persichetti et al., 2016; Ravagnan et al., 2017; Persichetti et al., 2018; Latrofa et al., 2020), Latvia (Bertzina et al., 2021), Malta (Mifsud et al., 2020), Portugal (Martinez-Diaz et al., 2013; Mesa-Sanchez et al., 2021), Romania (Imre et al., 2020), Serbia (Sarvani et al., 2018), Russia (Demkin and Kazakov, 2021), Spain (Roura et al., 2010; Ravicini et al., 2016; Diaz-Reganon et al., 2018; Mesa-Sanchez et al., 2021; Villanueva-Saz et al., 2023), Switzerland (Willi et al., 2006a), Turkey (Ural et al., 2009) and the UK (Tasker et al., 2003; Willi et al., 2006b; Peters et al., 2008).

Predisposing factors

Risk factors (Barker and Tasker, 2023) for haemoplasma infection have been studied in many cat populations. Varied results were obtained due to differences between the studies; these include sample size, which haemoplasma species was tested for, risk factor information collected and whether the information source was reliable, and the statistical analysis methods employed in the studies (e.g. univariable or multivariable analysis).

However, amongst the different studies, a number of characteristics have emerged as being risk factors for haemoplasma infection.

Being male was a risk factor for haemoplasma infection in many studies (Tasker et al., 2003; Luria et al., 2004; Tasker et al., 2004; Willi et al., 2006a; Willi et al., 2006b; Bauer et al., 2008; Sykes et al., 2008; Roura et al., 2010; Tanahara et al., 2010; Stojanovic and Foley, 2011; Georges et al., 2012; Lobetti and Lappin, 2012; Aquino et al., 2014; Ghazisaeedi et al., 2014; Raimundo et al., 2016; Walker Vergara et al., 2016; Bergmann et al., 2017; Ravagnan et al., 2017; Diaz-Reganon et al., 2018; Makino et al., 2018; Marcondes et al., 2018; Sarvani et al., 2018; Latrofa et al., 2020; Berzina et al., 2021; Demkin and Kazakov, 2021; Manvell et al., 2021; da Rosa Maciel et al., 2023; Villanueva-Saz et al., 2023) but gender was not a risk factor in others (Inokuma et al., 2004; Bauer et al., 2008; Gentilini et al., 2009; Nibblett et al., 2009; Juvet et al., 2010; Maher et al., 2010; Nibblett et al., 2010; Martinez-Diaz et al., 2013; Firmino et al., 2016; Rosenqvist et al., 2016; Sacristan et al., 2019; Do et al., 2020; Imre et al., 2020).

Non-pedigree breeds were at increased risk in some studies (Tasker et al., 2004; Nibblett et al., 2009; Rosenqvist et al., 2016; Makino et al., 2018; Sarvani et al., 2018), but not others (Gentilini et al., 2009; Nibblett et al., 2010; Roura et al., 2010; Martinez-Diaz et al., 2013; Makino et al., 2018; Imre et al., 2020).
Having outdoor access increased the risk of haemoplasma infection in most reports (Willi et al., 2006a; Sykes et al., 2007a; Roura et al., 2010; Walker Vergara et al., 2016; Attipa et al., 2017; Bergmann et al., 2017; Diaz-Reganon et al., 2018; Sarvani et al., 2018; Imre et al., 2020) but not all (Martinez-Diaz et al., 2013; da Rosa Maciel et al., 2023).

Increasing age was sometimes identified as a risk factor for infection (Tasker et al., 2003; Tasker et al., 2004; Willi et al., 2006a; Sykes et al., 2007a; Bauer et al., 2008; Maher et al., 2010; Georges et al., 2012; Rosengqvist et al., 2016; Walker Vergara et al., 2016; Attipa et al., 2017; Ravagnan et al., 2017; Diaz-Reganon et al., 2018; Persichetti et al., 2018; Sarvani et al., 2018; Do et al., 2020; da Rosa Maciel et al., 2023), although age was not a risk factor in others (Inokuma et al., 2004; Lobetti and Tasker, 2004; Gentili et al., 2009; Nibblett et al., 2009; Nibblett et al., 2010; Roura et al., 2010; Assarasakorn et al., 2012; Martinez-Diaz et al., 2013; Sacristan et al., 2019; Imre et al., 2020; Latrofa et al., 2020; Berzina et al., 2021).

Young cats were likely to develop more severe clinical disease than older cats following *M. haemofelis* infection (Harvey and Gaskin, 1978; Shaw et al., 2004).

Retrovirus infection (Sykes et al., 2008; Stojanovic and Foley, 2011; Georges et al., 2012; Martinez-Diaz et al., 2013; Attipa et al., 2017), especially feline immunodeficiency virus (FIV) (Luria et al., 2004; Sykes et al., 2007a; Macieira et al., 2008; Gentili et al., 2009; Roura et al., 2010; Tanahara et al., 2010; Walker Vergara et al., 2016; Bergmann et al., 2017; Ravagnan et al., 2017; Diaz-Reganon et al., 2018; Persichetti et al., 2018; Sarvani et al., 2018) but also feline leukaemia virus (FeLV) (Inokuma et al., 2004; Luria et al., 2004; Sykes et al., 2007a) are risk factors. However, retrovirus infections are not always a risk factor (Willi et al., 2006a; Georges et al., 2012; Marcondes et al., 2018; Imre et al., 2020) and some studies found that neither FIV (Inokuma et al., 2004; Macieira et al., 2008; Demkin and Kazakov, 2021) nor FeLV (Sykes et al., 2007a; Macieira et al., 2008; Gentili et al., 2009; Roura et al., 2010; Tanahara et al., 2010; Bergmann et al., 2017; Demkin and Kazakov, 2021; da Rosa Maciel et al., 2023) were risk factors for haemoplasma infection. Being anaemic or having a reduced packed cell volume can increase risk (Nibblett et al., 2010; Persichetti et al., 2018; Sarvani et al., 2018) (especially *M. haemofelis* infection (Jensen et al., 2001; Maher et al., 2010; Diaz-Reganon et al., 2018; da Rosa Maciel et al., 2023) but also ‘Ca. M. turicensis’ coinfection (Willi et al., 2006b)). Epidemiology studies suggest that the host phenotype (e.g. being an aggressive male phenotype) could drive some of these associations and risk factors, rather than infections being simple risk factors for each other (Carver et al., 2015).

Leishmania infection was associated with ‘Ca. M. turicensis’ coinfection in one study (Attipa et al., 2017).

Additionally, *Felis catus* gammaherpesvirus 1 (FcaGHV1), a potential feline pathogen, has been found to be significantly associated with haemoplasma infection in studies (McLuckie et al., 2016; Novacco et al., 2019), suggesting similar transmission routes, but the significance of FcaGHV1 in cats has not yet been elucidated (Beatty et al., 2014; see also the ABCD Guidelines on FcaGHV1).

Case reports of cats with haemophagocytic syndrome with concurrent ‘Ca. M. haemominutum’ (Strandberg et al., 2023) or *M. haemofelis* infection (Fonseca et al., 2023) have been described; this significance of the haemoplasma infection in these cases is not known although haemoplasma-associated haemophagocytic syndrome has been described in a human (Hattori et al., 2020).

No association between blood genotype or phenotype and haemoplasma infection has been found in a study evaluating UK and Italian cats (Spada et al., 2023).

**Transmission**

**Blood transfusion**

Transmission via contaminated blood transfusions has been reported (Willi et al., 2006a) and the use of freshly collected blood from a haemoplasma-infected blood donor for transfusion would very likely result in transmission of infection to the recipient cat. The risk of haemoplasma transmission when using stored blood for transfusions will depend on the viability of haemoplasmas in stored blood. A study using the canine haemoplasma species, *Mycoplasma haemocanis*, suggested that survival within stored blood was possible, although inoculation of blood into naïve dogs was not performed to confirm viability (Camargo Castillo et al., 2023). One study (Gary et al., 2006) evaluated the survival of haemoplasma organisms in blood collected into citrate-phosphate-dextrose-adenine (CPDA) anticoagulant using inoculation studies. Haemoplasma survival was assessed by the ability to transmit *M. haemofelis* or ‘Ca. M. haemominutum’ infection into naïve cats via the intravenous inoculation of infected blood which had been stored in CPDA at 4°C for 1 hour, 1 week or 1 month. *M. haemofelis* was only successfully transmitted to the naïve cat using the blood that had been stored for 1 hour, and in this cat there was evidence of subsequent in vivo amplification of *M. haemofelis* in the blood, as organism numbers increased during the 3-week post-inoculation monitoring period. Some evidence for ‘Ca. M. haemominutum’ transmission was also found using the blood stored for 1 hour, although organism numbers in the recipient naïve cat’s blood did not increase post-inoculation. ‘Ca. M. haemominutum’-infected blood that had been stored for 1 week resulted in a single positive PCR result in the naïve cat, in only one of the two PCR assays used to detect infection, so successful ‘persistent’ transmission was not seen. But this work suggested that ‘Ca. M. haemominutum’ may be able to survive for up to 1 week in CPDA. Interestingly, and in contrast to these results, experimental
studies at the University of Bristol have found that the viability of haemoplasma organisms in blood collected into EDTA or heparin anticoagulants is very short-lived (< 1 hour) as inoculations with blood stored for longer periods have failed (ST, personal communication).

Survival of haemoplasma organisms outside of the host is hard to research because of the current absence of a haemoplasma in vitro culture system meaning that only in vivo inoculation can prove organism viability. Nevertheless, it is important that blood donors are screened for all haemoplasma species (Tasker, 2010); in one study 3.7% of healthy indoor only retrovirus negative feline blood donors were haemoplasma-infected (Mesa-Sanchez et al., 2021).

Vertical

Vertical transmission of haemoplasmas in cats has not been definitively proven using molecular methods. No predilection for reproductive tissues was found in one study that reported haemoplasma detection rate in different feline tissues (Manvell et al., 2021). However, other haemoplasma species are vertically (likely transplacentally) transmitted, such as in pigs (Stadler et al., 2019; Ade et al., 2022), cattle (Sasaoka et al., 2015; Girotto-Soares et al., 2016; Niethammer et al., 2018) and beetles (Huclcr et al., 2012). Vertical transmission has been strongly suggested for M. haemocanis in dogs (Lashnits et al., 2019).

Fighting

Studies investigated the presence of haemoplasmas in the saliva and/or salivary glands of a small number of cats infected with ‘Ca. M. haemominutum’ or M. haemofelis: only a low proportion of samples from ‘Ca. M. haemominutum’ (Dean et al., 2008) or M. haemofelis (Tasker et al., 2009a) experimentally infected cats were found to be PCR positive. A more comprehensive study found ‘Ca. M. turicensis’ DNA in the saliva of cats during early experimental ‘Ca. M. turicensis’ infection, but failed to find either ‘Ca. M. haemominutum’ or M. haemofelis in the saliva of natural infected cats (Willi et al., 2007a). Others have found M. haemofelis DNA in occasional saliva samples collected from experimentally infected cats (Baumann et al., 2015), notably in those with high levels of bacteraemia. Transmission studies found that subcutaneous inoculation of blood containing ‘Ca. M. turicensis’, but not saliva containing ‘Ca. M. turicensis’ (both the blood and saliva contained the same copy numbers of ‘Ca. M. turicensis’), resulted in infection transmission (Museux et al., 2009), suggesting that social contact (saliva via mutual grooming etc.) is less likely to transmit haemoplasma (at least ‘Ca. M. turicensis’) than aggressive interactions (e.g. blood transmission during cat bites) (Museux et al., 2009). In that study as little as 10 µl of blood containing 10^3 copies of ‘Ca. M. turicensis’ was associated with transmission. It is possible that fighting can transmit feline haemoplasma species, particularly in cats with high levels of bacteraemia, and the fact that male outdoor cats are predisposed to feline haemoplasma infection may reflect an association with fighting.

Vectors

The clustered geographical distribution of infection in some studies supports the role of an arthropod vector in feline haemoplasma transmission (Sykes et al., 2007a). Huggins et al. (2023) found that a parasiticide use to kill fleas and ticks was ineffective at preventing haemoplasma transmission between dogs, suggesting that haemoplasmas were indeed transmitted by mechanisms that did not involve these vectors. Indeed, in that study (Huggins et al., 2023), dog aggression and fighting were frequently observed, highlighting fighting as a potential mode of transmission. It may well be that the situation is similar in cats.

Fleas

Of 153 cats in Bangkok, Thailand, around 33% were infested with fleas, and feline haemoplasma DNA (usually ‘Ca. M. haemominutum’ or M. haemofelis) was found in 34% of the 50 flea pools collected from infested cats (Assarasakorn et al., 2012). Additionally, cats had blood samples tested for haemoplasma DNA and of the 35 cats that were blood haemoplasma DNA positive, seven were found to have haemoplasma positive fleas (Assarasakorn et al., 2012). Interestingly, only 11% of haemoplasma-positive cats in this study had the same haemoplasma species in their blood and fleas, suggesting that the fleas may be feeding on more than one cat (Assarasakorn et al., 2012). Other studies have found evidence of feline haemoplasma DNA in fleas collected from cats (Shaw et al., 2004; Lappin et al., 2006; Willi et al., 2007a; Kamrani et al., 2008; Barrs et al., 2010; Homok et al., 2010; Abdulllah et al., 2019; Mifsud et al., 2020; Madder et al., 2022), although the rates of positivity in the fleas are not often high but can depend on whether flea pools or individual fleas were used for extraction and PCR. One study (Yamakawa et al., 2023) has reported an association between haemoplasma infection and flea infestation whilst another (Madder et al., 2022) found an association between M. haemofelis and Ctenocephalides felis (compared to the other fleas species, Echidnophaga, found on cats in the study). However, other studies have failed to document any statistical association between flea infestation and haemoplasma infection in cats (Assarasakorn et al., 2012; Martinez-Diaz et al., 2013; Sacristan et al., 2019).

Although the presence of haemoplasma DNA in fleas could suggest C. felis as a haemoplasma vector, evidence for this via transmission studies is very limited. ‘Ca. M. haemominutum’ and M. haemofelis have been shown to be ingested by C. felis when allowed to feed on experimentally infected cats, and the DNA of both haemoplasmas was detected in flea faeces or eggs (Woods et al., 2005). However,
only very transient *M. haemofelis* infection was reported (detected by PCR on day 12 [in only one of nine timepoints] after flea exposure) in only one of six cats exposed to the haematophagous activity of fleas that had previously fed on *M. haemofelis*-infected cats. Additionally, clinical and haematological signs of *M. haemofelis* infection were not induced in the recipient cat (Woods et al., 2005). Another study (Woods et al., 2006) evaluated whether ingestion, rather than the feeding, of fleas could transmit infection; but this study did not detect any evidence of transmission of either *M. haemofelis* (two cats) or ‘Ca. M. haemominutum’ (two cats) via ingestion of haemoplasma-infected fleas.

**Ticks**

Feline haemoplasma DNA has also been occasionally reported in ticks. In Switzerland ‘Ca. M. haemominutum’ was found in only two of 71 *Ixodes* sp. ticks collected from 39 cats (Willi et al., 2007a) whilst ‘Ca. M. haemominutum’ was found in only three (in two *Ixodes ricinus* and one *Ixodes trianguliceps*), *M. haemofelis* in only one (*I. trianguliceps*) and ‘Ca. M. turicensis’ in only one (*I. ricinus*) of 540 ticks collected from 540 cats in the UK (Duplan et al., 2018). Interestingly, one study (Taroura et al., 2005) reported ‘Ca. M. haemominutum’ DNA in three of eight pools of unfed *Ixodes ovatus* (both male and female) ticks and in some *Haemaphysalis flava* unfed ticks collected from vegetation in Japan. Both *I. ovatus* and *H. flava* are common ticks of cats in Japan. All the ticks in this study (Taroura et al., 2005) were adult stage, so the ixodid ticks might have been infected with ‘Ca. M. haemominutum’ by feeding on blood from animals infected with ‘Ca. M. haemominutum’ at their nymphal stage. Thus, transtidial transmission probably occurred in these ixodid ticks, but experimental transmission studies are needed to confirm these findings. Studies have reported the lack of any association between feline haemoplasma infection and tick infestations (Martinez-Diaz et al., 2013; Yamakawa et al., 2023).

**Mosquitoes**

Only one study evaluated mosquitoes for feline haemoplasma presence and transmission (Reagan et al., 2017). While 6.2% of 81 cats tested in feral colonies were ‘Ca. M. haemominutum’ or *M. haemofelis* positive, none of the pools of mosquitoes trapped near these cat colonies were haemoplasma PCR positive. In transmission studies ‘Ca. M. haemominutum’ or *M. haemofelis* DNA was amplified from *Aedes aegypti* mosquitoes immediately after taking a blood meal from haemoplasma-infected cats, but then DNA was no longer detected at 7 and 14 days after feeding. Additionally, neither of the two naïve cats that were subsequently exposed to the previously (7 days) *fed* *A. aegypti* mosquitoes became positive for either haemoplasma in the 10-week observation period. These results suggest that ‘Ca. M. haemominutum’ and *M. haemofelis* do not colonize *A. aegypti* and that this mosquito is not a biological vector for these haemoplasmas (Reagan et al., 2017).

**Lice**

One study (Sanchez-Montes et al., 2023) identified *M. haemofelis* by PCR in the lice (*Felicola subrostratus*) collected from one cat in Mexico. This work does not confirm the role of lice as biological vectors, as the PCR positivity could be due to the haematophagous activity of the lice only.

**Multiple modes of transmission**

As the natural route of transmission of feline haemoplasma species in the field has not yet been determined, it may be that different routes predominate for different host and haemoplasma species. Indeed, work on the transmission of ‘Ca. M. haemominutum’ in domestic and wild felids (Kellner et al., 2018) suggests that multiple transmission pathways exist concurrently. These include indirect spread (i.e. vector-borne) and direct spread (via predation [of larger wild felid species on smaller cats] or fighting) and it will be interesting for future work to evaluate other haemoplasmas using a similar approach. Others (Sacristan et al., 2019), evaluating both wild and domestic cats, have not found evidence for domestic cats being a reservoir for infection of the wild cats. These variable results make it difficult to make firm conclusions about transmission routes.

**Pathogenesis**

The attachment of haemoplasma organisms to erythrocytes can result in direct damage to the RBC membrane leading to haemolysis (Carney and England, 1993). Membrane damage can also result in an increase in osmotic fragility (OF) and a shortened erythrocyte lifespan. In one experimental study (Maede, 1975), it was found that the erythrocyte OF not only increased after the first appearance of haemoplasmas on blood smears, but continued to increase following the disappearance of organisms from blood smears. Although some intravascular haemolysis may occur due to direct damage to erythrocytes (Willi et al., 2005; Willi et al., 2006a), the majority of haemolysis in haemoplasma infection is thought to be extravascular in nature. Macrophage erythrophagocytosis occurs in the spleen, liver, lungs and bone marrow (Maede, 1978; Simpson et al., 1978).

*Mycoplasma haemofelis* is the most pathogenic feline haemoplasma species; immunocompetent cats with no other comorbidities can develop disease following infection. It can result in severe, sometimes fatal, haemolytic anæmia following acute infection although some cats develop only mild anæmia, so variability in outcome occurs. The haemolytic anaemia is primarily extravascular (such as within the spleen) in nature, but occasionally intravascular haemolysis is reported (Willi et al., 2005). This could be due to host response
differences or M. haemofelis strain variation, but severe disease can occur including in immunocompetent cats (Tasker et al., 2009b). Chronic infection is usually not associated with significant anaemia, and carrier cats exist which show no evidence of anaemia (Willi et al., 2006a; Laberke et al., 2010). In line with this, some epidemiological studies have not shown associations between anaemia and M. haemoplasma infection (Willi et al., 2006a; Bauer et al., 2008; Juvet et al., 2010; Munhoz et al., 2018), probably due to the inclusion of chronically M. haemofelis-infected cats without clinical signs.

Although ‘Ca. M. haemominutum’ infection can cause erythrocyte parameters (e.g. red blood cell count, haemoglobin, haematocrit) to decrease (Tasker et al., 2009b), anaemia is not commonly seen following infection unless the cat has concurrent problems, e.g. immunosuppression, undergoing chemotherapy, FeLV infection or concurrent disease (George et al., 2002; De Lorimier and Messick, 2004). However, splenectomised cats do not seem to be at an increased risk of developing disease (Sykes et al., 2007b). Many carrier cats of ‘Ca. M. haemominutum’ exist, which do not show any clinical signs (Willi et al., 2006a). ‘Ca. M. haemominutum’ has also been associated with the development of myeloproliferative disease in cats with FeLV infection in one experimental study (George et al., 2002). However, cases of anaemia have been reported in cats in which only ‘Ca. M. haemominutum’ infection was diagnosed, with other causes of the anaemia ruled out, and so it appears that in some cases, ‘Ca. M. haemominutum’ can cause anaemia in the absence of concurrent disease (Reynolds and Lappin, 2007; Weingart et al., 2016).

‘Ca. M. turicensis’ infection has caused anaemia or a mild decrease of erythrocyte parameters in some experimental studies (Willi et al., 2005), but generally anaemia is uncommon (Tasker et al., 2009b). Concurrent disease and immunosuppression are both thought to be involved in the pathogenesis of ‘Ca. M. turicensis’ disease (Willi et al., 2005; Willi et al., 2006b), similar to ‘Ca. M. haemominutum’.

Determining the pathogenicity of ‘Ca. M. haemominutum’ and ‘Ca. M. turicensis’ in naturally infected cats can be difficult as cats are often co-infected with other haemoplasma species, confounding disease associations.

Carrier cats often have subclinical infections, but reactivation of infection can occur, although rarely, and can result in clinical disease (Harvey and Gaskin, 1977; Harvey and Gaskin, 1978; Foley et al., 1998; Weingart et al., 2016). Reactivation can occur when the cat has failed to eliminate infection. One study found that cats that had previously recovered from M. haemofelis infection were protected from homologous re-challenge with M. haemofelis, confirming the presence of protective immunity (Hicks et al., 2014b), possibly in those that have previously eliminated the infection, and thus, reinfection seems unlikely. However, another study found that cats that had recovered from previous ‘Ca. M. turicensis’ infection actually showed more severe and rapid M. haemofelis infection signs than naive cats infected with M. haemofelis (Baumann et al., 2013). Thus, more research is required into the relationship between infection with different haemoplasma species and their pathogenesis and immunity.

Immunity

The existence of co-infections with dual and triple haemoplasma infections in cats suggests that cross-protection across the haemoplasma species does not occur. Indeed, a study has shown that not only were ‘Ca. M. turicensis’-recovered cats not protected against M. haemofelis challenge, they became PCR-positive for M. haemofelis significantly earlier than the naïve cats, suggesting possible antibody-dependent enhancement (Baumann et al., 2015). Furthermore, passive immunization via transfusion of a small volume of pooled plasma from M. haemofelis recovered cats failed to provide protection from infection with M. haemofelis and may have exacerbated clinical disease (Sugiarto et al., 2016). However, as mentioned earlier, protective immunity can develop following infection, and M. haemofelis- and ‘Ca. M. turicensis’-recovered cats were protected against re-challenge with the same species (Novacco et al., 2012; Hicks et al., 2014b), suggesting immunity due to previous infection. This may suggest that if cats do clear infection after acute haemoplasmosis, they may become immune to re-infection with the same species, although they may well still be susceptible to infection with other feline haemoplasma species, possibly with more severe disease developing.

Clinical signs

Common clinical signs associated with acute pathogenic haemoplasma infections are lethargy, weakness, reduced appetite, dehydratation, weight loss and intermittent pyrexia (with a temperature > 102.5°F [39.2°C]) (Tasker, 2010; Ameldev and Tresamol, 2017). Pallor, associated with anaemia, is also reported. Splenomegaly can be evident in some cats. Severe anaemia can result in tachycardia, tachypnoea and weak or bounding femoral pulses with haematic cardiac murmurs. Icterus is uncommon, despite the haemolytic nature of the anaemia, possibly because the haemolysis is not severe enough to cause significant elevations in bilirubin concentrations. The reasons for this are unknown as the haemolysis can be very severe in some cases. Lymphadenopathy with palpation of enlarged peripheral lymph nodes, such as submandibular or popliteal, is occasionally described (Willi et al., 2007b; Barker, 2019).

As mentioned earlier, chronic haemoplasma infection is not usually associated with clinical signs, although reactivation of infection is possible and can be associated with disease.
Diagnosis

Laboratory changes including haematology

Pathogenic haemoplasma infections typically cause a regenerative macrocytic hypochromic anaemia although pronounced reticulocytosis is not always evident (Kewish et al., 2004) and some cases present with a non-regenerative anaemia (da Rosa Maciel et al., 2023). Normoblasts (nucleated red blood cells) can be present. White blood cell changes can also occur including leukopenia, lymphopenia, eosinopenia and monocytosis.

Positive Coombs’ test results can occur, particularly with cold agglutinins, and persistent autoagglutination has been reported in acute haemoplasmosis, indicating the presence of erythrocyte-bound antibodies. However, in experimental studies (Tasker et al., 2009b) and clinical studies (da Rosa Maciel et al., 2023) these antibodies appear after the development of anaemia; the absence of erythrocyte-bound antibodies at the onset of development of anaemia could be due to reduced sensitivity for their detection or because erythrocyte-bound antibodies appear as a result of haemoplasma-induced haemolysis rather than mediating it. Indeed erythrocyte-bound antibodies disappear with antibiotic and supportive treatment alone, without glucocorticoid treatment (Tasker et al., 2009b).

Hyperbilirubinaemia is seen occasionally, due to haemolysis. Hypoxic liver damage can result in increased activities of alanine aminotransferase. A polyclonal hypergammaglobulinaemia is also sometimes seen (Baumann et al., 2015; Soto et al., 2017).

Detection of the infectious agent

Direct detection

Blood smear cytology

Cytology of blood smears, stained with Romanowsky type stains (e.g. Wright Giemsa or Diff-Quick), can reveal haemoplasmas on the surface of erythrocytes but this is known to be very unreliable for diagnosis. Sensitivity is a particular issue with figures of only 0% to 37.5% reported in various studies (Jensen et al., 2001; Westfall et al., 2001; Tasker et al., 2003; Ghazisaeedi et al., 2014; Firmino et al., 2016), with cytology revealing infection only when very high numbers of organisms are present in the blood (likely only in acute infections); indeed ‘Ca. M. turicensis’ has never been seen on blood smears due to the low numbers of organisms present in the blood during infection (Willi et al., 2006a; Willi et al., 2011). Specificity is usually higher, with values of 84 to 98% (Jensen et al., 2001; Westfall et al., 2001; Tasker et al., 2003; Ghazisaeedi et al., 2014), although, importantly, these figures are based upon board-certified clinical pathologists examining and interpreting blood smears (Tasker, 2010). The cytological detection of organisms during acute infection can be useful as a bench-side and immediate diagnostic test, although the expert interpretation of blood smears needed for this may only be available when sending blood smears to an external laboratory. However, in reality, many cases diagnosed as being haemoplasma-infected on the basis of blood smear interpretation in practice have been false positives, with stain precipitate, Howell-Jolly bodies and artefacts due to slow blood smear drying all being common reasons for error. Additionally, cytology cannot differentiate between haemoplasma species.

Culture

Haemoplasmas are currently unculturable in vitro including on bacteriological and cell culture media, despite numerous attempts in experimental studies (Schreiner et al., 2012; Filler, 2020) (personal communication RHL). This also means that antimicrobial sensitivity testing is not possible. A number of haemoplasmas have been subjected to whole genome sequencing, including sequencing of two feline haemoplasma species; M. haemofelis strain Langford 1 (Barker et al., 2011) and ‘Ca. M. haemominutum’ strain Birmingham 1 (Barker et al., 2012). These data have highlighted the limited metabolic capabilities of these important pathogens (glucose is their only energy source), which likely contribute to the haemoplasmas’ current uncultivable status. Such knowledge of haemoplasma metabolic capabilities has allowed studies to direct in vitro cultivation attempts but successful growth has not yet been possible (Schreiner et al., 2012; Baumann et al., 2013).

PCR

Polymerase chain reaction (PCR) assays, to detect haemoplasma DNA, are now the diagnostic method of choice for haemoplasma infection. PCR is performed in specialised diagnostic laboratories following DNA extraction from submitted EDTA blood samples (as little as 0.5 ml of EDTA blood is required). PCR is far more sensitive and specific than cytology and allows differentiation of the haemoplasma species present, as well as the detection of co-infections, when species-specific PCR assays are used. Currently available PCR assays for haemoplasmas are typically based on the detection of segments of the 16S rRNA gene. PCRs can be duplexed with a cat housekeeping gene PCR (Peters et al., 2008; Barker et al., 2010b) as an internal control, so that any false negative results due to the failure of DNA extraction, the presence of PCR inhibitors or PCR set-up errors are detected. Other PCR assays amplify cat housekeeping genes in a separate PCR (i.e. these are not duplex PCR assays) (Soto et al., 2017), which is less optimal as the control PCR is separate from the test PCR.
Quantitative PCR (qPCR) assays also allow quantification of haemoplasma DNA in the sample being analysed, allowing monitoring of response to treatment. Blood samples for PCR should be taken before antibiotic treatment is started as effective treatment can result in a rapid and dramatic fall in organism numbers within a few days, as shown in Figure 1, which could result in negative PCR results. It is also known that *M. haemofelis* blood organism numbers can fluctuate markedly in some cats for several months following infection; the reason for this is not known but could be related to antigenic variation (Tasker et al., 2006b; Tasker et al., 2009a). No evidence of significant tissue sequestration of *M. haemofelis*, to explain reduced blood organism numbers, has been found (Tasker et al., 2009a). This is in contrast to ‘Ca. *M. turicensis*’, in which evidence of tissue sequestration was found in PCR-negative cats (Novacco et al., 2013).

Fig. 1. Variation in *M. haemofelis* organism numbers (DNA copies) in the blood of a cat over time, measured by quantitative polymerase chain reaction (qPCR). The cat's packed cell volume (PCV) measurements are also shown over time, together with a pink shaded area illustrating PCV reference interval. The cat received doxycycline therapy (10 mg/kg q24h PO; grey shaded areas) on day 19 post-infection and then again for 21 days from day 28 post-infection. It can be seen that there is a marked drop in organism numbers in the blood with doxycycline treatment. Cycles of increasing and decreasing organism numbers occurred following completion of the 21-day course of doxycycline, but these were not associated with anaemia. Figure adapted from Tasker (2008).

In-house PCR is not available, although a point-of-care machine (Hawley et al., 2018) that uses isothermal non-quantitative amplification of DNA to diagnose *M. haemofelis* infection has been described. This method requires in-house extraction of DNA from blood and the extraction technique markedly influences sensitivity, precluding routine recommendation of this method.

Indirect detection
Haemoplasma antibody tests
Tests to detect antibodies to haemoplasma species have been difficult to develop due to the inability to culture haemoplasmas in vitro preventing the easy production of significant amounts of haemoplasma protein for use in antibody assays; they are currently only available for use in experimental studies. These antibody assays, based on a *M. haemofelis* dnaK protein, in experimental studies, have suggested that antibody levels can differentiate between acute and chronic infection with *M. haemofelis* (Barker et al., 2010a) and have been more sensitive than PCR in detecting haemoplasma exposure (as PCR-negative antibody-positive cats have been identified) (Novacco et al., 2011). However, these assays are not appropriate for use in naturally infected cats yet as their validation in field samples (particularly looking at their specificity) has not been completed.

Treatment
Antibiotic treatment

Haemoplasmosis generally has a good prognosis if prompt appropriate treatment is instigated (Tasker, 2022) (Table 1). However, as haemoplasmas lack a cell wall around their cell membrane, β-lactams (e.g. penicillins, cephalosporins) are not effective in treatment. Tetracyclines (primarily doxycycline) and fluoroquinolones (e.g. marbofloxacin, pradofloxacin) are effective for the treatment of haemoplasmosis. The majority of studies have evaluated the response of *M. haemofelis* only to treatment.

Table 1: Dosages of antibiotic that have been recommended for the treatment of feline haemoplasmosis

A 2-week course is usually adequate for uncomplicated haemoplasmosis – courses can be extended if only a partial clinical response occurs. Adapted from Tasker (2022). Enrofloxacin is not a preferred fluoroquinolone in cats as has the potential for irreversible retinal toxicity as an idiosyncratic reaction, although this is rare.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Dosage (mg/kg)</th>
<th>Route &amp; frequency</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline:</td>
<td>5</td>
<td>PO q12h</td>
<td>First line antibiotic for acute haemoplasmosis. Can be associated with gastrointestinal side effects when given q24h. Tablets have been associated with oesophagitis if incompletely swallowed, so always follow with food or water. Paste formulations of doxycycline may be used, if available, when tablets are difficult to administer.</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>10</td>
<td>PO q24h</td>
<td></td>
</tr>
<tr>
<td>Fluoroquinolone:</td>
<td>2-5.5</td>
<td>PO q24h</td>
<td>Reserve fluoroquinolones as second line antibiotics. Reported use in combination (sequentially) with doxycycline to clear <em>M. haemofelis</em> infection (Novacco et al., 2018)</td>
</tr>
<tr>
<td>Marbofloxacin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pradofloxacin</td>
<td>3-5</td>
<td>PO q24h</td>
<td>Reserve fluoroquinolones as second line antibiotics. May be more efficacious at clearing <em>M. haemofelis</em> than doxycycline (Dowers et al., 2009)</td>
</tr>
</tbody>
</table>

* Licensed dosages (e.g. for marbofloxacin) and drug availability vary by country and licensed product.

Doxycycline (10 mg/kg q24h PO or 5 mg/kg q12h PO) is often used as a first line treatment, typically for 2-4 weeks with the longer treatment courses recommended by some to increase the chance of eliminating infection. Straightforward cases which show a rapid response to doxycycline (typically within 3 days), a 2-week course of doxycycline is usually adequate with no further monitoring required. Administration of the hyclate pill preparation of doxycycline should always be followed by food or water because of the possibility of it inducing oesophagitis in cats with incomplete swallowing. Paste formulations of doxycycline, if available, may be preferable. Figure 1 above shows that when treatment is effective, it can be associated with a rapid fall in organism numbers in the blood.

One study (Dowers et al., 2009) suggested that 2 weeks of pradofloxacin (at two dosages; both the standard 5 mg/kg q24h PO, as well as a higher dosage of 10 mg/kg q24h PO) can be more effective at clearing *M. haemofelis* than doxycycline. It has been found that ‘Ca. M. haemominutum’ infection does not necessarily respond to antibiotics similarly to *M. haemofelis*; in one study (Tasker et al., 2006a) ‘Ca. M. haemominutum’ organism numbers in the blood fell only temporarily during marbofloxacin (2 mg/kg q24h PO) treatment, with organism numbers re-increasing to pre-treatment levels following completion of a 4 weeks course of treatment. Another study found that ‘Ca. M. haemominutum’ infection was not as effectively treated by doxycycline as *M. haemofelis* (Sykes et al., 2007b), again highlighting the varying response of different haemoplasma species to the same antibiotic. The response of ‘Ca. M. turicensis’ to antibiotic treatment has not been fully evaluated but doxycycline can be effective (Museux et al., 2009).

Azithromycin was not effective in the treatment of clinical haemoplasmosis in a partially controlled study of cats infected with *M. haemofelis* and/or ‘Ca. M. haemominutum’ (Westfall et al., 2001).

Novacco et al. (2018) described a method for clearance of *M. haemofelis* infection, should this be required. Here doxycycline treatment was given for 28 days followed by monitoring of copy numbers in the blood by quantitative PCR; if the cat remained PCR-positive, treatment was switched to a fluoroquinolone (marbofloxacin was used in the study) for 14 days and this was associated with apparent clearance of infection. So, this study suggests that the use of doxycycline followed by marbofloxacin could be useful for clearance of *M. haemofelis*. Attempts to clear infection can be considered when *M. haemofelis*-associated clinical disease is particularly severe and/or has been recurrent. Moreover, clearance of *M. haemofelis* infection may be required in certain situations, such as for cats living in multicat environments together with *M. haemofelis*-naïve cats, since acute primary infection can lead to severe haemolytic anaemia, for cats with immunodeficiency, for cats to be used as blood donors and for cats living with immunocompromised persons. However, treatment of carrier cats that do not show any clinical signs is not generally recommended.
Supportive care

Other supportive care for haemoplasma-infected cats can be important; correction of dehydration with fluid therapy is important, as well as tempting the cat to eat or assisted feeding if the cat is anorexic. Blood transfusions (ideally packed red blood cells if available) may be required if the anaemia is very severe. However, anaemic cats are at risk of fluid overload due to increased circulating volume, particularly in the presence of occult cardiac disease (Barker, 2019), so care should be taken with intravenous fluid treatment and blood administration. Oxygen therapy can be provided pending stabilization of the patient’s oxygen carrying capacity.

Corticosteroid treatment

Corticosteroids have been used as adjunct treatment for any immune-mediated component of haemoplasma-associated anaemia, although cats (even those with positive Coombs’ tests) usually recover without any need for corticosteroid treatment, as antibiotic and supportive care alone is usually adequate (Tasker et al., 2009b). Immunosuppressive dosages of glucocorticoids have been used experimentally to increase haemoplasma bacteraemia and induce reactivation of subclinical infection (Harvey and Gaskin, 1978; Dowers et al., 2002; Yuan et al., 2007; Dowers et al., 2009; Novacco et al., 2018), also suggesting that they should not be used in clinical haemoplasmosis. Thus, we do not recommend the use of corticosteroids for the treatment of haemoplasmosis unless a cat with a positive Coombs’ test fails to respond to appropriate antibiotic treatment alone and the diagnosis of haemoplasmosis is uncertain. In such cases, immune-mediated haemolytic anaemia could be the cause of the cat’s anaemia.

Prognosis

Haemoplasmosis generally has a good prognosis if prompt appropriate treatment is instigated quickly. If infection is not cleared, cats may have subsequent reactivation of infection with recurrence of clinical disease.

Vaccination

There are no vaccines against feline haemoplasmosis.

Prevention

As the natural route of transmission of haemoplasma infections is not known, it is difficult to be precise regarding how to prevent infection.

As biting may be involved in transmission, attempts to reduce any inter-cat aggression may be considered. Keeping cats indoors is also likely to prevent infection as outdoor status has been identified as a risk factor, but this is likely to be impractical and stressful for cats used to having outdoor access.

Blood donors should be screened for haemoplasma infection by PCR to prevent inadvertent transmission by blood transfusion from carrier cats that do not show any clinical signs (Pennisi et al., 2015; see also the ABCD Guidelines on Blood transfusion in cats). One study (Mesa-Sanchez et al., 2021) describing the screening of healthy, client-owned, indoor cats to become blood donors in Spain and Portugal found that in 4,880 retroviral (FIV antibody and FeLV antigen) seronegative cats, haemoplasmas were detected in 3.7% of cats (1.3% were positive for *M. haemofelis*; 2.3% for ‘*Ca. M. haemominutum*’ and 0.12% for ‘*Ca. M. turicensis*’) showing that haemoplasma infections should be screened for even in cats deemed to be of low risk for infections.

Although vectors have not been proven to transmit haemoplasma infection, preventative flea and tick treatment is probably wise to help prevent infection in case vectors are involved.

Zoonotic risk

Haemoplasma infections with novel haemoplasma species have been described in humans (Steer et al., 2011; Maggi et al., 2013; Alcorn et al., 2020; Hattori et al., 2020), as well as with species that have possibly originated in animals, including one case in an immunocompromised human with *M. haemofelis* (Santos et al., 2008), raising the possibility of zoonotic infections. We do not believe that there is a significant risk of human infection with feline haemoplasma species. As with the routine handling of clinical samples, vets should handle the blood and tissues from cats suspected to be haemoplasma-infected with caution.

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