INTRODUCTION

The haemotropic mycoplasmas (haemoplasmas) are small bacteria that parasitise red blood cells and can induce haemolysis, causing anaemia.

Feline haemoplasma species, their prevalence and pathogenicity

<table>
<thead>
<tr>
<th>Haemoplasma species</th>
<th>Reported prevalence</th>
<th>Pathogenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Mycoplasma haemofelis</em></td>
<td>0 – 46.6% (median 4.8%)</td>
<td>Acute infection often results in haemolytic anaemia</td>
</tr>
<tr>
<td>‘Candidatus haemominutum’</td>
<td>0 – 46.7% (median 14.4%)</td>
<td>Acute infection can induce a fall in erythrocyte parameters but not usually severe enough to cause anaemia unless cat has concurrent disease or is immunocompromised e.g. chemotherapy</td>
</tr>
<tr>
<td>‘Candidatus Mycoplasma turicensis’</td>
<td>0 – 26% (median 2.0%)</td>
<td></td>
</tr>
</tbody>
</table>

In most studies, feline haemoplasma infections are more common in male, non-pedigree cats with outdoor access. Infection with ‘Ca. M. haemominutum’ is usually more prevalent in older cats, presumably because the chance of acquiring chronic subclinical infection increases with time. Some studies have shown an association between haemoplasma infection and feline immunodeficiency virus (FIV) infection whereas others have not. Most studies have failed to show an association between haemoplasma infection and feline leukaemia virus (FeLV) infection, but variable results are seen in different reports. Recent research suggests that the host phenotypic traits, such as being male and/or older, are more important in driving multiple exposures to pathogens compared to pathogen-pathogen interactions. *Felis catus* gammaherpesvirus 1 (FcaGHV1), a potential feline pathogen, has been found to be significantly associated with haemoplasma infection in a recent study, but the significance of FcaGHV1 in cats is not known.

OUTCOME OF HAEMOPLASMA INFECTION

*Mycoplasma haemofelis* is the most pathogenic of the feline haemoplasma species. Acute infection often results in severe haemolytic anaemia although in some cases only mild anaemia results. A regenerative macrocytic hypochromic anaemia often results, although pronounced reticulocytosis is not always evident. Normoblasts may be present. Chronic infection is not usually associated with significant anaemia. Cats do not need to be immunocompromised or splenectomised to succumb to clinical disease with *M. haemofelis*.

Although ‘Ca. M. haemominutum’ infection can cause a drop in red blood cell parameters, anaemia is not usually induced except in cats with concurrent problems e.g. FeLV infection. ‘Ca. M. haemominutum’ has also been associated with the development of myeloproliferative disease in cats with FeLV infection in one experimental study. Although concurrent problems are usually present in ‘Ca. M. haemominutum’ infected cats that develop anaemia, cases of so-called primary ‘Ca. M. haemominutum’ anaemia, without any apparent concurrent disease or infection present, have also been reported.

‘Candidatus Mycoplasma turicensis’ infection has resulted in anaemia or a small drop in red blood cell parameters in some experimental studies, but generally anaemia is uncommon following infection. Concurrent disease and immunosuppression are both thought to be involved in the pathogenesis of ‘Ca. M. turicensis’ disease, in a similar way to the pathogenesis described for ‘Ca. M. haemominutum’. Determining the pathogenicity of ‘Ca. M. turicensis’ in naturally infected cats has been difficult in
epidemiological studies of naturally infected cats, as they are often co-infected with other haemoplasma species, confounding disease associations, but anaemia has been induced experimentally\(^9\).

Different strains of each of the feline haemoplasma species may also exist, as well as these different species, and these may also vary in pathogenicity. This might explain the conflicting data in different studies. However, other factors, such as the health status of the cat and its age, are also likely to play an important role in the outcome of haemoplasma infection. Long-term asymptomatic carrier status can occur, especially with ‘Ca. M. haemominutum’ and *M. haemofelis* infection. Clinical disease associated with reactivation of infection in carrier cats has been reported\(^7\).

**TRANSMISSION**

The natural route of transmission of haemoplasma infection between cats in the field has not yet been determined, and it may be that different routes predominate for the different haemoplasma species that exist. Fleas and ticks have been implicated, however this could reflect their haematophagous activity on infected hosts rather than signifying their role as a vector. The cat flea has been implicated in feline haemoplasma transmission, but only very transient *M. haemofelis* infection has been reported via the haematophagous activity of fleas, and clinical and haematological signs of *M. haemofelis* infection were not induced in the recipient cat\(^9\). Additionally, a recent study found no evidence of haemoplasma transmission by fleas in an experiment involving the introduction of fleas into groups of cats housed together\(^10\). A recent study evaluating haemoplasma prevalence in ticks removed from cats in the UK\(^11\) found feline haemoplasma DNA in 5 of 540 ticks; interestingly 2 of these 5 ticks were *Ixodes trianguliceps*, the vole or shrew tick, which comprised quite an uncommon tick in the study (only 8 of the 540 ticks were *I. trianguliceps*). Recently, a bovine haemoplasma species was found in wild caught mosquitoes in the US, but although *Aedes aegypti* mosquitoes were shown to ingest *M. haemofelis* or ‘Ca. M. haemominutum’ in an experimental study, transmission to naïve cats was not documented, suggesting this mosquito is not a biological vector for these feline haemoplasma species\(^12\).

Cat fights may be involved in haemoplasma transmission. Subcutaneous inoculation of ‘Ca. M. turicensis’-containing blood resulted in infection transmission, whereas the same inoculation method using ‘Ca. M. turicensis’-containing saliva, did not\(^13\). This suggests that haemoplasma transmission by social contact (saliva via mutual grooming etc.) is less likely than transmission by aggressive interaction (blood transmission during a cat bite incident)\(^14\). However, a recent study\(^15\) found evidence of horizontal transmission of ‘Ca. M. haemominutum’, but not *M. haemofelis*, by direct contact between cats in the absence of aggressive interaction and vectors. Vertical transmission has not been definitively shown using molecular methods with feline haemoplasma infections but has been suggested for other haemoplasma species\(^14,15\). Blood transfusion is another potential route of transmission, and blood donors should be screened for haemoplasma infection.

**CLINICAL SIGNS**

Common clinical signs associated with pathogenic haemoplasma infections are lethargy, weakness, reduced appetite, dehydration, weight loss and intermittent pyrexia (which can be high). Pallor, associated with anaemia, is also reported. Splenomegaly may be evident in some cats. Severe anaemia may result in tachycardia, tachypnoea and weak or bounding femoral pulses with haemic cardiac murmurs. Icterus is uncommon despite the haemolytic nature of the anaemia.

**DIAGNOSIS**

As mentioned earlier, pathogenic haemoplasma infections typically cause a regenerative macrocytic hypochromic anaemia although pronounced reticulocytosis is not always evident. Normoblasts may be present. 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\(^16\) 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. Hyperbilirubinaemia can occur due to haemolysis, and hypoxic liver damage may result in increased activities of alanine aminotransferase.
Haemoplasmas are currently unculturable in vitro despite numerous attempts in our and other laboratories. Recently a number of haemoplasmas have been subjected to whole genome sequencing, including work performed by our group in sequencing two feline haemoplasma species; *M. haemofelis* strain Langford 117 and ‘Ca. M. haemominutum’ strain Birmingham 119. 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 uncultivatable status.

**Cytology** of blood smears may show haemoplasmas on the surface of erythrocytes but this is known to be very insensitive for diagnosis, and cytology cannot easily differentiate between haemoplasma species. The untrained eye may also fail to distinguish stain precipitate and Howell-Jolly bodies from true haemoplasma organisms, although those confident in identifying haemoplasma organisms may be able to diagnose infection cat-side by examination of a blood smear as a screening tool, but organism numbers need to be extremely high in the blood to allow visualization on cytology.

**Polymerase chain reaction (PCR)** assays are now the diagnostic method of choice for haemoplasma infection. PCR is far more sensitive and specific than cytology. Real-time quantitative PCR (qPCR) assays allow quantification of haemoplasma DNA in the sample being analysed (usually a defined volume of blood, which is subjected to extraction and then PCR) so we can monitor haemoplasma infection and evaluate response to treatment e.g. a decrease in the level of haemoplasma DNA in the blood following institution of effective antibiotic treatment. Quantitative PCRs have enabled us to describe the *in vivo* kinetics of experimental haemoplasma infection. Cats experimentally infected with haemoplasmas initially show a rapid increase in copy number with peak numbers typically being reached around 2 to 4 weeks after infection, although *M. haemofelis* copy numbers can fluctuate greatly, especially in the first few weeks post-infection. Some (up to a third) *M. haemofelis*-infected cats continue to show very large fluctuations in *M. haemofelis* copy number for several months following initial experimental infection; this should be considered when interpreting qPCR results. In contrast, ‘Ca. M. haemominutum’- and ‘Ca. M. turicensis’-infected cats show little fluctuation in copy number over time. The reasons for the marked fluctuations in blood *M. haemofelis* copy number over time is not known. We have not been able to show any evidence of sequestration of *M. haemofelis* organisms in tissues (e.g. spleen, liver) at times of cyclical low copy number19. The marked increase in *M. haemofelis* copy number seen in the blood immediately after initial infection confirms that rapid multiplication of organisms is possible in infected cats, so this can explain the marked rapid increases in *M. haemofelis* copy number seen during *M. haemofelis* cycling. The rapid decreases in copy number could then arise due to autolysis of bacteria and subsequent rapid clearance from the blood. Antigenic variation may mediate such fluctuations. Indeed analysis has shown that a very large portion (>70%) of the *M. haemofelis* genome encodes a set of uncharacterized hypothetical proteins arranged in multiple series of paralogous repeats; these could mediate antigenic variation through differing expression of haemoplasma surface proteins over time (we have confirmed *in vivo* expression of some of these proteins), thus enabling *M. haemofelis* to evade the host’s immune response17. Although, as described above, no evidence of sequestration in tissues was found in experimental studies with *M. haemofelis*, evidence of tissue sequestration was found for ‘Ca. M. turicensis’ infection in blood PCR negative cats10.

The development of *haemoplasma protein-based serological assays* has been limited by our inability to culture haemoplasmas in vitro preventing the easy acquisition of adequate amounts of haemoplasma proteins for use in such assays. We have evaluated the feline serological response to haemoplasma infection using an ELISA developed based on recombinant *M. haemofelis* DnaK21 in experimentally infected cats. Experimentally infected cats became seropositive following infection, with a greater antibody response recorded in those cats inoculated with *M. haemofelis*, compared to ‘Ca. M. haemominutum’ and ‘Ca. M. turicensis’. This could be due to the humoral immune response being directed against conserved, haemoplasma clade-specific, and/or species-specific epitopes on *M. haemofelis* DnaK, or a measure of the degree to which the immune response to DnaK is triggered by the infecting haemoplasma species due to the severity of disease. Antibody levels were maximal in the early (~2-4 weeks) post-infection period, suggesting that antibody levels may help differentiate acute from chronic *M. haemofelis* infection. Such differentiation could be useful to the veterinarian trying to evaluate if a haemoplasma infection is likely to be the cause of disease in an anaemic cat, due to the existence of asymptomatic carrier cats and anaemia being more common in cats acutely infected with *M. haemofelis*.
The cross-reactivity between the haemoplasma species seen on existing serological assays limits its usefulness, but since serology can be more sensitive than PCR in detecting haemoplasma exposure (PCR negative seropositive cats have been identified), the development of further serological assays should be investigated.

**TREATMENT**

Antibiotic treatment is indicated for cats with clinical signs and clinicopathological abnormalities consistent with haemoplasmosis. Treatment should also be considered for cats that test positive for *M. haemofelis* in view of the particular potential for recrudescence of anaemia with this haemoplasma species, which is the most pathogenic. Antibiotics are typically given for 2-4 weeks.

Doxycycline (10 mg/kg daily PO) is often used as 1st line treatment for haemoplasmosis. This is usually adequate to induce a clinical response in *M. haemofelis* cases, but it has been shown that a 2 week course of doxycycline (5 mg/kg BID PO) does not consistently eliminate infection. Similar results were reported in another controlled study with doxycycline. Unfortunately controlled antibiotic doxycycline treatment studies have not been performed for either *Ca. M. haemominutum* or *Ca. M. turicensis* infection, although one uncontrolled study reported the failure of 3 weeks of doxycycline to eliminate *Ca. M. haemominutum* infection in cats, and a single *Ca. M. turicensis* infected cat became PCR negative after 2 weeks of doxycycline treatment in another observational study. Longer treatment courses are recommended by some to help eliminate infection, although controlled experiments to confirm this have not been performed.

Fluoroquinolones are usually used as 2nd line treatments for haemoplasmosis. A 2 week course of enrofloxacin (5 mg/kg q24 hrs PO) has been successfully used to treat clinical *M. haemofelis* infection in controlled studies. However, most of the enrofloxacin treated cats were still PCR positive for *M. haemofelis* in the 3 weeks after enrofloxacin treatment was stopped, so clearance of infection was not documented. In controlled studies using 4 weeks of marbofloxacin treatment (2 mg/kg q24 hrs PO), significantly lower *Ca. M. haemominutum* copy numbers were observed in treated cats c.f. untreated control cats, with similar statistical results seen with *M. haemofelis* copy numbers. However *Ca. M. haemominutum* copy numbers only plateaued during treatment and copy numbers rose back to near pre-treatment levels within 7-10 days of finishing marbofloxacin treatment. Conversely the fall in *M. haemofelis* copy numbers was progressive during the treatment period with intermittent negative PCR results obtained at the end of the marbofloxacin treatment period and in the 6 weeks following it, although clearance of infection (as indicated by repeated negative PCR results) was not consistently documented in any cat. Another study similarly documented that marbofloxacin was not effective at eliminating *M. haemofelis* infection. One controlled study has evaluated 2 weeks of pradofloxacin (either 5 mg/kg q24 PO or 10 mg/kg q24 PO) or doxycycline (5 mg/kg q12 hrs PO) treatment for *M. haemofelis* infection. Copy numbers of *M. haemofelis* were significantly lower in all three treatment groups c.f. the untreated control cats, and at various time points *M. haemofelis* copy numbers were significantly lower in the pradofloxacin treated cats c.f. the doxycycline treated cats, with intermittent negative PCR results obtained in only the pradofloxacin, and not the doxycycline, treated cats. Thus, pradofloxacin may be more effective at clearing *M. haemofelis* than doxycycline.

Response to antibiotics can be monitored by qPCR to ensure organism numbers are decreasing appropriately with therapy, especially in severe cases, those in which a clinical improvement is not seen, and/or cases that have had previous antibiotic therapy. A goal of treatment should be to eliminate infection, although proving infection has been eliminated is difficult without performing PCR on the whole host! Repeatedly negative PCR results on blood samples are probably most reliable to indicate elimination, although recent studies have suggested that serology, as mentioned above, has increased sensitivity over PCR in the detection of infection. If negative PCR results do not result from treatment, control of clinical signs and a reduction of copy numbers in the blood indicates efficacy of treatment even if elimination is not possible. However, recrudescence of disease remains possible in cats that remain haemoplasma positive. A recent study has reported that to facilitate clearance of *M. haemofelis*, when this is required, doxycycline treatment (5 mg/kg q12 hrs PO) is given for 28 days followed by monitoring of copy numbers in the blood by quantitative PCR. If the cat remains PCR positive and clearance is needed, treatment should be switched to a fluoroquinolone (marbofloxacin was used in the published study at 2 mg/kg q24 hrs PO) for 14 days as this was associated with apparent clearance of infection. So,
although no antibiotic treatment regime that predictably eliminates haemoplasma infection with any species has yet been described, this study suggests that the use of doxycycline followed by marbofloxacin may be useful for clearance of *M. haemofelis*.

Corticosteroids have been recommended as adjunct treatment for haemoplasmosis, to treat any immune-mediated component of anaemia, although their efficacy has not yet been proven. In our experience, clinically ill cats, including those that are Coombs’ positive, respond to antibiotic treatment and supportive care alone without the need for corticosteroids. Indeed immunosuppressive doses of corticosteroids have been used experimentally to exacerbate haemoplasma infection, so their routine use is not advised.

Supportive care is also required for acute haemoplasmosis treatment. This should include correction of dehydration with fluid therapy, and blood transfusion if the anaemia is severe.

**PREVENTION**

Blood donors should be screened for haemoplasma infection by PCR to help prevent inadvertent transmission by blood transfusion from asymptomatic carrier cats. In view of the potential for vector transmission, preventative flea and tick treatment is recommended. Recent work suggests that protective immunity develops following *M. haemofelis* infection\(^1\), opening the way for future haemoplasma vaccination.

**REFERENCES**


