Haemoplasmosis in cats.
European guidelines from the ABCD on prevention and management

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Synopsis

- *Mycoplasma haemofelis* is the most pathogenic of the three feline haemoplasma species.
- ‘ M. haemominutum’ and ‘*Ca.* M. turicensis’ infections are less pathogenic but can result in disease in immunocompromised cats.
- Male non-pedigree cats with outdoor access are more likely to be haemoplasma infected.
- ‘ M. haemominutum’ is more common in older cats.
- The natural mode of transmission of haemoplasma infection is not known; aggressive interactions and vectors are possibilities.
- Transmission by blood transfusion is possible and all blood donors should be screened for haemoplasma infection.
- Polymerase chain reaction assays are the preferred diagnostic method for haemoplasma infections.
- Asymptomatic carrier cats exist for all feline haemoplasma species.
- Treatment with doxycycline for 3 weeks is usually effective for *haemofelis*-associated clinical disease (but this may not clear infection).
- 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 (Figure 1) and can induce haemolytic anaemia. They are currently classified within the genus *Mycoplasma* in the *Mycoplasmataceae* family of bacteria. However, recent work suggests that although the haemoplasmas probably do belong to this family, they may 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, not *in vitro*.

Three main haemoplasma species known to infect cats are *Mycoplasma haemofelis*, '*Candidatus* Mycoplasma haemominutum' and '*Candidatus* Mycoplasma turicensis'. These mycoplasmas have a worldwide distribution.

Epidemiology

Feline haemoplasma infections are usually more common in male, non-pedigree cats with outdoor access and cat bite abscesses. Infection with '*Ca.* M. haemominutum' is usually more prevalent in older cats, presumably because cats have an increasing chance of acquiring chronic subclinical infection over their lifetime. Some studies have found an association between haemoplasma and feline immunodeficiency virus (FIV) infection (Gentilini et al., 2009, Macieira et al., 2008), whereas others have not (Willi et al., 2006), and most studies have failed to show an association between haemoplasma infection and feline leukaemia virus (FeLV) infection (Gentilini et al., 2009, Macieira et al., 2008, Willi et al., 2006). However, variable results have been seen regarding retroviruses as risk factors for haemoplasma infections. Recent epidemiology studies suggest that the host phenotype (e.g. aggressive male phenotype) may drive some of these associations rather than infections being simple risk factors for each other (Carver et al., 2015).

The prevalence of the feline haemoplasma species varies geographically. In general '*Ca.* M. haemominutum' is most prevalent (0-46.7% of cats in prevalence studies found to be infected), followed by *M. haemofelis* (0-46.6% of cats) and '*Ca.* M. turicensis' (0-26.0% of cats) in domestic cats. Reported prevalences vary not only due to geographical variation but also because the cats sampled in different studies

are very variable, i.e. some test only ill anaemic cats, whereas others sample healthy cats only, and some test stray feral cats whereas others focus on owned cats.

The clustered geographical distribution of infection in some studies supports the role of an arthropod vector in haemoplasma transmission (Sykes et al., 2007). 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 (Woods et al., 2005). 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 (Lappin, 2014). Some observations have suggested that cat fights are involved in 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 (Museux et al., 2009). 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) (Museux et al., 2009). However, a recent study (Lappin, 2014) found evidence of horizontal transmission of '*Ca.* M. haemominutum', but not *M. haemofelis*, by direct contact between cats in the absence of any apparent significant aggressive interaction and vectors. Blood transfusion is another potential route of transmission, and blood donors should be screened for haemoplasma infection (Pennisi et al., 2015).

Pathogenesis

Mycoplasma haemofelis is the most pathogenic feline haemoplasma species (Figure 2) as it can result in severe, sometimes fatal, haemolytic anaemia following acute infection in some cats, although others may develop only mild anaemia, so variability in outcome occurs. This may be due to host response differences or *M. haemofelis* strain variation, but disease can occur in immunocompetent cats. Chronic infection is usually not associated with significant anaemia and carrier cats exist which show no evidence of anaemia. In line with this, some epidemiological studies

have not shown associations between anaemia and *M. haemofelis* infection, probably due to the inclusion of chronically *M. haemofelis*-infected asymptomatic cats.

Although '*Ca.* M. haemominutum' infection can cause erythrocyte parameters (e.g. red blood cell count, haemoglobin, haematocrit) to lower (Figure 2), anaemia is not commonly seen following infection unless the cat has concurrent problems, e.g. immunosuppression or is undergoing chemotherapy. Many asymptomatic carrier cats of '*Ca.* M. haemominutum' exist. '*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 which only '*Ca.* M. haemominutum' infection was diagnosed and so it appears that in some cases '*Ca.* M. haemominutum' can cause anaemia in the absence of concurrent diseases (Weingart et al., 2015).

'*Ca.* M. turicensis' infection has caused anaemia or a small lowering in erythrocyte parameters in some experimental studies (Figure 2), but generally anaemia is uncommon. Concurrent disease and immunosuppression are both thought to be involved in the pathogenesis of '*Ca.* M. turicensis' disease, 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 and may result in clinical disease (Weingart et al., 2015). This may occur when the cat has failed to eliminate infection as a recent 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. 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 naïve cats infected with *M. haemofelis* (Baumann et al., 2015). Thus more research is required into the relationship between infection with different haemoplasma species and their pathogenesis and immunity.

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 (Figure 3). 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

Pathogenic haemoplasma infections typically cause a regenerative macrocytic hypochromic anaemia although pronounced reticulocytosis is not always evident (Kewish et al., 2004). Normoblasts may be present. White blood cell changes may 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) 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 is seen occasionally, due to haemolysis, and hypoxic liver damage may result in increased activities of alanine aminotransferase. A polyclonal hypergammaglobulinaemia is also sometimes seen.

Cytology of blood smears, stained with Romanowsky type stains, may reveal haemoplasmas on the surface of erythrocytes but this is known to be very insensitive for diagnosis, and cytology cannot easily differentiate haemoplasma species. The

untrained eye may also fail to distinguish stain precipitate and Howell-Jolly bodies from haemoplasmas.

As haemoplasmas do not grow on bacteriological media, *in vitro* culture is currently not possible.

Polymerase chain reaction (PCR) assays are now the diagnostic method of choice for haemoplasma infection. PCR is far more sensitive and specific than cytology. Quantitative PCR (qPCR) assays (Figure 4) allow quantification of haemoplasma DNA in the sample being analysed, allowing monitoring of response to treatment. It is 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 may be related to antigenic variation. 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).

Haemoplasma serological tests have been difficult to develop due to our inability to culture haemoplasmas *in vitro* preventing the easy acquisition of significant amounts of haemoplasma proteins for use in serological assays. Serological assays are currently only available for use in experimental studies. These serological assays, based on a *M. haemofelis* dnaK protein, have suggested that antibody levels may differentiate between acute and chronic infection with *M. haemofelis* (Barker et al., 2010) and have been more sensitive than PCR in detecting haemoplasma exposure (as PCR negative seropositive cats have been identified) (Novacco et al., 2011), but these assays are not appropriate for use in field cats yet due to cross-reactivity.

Treatment

Haemoplasmosis generally has a good prognosis if prompt appropriate treatment is instigated. However, as haemoplasmas lack a cell wall around their cell membrane, ß-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. Doxycycline (10 mg/kg SID PO or 5 mg/kg BID PO) is often used as a 1st line treatment, typically for 3 weeks although longer treatment courses are recommended by some to increase the chance of eliminating infection, although longer antibiotic treatment courses have not been evaluated for the clearance of infection. One study (Dowers et al., 2009) suggested that pradofloxacin (at two doses; both the standard 5 mg/kg daily PO, as well as a higher dose of 10 mg/kg daily PO) may be more effective at clearing *M. haemofelis* than doxycycline. Sometimes dual (Tasker, 2010) or sequential therapy with doxycycline and a fluoroquinolone can be helpful. 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., 2006) '*Ca.* M. haemominutum' organism numbers in the blood fell only temporarily during marbofloxacin (2 mg/kg SID PO) treatment, with organism numbers re-increasing to pre-treatment levels following completion of a 4 weeks course of treatment. The response of '*Ca.* M. turicensis' to antibiotic treatment has not been fully evaluated but doxycycline can be effective (Museux et al., 2009).

Corticosteroids have been recommended as adjunct treatment for any immune-mediated component of haemoplasma-associated anaemia, although cats usually recover without any need for corticosteroid treatment, as antibiotic and supportive care alone is usually adequate. Supportive care can be important; 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 in order to prevent inadvertent transmission by blood transfusion from asymptomatic carrier cats. There are no vaccines against feline haemoplasmosis. Keeping cats indoors is also likely to prevent infection as outdoor status has been identified as a risk factor (but may be impractical). Although vector transmission has not been proven, preventative flea and tick treatment is probably wise to help prevent infection in case vectors are involved.

Zoonotic infections

Haemoplasma infections with novel haemoplasma species have been described in humans (Maggi et al., 2013, Steer et al., 2011), as well as with species that have possibly originated in animals, including the cat (Santos et al., 2008), raising the possibility of zoonotic infections.

Figure Legends

Figure 1

Scanning electron micrograph showing two *Mycoplasma haemofelis* organisms attached to the surface of a feline erythrocyte
Image courtesy of Séverine Tasker

Figure 2

Graph showing mean haemoglobin values post-infection for cats infected with each of the three main feline haemoplasma species. Significant anaemia is only induced in the cats infected with *Mycoplasma haemofelis*. Although a fall in haemoglobin concentration does occur following infection with both '*Candidatus* Mycoplasma haemominutum' and '*Candidatus* Mycoplasma turicensis', anaemia is usually only induced if the infected cat is also immunocompromised
Image courtesy of Séverine Tasker

Figure 3

Pallor can be seen due to the anaemia induced by haemoplasma infections. This cat was infected with both FeLV and *Mycoplasma haemofelis* and shows very pale conjunctival mucous membranes.
Image courtesy of Tadeusz Frymus

Figure 4

Quantitative PCR allows the amount of haemoplasma DNA present in a sample to be quantified. Species-specific qPCRs are offered by many diagnostic laboratories. Quantification is performed by measurement of the change in fluorescence in the PCR tube by the qPCR machine. The more DNA present in the PCR (and thus sample), the earlier a change in fluorescence occurs which reaches the threshold level, as show in the graph. The cycle number at which each sample reaches the threshold is called the threshold cycle value and this figure may be reported to allow comparison of haemoplasma DNA levels in different samples
Image courtesy of Séverine Tasker

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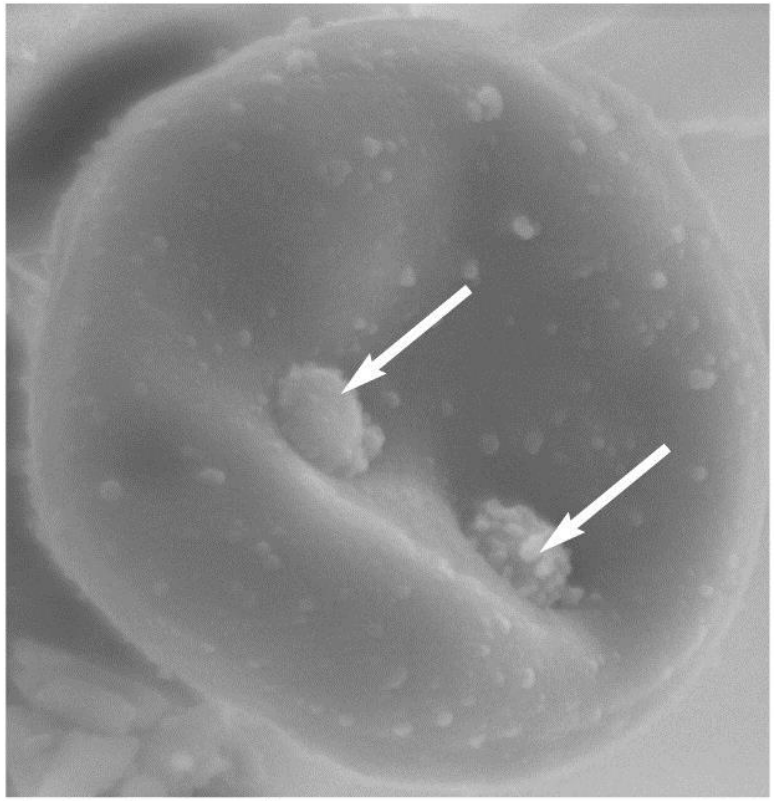
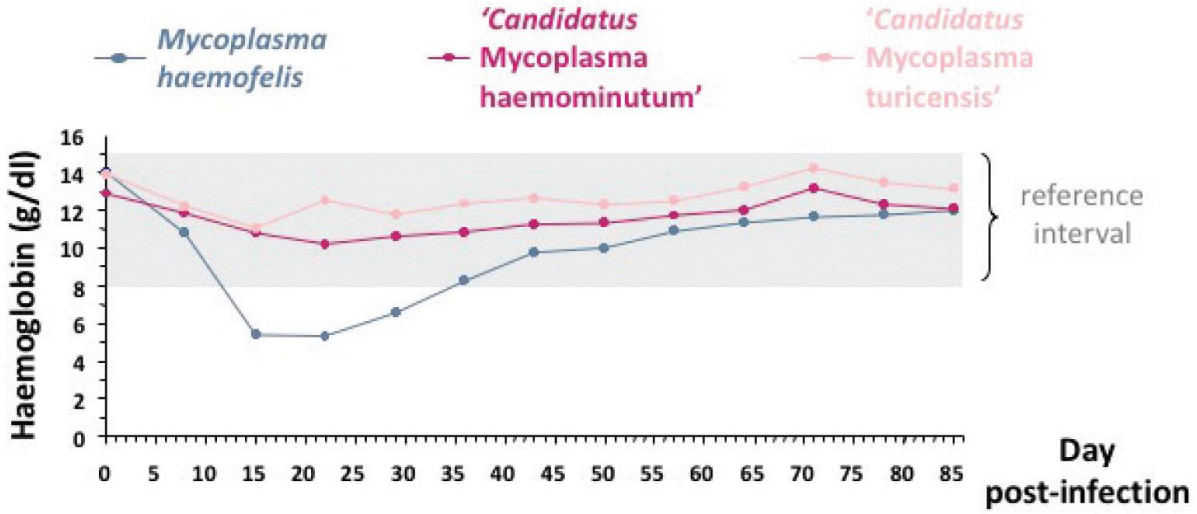
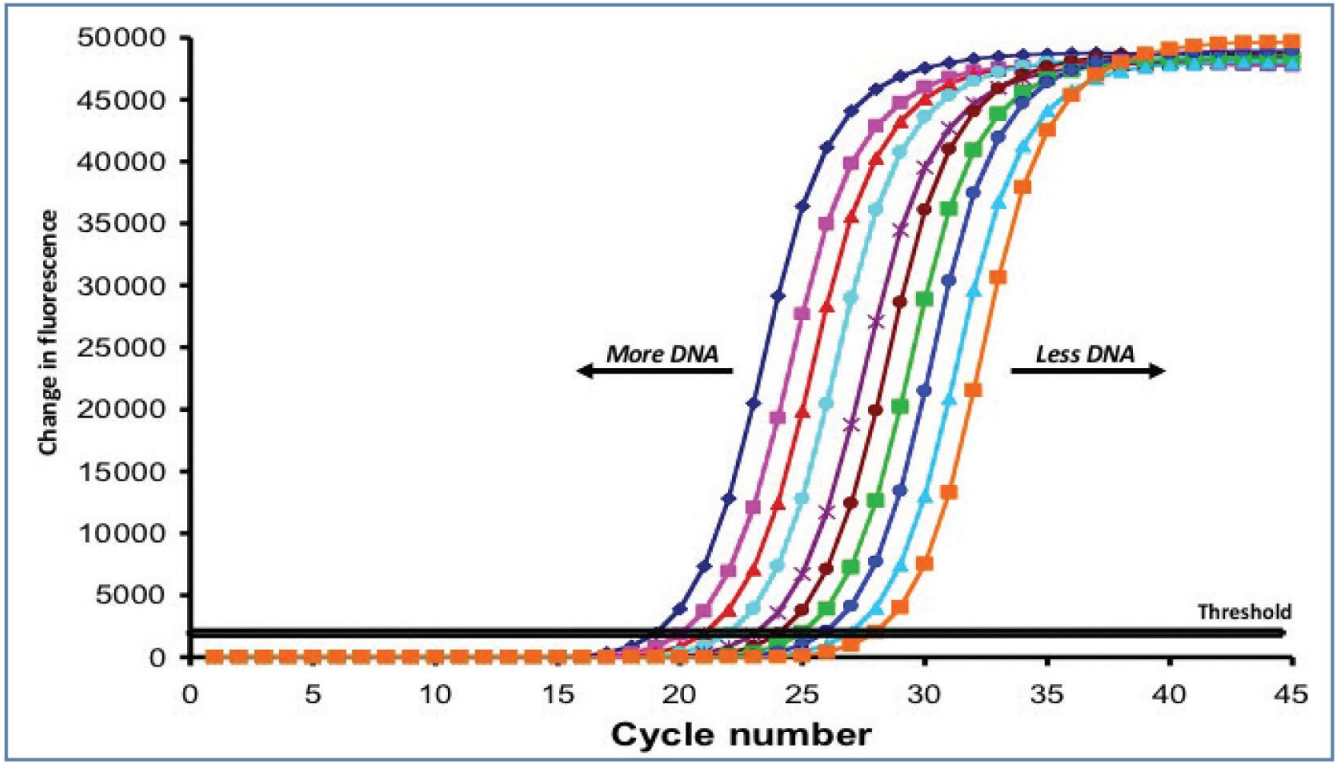




M haemofelis – can cause haemolytic anaemia in immunocompetent cats

***'Candidatus* M haemominutum'**
'Candidatus M turicensis'

} anaemia *usually* only results from infection in immunocompromised cats; for example, those undergoing chemotherapy, or if concurrent disease is present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