

GUIDELINE for Anaplasma, Ehrlichia, Rickettsia infections

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Key points

- *Anaplasma* spp., *Ehrlichia* spp. and *Rickettsia* spp. are vector-borne pathogens infecting many mammalian species, but causing disease in very few of them. *Anaplasma phagocytophilum* is the most important feline pathogen among them and co-infections are possible.
- The geographical distribution of *Anaplasma*, *Ehrlichia* and *Rickettsia* pathogens overlaps with that of their competent vectors (ticks and fleas).
- Little information is available on the pathogenesis of these agents in cats.
- Infection can be asymptomatic, or clinical signs are usually reported soon after tick infestation.
- The most frequent clinical signs are non-specific and consist of fever, anorexia and lethargy. Joint pain may occur.
- Blood or buffy-coat smear evaluation may be useful for cytological diagnosis of infections with *Ehrlichia* and *Anaplasma* spp.
- Blood PCR analysis is a sensitive and specific method for confirming diagnosis at the onset of acute clinical signs, provided samples are obtained before starting therapy.
- Cats with clinical signs are usually antibody negative due to inadequate time for seroconversion.
- Antibodies to rickettsial infections can be detected by immunofluorescence (IF) test and ELISA, but cross-reactions exist between organisms of the same genus.
- Doxycycline is the first choice antibiotic for treating rickettsial infections.
- Regular treatment with appropriate ectoparasiticides protects cats from transmission of infection by the competent vectors.
- In endemic areas blood donors should be tested for rickettsial blood-borne infections.
- Some species (*A. phagocytophilum*, *Ehrlichia chaffeensis*, *Ehrlichia ewingii*, *Rickettsia conorii*, *Rickettsia rickettsii*, *Rickettsia felis*, *Rickettsia typhi*) are of zoonotic concern.
- Infected cats are "sentinels" for the presence of rickettsial pathogens in ticks and fleas in a given geographical area and they signal a risk for people exposed to vectors.
- Direct contact with cat saliva should be avoided because of the potential contamination by *R. felis* as well as by other zoonotic pathogens.

Agent properties

Obligate intracellular Gram negative coccoid organisms of the *Anaplasma*, *Ehrlichia* and *Rickettsia* genera are vector-borne members of the *Rickettsiales* order infecting humans and a wide variety of domestic and wild animals worldwide (Allison and Little, 2013). They generally have a low host specificity, considering that many mammalian species can be infected. Importantly, some hosts might serve as reservoirs of infection; however, susceptibility to disease is usually more restricted.

Anaplasma, *Ehrlichia* and *Rickettsia* are difficult to culture *in vitro*; molecular genetics have opened new avenues to study their infection biology (Allison and Little, 2013). Compared to dogs, these pathogens have been generally less studied in cats (Table 1).

<i>Ehrlichia</i> genus	Countries in which cat infection has been detected
<i>E. canis</i>	EUROPE: Portugal Spain Italy AFRICA: Angola ASIA: Qatar AMERICAS: US Brazil Saint Kitts OCEANIA: Guam (Micronesia)
<i>E. chaffeensis</i>	AMERICAS: US Brazil
<i>E. ewingii</i>	AMERICAS: US
<i>Anaplasma</i> genus	
<i>A. phagocytophilum</i>	EUROPE: Sweden Finland Poland Switzerland Germany Austria Italy Spain AMERICAS: US Brazil ASIA: Korea
<i>A. platys</i>	EUROPE: Cyprus Turkey AMERICAS: US Brazil Chile
<i>A. platys</i> -like	EUROPE: Italy AMERICAS: Brazil
<i>A. bovis</i>	ASIA: Japan AFRICA: Angola
<i>Rickettsia</i> genus	
<i>R. rickettsii</i>	AMERICAS: US Saint Kitts
<i>R. conorii</i>	EUROPE: Spain Portugal
<i>R. massilliae</i>	EUROPE: Spain Portugal
<i>Rickettsia</i> spp.	EUROPE: Italy

Table 1: Members of the Ehrlichia, Anaplasma and Rickettsia genera detected in cats in various countries. Please note that the rickettsial infections may occur even in additional countries, however, not published so far. Therefore it is possible that cats are infected in a larger range of countries, not listed in this table, particularly in areas where the competent tick vectors are abundant (see the text).

Anaplasma and *Ehrlichia* spp. are tick-borne pathogens belonging to the *Anaplasmataceae* family and are pleomorphic intravacuolar organisms that replicate in haemopoietic cells. They give rise to cytoplasmic inclusion bodies: small elementary bodies (0.2-0.4 µm diameter), larger reticulate bodies and *morulae* (up to 2-6 µm diameter). *Anaplasma phagocytophilum* replicates in myeloid cells (mostly in neutrophils; Fig. 1) and is the agent of granulocytotropic anaplasmosis. It infects people and a wide range of animal species worldwide, especially in the Northern hemisphere. It is the most important feline pathogen of the *Anaplasma* genus. Wild small mammals are the natural reservoirs of infection.

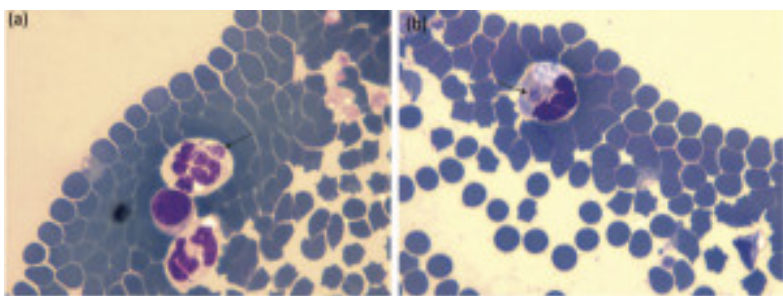


Fig. 1. Presence of *Anaplasma spp morulae* (arrows) in neutrophils.
Reprinted with permission from Adaszek et al., 2013.

Anaplasma platys replicates in mature platelets and is the agent of thrombocytotropic anaplasmosis, a disease well documented in dogs worldwide (Sainz et al., 2015).

Anaplasma bovis is responsible for a severe tick-borne disease in cattle and in 2012 was reported for the first time in cats in Japan (Sasaki et al., 2012). A novel, unclassified *A. platys*-like strain from cats was characterized in Sardinia (Italy). This strain, despite its tropism for platelets, is closely related to other *Anaplasma* spp. identified in ruminants (Zobba et al., 2015). ‘*Candidatus Anaplasma amazonensis*’ described in sloths from northern Brazil, was detected in the blood of two cats from the Minas Gerais state of Brazil (André et al., 2022). An *A. platys*-like strain detected in gray-pocket deer (*Mazama gouazoubira*) from Brazil was found in the blood from a cat of the São Paulo state of Brazil (André et al., 2022).

Ehrlichia canis is the agent of canine monocytotropic ehrlichiosis. This disease is described in tropical and temperate areas worldwide, with the exception of Australia. In endemic areas for the canine disease, feline infection is reported (Braga et al., 2012; Braga et al., 2021). In Brazil the possible existence of a new *E. canis* genotype infecting cats was supported based on ELISA antibody detection of anti-*E. canis* specific peptide antibodies (Braga et al., 2021).

Ehrlichia chaffeensis is the agent of human monocytotropic ehrlichiosis reported mainly in the USA where it was detected in ticks collected from cats (*Amblyomma americanum*) as well as dogs (Little et al., 2018).

The granulocytotropic *Ehrlichia ewingii* has been evidenced in dogs and humans in the Midwestern and Southern United States.

Another member of the *Anaplasmataceae* family that leads to neoehrlichiosis is *Neoehrlichia mikurensis*. This emerging tick-borne agent has been found mainly in immunocompromised patients, in ticks and rodents and also in dogs (Diniz et al., 2011; Hofmann-Lehmann et al., 2016), but so far not in cats. However, *N. mikurensis* has been detected in ticks collected from cats and infection may be underdiagnosed because diagnostic assays are not yet widely available (Kooyman et al., 2022).

The *Rickettsiaceae* family includes the spotted fever group (SFG) and the typhus group agents (Allison and Little, 2013). More than 20 species are included in the SFG of the *Rickettsia* genus, some of them being important human pathogens. Historically, the most important zoonotic agents are *R. conorii* (the cause of Mediterranean spotted fever) in the Old World, and *R. rickettsii* (the agent of Rocky Mountains spotted fever) in the Americas. However, molecular studies have increasingly focused on other rickettsial species that may be involved in human clinical disease. *Rickettsia massiliae*, for example, is now recognised as the most widely distributed *Rickettsia* species that affects humans, being found worldwide (Brouqui et al., 2007).

Rickettsia felis is a worldwide emerging flea-borne SFG human pathogen, frequently detected in *Ctenocephalides felis*, less often in other flea species (Brouqui et al., 2007; Abdullah et al., 2020).

Rickettsia typhi is a worldwide flea-borne rickettsia of the typhus group. It is the agent of murine or endemic typhus transmitted from rats by *Xenopsylla* fleas, or from cats to humans by *C. felis*, as well as to cats or wild animal reservoirs (Blanton et al., 2019).

Epidemiology

Prevalence

Anaplasma spp.

In Europe, feline antibody prevalence to *A. phagocytophilum* has been reported as 4.5-33.3% in Italy (Ebani and Bertelloni, 2014; Persichetti et al., 2016, 2018), 2.0-8.0% in Spain (Solano-Gallego et al., 2006; Ayllón et al., 2012), 16.2-23.0% in Germany (Hamel et al., 2012; Schäfer et al., 2022), 30.0% in Austria (Schäfer et al., 2022), 24.0% in Switzerland (Schäfer et al., 2022), and 22.1% in Sweden (Elfving et al., 2015). In the Americas, the prevalence was 1.8-38.0% in studies performed in the US (Magnarelli et al., 2005; Billeter et al., 2007; Hegarty et al., 2015) and 23.0% in Brazil (Pedrassani et al., 2019).

Anaplasma phagocytophilum DNA was amplified in blood samples from cats admitted to veterinary clinics in Germany (0.4-3.0%), Austria (8.0%), and Switzerland (10.0%) (Bergmann et al., 2015; Schäfer et al., 2022). Blood PCR positivity percentage for *A. phagocytophilum* was 0.33% in apparently healthy cats admitted for neutering in Brazil (Pedrassani et al., 2019), 0.9% in shelter cats from Korea (Lee et al., 2016), and 1.0% of anaemic cats in the US (Chan et al., 2021). The DNA of *Anaplasma* spp. closely related to *A. phagocytophilum* was detected in the blood of cats in Brazil (André et al., 2014, 2017).

Schäfer et al. (2022) reported the annual antibody and PCR prevalence of *A. phagocytophilum* in Germany. Antibody positivity increased from 8.0-12.0% of cats tested between 2008 and 2012 to 35.0-45.0% between 2018 and 2020. Results of blood PCR positivity increased from 0-2.0% between 2009 and 2013 to 2.0-10.0% between 2017 and 2020. Changes in the presence of vectors are much feared factors for these increases in positivity, however they may be influenced also by an increased awareness of practitioners about feline anaplasmosis (Schäfer et al., 2022).

In Europe, feline *A. platys* infection has been detected by blood PCR analysis in Cyprus (0.6% of 174 samples tested) (Attipa et al., 2017) and in Turkey (30.5% of 167 samples analysed) (Muz et al., 2021). In the Americas, PCR positivity for *A. platys* was 0.4-1.0% in the US (Hegarty et al., 2015; Chan et al., 2021), 3.3% in Chile (Sacristán et al., 2019) and a case was reported in Brazil (Lima et al., 2010).

Anaplasma bovis DNA has been detected in the blood of two feline immunodeficiency virus (FIV) positive cats from Japan (Sasaki et al., 2012). These were the only samples found to be positive out of 1764 samples tested from outdoor cats in 2008 throughout Japan (Sasaki et al., 2012). In Africa, one apparently healthy cat out of 102 tested in Angola was PCR positive in blood for *A. bovis* (Oliveira et al., 2018).

Ehrlichia spp.

In Europe, *E. canis* DNA has been detected by PCR in 5.4% (35/649) blood samples of cats from Portugal (Maia et al., 2014) and serum antibody positive cats were found in 9.9%-17.9% of tested samples in Spain (Aguirre et al., 2004; Ortuño et al., 2005; Solano-Gallego et al., 2006; Ayllón et al., 2012), and 6.4-16.2% of samples in Italy (Ebani and Bertelloni, 2014; Persichetti et al., 2016, 2018). In Germany, among 320 cats imported or travelling abroad, 40 were found antibody positive to *E. canis* (Schäfer et al., 2021).

In the Americas, *E. canis* DNA has been detected in the blood of three cats from the US (Breitschwerdt et al., 2002) and in 0.1% (Hegarty et al., 2015) and 1.0% (Chan et al., 2021) of tested cats in other US studies. Additionally, PCR positivity was obtained in 5.0% of cats in the Caribbean archipelago of Saint Kitts (Kelly et al., 2017). In Brazil studies have found variable rates of positivity, ranging from 0.5% to 20.0% (Oliveira et al., 2009; Braga et al., 2012, 2013, 2014; Guimarães et al., 2019; Pedrassani et al., 2019; André et al., 2015, 2022). In Africa, the blood samples of three of 102 apparently healthy cats (2.9%) from Angola were found to be PCR positive to *E. canis* (Oliveira et al., 2018). In the tropical island of Guam (a territory of US located in Micronesia in the Western Pacific Ocean) the blood of a cat was found to be PCR positive for *E. canis* (Weaver et al., 2022). In Qatar cats, PCR positivity was 2.9% (Alho et al., 2017). Antibody positivity to *E. canis* has been reported in the US (0.7%), Brazil (5.5-26.4%), and Saint Kitts (10.0%) (Braga et al., 2012, 2021; Hegarty et al., 2015; Kelly et al., 2017; Guimarães et al., 2019; Pedrassani et al., 2019).

Ehrlichia chaffeensis and *E. ewingii* have been rarely found in cats in the US (Hegarty et al., 2015) and *E. chaffeensis* has been rarely reported in Brazil (Braga et al., 2012).

Rickettsia spp.

Feline infections caused by *R. massiliae* and *R. conorii* have been confirmed by both PCR and antibody testing in endemic areas of Spain and Portugal (Solano-Gallego et al., 2006; Alves et al., 2009; Vilhena et al., 2013; Segura et al., 2014), whereas cats seropositive for *R. rickettsii* have been reported in the US and Saint Kitts in West Indies (Case et al., 2006; Kelly et al., 2017). In a retrospective evaluation of cats tested in Germany between 2015 and 2020, 11.0% of cats had anti-*Rickettsia* spp. antibodies (Schäfer et al., 2022).

Just as in humans and dogs, co-infections with multiple vector-borne pathogens can occur in cats (Maia et al., 2014; Hegarty et al., 2015; Attipa et al., 2017; Oliveira et al., 2018; Schäfer et al., 2022; André et al., 2022). For instance, amongst stray cats from Northern Italy, blood PCR positivity for *A. phagocytophilum*, *Ehrlichia* spp. and *Rickettsia* spp. (17.7%, 5.4% and 31.9%, respectively) showed that 5.4% of tested cats were positive for both *A. phagocytophilum* and *Rickettsia* spp. and 0.8% were positive for all three pathogens

(Spada et al., 2014). In Brazil, two cats with non specific signs were found to be blood PCR positive for *Anaplasma* spp., *Ehrlichia* spp., *Cytauxzoon felis* and “*Candidatus Mycoplasma haemominutum*” (André et al., 2017). Host co-infection can be caused by concurrent transmission from vectors carrying multiple pathogens (Noden et al., 2017; Calvani et al., 2020).

Transmission

The geographical distribution of *Anaplasma*, *Ehrlichia* and *Rickettsia* pathogens overlaps with that of the competent vectors. In Europe, two tick species are mainly involved: *Rhipicephalus sanguineus* sensu lato (the brown dog tick or kennel tick), which is the main tick vector of *E. canis* and *R. conorii* (and suspected for *A. platys*); and *Ixodes ricinus*, the vector of *A. phagocytophilum*. Both species can transmit also other pathogens (*Babesia* spp., *Borrelia* spp.) as single or multiple infections, and both species are found also on cats (Jameson and Medlock, 2011; Claerebout et al., 2013; Pennisi et al., 2015a; Król et al., 2015; Duplan et al., 2018; Jongejan et al., 2019; Kooyman et al., 2022). In a large-scale survey performed in the UK evaluating ticks collected from cats for pathogen DNA by PCR, 0.9% of 540 ticks were positive for *A. phagocytophilum* DNA. Four positive samples were from *I. ricinus* ticks and one from a *I. hexagonous* tick (Duplan et al., 2018). In Canada exposure to *A. phagocytophilum* was found in cats carrying infected *Ixodes scapularis* ticks (Duplaix et al., 2021). In Asia infection of ticks collected from cats was documented for *E. canis* in *R. sanguineus* (Indonesia, the Philippines) and *A. platys* in *Ixodes* spp. (Taiwan) (Nguyen et al., 2020). However, *A. phagocytophilum* and *A. platys* DNA were also detected in fleas collected from cats (Pawelczyk et al., 2019; Calvani et al., 2020).

Ixodes ricinus has a wide distribution, from the Mediterranean area to Scandinavian countries, and from Portugal to Ukraine (<http://ecdc.europa.eu/en/healthtopics/vectors/vector-maps/Pages/VBORNET-maps-tick-species.aspx>). In the Eastern part of Europe a closely related species, *Ixodes persulcatus* is found (Sainz et al., 2015). *Ixodes hexagonus* was reported in cats from the UK (Duplan et al., 2018) and the Netherlands (Kooyman et al., 2022).

Rhipicephalus sanguineus sensu lato is common in the Mediterranean basin; it is not indigenous in northern countries but it can hibernate sheltered in cracks of kennel structures, so the area of its distribution is expanding northwards.

Based on vector distribution, *E. canis* and *A. platys* are considered endemic in Mediterranean countries but are spreading northwards, whereas *A. phagocytophilum* is reported mainly in Northern and Central Europe (Sainz et al., 2015).

Rickettsia rickettsii and *R. conorii* are both transmitted by ticks, infect dogs and may cause an acute febrile clinical disease in dogs (Solano-Gallego et al., 2015). Less information is available about the effect of these agents in cats.

Similar rates of positivity to *Ehrlichia* spp., *Anaplasma* spp., and *Rickettsia* spp. were found in healthy cats as compared to those showing clinical signs (Spada et al., 2014). Blood transfusion consequently may be a non-vectorial mode of transmission for rickettsial agents in cats, as it is well known in dogs (Sainz et al., 2015).

Ctenocephalides felis is the vector and the recognised reservoir of *R. felis*, which is vertically transmitted to successive generations of fleas (Wedincamp and Foil, 2002). However, dogs can be considered a reservoir as well because they have prolonged rickettsiaemias without showing clinical or haematological manifestations, and maintain horizontal transmission from infected to uninfected fleas (Ng-Nguyen et al., 2020). Studies carried out in some parts of Europe have shown that *R. felis* infection rates of *Ctenocephalides* range from 2.8% in Albania (Silaghi et al., 2012) to 54.2% in Andalusia (Márquez et al., 2006).

Pathogenesis

Little information is available on the pathogenesis of rickettsial diseases in cats. A limited number of studies on experimental infections or exposure with *A. phagocytophilum* or *R. felis* in cats exist. The intraperitoneal experimental infection with *A. phagocytophilum* infected blood in a small number of cats resulted in mild disease with transient fever not associated with changes in appetite nor general appearance. However, a mild reduction in total leukocyte, neutrophil and lymphocyte counts, a marked reduction in PCV values, and transient increase of ALT and AST values, were detected (Foley et al., 2003). Anti-nuclear antibodies and increased expression of γ IFN mRNA were also found in infected cats but they had normal antibody responses to feline herpesvirus and feline leukaemia virus vaccination two weeks post infection (p.i.). When experimental infection with *A. phagocytophilum* was performed in FIV infected cats, upregulation of IL-10 expression was observed instead of γ IFN, but the clinical course of disease was similar (Foley et al., 2003). The experimental exposure of four cats with wild-caught adult *Ixodes scapularis* induced a subclinical dual infection with *A. phagocytophilum* and *Borrelia burgdorferi* with no abnormalities concerning general appearance, appetite, and body temperature (Lappin et al., 2015).

In two reported cases of *A. platys* infection, the pathogenic role of the organism was not clearly established (Lima et al., 2010; Quorollo et al., 2014). One of the cats was diagnosed with a multiple myeloma, and coinfections with *Bartonella henselae*, *B. koehlerae* and ‘*Ca. M. haemominutum*’ (Quorollo et al., 2014). In this case, immunosuppression due to the severe monoclonal gammopathy could have been responsible for increased susceptibility to the co-infections.

Experimental subcutaneous inoculation of cats with a canine strain of *E. canis* was not successful and the pathogenesis of feline monocytotropic ehrlichiosis in cats is not known (Lappin and Breitschwerdt, 2012).

Cats are susceptible to *R. felis* infection and seroconvert after exposure to infected fleas. In rare cases, *R. felis* DNA can be amplified also from the skin or gingiva of cats whilst blood PCR testing is negative (Lappin and Hawley, 2009). It is unknown whether *R. felis* is present in other tissues of seropositive cats and whether it should be considered to be a feline pathogen. A short-term bacteraemia does occur in cats infected by *R. felis* but blood PCR testing is usually negative in antibody positive cats (Wedincamp and Foil, 2000; Hawley et al., 2007; Segura et al., 2014; Persichetti et al., 2016).

Clinical signs

In naturally exposed cats, clinical signs of feline granulocytotropic anaplasmosis are usually reported soon after tick infestation. They are mostly non-specific and consist of fever, anorexia, lethargy, conjunctivitis, and dehydration (Schäfer and Kohn, 2020). Lymphadenomegaly, lameness and swollen joints, epistaxis, and pain on abdominal palpation were less frequently reported (Bjöersdorff et al., 1999; Tarello, 2005; Adaszek et al., 2013; Savidge et al., 2016; Schäfer et al., 2022). The clinical course is usually short and not severe, and abnormalities resolve quickly, particularly when antibiotic treatment is given.

In Brazil, a natural infection with *A. platys* was found associated with anorexia and lethargy in a cat with a concurrent urinary infection (Lima et al., 2010).

Cats with monocytotropic ehrlichiosis manifest non-specific signs such as fever, anorexia and lethargy, but more rarely hyperesthesia, joint pain, pale mucous membranes, lymph node and spleen enlargement, and haemorrhagic diathesis (petechiae, vitreous haemorrhage) (Lappin and Breitschwerdt, 2012).

In US a case-control study comparing *Rickettsia* species positivity in cats with and without fever found more cats seropositive for *R. felis* and *R. rickettsii* among the cats with fever, but the difference was not statistically significant (Bayliss et al., 2009).

Laboratory and diagnostic imaging findings

Laboratory changes

Clinicopathological abnormalities reported in cats with granulocytotropic anaplasmosis include complete blood count (CBC) abnormalities as mild or moderate thrombocytopenia, anaemia, lymphopenia, and eosinopenia (Bjöersdorff et al., 1999; Lappin et al., 2004; Schaarschmidt-Kiener et al., 2009; Heikkilä et al., 2010; Adaszek et al., 2013; Savidge et al., 2016; Schäfer et al., 2022). However, in 13 cases with no known comorbidities, the CBC of five cats showed leukocytosis and in one case a severe haemolytic anaemia was observed (Schäfer et al., 2022). A transient lymphopenia was the only CBC abnormality detected during a 13 weeks of observation after an experimental exposure of four cats with wild-caught adult *Ixodes scapularis* (Lappin et al., 2015). In a few cases without known comorbidities biochemical investigations were available, and changes included increased values of serum proteins (3/10), globulins (2/9), ALT (1/8), and bilirubin (2/8) (Schäfer et al., 2022).

Mild thrombocytopenia was reported in a cat with *A. platys* infection and a concurrent urinary tract infection (Lima et al., 2010). One month later the platelet count was within the reference range and leukocytosis was found.

Clinicopathological changes seen in cats with monocytic ehrlichiosis included non-regenerative anaemia, thrombocytopenia, pancytopenia and increased or decreased white cell counts (Lappin and Breitschwerdt, 2012). Bone marrow hypoplasia was found in cats with pancytopenia or anaemia and thrombocytopenia on CBC (Breitschwerdt et al., 2002). The most consistent biochemical abnormality seen with feline monocytotropic ehrlichiosis was hyperproteinaemia and polyclonal or monoclonal gammopathy, which is also typical of the chronic course of canine monocytotropic ehrlichiosis (Lappin and Breitschwerdt, 2012). Anti-nuclear antibodies were found in some cats and neutrophilic polyarthritis was diagnosed in a cat with joint signs (Breitschwerdt et al., 2002).

In a study conducted in Brazil, anaemia was found associated in cats with antibody positivity to *E. canis* (Guimarães et al., 2019). Another study, however, which explored the associations between *Ehrlichia* spp. or *A. phagocytophilum* infections and anaemia, did not detect any significant associations (Ishak et al., 2006).

Diagnostic imaging findings

Little information is available on diagnostic imaging findings in feline Rickettsial infections. Splenomegaly was documented by abdominal imaging in three out of 13 cats with granulocytotropic anaplasmosis with no known comorbidities (Schäfer et al., 2022).

Abdominal ultrasound evaluation was performed at diagnosis in a cat with *A. platys* and no abnormality was reported (Lima et al., 2010).

Diagnosis

The clinical suspicion for Rickettsial diseases mostly arises in cases of a febrile syndrome affecting cats exposed to ticks and fleas in endemic areas, especially stray or outdoor pet cats not protected by the regular use of appropriate ectoparasitocides (Lappin et al., 2020a, 2020b).

Detection of the infectious agent

Blood or buffy-coat smear evaluation may provide a cytological diagnosis of infections with *Ehrlichia* and *Anaplasma* spp. In general, intracytoplasmic inclusion bodies are more frequently found in granulocytotropic anaplasmosis than in monocytotropic ehrlichiosis and in animals with fever. *Anaplasma phagocytophilum* inclusion bodies are found in 1-24% of circulating neutrophils in cats with natural granulocytotropic anaplasmosis. In experimentally infected cats they appear 7-9 days p.i. (Foley et al., 2003) or over the first 10 weeks after tick infestation (Lappin et al., 2015). After antibiotic therapy they are no longer visible (Bjöersdorff et al., 1999; Heikkilä et al., 2010; Lappin et al., 2015). With *A. platys*, bacteraemia is cyclical in dogs at 1 to 2-week intervals, with a higher percentage of circulating infected platelets occurring during the initial cycles (Harvey et al., 1978), but no information is available in cats.

Blood PCR analysis is a sensitive and specific method for confirming diagnosis at the onset of acute clinical signs when antibody testing is usually still negative (Foley et al., 2003; Lappin et al., 2015). Because of overlapping clinical signs, the use of genus-inclusive primers for *Ehrlichia-Anaplasma* and *Rickettsia* spp. genera in PCRs has been suggested as best practice, followed by sequencing of any resulting PCR products to determine the infecting species (Allison and Little, 2013). However, a study demonstrated that some genus-specific PCRs also detect *Pseudomonas* sequences and may lead to false positive results that may only be recognized after sequencing analysis (Hofmann-Lehmann et al., 2016). Alternatively, the use of species-specific real-time TaqMan assays may be faster and more sensitive options for the molecular detection of rickettsaemia.

Blood samples for microscopic detection or PCR should be collected prior to the initiation of antibiotic treatment.

A splenic aspirate was performed in one cat with granulocytic anaplasmosis and splenomegaly. The cytological evaluation showed reactive hyperplasia of the white pulp, pyogranulomatous inflammation and morulae in neutrophils (Schäfer et al., 2022). A prospective *post-mortem* investigation of 37 cats subjected to necropsy evaluated the occurrence of the *Anaplasma* spp. and *Ehrlichia* spp. DNA in different tissues such as spleen, bone marrow, blood clot, and hair (Balboni et al., 2021). Interestingly, positive results were obtained from spleen (one cat) and hair (two cats) samples, and none of the cats was positive in bone marrow nor blood (Balboni et al., 2021).

Detection of antibodies

Antibodies to rickettsial infections can be detected by immunofluorescence (IF) testing and ELISA. Cross-reaction is possible between *A. phagocytophilum* and *A. platys* but not with *E. canis*, although *E. canis* can cross react with other *Ehrlichia* spp. Rapid in-house ELISAs are available for detecting canine antibodies against *A. phagocytophilum*. These ELISAs were used in feline infection cases, comparing results with a commercial IF assay, but discrepancies were found (Hegarty et al., 2015).

Antibodies against *A. phagocytophilum* were detected in an experimental study within 14 days p.i. and seroconversion also occurs in natural infections, even in antibiotic treated cats (Foley et al., 2003). In cats experimentally exposed to infected ticks, antibodies against *A. phagocytophilum* appeared 2-6 weeks after infestation (Lappin et al., 2015). In the case of a positive IF test, a 4-fold increase of the titre over about four weeks is needed to confirm the acute course of the infection (Bjöersdorff et al., 1999; Foley et al., 2003; Lappin et al., 2004; Heikkilä et al., 2010). Moreover, some cats testing positive to *E. canis* by PCR were found to be antibody negative despite the advanced course of their disease, suggesting that a negative antibody test does not exclude the diagnosis (Breitschwerdt et al., 2002). When possible, both serological and blood PCR test should be performed in cats with compatible clinical signs.

Treatment

There are no controlled studies evaluating the efficacy of drugs used for treating rickettsial diseases in cats. However, doxycycline is considered the first choice antibiotic administered at 10 mg/kg orally q24h (or 5 mg/kg orally q12h) for 28 days. Available formulations of doxycycline vary but care must be taken with the administration of doxycycline hyclate tablets due to the risk of oesophagitis occurring with their permanence in the oesophagus after swallowing; oral administration should be followed by food and/or water to ensure passage of the tablet into the stomach.

In cases testing negative by microscopy, PCR or antibody testing, or when results of diagnostic tests are pending, but where there is a strong clinical suspicion of rickettsial disease, treatment should be initiated soon after blood collection to prevent the potential of rapid progression of clinical disease.

Management of infected patients

There is no evidence of the efficacy of antibiotic treatment for clearing infection in clinically healthy cats; therefore doxycycline or other antibiotics should not be given in these cases.

Prognosis

Clinical improvement is seen in the first 24-48 hours unless comorbidities or co-infections not susceptible to doxycycline are present, such as protozoal vector-borne agents, or if other complications develop such as severe bleeding (Savidge et al., 2016; Schäfer et al., 2022). Animals generally respond well to treatment but may remain persistently infected. Three cats diagnosed with granulocytotropic anaplasmosis (blood PCR positive) and treated with doxycycline for two or three weeks, had a clinical recurrence and tested again positive for *A. phagocytophilum* DNA at four and five weeks and two years, respectively, after the end of treatment (Schäfer et al., 2022). Persisting infections or reinfections may therefore occur after doxycycline treatment.

Vaccination

No vaccine is available for preventing disease caused by Rickettsiales in humans and animals.

Prevention

As vectors are the main routes of transmission of rickettsial infections, regular treatment with appropriate topical (spot ons, collars) or oral ectoparasiticides may protect cats from becoming infected, as it is well recognised in dogs.

In endemic areas, blood donors should be tested for rickettsial blood-borne infections to confirm they are negative before being used as donors (Pennisi et al., 2015b).

Zoonotic risk

Rickettsial pathogens are transmitted to humans by competent vectors. Infected cats, as well as dogs, are “sentinels” of the presence of rickettsial pathogens in ticks and fleas in a given geographical area and they signal a risk for people exposed to vectors (Król et al., 2015; Persichetti et al., 2016; Jongejan et al., 2019; Kooyman et al., 2022). Regular application of ectoparasiticides to pets reduces the risk of exposure of humans to vectors of rickettsial agents.

Direct contact with cat saliva should be avoided because of the potential contamination by *R. felis* as well as by other zoonotic pathogens.

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