Haemotropic mycoplasmas of cats and dogs: transmission, diagnosis, prevalence and importance in Europe

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Summary

Haemotropic mycoplasmas (or haemoplasmas) are the causative agents of infectious anaemia in many mammalian species. They were previously known as Haemobartonella and Eperythrozoon species. The development of sensitive, specific PCR assays has expanded our knowledge of these agents and PCR is the method of choice to diagnose and differentiate haemoplasma infections. In felids, Mycoplasma haemofelis, ‘Candidatus Mycoplasma haemominutum’ and ‘Candidatus Mycoplasma turicensis’ have been described. They vary strongly in their pathogenic potential and co-factors may influence the disease severity. In dogs, Mycoplasma haemocanis and ‘Candidatus Mycoplasma haemotarvum’ are known; clinical signs are mainly found in immunocompromised dogs. Transmission of haemoplasmas may occur via infected blood (aggressive interaction, transfusion) or blood-sucking arthropods. Infections can be treated with Doxycycline, although it is disputable whether the infection is completely eliminated. Feline haemoplasmas must be expected in cats all over Europe, while canine haemoplasmas are mainly encountered in dogs in Mediterranean countries but should also be considered in Swiss dogs with a travel history.

Key words: haemotropic mycoplasma, transmission, prevalence, diagnostics, companion animals

Haemotrope Mykoplasmen bei Hund und Katze: Übertragung, Diagnose, Prävalenz und Bedeutung in Europa


Schlüsselwörter: Haemotrope Mykoplasmen, Übertragung, Prävalenz, Diagnose, Kleinrindertiere

Introduction

In recent years, there has been a growing interest in haemotropic mycoplasmas, also known as haemoplasmas. Haemotropic mycoplasmas are small ( < 1 μm), cell wall-less, discoid-shaped bacteria, closely attached to red blood cells (RBC) of infected animals (Fig. 1). They were formerly known as Haemobartonella and Eperythrozoon species and are the causative agents of infectious anaemia in a variety of mammalian species. Increasing knowledge on the bacterial genomic sequences and phylogenetic relationship led to the reclassification of these organisms within the genus Mycoplasma as haemotropic mycoplasmas (Neimark et al., 2001). Infections with haemoplasmas can induce acute haemolysis, associated with anorexia, lethargy, dehydration, weight loss and sudden death of infected animals. The inability to culture these agents in vitro has limited the possibilities to investigate
haemotropic mycoplasmas. However, in recent years species-specific conventional and real-time TaqMan PCR assays have been developed for sensitive detection of feline and canine haemoplasmas. The application of these assays has been allowing investigating the pathogenesis and epidemiology of haemoplasma infections in cats and dogs in more detail.

**Haemotropic mycoplasmas in cats**

In cats, early studies described two distinct haemoplasma species: the Ohio isolate (large form) and the California isolate (small form) of *Haemobartonella felis* (Berent et al., 1998; Foley et al., 1998; Messick et al., 1998). Along with the suggested reclassification within the genus *Mycoplasma*, these isolates were renamed *Mycoplasma haemofelis* (Mhf) (Neimark et al., 2001) and 'Candidatus Mycoplasma haemominutum' (CMhm) (Foley und Pedersen, 2001; Fig. 2). In 2002, a third haemotropic *Mycoplasma* species was identified in a privately owned Swiss cat that presented with haemolytic anaemia; this third species was designated 'Candidatus Mycoplasma turicensis' (CMt) (Willi et al., 2005; Willi et al., 2006a; Figure 1: Giemsa-stained blood smear from a cat infected with Mhf. Multiple basophilic coccoid structures (black arrows), around 0.5 μm in diameter, some of them arranged in pairs or chains, are seen attached to RBC.

Figure 1: Giemsa-stained blood smear from a cat infected with Mhf. Multiple basophilic coccoid structures (black arrows), around 0.5 μm in diameter, some of them arranged in pairs or chains, are seen attached to RBC.

**Figure 2:** Phylogenetic tree of common haemoplasma species. The main host species are indicated in parenthesis. Bootstrap values are given at the nodes of the tree. Evolutionary distances are shown to the scale. GenBank accession numbers are available upon request from the authors.

Figure 2: Phylogenetic tree of common haemoplasma species. The main host species are indicated in parenthesis. Bootstrap values are given at the nodes of the tree. Evolutionary distances are shown to the scale. GenBank accession numbers are available upon request from the authors.
Haemotropic mycoplasmas of cats and dogs

Haemobartonella canis infections associated with anaemia have been sporadically reported in dogs. Subsequently, H. canis was reclassified as Mycoplasma haemocanis (Mhc), and a second canine haemoplasma, CMhp (Fig. 2) has been described in an anaemic splenectomised dog undergoing chemotherapy (Messick et al., 2002; Sykes et al., 2004). Both agents seem to exhibit worldwide distribution, but only limited prevalence data based on molecular detection methods are yet available. Using specific PCR assays to investigate the prevalence and clinical importance of canine haemoplasma infections in Europe, a higher prevalence was recently reported in countries with Mediterranean and sub-Mediterranean climate when compared to Switzerland (Kenny et al., 2004; Wengi et al., 2008; Novacco et al., 2009). Additionally, in some populations young animals and male dogs seemed more susceptible to canine haemoplasma infections than adult and female dogs, respectively (Novacco et al., 2009; Barker et al., 2010). Severe haemolytic anaemia has only occasionally been described in haemoplasma-infected dogs, mainly in immune-compromised or splenectomized animals. PCR-based investigations of canine haemoplasmosis in Europe also support the low pathogenic potential of these agents (Wengi et al., 2008; Novacco et al., 2009). Early studies suggest that co-infections with parvovirus, Ehrlichia and Babesia species, or concurrent neoplasia may play an aggravating role for canine haemoplasma infections. More recently, mange infection was associated with canine haemoplasma infections (Novacco et al., 2009). The mites may play a role in the mechanical transmission of haemoplasmas, or mange infection may signal a compromised immune system in affected animals. Most haemoplasma-infected dogs present with chronic, asymptomatic infections. These animals seem unable to clear the infection. In a recent study using quantitative real-time PCR, all samples collected from three infected dogs throughout a follow-up period of up to 13 months tested positive (Wengi et al., 2008). As described for other haemoplasma infections, antibiotic treatment may be unable to eliminate canine haemoplasma infections completely, but was found to reduce clinical signs of infection.

Transmission

The natural mode of transmission of feline and canine haemoplasmas has not been definitely elucidated. Blood transfusions have been reported as a source of Mhf and CMhm infections (Gary et al., 2006; Willi et al., 2006b). Furthermore, blood-sucking arthropods may be involved in the transmission of feline and canine haemoplasmas. Mhf and CMhm DNA was detected in the cat flea, Ctenocephalides felis, and in flea faeces, but a recent experimental transmission study for Mhf and CMhm via C. felis was not conclusive (Shaw et al., 2004; Woods et al., 2005; Lappin et al., 2006; Woods et al., 2006; Willi et al., 2007a; Kamrani et al., 2008). In Switzerland, positive results for CMhm and CMt were obtained from some Ixodes spp. ticks collected directly from animals but no haemoplasma DNA was detected in Ixodes ticks collected from the vegetation, which indicates that Ixodes ticks are not a major reservoir for haemotropic mycoplasmas.
in Switzerland (Willi et al., 2007a; Willi et al., 2009). In contrast, the brown dog tick, *Rhipicephalus sanguineus*, is likely to play a role as a vector and reservoir for canine haemoplasmas (Seneviratna et al., 1973). In Europe, the brown dog tick is commonly encountered in areas with Mediterranean and sub-Mediterranean climate and the high prevalence of canine haemoplasma infections found in these countries supports the hypothesis of it being a possible tick vector for the transmission of the infection (Kenny et al., 2004; Novacco et al., 2007b; Barker et al., 2010). CMt was detected by PCR in saliva and faeces of infected cats during early infection and CMhm was detected in the saliva and salivary glands of experimentally infected cats (Willi et al., 2007a; Dean et al., 2008; Museux et al., 2009) indicating that direct transmission of haemoplasmas via saliva may be important. However, a recent *in vivo* study that modelled CMt transmission via social contact among cats was unable to infect cats by subcutaneous or oral inoculation of CMt PCR-positive saliva (Museux et al., 2009). In contrast, transmission by subcutaneous inoculation of as little as 10 μl of PCR-positive blood was successful, which may indicate that aggressive interaction is necessary to transmit CMt between cats (Museux et al., 2009). This would also be in agreement with observations for Mhc, for which the prevalence was higher in Japanese fighting dogs compared to other breeds (Sasaki et al., 2008). The latter was attributed to direct transmission of Mhc via infected blood during aggressive contact (dogfights).

### Diagnosis

Specific conventional and quantitative real-time TaqMan PCR systems have been introduced and are now considered the gold standard for the detection and differentiation of feline and canine haemoplasma species (Tasker et al., 2003b; Willi et al., 2007b; Peters et al., 2008; Wengi et al., 2008; Willi et al., 2009; Barker et al., 2010). They were also applied to investigate the pathogenesis and haemoplasma tissue loads in experimental studies (Tasker et al., 2009). No *in vitro* culture system has been established to date to propagate feline and canine haemoplasmas outside their hosts. The light microscopic investigation of Giemsa-stained blood smears from infected animals was shown unreliable to diagnose haemoplasma infections. A diagnostic sensitivity of less than 20 % has been reported for this method, and the diagnostic specificity is often hampered by confusing organisms with stain precipitates or Howell-Jolly bodies. In particular, light microscopy is unfit to diagnose CMt infection because of the usually low CMt blood loads. Even at peak CMt bacteremia

### Table 1: Percentage of feline and canine haemoplasma PCR-positive animals in the sampled populations in Europe.

<table>
<thead>
<tr>
<th>Feline haemoplasmas</th>
<th>Switzerland</th>
<th>Germany</th>
<th>UK</th>
<th>Spain</th>
<th>Italy</th>
<th>Portugal</th>
<th>France</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 615</td>
<td>n = 397a</td>
<td>n = 2011</td>
<td>n = 30</td>
<td>n = 30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Mhf'</td>
<td>0.5 %</td>
<td>5.3–7.4 %</td>
<td>1.6–2.8 %</td>
<td>0 %</td>
<td>20 %b</td>
<td>5.9 %</td>
<td></td>
</tr>
<tr>
<td>- CMhm*</td>
<td>8.5 %</td>
<td>8.9–23.3 %</td>
<td>11.2–17.1 %</td>
<td>10 %b</td>
<td>17.3 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- CMt</td>
<td>1.0 %</td>
<td>2.2 %</td>
<td>1.7–2.3 %</td>
<td>nt</td>
<td>1.3 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Co-infected</td>
<td>1.0 %</td>
<td>0.8–3.0 %</td>
<td>1.6–1.9 %</td>
<td>nt</td>
<td>0.8 %</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

References: (Willi et al., 2006b)

<table>
<thead>
<tr>
<th>Canine haemoplasmas</th>
<th>Switzerland</th>
<th>Germany</th>
<th>UK</th>
<th>Spain</th>
<th>Italy</th>
<th>Portugal</th>
<th>France</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 889</td>
<td>n = 200</td>
<td>n = 600</td>
<td>n = 50</td>
<td>n = 460</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Mhc'</td>
<td>0.9 %</td>
<td>0.5 %</td>
<td>4.5 %</td>
<td>0 %</td>
<td>4.5 %</td>
<td>5.8 %</td>
<td></td>
</tr>
<tr>
<td>- CMhp</td>
<td>0.1 %</td>
<td>20 %</td>
<td>20 %</td>
<td>nt</td>
<td>0 %</td>
<td>12.2 %</td>
<td></td>
</tr>
<tr>
<td>- Co-infected</td>
<td>0 %</td>
<td>0 %</td>
<td>0 %</td>
<td>0 %</td>
<td>2.6 %</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

References: (Wengi et al., 2008)

Table 1: Percentage of feline and canine haemoplasma PCR-positive animals in the sampled populations in Europe.

# including some pre-selected samples (anaemic cats).

# all samples pre-selected (cats with suspected haemoplasma infection).

# including co-infected animals. nt = not tested
Prevalence and importance in Europe

The sample prevalence of haemoplasma infections in dogs and cats in Europe based on real-time PCR investigations are listed in Table 1. Feline haemoplasma infections were found in all investigated populations. In Switzerland, canine haemoplasma infections are less prevalent than in most of the other examined countries, particularly those with a Mediterranean climate (Wengi et al., 2008; Novacco et al., 2009). Moreover, the infected dogs in Switzerland had either been imported from or visited regions where R. sanguineus is indigenous (Wengi et al., 2008). This observation supports the hypothesis that canine haemoplasmas may be indirectly transmitted by blood-sucking arthropods, in particular those that rely on a warm climate for their survival. In a recent study on the situation in Europe, we identified several risk factors for canine haemoplasma infections, e.g. living in kennels, young age, and non-pedigree lineage (Novacco et al., 2009). A higher prevalence of canine haemoplasma infections in kennel-kept dogs may be due to the fact that dogs in kennels are group housed, which could increase the risk of direct haemoplasma transmission among dogs and their risk of exposure to fleas and ticks. The close relationship between CMt and rodent haemoplasmas as well as among certain feline and canine haemoplasmas suggests a potential interspecies transmission of these agents (Sykes et al., 2004; Willi et al., 2005). While CMt has not yet been detected in rodents (Willi et al., 2007a), CMhm infection has been reported in a dog in China (Zhuang et al., 2009). Moreover, Mhf has recently been detected by PCR in the blood of a human AIDS patient (dos Santos et al., 2008), and PCR-positive results for porcine haemoplasma-like organisms have been obtained from blood of Chinese farm workers and swine veterinarians (Yuan et al., 2009). This substantiates the risk of an interspecies transmission of haemoplasmas. Nevertheless, so far the zoonotic potential of haemoplasma infections has been largely neglected.

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Mycoplasma hémotropique chez le chat et le chien: mode de transmission, diagnostic, prévalence et incidence en Europe

Le mycoplasme hémotropique (anc. Hémoplasmose) est l’agent responsable de l’anémie infectieuse chez de nombreux mammifères. Il était préalablement décrit en tant qu’Hémobartonelle et Éperythrozoon. Le développement d’un test PCR sensible et spécifique a nettement amélioré nos connaissances de cet agent, et la PCR est maintenant la méthode de choix pour le diagnostic et le différentiel des infections à hémoplasmes. Chez le chat, les mycoplasma haemofélis, candidatus mycoplasma haemominutum et candidatus mycoplasma turicensis ont été décrits. Leur potentiel pathogénique est très variable, ainsi que leur importance en tant que co-facteurs pathogènes. Chez le chien, les mycoplasma haemocanis et candidatus mycoplasma haematoparum sont connus, les symptômes cliniques étant plus fréquents chez les animaux immunodéprimés. La transmission des hémoplasmes par voie hémotogène est possible (transfusion, agression ou par le biais des arthropodes hématopha-

Mycoplasmi emotropi nel cane e nel gatto: trasmissione, diagnosi, prevalenza e importanza a livello Europeo

I micoplasmi emotropi (o emoplasmi) rappresentano l’agente patogeno dell’anemia infectiva in molte specie di mammiferi. In passato erano conosciuti come Haemobartonella ed Éperythrozoon. Il recente sviluppo di specifiche e sensibili metodiche basate sulla PCR ha permesso di approfondire le conoscenze riguardanti questi agenti infettivi. Tali metodiche sono attualmente considerate la prima scelta per la diagnosi e la differenziazione delle infezioni da emoplasmi. Nei felini sono stati descritti i seguenti emoplasmi: Mycoplasma haemofélis, ‘Candidatus Mycoplasma haemominutum’ e ‘Candidatus Mycoplasma turicensis’. La loro potenziale patogenicità è molto differente e possibili fattori concomitanti possono influenzare la gravità della patologia. Nel cane sono noti Mycoplasma haemocanis e ‘Candidatus Mycoplasma haemominutum’. La manifestazione clinica della patologia è prevalente nei soggetti immunodepressi. La trasmissione degli emoplasmi avviene attraverso sangue inietto (interazioni
References


Messick J.B., Walker P.G., Raphael W., Berent L., Shi X.: 'Candidatus mycoplasma haemodidelphidis' sp. nov., 'Candidatus mycoplasma haemolamae’ sp. nov. and Mycoplasma haemocanis


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