Original Article

jfms Journal of Feline Medicine and Surgery

Journal of Feline Medicine and Surgery
15(10) 879–885

DOI: 10.1177/1098612X13480985
jfms.com
SAGE

Prevalence and co-infection of haemotropic mycoplasmas in Portuguese cats by real-time polymerase chain reaction

Verónica L Martínez-Díaz[1], Ana Cristina Silvestre-Ferreira[2], Hugo Vilhena[3,4], Josep Pastor[5], Olga Francino[6] and Laura Altet[6]

Abstract

The diagnosis of feline haemoplasmosis has improved over the years, with several techniques enabling a clear and specific diagnosis, and where polymerase chain reaction (PCR) is considered as the 'gold standard'. The aim of this study was to survey the prevalence of feline haemoplasmas in 320 cats from the north-central region of Portugal by the use of real-time PCR, as well as to evaluate any associations between infection, clinical presentation and risk factors. The overall prevalence of infection by feline haemoplasmas was 43.43% (139/320), where 41.56% (133/320) corresponded to *Candidatus* Mycoplasma haemominutum (*CMhm*), 12.81% (41/320) to *Mycoplasma haemofelis* (*Mhf*), 4.38% (14/320) to *Candidatus* Mycoplasma haematoparvum and 1.25% (4/320) to *Candidatus* Mycoplasma turicensis. Almost 13% (47/320) of the samples were co-infected, with the most common co-infection being *CMhm* and *Mhf* (23.74%). Infection was found statistically significant with feline immunodeficiency/feline leukaemia virus status ($P = 0.034$), but no significant association was found for breed, sex, fertility status (neutered/spayed/entire), age, clinical status, living conditions (in/outdoor), anaemia status, or the presence/absence of ticks or fleas. Cats from north-central Portugal are infected with all the known feline haemoplasma species, with *CMhm* being the most common one. Prevalence of all feline haemoplasmas was higher than that reported previously in cats from other European countries, but similar to that described in Portugal for dogs. These data provide a better perspective regarding *Mycoplasma* species infection in Europe, and new information that helps us better understand feline haemoplasmosis.

Accepted: 1 February 2013

Introduction

Haemotropic haemoplasmas are Gram-negative epicellular parasites of erythrocytes that have been re-classified to the genus *Mycoplasma* owing to results from sequencing of the 16S rRNA genes.[1,2] Three different haemoplasma species have been identified in cats:[3–5] (i) *Mycoplasma haemofelis* (*Mhf*), known previously as *Haemobartonella felis* large variant; (ii) *Candidatus* Mycoplasma haemominutum (*CMhm*), known previously as *Haemobartonella felis*; and (iii) *Candidatus* Mycoplasma turicensis (*CMt*), discovered in Switzerland in 2005. In 2007, a fourth haemoplasma called *Candidatus* Mycoplasma haematoparvum-like was identified in cats.[6] In dogs, two different haemotropic mycoplasmas have been identified: *Mycoplasma haemocanis* (formerly *Haemobartonella canis)* and *Candidatus* Mycoplasma haematoparvum (*CMhp*).[7] Overall, phylogenetic studies

[1]Department of Animal and Food Science, Center for Research in Agricultural Genomics (CRAG), Autonomous University of Barcelona (UAB), Barcelona, Spain
[2]Department of Veterinary Sciences, University of Trás-os-Montes e Alto Douro, Vila Real, Portugal
[3]Department of Veterinary Medicine, Vasco da Gama University School Coimbra, Coimbra, Portugal
[4]Baixo Vouga Veterinary Hospital, Águeda, Portugal
[5]Department of Animal Medicine and Surgery, Autonomous University of Barcelona (UAB), Barcelona, Spain
[6]Molecular Genetics Veterinary Service (SVGM), Department of Animal and Food Science, Autonomous University of Barcelona (UAB), Barcelona, Spain

Corresponding author:
Verónica L Martínez-Díaz DVM MRes, Autonomous University of Barcelona (UAB) Campus, CRAG Building, Office 301, 08193 Bellaterra, Barcelona, Spain
Email: veronica.martinez@cragenomica.es

have shown that although *Mhf* and *M haemocanis* are distinct species, they are closely related, as well as to *CMhm* and *CMhp*.[8]

Feline haemoplasmosis can be diagnosed by cytology examination of stained blood smears, but cytological diagnosis has poor sensitivity and specificity owing to intrinsic limitations, such as stain precipitates, Howell–Jolly bodies and artifacts caused by slow blood smear drying. Moreover, blood smears can't differentiate between haemoplasma species, and, because of the cyclic nature of the parasitaemias, the absence of *Mhf* organisms in blood films does not rule out the diagnosis of haemotrophic mycoplasmosis.[2] Currently, polymerase chain reaction (PCR) techniques have been developed to diagnose *Mycoplasma* species infections, offering better reliability as they are more sensitive than cytology examination.[9] The use of real-time PCR (qPCR) has been extended as it is more sensitive than conventional PCR and it allows quantification of the amount of haemoplasma DNA present in a cat's blood, helping to determine the significance of the haemoplasma infection and to monitor the response to treatment. qPCR does not need post-PCR treatment therefore reducing the risk of sample contamination at the diagnostic laboratory. qPCR testing has been beneficial in testing clinically healthy cats being screened as blood donors or for entry into specific pathogen-free colonies.[10]

In Europe, prevalence studies carried out in Italy,[11] UK,[12,13] Germany,[14–16] Greece,[17] Switzerland[18,19] and Spain[20] based on PCR results report an overall prevalence of *Mycoplasma* species ranging from 7.2% to 30%. *CMhm* is the most common type of *Mycoplasma* species ranging from 9.6% to 66.6%, followed by *Mhf* and *CMt* with prevalences of infection of 1.3–33.3% and 0.3–2.1%, respectively. *CMt* infection is associated with co-infection with either, and occasionally both, of the two other feline haemoplasma species, particularly with CMhm,[8] inducing moderate-to-severe anaemia.[19] The nature of the cat's sampled for these studies included samples from healthy cats to cats suspected of having haemoplasmosis. They also belonged to different geographical locations — a variable that is believed to play an important role in the rates of infection as warmer climates have higher rates of prevalence.[18,20] To our knowledge, no previous studies on feline haemoplasmas have been carried out in Portugal. Therefore, the aims of this study were to survey the prevalence of feline mycoplasmas in 320 Portuguese cats by the use of qPCR, as well as to evaluate any associations between infection, clinical presentation and risk factors.

Materials and methods

Sample collection

Three hundred and twenty ethylenediamine tetra-acetic acid-anticoagulated blood samples were collected from both stray cats (rescued for animal shelters) and client-owned cats with indoor/outdoor access in Portugal. These samples were collected by veterinarians at the time of clinical presentation and frozen at –20°C until DNA extraction. Data obtained at the time of presentation included age, breed, gender, living condition, geographical region and clinical status (healthy vs unhealthy was determined based on the reason for presentation and physical examination). Data collected in a retrospective manner if available were feline leukaemia virus (FeLV)/feline immunodeficiency virus (FIV) status (determined by SNAP FIV/FeLV Combo Test) and the presence of ticks and/or fleas. Haematology was performed when requested by the attending veterinarian. Packed red cell volume (PCV) value was considered normal when values ranged from 25% to 45%, and anaemia was defined as PCV <25% according to the reference interval of our laboratory.

DNA isolation and qPCR amplification

DNA was obtained from 0.5 ml of peripheral whole blood as described previously.[21] Amplification of *Mycoplasma* species and the different species in positive samples (*Mhf, CMhm, CMt* and *CMhp*) were carried out in a final volume of 20 µl using FastStart Universal SYBR Green Master (Roche), 0.3 µM of each primer (with the exception of reverse primer of *CMt* at 0.5 µM) and 4 µl of diluted DNA. The thermal cycling profile was 50°C for 2 mins and 95°C for 10 mins followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. The PCR primers were designed with Primer Express 2.0 (Applied Biosystems) based on conserved regions of 16S ribosomal RNA gene for *Mycoplasma* species. The specificity of each PCR primer pair included (i) in silico analysis; (ii) sequencing the positive controls; and (iii) performing a cross-amplification study in which amplification with specific primers was performed over all the positives of other pathogens available in our laboratory (pathogens that could be in co-infection in cats, mostly vector borne diseases). Expected results were amplification of the specific *Mycoplasma*-positive sample and no amplification of any of the other pathogens tested. Validation of the PCR included the analysis of clinical samples suspected of being infected by the pathogen and the sequencing of any spurious or unspecific amplification that could occur.

Internal controls were added to all the samples analysed and all the PCRs performed. Negative controls include negative extraction control for the DNA extraction and a negative template control for the PCR. Positive qPCR controls were obtained from clinical samples that had been amplified previously and sequenced to confirm the pathogen. The sequences of the primers used and assay temperatures (dissociation curve analysis) are shown in Table 1.

Table 1 Primer sequences for *Mycoplasma* real-time PCR assays and species-specific melting temperatures

Primer	Pathogen	Sequence (5′–3′)	PCR, μM	Melting Temperature (°C)*
Mycoplasma species — F†	*Mycoplasma* species	GGAATCACTAGTAATCCYGTGTCAGCTATAT	0.3	75.1 ± 0.4
Mycoplasma species — R	*Mycoplasma* species	GGCGGTGTGTACAAGACCTGG	0.3	
Mycoplasma haemofelis — R	*M haemofelis*	CAAATRAATGTATTTTTAAATGCCCAC	0.3	77.1 ± 0.6
Candidatus Mycoplasma haemominutum — R	*Candidatus* M haemominutum	GTAATTAAATACGGTTTCAACTAGTACTTTCTCC	0.3	76.3 ± 1.0
Candidatus Mycoplasma haematoparvum — R	*Candidatus* M haematoparvum	AATTAAATACGGTTTCAACTAGTACGTTTCTTT	0.3	75.4 ± 0.9
Candidatus Mycoplasma turicensis — R	*Candidatus* M turicensis	TGAATRTGTTTTYAAATGCYCCTT	0.5	76.3 ± 0.3

*PCR performed with HT7900 (AB Life Technologies) and SYBRGreen (Roche)
†The same forward primer was used for all the *Mycoplasma* species PCR assays
F = forward; R = reverse

The eukaryotic 18S RNA Pre-Developed TaqMan Assay Reagents (Applied Byosystems) was used as internal reference for cat genomic DNA amplification to ensure proper qPCR amplification of each sample and that negative results corresponded to true negative samples rather than to a problem with DNA loading, sample degradation or PCR inhibition. The use of an endogenous control (18S RNA) that amplified between cycle threshold 19–21 in all samples corroborate that similar amounts of genomic DNA were used in all PCRs.

Statistical analysis

Difference between groups was tested for significance by χ^2 analysis (cell frequencies >5) or Fisher's exact test (cell frequencies ≤5). A *P*-value <0.05 was considered statistically significant. For the observed prevalence, the 95% confidence interval (CI) was calculated. Potential risk factors were evaluated by calculating the odds ratio with a 95% CI, and *P*-value <0.05 was considered statistically significant. All available data were analysed using MedCalc Software.[22]

Results

A total of 320 cats (175 males and 136 females), ranging in age from 0.5 to 20 years (median 4.23 years) from the north and north-central regions of Portugal were included in the study. Most samples came from the district of Aveiro (36.87%; 118/320). The other samples came from Oporto 14.37% (46/320), Coimbra 13.12% (42/320) and 35.62% from Braga, Bragança, Leiria, Viana do Castelo, Vila Real and Viseu (114/320).

Pedigree cats represented only 14.6% of the cohort, which included 26 Persian, 11 Siamese, three Norwegian Forest, two Sphynx, one Birman and one Chartreaux. The remaining 85.4% were domestic shorthair cats. Females represented 43.7% (136/311) of the population, whereas males represented 56.2% (175/311); 40.4% (55/136) of the females and 46.8% of the males were PCR-positive. Over half the population of cats had outdoor access [61.1% (189/320)] and 60.5% (184/320) were classified as sick when presented to the veterinary clinic, 44.5% (82/184) of whom were PCR-positive for feline haemoplasmas. Regarding antibiotic treatment, information was available for only 21 cats (6.25%). Antibiotics used in these 21 cats were amoxicillin, esperamicin, enrofloxacin, doxycycline and cefalexin, but only eight cats were receiving enrofloxacin or doxycycline as treatment for mycoplasmosis.

Table 2 summarises all the data for breed, sex, fertility status (neutered/spayed/entire), indoor/outdoor status, age, clinical status, PCV, presence/absence of ticks or fleas and FIV/FeLV status.

The overall sample prevalence for *Mycoplasma* species infection was 43.44% (139/320): 41.56% (133/320) were infected with *CMhm*; 12.81% (41/320) with *Mhf*; 4.38% (14/320) with *CMhp* and 1.25% (4/320) with *CMt*. Forty-two samples were co-infected with two types of *Mycoplasma* species, with the most common co-infection being *CMhm* + *Mhf*; four samples were co-infected with three types of *Mycoplasma* species and one sample presented positive results for all the *Mycoplasma* species tested. Overall co-infection sample prevalence was 14.68% (47/320). The results are summarised in Table 3.

Table 2 Sample characteristics. Data from animals that had no available information were not analysed

	Total number	% Total	% Positive	Positive	Odds ratio	95% CI	*P*
Breed							
Pedigree	44	14,06	43.18	19	0.94	0.49–1.79	0.86
Non-pedigree	269	85.94	44.61	120			
Gender							
♀	136	43.73	40.44	55			
♂	175	56.27	46.86	82	0.77	0.48–1.21	0.25
Spayed ♀	63	20.26	38.10	24			
Entire ♀	73	23.47	42.47	31	0.83	0.41–1.65	0.60
Neutered ♂	93	29.90	43.01	40			
Entire ♂	82	26.37	51.22	42	0.71	0.39–1.30	0.27
Living condition							
Indoor	120	38.83	40.00	48			
Outdoor	189	61.17	47.09	89	0.74	0.47–1.19	0.22
FeLV/FIV status							
Positive	26	24.53	61.54	16			
Negative	80	75.47	37.50	30	2.6	1.07–6.6	0.03
Age (years)							
<1	87	28.52	42.53	37	0.88	0.53–1.46	0.64
>1–3	84	27.54	41.67	35	0.84	0.51–1.4	0.52
4–9	97	31.80	47.42	46	1.18	0.72–1.91	0.49
>10	37	12.13	48.65	18	1.20	0.60–2.39	0.59
Clinical status							
Healthy	120	39.47	41.67	50			
Unhealthy	184	60.53	44.57	82	0.88	0.55–1.41	0.61
PCV							
Anaemia	35	59.32	57.14	22			
Normal	24	40.68	41.67	10	2.36	0.81–6.85	0.11
Ticks/fleas							
Yes	51	28.81	49.02	25			
No	126	71.19	44.44	56	1.20	0.62–2.30	0.58
Geographical region							
Interior	94	29.38	45.74	43			
Coastal	219	68.44	43.84	96	1.08	0.66–1.75	0.75

CI = confidence interval; FeLV = feline leukaemia virus; FIV = feline immunodeficiency virus; PCV = packed cell volume

The highest sample prevalence of infected cats was found in Viseu (73.33%, $P = 0.02$, odds ratio: 3.65, 95% CI = 1.13–11.73) (Table 4). FIV/FeLV status was statistically significant ($P = 0.03$, odds ratio: 2.6, 95% CI = 1.07–6.62) when comparing clinically sick cats (15/82) and healthy cats (1/50) with positive *Mycoplasma* species qPCR (Table 2). No significant *P*-value was found for breed, sex, fertility status (neutered/spay/entire), age, clinical status, presence/absence of ticks or fleas, living conditions or anaemia status in the overall population.

The majority of the samples that amplified *Mhf* were co-infected with *CMhm* alone (33/41), although multiple co-infections with additional species were also detected (3/41 *CMhm* + *Mhf* + *CMhp* and 1/41 *Mhf* + *CMhm* + *CMhp* + *CMt*). There was a statistical significance when comparing breeds (pedigree vs non-pedigree) with an odds ratio of 2.25 (95% CI 1.01–5.01) and a *P* value of 0.04 for cats infected with *Mhf*.

Only 14 samples were positive for *CMhp*. All the samples that amplified for this type of haemoplasma were co-infected (14/320): nine with *CMhm*, one with *CMt*+*CMhm* and three with *CMhm*+*Mhf*. Only four samples were positive for *CMt*. Two of these samples were co-infected, and 3/4 samples belonged to sick cats with outdoor access.

Discussion

This study documents the occurrence of *Mhf*, *CMhm*, *CMhp* and *CMt* in naturally-infected cats from the north-central area of Portugal. To our knowledge, no previous studies have reported on the prevalence of feline haemoplasmas in Portugal. Previous studies have only been

Table 3 Haemoplasma detection by real-time PCR

Pathogen	Number of infected cats	Prevalence	95% CI
Mycoplasma species	139/320	0.434	0.365–0.512
Mycoplasma haemofelis (*Mhf*)	4/320	0.028	0.007–0.07
Candidatus Mycoplasma Haemominutum (*CMhm*)	86/320	0.618	0.494–0.764
Candidatus Mycoplasma turicensis (*CMt*)	2/320	0.014	0.001–0.051
Candidatus Mycoplasma Haematoparvum (*CMhp*)	0/320	0	0–0.026
Dual *CMhm* + *CMhp*	9/320	0.064	0.029–0.122
Dual *CMhm* + *CMhp*	33/320	0.024	0.163–0.333
Triple *CMhm* + *Mhf* + *CMhp*	3/320	0.022	0.004-–0.063
Triple *CMt* + *CMhp* + *CMhm*	1/320	0.007	0.001–0.04
Quadruple *Mhf* + *CMhm* + *CMhp* + *CMt*	1/320	0.007	1E^{-4}0.0001–0.04

Table 4 Prevalence of *Mycoplasma* infection by geographical region

District n = 320	Total	Positive	Negative	Prevalence per district	95% CI prevalence per district	Prevalence of total population	95% CI for prevalence of total population
Aveiro	118	51	67	0.43	0.32–0.56	0.37	0.27–0.48
Braga	7	2	5	0.28	0.03–1.03	0.01	1 E^{-3}–0.05
Braganza	8	4	4	0.5	0.13–1.28	0.03	0.00–0.07
Coimbra	42	23	19	0.54	0.34–0.82	0.17	0.10–0.21
Leiria	2	1	1	0.5	0.01–2.78	0.01	0.00–0.04
Madeira	1	0	1	0	0.00–3.68	0	0.00–0.02
Oporto	46	16	30	0.31	0.19–0.56	0.11	0.06–0.18
Viana do Castelo	3	3	0	1	0.20–2.92	0.02	4 E^{-3}–0.06
Vilareal	71	28	43	0.39	0.26–0.57	0.20	0.13–0.29
Viseu	15	11	4	0.73	0.36–1.31	0.08	0.03–0.14
ND	7	2	5				

CI = confidence interval; ND = no data

conducted on canine samples, reporting a prevalence for haemoplasma infection of 40%[23] which is similar to that found in our study for the cat population. The prevalence rates observed in Portugal are higher than those of other European countries, such as Spain[20,24] Greece,[17] Italy,[11] the UK,[12,13,19] Switzerland[18] or Germany[14–16] which range from 7.2% to 30%. Differences in prevalence among countries could be explained by geographical variations, such as climate, vector distribution and the cat population per se. Moreover, direct comparisons of prevalence results are of limited value because of sample characteristics, inclusion criteria, diagnostic techniques, different statistical methods or a combination of them all, resulting in discrepancies between studies. Most of the cats in our study were cats with outdoor access, which means that they have a higher chance of *Mycoplasma* species infection as a result of their living style (more exposure to vectors like ticks and fleas which could transmit *Mycoplasma* species, higher chance of FIV/FeLV transmission, etc).

In our Portuguese samples *CMhm* was the most prevalent haemoplasma found, as has been described previously.[25,26] In fact, Reynolds and Lappin[27] suggest that *CMhm* may be a primary pathogen in some naturally-infected cats. Some studies indicate that *CMhm* infection is less pathogenic when compared with other feline mycoplasmas,[4,18] and some reports, such as one from Spain,[20] as well as our study, have failed to demonstrate an association between anaemia and *Mycoplasma* species infection. A limitation to our study was that a complete blood count was not performed for all 320 cats.

Mhf has been classified as the most pathogenic *Mycoplasma* species in cats.[9] Cats during the acute phase of infection with *Mhf* develop anaemia as a result of extravascular erythrophagocytosis by macrophages in the liver, spleen, lungs and bone marrow. In chronic phases of infection anaemia may not be present.[2] Conversely, various studies of naturally-infected cats, including ours, have failed to demonstrate an association between anaemia and *Mhf* infection.[12,20,25] Different

results might occur owing to different cat populations, the presence of different isolates of *Mhf* involved co-infections with other *Mycoplasma* species and the underlying health status of the cats. Most of the cats infected with *Mhf* in our study were co-infected with other types of *Mycoplasma* species, making it difficult to explain any association with the risks factors included. In our study, *Mhf* + *CMhm* co-infection was the most common co-infection, with a sample prevalence of 23.7% (95% CI 16.3–33.3%), although it was considered rare in previous articles with prevalences ranging from 0.2% to 4.9%.[11,12,15,16,18,28,29]

Regarding *CMhp* infection, we have detected, by means of a qPCR assay, molecular evidence of infection in 4.37% (14/320) of cats. All of the qPCR *CMhp*-positive cats were co-infected with other types of *Mycoplasma* species, with the most common co-infection being *CMhp* + *CMhm* (9/14) followed by triple co-infections with *CMhp* + *CMhm* + *Mhf* (3/14). These results are higher than the prevalence rate reported by Sykes et al,[6] who reported a 0.7% prevalence in anaemic and non-anaemic cats from the USA. Other studies performed in the USA[30] or Italy[11] have failed to find any evidence of *CMhp* infection in cats. Further prevalence studies should be performed in different countries to understand the pathogenicity of this species of *Mycoplasma*.

In the case of *CMt* infection, 2.8% (4/139) of cats were positive for *CMt*, a sample prevalence rate similar to that reported in UK[19] and greater than those observed in other European studies of 1.1%,[16] 0.8%,[13] 0.7%,[15] 0.5%,[20] 0.4%[5] and 0.3%.[11] It has been described that cats infected with *CMt* have significantly lower blood copy numbers than cats infected with *Mhf* or *CMhm* as these cats become qPCR-negative as early as 45 days after infection, while cats infected with *Mhf* or *CMhm* remain qPCR-positive even after 85 days after infection.[9] In fact, eight cats in our study were receiving or had received antibiotic (enrofloxacin, doxycycline) treatment before qPCR analysis, and five of these cats remained qPCR-positive for *CMhm*, indicating that long-term carrier status is common in comparison to infection with other *Mycoplasma* species, as reviewed by Tasker.[9]

There was no significant difference in overall haemoplasma sample prevalence between healthy and unhealthy cats, which could be explained by chronic infection detection rather than an acute infection, and the presence/absence of other diseases which may not have been reported. It has been reported that 10% of healthy cats can be infected with feline haemoplasma at any point in their lives.[9] These data should be kept in mind when interpreting qPCR results, as a positive result does not always correlate with clinical disease. A diagnosis of mycoplasmosis should be based on the patient's physical findings, biochemistry and haematological tests, as well as qPCR results.[31]

Results regarding the geographical region have to be interpreted with caution. Although microclimate variations within the same climate area could influence the epidemiology of haemoplasma infections,[11] it is not safe to assume that one district is more at risk of having a higher prevalence of a specific type of haemoplasma because not all districts had the same sample size (sample size was relatively low in most of the districts). Furthermore, indoor or outdoor habitat may function as a cofactor in different geographic areas.

Contradictory results have been reported on whether concomitant FIV and FeLV infections could predispose to haemoplasma infections, favouring the chronic carrier state or worsen the clinical course.[6,11,13,32,33] In our study, being FIV- or FeLV-positive was a risk factor for haemoplasma infection, which is in agreement with previous studies,[11] but results should be interpreted carefully as FIV/FeLV status was only determined in 33.12% of the cats in this study.

Conclusions

Cats from north-central Portugal are infected with all the known feline haemoplasma species, with *CMhm* being the most common one. The sample prevalence of all feline haemoplasmas was higher than that reported previously in cats from other European countries, but similar to that described in Portugal for dogs. These data provide a better perspective regarding *Mycoplasma* species infection in Europe and new information that helps us better understand feline haemoplasmosis.

Acknowledgements Thanks to the veterinarians, cat owners and veterinary clinics that willingly provided the samples for this research. Special thanks to Lisete Vieira who collaborated with the collection of blood samples.

Funding This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of interest The authors do not have any potential conflicts of interest to declare.

References

1 Rikihisa Y, Kawahara M, Wen B, Kociba G, Fuerst P, Kawamori F, et al. **Western immunoblot analysis of *Haemobartonella muris* and comparison of 16S rRNA gene sequences of *H muris*, *H felis*, and *Eperythrozoon suis***. *J Clin Microbiol* 1997; 35: 823–829.

2 Harvey JW. **Hemotrophic mycoplasmosis (hemobartonellosis)**. In: Green CE (ed). Infectious diseases of the dog and cat. Philadelphia: Saunders Elsevier, 2006, pp 252–260.

3 Flint JC and Moss LC. **Infectious anemia in cats**. *J Am Vet Med Assoc* 1953; 122: 45–48.

4 Foley JE and Pedersen NC. **'*Candidatus* Mycoplasma haemominutum', a low-virulence epierythrocytic parasite of cats**. *Int J Syst Evol Microbiol* 2001; 51: 815–817.

5 Willi B, Boretti FS, Cattori V, Tasker S, Meli ML, Reusch C, et al. **Identification, molecular characterization, and experimental transmission of a new hemoplasma isolate from a cat with hemolytic anemia in Switzerland**. *J Clin Microbiol* 2005; 43: 2581–2585.

6 Sykes JE, Drazenovich NL, Ball LM and Leutenegger CM. **Use of conventional and real-time polymerase chain reaction to determine the epidemiology of hemoplasma infections in anemic and nonanemic cats**. *J Vet Intern Med* 2007; 21: 685–693.

7 Sykes JE, Ball LM, Bailiff NL and Fry MM. **'*Candidatus* Mycoplasma haematoparvum', a novel small haemotropic mycoplasma from a dog**. *Int J Syst Evol Microbiol* 2005; 55: 27–30.

8 Tasker S. **Canine and feline hemotropic mycoplasmosis**. In: Bonagura JD and Twedt D (eds). Kirk's current veterinary therapy XIV. London: Elsevier, 2008, pp 1245–1248.

9 Tasker S. **Haemotropic mycoplasmas: what's their real significance in cats?** *J Feline Med Surg* 2010; 12: 369–381.

10 Tabar MD, Altet L, Francino O, Sanchez A, Ferrer L and Roura X. **Vector-borne infections in cats: molecular study in Barcelona area (Spain)**. *Vet Parasitol* 2008; 151: 332–336.

11 Gentilini F, Novacco M, Turba ME, Willi B, Bacci ML and Hofmann-Lehmann R. **Use of combined conventional and real-time PCR to determine the epidemiology of feline haemoplasma infections in northern Italy**. *J Feline Med Surg* 2009; 11: 277–285.

12 Tasker S, Binns SH, Day MJ, Gruffydd-Jones TJ, Harbour DA, Helps CR, et al. **Use of a PCR assay to assess the prevalence and risk factors for *Mycoplasma haemofelis* and '*Candidatus* Mycoplasma haemominutum' in cats in the United Kingdom**. *Vet Rec* 2003; 152: 193–198.

13 Peters IR, Helps CR, Willi B, Hofmann-Lehmann R and Tasker S. **The prevalence of three species of feline haemoplasmas in samples submitted to a diagnostics service as determined by three novel real-time duplex PCR assays**. *Vet Microbiol* 2008; 126: 142–150.

14 Bauer N, Balzer HJ, Thure S and Moritz A. **Prevalence of feline haemotropic mycoplasmas in convenience samples of cats in Germany**. *J Feline Med Surg* 2008; 10: 252–258.

15 Laberke S, Just F, Pfister K and Hartmann K. **Prevalence of feline haemoplasma infection in cats in Southern Bavaria, Germany, and infection risk factor analysis**. *Berl Munch Tierarztl Wochenschr* 2010; 123: 42–48.

16 Morgenthal D, Hamel D, Arndt G, Silaghi C, Pfister K, Kempf VA, et al. **Prevalence of haemotropic *Mycoplasma* spp., *Bartonella* spp and *Anaplasma phagocytophilum* in cats in Berlin/Brandenburg (Northeast Germany)**. *Berl Munch Tierarztl Wochenschr* 2012; 125: 418–427.

17 Maher IE, Tasker S, Polizopoulou Z, Dasopoulou A, Egan K, Helps CR, et al. **Polymerase chain reaction survey of feline haemoplasma infections in Greece**. *J Feline Med Surg* 2010; 12: 601–605.

18 Willi B, Boretti FS, Baumgartner C, Tasker S, Wenger B, Cattori V, et al. **Prevalence, risk factor analysis, and follow-up of infections caused by three feline hemoplasma species in cats in Switzerland**. *J Clin Microbiol* 2006; 44: 961–969.

19 Willi B, Tasker S, Boretti FS, Doherr MG, Cattori V, Meli ML, et al. **Phylogenetic analysis of 'Candidatus Mycoplasma turicensis' isolates from pet cats in the United Kingdom, Australia, and South Africa, with analysis of risk factors for infection**. *J Clin Microbiol* 2006; 44: 4430–4435.

20 Roura X, Peters IR, Altet L, Tabar MD, Barker EN, Planellas M, et al. **Prevalence of hemotropic mycoplasmas in healthy and unhealthy cats and dogs in Spain**. *J Vet Diagn Invest* 2010; 22: 270–274.

21 Francino O, Altet L, Sanchez-Robert E, Rodriguez A, Solano-Gallego L, Alberola J, et al. **Advantages of real-time PCR assay for diagnosis and monitoring of canine leishmaniosis**. *Vet Parasitol* 2006; 137: 214–221.

22 Stephan C, Wesseling S, Schink T and Jung K. **Comparison of eight computer programs for receiver-operating characteristic analysis**. *Clin Chem* 2003; 49: 433–439.

23 Novacco M, Meli ML, Gentilini F, Marsilio F, Ceci C, Pennisi MG, et al. **Prevalence and geographical distribution of canine hemotropic mycoplasma infections in Mediterranean countries and analysis of risk factors for infection**. *Vet Microbiol* 2010; 142: 276–284.

24 Criado-Fornelio A, Martinez-Marcos A, Buling-Sarana A and Barba-Carretero JC. **Presence of *Mycoplasma haemofelis, Mycoplasma haemominutum* and piroplasmids in cats from southern Europe: a molecular study**. *Vet Microbiol* 2003; 93: 307–317.

25 Tasker S, Helps CR, Day MJ, Gruffydd-Jones TJ and Harbour DA. **Use of real-time PCR to detect and quantify *Mycoplasma haemofelis* and '*Candidatus* Mycoplasma haemominutum' DNA**. *J Clin Microbiol* 2003; 41: 439–441.

26 Jensen WA, Lappin MR, Kamkar S and Reagan WJ. **Use of a polymerase chain reaction assay to detect and differentiate two strains of *Haemobartonella felis* in naturally infected cats**. *Am J Vet Res* 2001; 62: 604–608.

27 Reynolds CA and Lappin MR. **"*Candidatus* Mycoplasma haemominutum" infections in 21 client-owned cats**. *J Am Anim Hosp Assoc* 2007; 43: 249–257.

28 Kewish KE, Appleyard GD, Myers SL, Kidney BA and Jackson ML. ***Mycoplasma haemofelis* and *Mycoplasma haemominutum* detection by polymerase chain reaction in cats from Saskatchewan and Alberta**. *Can Vet J* 2004; 45: 749–752.

29 Sykes JE, Drazenovich NL, Kyles AE, Ball LM and Leutenegger CM. **Detection of mixed infections with '*Candidatus* Mycoplasma haemominutum' and *Mycoplasma haemofelis* using real-time TaqMan polymerase chain reaction**. *J Vet Diagn Invest* 2007; 19: 250–255.

30 Sykes JE, Terry JC, Lindsay LL and Owens SD. **Prevalences of various hemoplasma species among cats in the United States with possible hemoplasmosis**. *J Am Vet Med Assoc* 2008; 232: 372–379.

31 Tasker S and Lappin MR. ***Haemobartonella felis*: recent developments in diagnosis and treatment**. *J Feline Med Surg* 2002; 4: 3–11.

32 Bobade PA, Nash AS and Rogerson P. **Feline haemobartonellosis: clinical, haematological and pathological studies in natural infections and the relationship to infection with feline leukaemia virus**. *Vet Rec* 1988; 122: 32–36.

33 Tasker S, Caney SM, Day MJ, Dean RS, Helps CR, Knowles TG, et al. **Effect of chronic FIV infection, and efficacy of marbofloxacin treatment, on *Mycoplasma haemofelis* infection**. *Vet Microbiol* 2006; 117: 169–179.