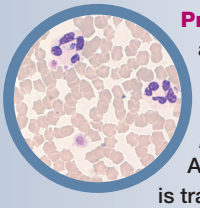
Journal of Feline Medicine and Surgery (2020) **22**, 428–441

CLINICAL REVIEW

ANAPLASMA PHAGOCYTOPHILUM INFECTION IN CATS
A literature review to raise clinical awareness

Ingo Schäfer and Barbara Kohn



Practical relevance: Granulocytic anaplasmosis is a disease in humans and animals caused by the Gram-negative bacterium *Anaplasma phagocytophilum* within the family Anaplasmataceae. The pathogen is transmitted by ticks of the *Ixodes* species. Infections with *A phagocytophilum* have often been described in dogs but reports on natural infections in cats are rare. An infection with *A phagocytophilum* should be considered as a differential diagnosis in cats if the history reveals tick infestation and/or outdoor access in combination with the relevant clinical signs.

Global importance: *A phagocytophilum* is also important in human medicine because of its zoonotic potential. Due to the risk of vector-borne infections for both feline and public health, cats should be protected with ectoparasiticides, especially in endemic areas.

Aim: The aim of this review is to give an overview of the published data and summarise the epidemiology, pathogenesis, diagnosis, clinical signs and therapy of feline granulocytic anaplasmosis. As clinical signs are vague and non-specific, this review aims to raise awareness of *A phagocytophilum* infection, both among clinicians, so that they consider testing potentially exposed cats, and scientists, in order to prompt further research.

Evidence base: Sixteen publications describing 55 cats have been reviewed. Thirty-four cats were well diagnosed based on guidelines of the European Advisory Board on Cat Diseases and blood analyses were performed to varying extents for these cats. Because of the limited number of studies and a lack of knowledge in cats, clinical signs and blood analyses are compared with available data in dogs.

Keywords: *Anaplasma phagocytophilum*; vector-borne; anaplasmosis; granulocytic anaplasmosis; zoonosis

Introduction

Anaplasma phagocytophilum is a Gram-negative, obligate intracellular bacterium within the family Anaplasmataceae.[1] The pathogen was formerly variously known as *Ehrlichia equi*, *Ehrlichia phagocytophila* and human granulocytic ehrlichiosis (HGE) agent, thus making literature reviews challenging.[2] *A phagocytophilum* causes granulocytic anaplasmosis in humans and animals[3] and is transmitted by ticks of the *Ixodes* species within 24–48 h of tick attachment.[4] Rodents and wild ruminants are the most common reservoirs.[3]

Anaplasma phagocytophilum causes granulocytic anaplasmosis in humans and animals, and is transmitted by ticks from the _Ixodes_ genus within 24–48 h of tick attachment.

While infections with *A phagocytophilum* occur commonly in dogs, the literature only rarely describes natural infections in cats. Case reports of *A phagocytophilum* infection in cats, based on detection by PCR, have been published in Germany,[5–8] Austria,[9] Poland,[10,11] Switzerland,[12] Italy,[13] the UK,[14] Finland,[15] Sweden[16] and the USA.[17,18]

Epidemiology

Infections with *A phagocytophilum* have been described in humans and a number of animal species including cats. The first case report of an infected cat was published in Sweden in 1999.[16] Prior to this, the pathogen had already been described via microscopic detection of morulae in sheep in Scotland in 1932 (cited by Woldehiwet and Scott[19] and Foggie[20]), in cattle in the UK in 1950,[21] as well as in other domestic ruminants such as goats[22] and deer,[23] in horses in the USA in 1968,[24] in dogs in the USA in 1982[25] and in humans in the USA via PCR in 1994.[26]

Ingo Schäfer
Dr med vet, MSc*
Barbara Kohn
Prof Dr med vet, DECVIM-CA
Faculty of Veterinary Medicine,
Clinic for Small Animals,
Freie Universität Berlin,
Oertzenweg 19b, Building 1,
14163 Berlin, Germany

*Corresponding author:
ingo.schaefer@fu-berlin.de

DOI: 10.1177/1098612X20917600

The species of tick that transmits *A phagocytophilum* varies geographically.

A phagocytophilum is transmitted by ticks, with the species of tick varying based on geography. In the USA, *Ixodes pacificus* (West) and *Ixodes scapularis* (Midwest and Northeast) have been described as vectors.[27,28] In Europe, *Ixodes ricinus* is the most important vector,[3] followed by *Ixodes trianguliceps*, *Ixodes hexagonus* and *Ixodes ventalloi*.[29–31] In Asia and Russia, *Ixodes persulcatus* and *Dermacentor silvarum* are the most common vectors.[27,28,32]

In humans, rare infections without vector contact have been diagnosed; for example, nosocomial infections in China,[33] infections transmitted via blood transfusion[34] and transplacental infections.[35] Transplacental infections have also been described in cows[36] but not in cats. The natural and experimental transmission of *A phagocytophilum* via blood/blood transfusion has been described in dogs,[37,38] as well as in cats (MR Lappin, unpublished data). In a study performed in Berlin, Germany, 5/42 clinically healthy blood donor cats were serologically positive for *A phagocytophilum*; direct pathogen detection via PCR was negative in all cats.[6] Consensus guidelines from the American College of Veterinary Internal Medicine (ACVIM),[39] as well as the European Advisory Board on Cat Diseases (ABCD),[40,41] recommend methods of detection for *A phagocytophilum* in blood donor cats (see later).

Screening for *A phagocytophilum* in blood donor cats is recommended using both direct and indirect methods.

In European cats, antibody prevalences for *A phagocytophilum* have been described, ranging from 0% to 33.3%, and in the USA prevalences from 4.3% to 37.6% have been reported (Table 1). Direct pathogen detection via PCR or within a blood smear was positive in 0–23.1% of cats in Europe, and in 0–6.9% of cats in the USA (Table 1). Direct methods of detection of *A phagocytophilum* in European dogs showed a prevalence of 0–21.7% in different countries;[72–74] via indirect detection methods, 2.7–56.5% of dogs tested positive.[72,74] In the USA, prevalences in dogs ranged from 3% to 37% using direct detection methods and 0% to 55.4% via antibody testing.[72,74]

These wide ranges in prevalence of *A phagocytophilum* infection in dogs and cats could be explained by the large geographical areas studied, with their varying climates and environments, tick populations and reservoir host populations. The different study populations also have an impact on the prevalence rates (Table 1). Stray cats and dogs will have received little or no veterinary care and prophylactic measures against vector-borne infections will not have been implemented. Living outdoors all the time, they also have an increased risk of vector contact and infection. Some studies have been performed in areas not endemic for *Ixodes* species and, as expected, these studies showed a dramatically lower prevalence compared with areas in which *Ixodes* species are endemic. Cats with and without outdoor access have also been studied (Table 1). Compared with cats with outdoor access (and also with dogs), cats living only indoors are less likely to have vector contact. Moreover, the intensive grooming behavior of cats might lead to the removal of ticks before the transmission of pathogens.[4] Furthermore, cats may show lower numbers of *A phagocytophilum* in circulating neutrophilic granulocytes in comparison with dogs, potentially leading to false-negative PCR results.[5,17]

Pathogenesis

Based on studies in humans and dogs, *A phagocytophilum* is known to be spread by *Ixodes* species ticks via transstadial transmission. For the pathogen to be transmitted, it is assumed that the vector has to be in direct contact with the host for 24–48 h.[4,75] The pathogen then spreads via blood and lymphatic circulation,[76] and the incubation time ranges from 1 to 2 weeks.[37] Neutrophilic granulocytes are infected via endocytosis following P-selectin-mediated adhesion.[77–80] After the pathogen has penetrated the cell membrane of the phagosomes, it proliferates by forming morulae.[81,82] The pathogen inhibits some of the vital functions of the neutrophilic granulocytes, such as neutrophilic motility, phagocytosis, release of reactive oxygen radicals (oxidative burst) and interaction of neutrophilic granulocytes with endothelial cells, in order to survive and ensure its own proliferation.[80,83–85] The breakdown of the phagosomes and the host's cell membrane releases the pathogen and leads to infection of further cells and organs.[76] The bacterium is able to prevent its recognition by the immune system by activating certain pathogenic mechanisms[86] and delaying apoptosis.[87]

There is little information on the specific pathogenesis of *A phagocytophilum* infection in cats. In an experimental study with six cats, mild clinical signs (transient fever) were triggered by intraperitoneal administration of infected blood. Blood tests showed a slight decrease in leukocytes (neutrophilic granulocytes and lymphocytes), significant reduction of mean cell volume and elevated liver enzymes (alanine aminotransferase and aspartate trans-

Cats may show a lower number of *A phagocytophilum* in circulating neutrophilic granulocytes in comparison with dogs, potentially leading to false-negative PCR results.

Table 1 Prevalence of feline *Anaplasma phagocytophilum* infections in selected studies

	Country	Region	n	Study population	Detection method	Prevalence	Period	Reference
Europe	Germany	North East Germany (Berlin/Brandenburg)	265	150/265 indoor, 99/265 outdoor access, 16/265 stray cats 49/265 clinically healthy, 216/265 with clinical signs (96/265 with ectoparasitic treatment), 72/265 with tick infestation	IFAT PCR	24/265 (9.1%) 1/265 (0.4%)	11/2007–11/2008	6
		Bavaria, Lower Saxony	326	238/326 domestic cats, 58/326 animal shelter cats, 20/326 laboratory cats, 10/326 suspected *A phagocytophilum* infection 24 domestic cats, 6 animal shelter cats and 2 cats with suspected infection tested positive	IFAT	53/326 (16.3%)	05/2006–08/2008	7
			306	238/306 domestic cats, 58/306 stray cats (clinically healthy), 10/306 suspected *A phagocytophilum* infection; 1 cat, with a history of travel to Denmark 6 months earlier, tested positive	PCR	1/306 (0.3%)	05/2006–08/2008	
		Southern Germany	479	Domestic cats	PCR	2/479 (0.4%)	–	5
	UK	England	60	Domestic cats with clinical signs indicative of suspected vector-borne infections	PCR	1/60 (1.7%)	08/2001–10/2001	14
	Ireland	Dublin and surrounding area	116	75/116 stray cats, 41/116 domestic cats	PCR	0/116	01/2008–05/2008	42
	Sweden	Central Sweden	90	Domestic cats presented to a veterinary clinic*	IFAT	19/90 (21.1%)	2010–2011	43
	Greece	Crete, Mykonos, Skopelos, Athens	148	Cats with outdoor access	PCR	0/148	Summer 2015	44
		Northern and central Greece	100	Domestic cats: 40/100 indoor, 60/100 with outdoor access 50/100 clinically healthy, 50/100 with clinical signs	IFAT PCR	0/100 0/100	–	45
	Italy	Northern and central Italy	250	Cats with outdoor access	Morulae	15/250 (6%)	1997–2000	46
		Central Italy	560	Clinically healthy cats: 176/560 animal shelter cats, 384/560 domestic cats 7/176 animal shelter cats and 18/384 domestic cats tested positive (21 cats with tick attachment in the previous 3–6 months)	IFAT	25/560 (4.5%)	01/2005–12/2011	47
		Northern Italy (Milan)	260	Stray cats	PCR	60/260 (23.1%)	01/2008–01/2010	48
		Southern Italy	42	Domestic cats with outdoor access and ectoparasites; 20/42 clinically healthy, 22/42 with clinical signs	IFAT PCR	14/42 (33.3%) 0/42	03/2012–01/2013	49
		Southern Italy	197	134/197 cats with outdoor access (29.1% serologically positive), 63/197 indoor cats with ectoparasitic treatment (22.1% serologically positive); 10/197 clinically healthy, 187/197 with clinical signs	IFAT PCR	53/197 (26.9%) 0/197	03/2012–03/2013	50
	Portugal	Lisbon and Évora	37	22/37 domestic cats, 13/37 animal shelter cats, 2/37 stray cats (3 animal shelter cats and 2 domestic cats tested serologically positive)	IFAT PCR	5/37 (13.5%) 0/37	08/2007–04/2008	51
		Northern and central Portugal	320	Domestic cats: 192/320 outdoor access, 124/320 indoor, 4/320 unknown 2/192 cats with outdoor access clinically healthy and tested positive	PCR†	2/320 (0.6%)	–	52
		Southern Portugal	649	329/649 stray cats, 320/649 domestic cats 35/649 tested positive (26/35 stray cats, 9/35 domestic cats; 8/35 clinically healthy, 3/35 with suspected infection)	PCR†	35/649 (5.4%)	01/2012–08/2013	53

Table 1 Prevalence of feline *Anaplasma phagocytophilum* infections in selected studies (continued)

	Country	Region	n	Study population	Detection method	Prevalence	Period	Reference
Europe (continued)	Spain	Northeastern regions	168	Domestic cats: 70/168 clinically healthy, 60/168 with clinical signs 47/168 additionally examined via PCR (27/47 clinically healthy, 20/47 with clinical signs)	IFAT PCR	3/168 (1.8%) 0/47	–	54
		Barcelona and surrounding area	100	48/100 clinically healthy, 52/100 with clinical signs (1/52 tested positive)	PCR†	1/100 (1%)	01/2006–12/2006	55
		Madrid and surrounding area	52	Domestic cats; 28/52 clinically healthy, 24/52 with clinical signs 1/4 cats serologically positive with clinical signs	IFAT PCR + culture	4/52 (7.7%) 0/52	10/2005–06/2007	56
		Madrid and surrounding area	680	539/680 domestic cats, 141/680 stray cats 247/501 with outdoor access, 34/440 with tick infestation, 117/420 with ectoparasitic treatment	IFAT PCR	57/680 (8.4%) 0/680	09/2005–08/2008	57
		Catalonia	116	Animal shelter cats with outdoor access	IFAT PCR	0/116 0/116	09/2012–11/2012	58
USA	USA	Florida	484	Clinically healthy stray cats	PCR	0/484	06/1999–02/2000	59
		Northeastern regions	93	Domestic cats with outdoor access (84/93 clinically healthy, 9/93 with clinical signs)	IFAT ELISA	28/93 (30.1%) 35/93 (37.6 %)	1985–1989	60
		Arizona	112	57/112 animal shelter cats, 50/112 stray cats, 5/112 cats living in a veterinary clinic	PCR‡	0/112	03/2004–07/2004	61
		USA	146	Clinically healthy cats presented for blood donation	PCR	0/146	–	62
		Alabama, Maryland, Texas	92	54/92 humane shelter cats, 38/92 domestic cats	PCR‡	0/92	–	63
		Florida, California, Michigan	460	373/460 stray cats, 65/460 animal shelter cats, 22/460 domestic cats Examination via PCR in 158/460 cats with IFAT ⩾1:50	IFAT PCR	20/460 (4.3%) 0/158	–	64
		Colorado	133	Cats with anaemia of unknown origin	PCR	0/133	01/2001–11/2004	65
		Maine	159	42/159 cats with clinical signs, 117/159 clinically healthy	SNAP§	10/159 (6.3%)	–	66
		California, Illinois, Massachusetts	5416	Domestic cats	Specific peptide immunoassay	9.7%	09/2014–02/2015	67
		Northeastern regions	4334	4334 blood samples of cats sent to a commercial laboratory	PCR∞	40/4334 (0.92%)	05/2009–05/2011	18
		Maryland	25	70 clinically healthy domestic cats (SNAP results from 25/70 cats)	SNAP§	1/25 (4%)	04/2011–04/2014	68
		Massachusetts	175	175 clinically healthy stray cats (in 2/175 cats no examination via PCR possible)	SNAP§ PCR	17/175 (9.7%) 12/173 (6.9%)	06/2015–12/2015	69
Other countries	USA, Canada, Caribbean	USA, Canada, Caribbean	858	827/858 cats from the USA, 28/858 cats from Canada, 3/858 cats from the Caribbean Examination via SNAP test in 715/858 cats, via PCR in 406/858 cats	SNAP# PCR†	13/715 (1.8%) 13/406 (3.2%)	2008–2013	70
	Korea	Seoul	222	Animal shelter cats	PCR	2/222 (0.9%)	–	71

IFAT = immunofluorescence antibody test; morulae = detection of inclusion bodies in blood smears
*No history/anamnesis available
†*Anaplasma* species/*Ehrlichia* species PCR without species differentiation
‡Multiplex PCR with species differentiation (*Ehrlichia* species, *Anaplasma phagocytophilum*, *Neorickettsia risticii*, *Mycoplasma haemofelis*, '*Candidatus* Mycoplasma haemominutum')
§SNAP 4Dx Plus Assay (IDEXX)
¶Coinfection with *Borrelia burgdorferi*
∞Multiplex PCR with species differentiation (*Anaplasma phagocytophilum*, *Bartonella henselae*, *Bartonella clarridgeiae*, *Bartonella quintana*, *Ehrlichia* species, *Mycoplasma haemofelis*, '*Candidatus* Mycoplasma haemominutum', '*Candidatus* Mycoplasma turicensis', *Rickettsia rickettsii* and *Rickettsia felis*)
#SNAP Multi-Analyte Test (detection of antibodies against *Anaplasma* species, *Borrelia* species, *Ehrlichia* species); in the case of enough sample material, specific IFAT and SNAP 4Dx Plus Assay (IDEXX)

Infections with *A phagocytophilum* most often produce an acute disease. There are also reports of asymptomatic infections in cats.

aminase).[88] Mild to severe thrombocytopenia is a common – and the most diagnostically relevant – laboratory finding in *A phagocytophilum* infections in both cats and dogs (Table 2).[90,91] Mechanisms of induced thrombocytopenia could include reduced production of platelets, increased consumption due to disseminated intravascular coagulopathy, shortened platelet lifespan due to immune-mediated destruction or sequestration of platelets in an enlarged spleen.[90] In humans and dogs, antiplatelet antibodies have been detected, indicating that immune-mediated factors may also play an important role.[90,92] Antinuclear antibodies, as well as an elevated release of interferon gamma-messenger ribonucleic acid, has been noted in cats,[88] which could indicate an immunological pathogenesis, eventually leading to the development of clinical signs.[3]

In a study from Colorado, USA, wild-caught *I scapularis* ticks were transferred onto four cats, resulting in a subclinical coinfection with *A phagocytophilum* (detection via PCR and antibody ELISA) and *Borrelia burgdorferi* (detection via antibody ELISA).[93] The ticks were collected in a region in which previous examinations had detected *A phagocytophilum* DNA in 15% and *B burgdorferi* DNA in 50% of ticks. The cats showed transient lymphopenia postinfection. In the following 13 weeks, no changes in general condition, appetite, body weight or blood cell count, besides lymphopenia, were detected. The failure of the cats to develop clinical anaplasmosis may have been related to the immune status of the cats, the dose of the organism or the strain of *A phagocytophilum*.[93]

Infections with *A phagocytophilum* most often produce an acute disease (Table 2). To date, there are only a few reports in the literature supporting persistent infections in dogs, sheep and horses.[94–97] In cats, there are two cases reported where persistent infection was documented, with one cat still PCR positive on day 120 after the initiation of treatment and the other cat being PCR positive until day 37 and negative on day 139 after the initiation of treatment.[17] There are reports of asymptomatic infections with *A phagocytophilum* in cats.[93,98] Subclinical and self-limiting infections have been described after natural exposure in dogs,[99,100] and have also been experimentally confirmed in studies with sheep and horses.[101,102] PCR-positive dogs may also be clinically healthy.[99] There is widespread serological detection of the pathogen in naturally infected dogs without the development of clinical signs, especially in endemic areas.[99,100,103–105]

Clinical signs often occur shortly after tick contact and rapidly improve with antimicrobial therapy.

Clinical and laboratory findings

Thirty of 34 cats previously reported in the literature to have *A phagocytophilum* infection (see box below) had outdoor access. Twelve of the 34 cats (35%) were infested with ticks; of those cats, outdoor access was available in nine and unknown in three. Clinical signs were described in 33/34 cats; the remaining cat (3%) was clinically asymptomatic. Cats mostly showed non-specific clinical signs such as lethargy (31/33 cats, 94%), increased rectal temperature ranging from 39.1°C to 41.5°C (29/33 cats, 88%), anorexia or reduced appetite (25/33, 76%), conjunctivitis (12/33, 36%) and dehydration (5/33, 15%). Ten of 33 cats (30%) had a painful abdomen or painful limbs. Further clinical signs included pale mucous membranes (3/33, 9%), respiratory signs (3/33, 9%) and tachycardia (3/33, 9%). Neurological signs (2/33, 6%), weight loss (2/66, 6%) and dental calculus (2/33, 6%), with or without gingivitis, were also described in a few cats. Rare clinical signs included recurrent epistaxis, polyuria and polydipsia, and hypothermia, as well as abnormal lung sounds on auscultation.

Clinical signs often occur shortly after tick contact and rapidly improve with antimicrobial therapy.[40] For example, in the study by Adaszek et al, the owners of three cats reported the development of clinical signs 3–7 days after vector contact.[10]

In a recent study by Chirek et al of 63 dogs in Germany with granulocytic anaplasmosis, lethargy was listed as the most common clinical sign, with 83% of dogs affected, followed by fever (67%) and inappetence (63%);[91] these rates are comparable to those in cats (Table 2). Haemorrhage was reported in 13% of dogs, but has been rarely described in cats.

Publications reporting *A phagocytophilum* infections in cats

To the authors' knowledge, there are 16 publications describing infections with *A phagocytophilum* in 55 cats (Table 2). Eighteen of these 55 cats, from two study populations,[46,89] were diagnosed based on the detection of morulae in neutrophilic granulocytes. Those cats were not included in the analysis in the 'Clinical and laboratory findings' section of this article, because PCR analysis had not been performed in order to confirm *A phagocytophilum* infection. One cat from the UK,[14] as well as one cat from Italy,[13] were also not included in the analysis due to an inadequate description of the clinical signs and laboratory findings. In one cat, a urethral obstruction was the cause for presentation to the clinic;[6] this cat was also not considered for further analysis because the laboratory changes were most likely caused by the underlying urinary disease and not by infection with *A phagocytophilum*. This left 34 cats included in the analysis that had been diagnosed with *A phagocytophilum* infection based on the guidelines of the ABCD.[40]

Table 2 Case reports of feline *Anaplasma phagocytophilum* infections (n = 55, 1989–2019)

	Country	n	Signalment	History	Clinical signs	Laboratory results	Diagnostic methods	Therapy	Outcome	Reference
Europe	Sweden	1	ESH, 1 year, MN	Lethargy, anorexia, tachypnoea	Depression, fever (41.3°C), dehydration, tick infestation	Neutrophilia, lymphopenia	Morulae*, PCR positive	Doxycycline	Very good	16
	UK	1	Unknown	Acute pyrexia, weakness, lethargy	Fever	Unknown	PCR positive	Unknown	Unknown	14
	Italy	15	Unknown	Anorexia/poor appetite (13/15); weight loss (5/15); vomiting (4/15); incoordination (3/15); tick infestation, haematuria, polydipsia, dyspnoea, hiding (2/15 each)	Pain (8/15); lethargy, lymphadenomegaly, poor coat condition (6/15 each); gingivitis, periodontitis, conjunctivitis (5/15 each); fever (3/15); pharyngitis, dehydration, pale mucous membranes (2/15 each)	Thrombocytopenia in most cats†, monoclonal gammopathy (2/15)	Morulae*	Doxycycline	Very good	46‡
	Austria	2	ESH, 3 years, FN	Tick infestation, lethargy, poor appetite, incoordination	Dehydration, conjunctivitis, fever (40.4°C)	Thrombocytopenia†, elevated lactate dehydrogenase, lymphocytosis	Morulae*, PCR positive, IFAT positive	Doxycycline	Very good	9
			ELH, 4 years, MN	Tick attachment, anorexia, lethargy, pain	Fever (40.3°C), dehydration, conjunctivitis, serous nasal secretion, tachypnoea	Anaemia, thrombocytopenia†, eosinophilia	No morulae*, PCR negative, IFAT positive	Doxycycline	Very good	
	Italy	1	Unknown	Unknown	Clinical signs indicative of vector-borne infections§	Unknown	PCR positive, IFAT positive	Unknown	Unknown	13‡
	Switzerland	1	ESH, 14 years, MN	Lethargy, anorexia	Fever (40.1°C), minor dehydration, gingivitis	Thrombocytopenia, leukocytosis, hypoalbuminaemia, hypokalaemia, low iron	Morulae*, PCR positive, IFAT positive	Doxycycline	Very good	12
	Finland	1	Maine Coon, 3.5 years, FN	Tick infestation, reduced appetite, hiding, lethargy, ocular discharge	Fever (39.5°C), tachypnoea, painful cranial abdomen, bilateral increased lung sounds	Lymphopenia, hyperglycaemia	Morulae*, PCR positive, IFAT positive	Doxycycline	Very good	15
	Poland	3	ESH, 2.5 years, M	Loss of appetite and thirst, lethargy	Pale mucous membranes, fever (39.8°C), pain	Anaemia, thrombocytopenia†, leukopenia	Morulae*, PCR positive	Doxycycline	Very good	10
			ESH, 3 years, M	Tick infestation, pain, reduced appetite	Lethargy, pale mucous membranes	Anaemia, thrombocytopenia†, leukopenia	Morulae*, PCR positive	Doxycycline	Very good	
			ESH, 6 years, F	Tick infestation, lethargy, reduced thirst and appetite	Fever (39.6°C)	Anaemia, thrombocytopenia†, leukopenia	No morulae*, PCR positive	Doxycycline	Very good	
	Poland	1	ESH, 2.5 years, M	Loss of appetite, lethargy, tick attachment	Pale yellow mucous membranes, pain	Anaemia, thrombocytopenia†, elevated liver enzymes	PCR positive, IFAT positive	Doxycycline	Very good	11
	Germany	2	ESH, FN	Fever, loss of appetite, weight loss, polyuria, polydipsia, ocular lesions	Dehydration, hypothermia (37.4°C)	Anaemia, neutrophilia with left shift, monocytosis, lymphocytosis renal azotaemia, electrolyte shift	PCR positive	Unknown	Unknown	5‡
			ESH, M	Unknown	No clinical signs	Thrombocytopenia†, leukocytosis, monocytosis, lymphocytosis, neutrophilia	PCR positive (coinfection with haemotrophic mycoplasma)	Unknown	Unknown	

Table 2 Case reports of feline *Anaplasma phagocytophilum* infections (n = 55, 1989–2019) (continued)

	Country	n	Signalment	History	Clinical signs	Laboratory results	Diagnostic methods	Therapy	Outcome	Reference
Europe (continued)	Germany	1	Persian, 7 years, MN	Tick attachment, ocular discharge	Pain, gingivitis	Thrombocytopenia†, leukocytosis, eosinophilia, hyperproteinaemia	PCR positive	Unknown	Unknown	7‡
	Germany	1	Unknown	Unknown	Obstructive feline lower tract disease	Azotaemia	PCR positive	Unknown	Unknown	6‡
	Germany	1	LaPerm longhair, 7 years, MN	Tick attachment, loss of appetite, lethargy	Fever (40.8°C)	Leukopenia, thrombocytopenia¶, hyperproteinaemia, hyperglobulinaemia, hyperglycaemia, lymphopenia	Morulae*, PCR positive, IFAT positive	Doxycycline	Very good	8
USA	USA	5	DSH; 9 months to 3 years; 3 MN, 2 FN	Lethargy (5/5), tick attachment (3/5)	Fever (5/5, 39.7–40.9°C)	Thrombocytopenia† (3/5, 1/5 thrombocytic aggregation), hyperglycaemia 1/5	PCR positive, IFAT positive (1/5 positive for *Bartonella henselae*, 3/5 positive for *Toxoplasma gondii* IgG)	Doxycycline	Very good	17
	USA	16	Median 2 years old (4 months to 13 years); 9 MN, 1 M, 6 FN	Lethargy (16/16), loss of appetite (14/16), ocular signs (7/16), ataxia (1/16)	Fever (15/16, 39.6–41.5°C), pain (4/16), tachycardia (3/16), proteinuria (2/16), hepatosplenomegaly (1/16)	Thrombocytopenia† (7/16, thrombocyte aggregation); lymphopenia (6/16); hyperglycaemia, anaemia, neutropenia (2/16 each); leukopenia (1/16)	PCR positive (1/16 positive for *Mycoplasma haemominutum* and *Bartonella clarridgeiae*), morulae* (3/16)	Doxycycline	Very good	18‡
Africa	Kenya	3	10 years, M	Loss of appetite, weight loss, dyspnoea	Tick infestation, fever (40.1°C), splenomegaly	Normocytic, normochromic anaemia, hyperproteinaemia, hyperglobulinaemia	Morulae*	Tetracycline hydrochloride	Very good	89
			4 years, M	Loss of appetite, weight loss	Tick infestation, fever (39.7°C), splenomegaly	Normocytic, normochromic anaemia	Morulae*	Imidocarb-dipropionate	Very good	
			2 years, F	Loss of appetite, weight loss, dyspnoea	Tick attachment, fever (40°C), splenomegaly, lymphadenomegaly	Normocytic, normochromic anaemia, leukopenia, neutropenia	Morulae*	Imidocarb-dipropionate	Very good	

ESH = European shorthair; ELH = European longhair; DSH = domestic shorthair; M = male; MN = male neutered; F = female; FN = female neutered; IFAT = immunofluorescence antibody test
*Microscopic detection in blood smears
†No manual count of platelets with a haemocytometer
‡Prevalence study with additional case report content, providing further description of infected cats
§No further definition of clinical signs
¶Confirmed by manual count of platelets with haemocytometer

Mild to severe thrombocytopenia is a common – and the most diagnostically relevant – laboratory finding in *A phagocytophilum* infections in both cats and dogs.

In all of the 34 cats with *A phagocytophilum* infections from the literature that are analysed here (see box on page 432), haematological examination was performed (Table 2). Thrombocytopenia was diagnosed in 20/34 cats (59%); however, low platelet counts in cats must be interpreted with caution (see box), and in six of these 20 cats platelet aggregation was present. Nine out of 34 cats (26%) were anaemic.

Interpreting thrombocytopenia with caution

Thrombocytopenia is the most diagnostically relevant laboratory finding in *A phagocytophilum* infections in cats. However, in general, low platelet counts must be interpreted with caution, because the impedance measurement is influenced by platelet aggregates, giant platelets or inadequate separation of erythrocytes and platelets, all of which can lead to falsely low values.[106] It is therefore recommended that feline platelets are counted manually with a haemocytometer.

Five out of 34 cats (15%) were leukopenic and 3/34 cats (9%) had leukocytosis. Similarly to cats, in the 63 dogs with granulocytic anaplasmosis investigated by Chirek et al, thrombocytopenia was the most common laboratory abnormality (86%), followed by anaemia (70%) and leukocytosis (27%), as well as leukopenia (14%).[91] Leukopenia occurred more often in cats than leukocytosis. A differential blood count was available in 25/34 cats (74%). Nine of 25 (36%) cats were lymphopenic and 3/25 (12%) had a lymphocytosis or a neutrophilia (2/3 with a left shift). Further abnormalities included neutropenia (2/25, 8%), eosinophilia (2/25, 8%) and monocytosis (2/25, 8%). Again, in Chirek et al's study of 63 dogs, similar laboratory abnormalities such as lymphopenia (44%), monocytosis (43%), neutrophilia (35%), eosinophilia (10%), lymphocytosis (8%) and neutropenia (2%) were described.[91]

Blood chemistry was performed to varying extents in 27 of the 34 cats (79%) (Table 2). The most common finding was hyperglycaemia, which was found in 6/27 cats (22%). Two out of 27 cats (7%) showed azotaemia, one of them due to an underlying disease (chronic renal insufficiency with a suspected acute component) and one during the course of disease while under intensive care. Electrolyte imbalances and increased liver enzymes were detected in 2/27 cats (7%). Further abnormalities included an increase in lactate dehydrogenase (1/27, 4%) and an abnormal albumin concentration (1/27, 4%), as well as a reduction in serum iron levels (1/27, 4%). Hyperproteinaemia with corresponding hyperglobulinaemia was detected in 1/27 cats (4%), and hyperproteinaemia without hyperglobulinaemia in another cat. In Chirek et al's 63 dogs with granulocytic anaplasmosis, the proportion of animals with increased liver enzymes and hyperbilirubinaemia was considerably higher, at 75%.[91] Hyperproteinaemia was detected in 43% of dogs and hypoproteinaemia in 2%. Hypoalbuminaemia was reported more commonly in the dogs (62%). Electrolyte imbalances such as hypernatraemia (10%), hyperkalaemia (2%), hyponatraemia (24%) and hypokalaemia (19%) were additionally recorded. Azotaemia was only documented in a small number of dogs in the study (3%).[91]

Diagnosis

Several direct and indirect methods have been described for diagnosing infections with *A phagocytophilum*.[40,107] The detection of morulae in neutrophilic granulocytes in a blood or buffy-coat smear is one such method and is highly indicative of an infection with *A phagocytophilum*. However, these morulae cannot be differentiated from those of *Ehrlichia ewingii*; hence, further tests are necessary for confirmation of an infection with *A phagocytophilum*. In addition, there is always the possibility of falsely interpreting stain residues, nuclei or basophil precipitates in the blood smear as morulae.[108] In experimentally infected cats, morulae were detectable 7–9 days postinfection[88] or within the first 10 weeks after tick infestation.[93] In experimentally infected dogs, morulae were detectable 4 days postinfection and persisted for 4–8 days.[37]

Detection of morulae in neutrophilic granulocytes is highly indicative of an infection with *A phagocytophilum*, although further tests are necessary for confirmation.

PCR examination detects the pathogen's DNA in peripheral blood, buffy coat, bone marrow or splenic tissue. Some protocols also include the detection of DNA from other pathogens such as *A platys* or *Pseudomonas* species, meaning that further sequencing is necessary for the confirmation of *A phagocytophilum* infection. In dogs, the detection of *Pseudomonas* sequences has been reported to cause false-positive results, which will not be apparent until further sequencing has been implemented.[109] To the authors' knowledge, there are no similar experiences in cats. Another study in cats described direct antigen detection via PCR, which has a high sensitivity and specificity in acute cases but can be falsely negative in chronic infections due to the absence of the pathogen in blood.[93]

The detection of antibodies via immunofluorescence antibody test (IFAT) or ELISA also indicates exposure to *A phagocytophilum*. However, an acute infection is only confirmed if the antibody titre increases or decreases four-fold within 4 weeks.[40] In general, IFAT and ELISA have a high sensitivity and specificity, but it is important to give consideration to the limitations of these tests, which include, for example, possible cross reactions with *Ehrlichia* species and *A platys* (see box on page 436).[111,112]

SNAP tests, for example the SNAP Multi-Analyte Test and the SNAP 4Dx Plus Assay (IDEXX), are used as rapid in-house ELISAs in veterinary medicine. Both tests have been developed as canine assays, but have also successfully detected antibodies against *A phagocytophilum* in domestic cats.[70,93,113] A comparison between the two SNAP tests and a commercial IFAT for the detection of *A phagocytophilum* in cats showed discrepancies between the different assays.[70] Reasons for this could include the lack of specificity of peptides chosen in the design of the assays, the lack of sensitivity of commercial ELISA and/or IFAT and/or an enhanced analytic sensitivity of p16 analytes for testing cat sera. In this study, the IFAT was slightly more sensitive than the ELISA.[70]

Acute infection with *A phagocytophilum* is confimed on immunofluorescence antibody test or ELISA if the antibody titre increases or decreases four-fold within 4 weeks.

Considerations when using IFAT or ELISA

The limitations of serological tests extend to the premature implementation of tests postinfection before the beginning of seroconversion, the cross-reactions with other pathogens and the possibility of false-negative results, as seen in young or immunosuppressed dogs.

Antibodies might not be detectable in acute cases; for example, if tested before seroconversion.[17] In an experimental study, antibodies against *A phagocytophilum* were detected in cats within 14 days postinfection.[88] In experimentally infected cats, antibodies were detectable for a duration of 2–6 weeks postinfection.[93] Under natural conditions, seroconversion can also be seen in cats treated with antibiotics.[88] Antibodies can persist for several months after pathogen contact.[94,110]

A measurable antibody titre may also be due to a cross reaction with *A platys* or *Ehrlichia* species.[40,111] In Europe, *A platys* and *E canis* are transmitted by the vector *Rhipicephalus sanguineus*. Owing to the vector distribution, there are no autochthonous infections in northern and central European areas. Of course, this does not account for animals with a stay abroad; for example, those imported from or travelling to endemic regions. This underlines the importance of a thorough history including information on stays abroad.

It is important to obtain a thorough history; information on periods of time the cat has spent abroad (travelling or before importation) is especially pertinent.

Treatment and management

A phagocytophilum is resistant to several antimicrobial agents.[110,114–116] Doxycycline is the antibiotic of choice for treating rickettsial infections in cats, although currently there are only retrospective case reports supporting this recommendation. It is administered at 10 mg/kg PO q24h for 28 days.[40] It is recommended that the tablets be dissolved in water or administered with food in order to prevent oesophagitis.[117]

The ABCD guidelines describe rapid clinical improvement in patients within the first 24–48 h after initiation of antimicrobial treatment with doxycycline.[40] One cat tested negative as soon as 1 day after the initiation of treatment with doxycycline.[9] In contrast, however, some studies have described that the pathogen was no longer detectable in blood via PCR after treatment with doxycycline on day 15,[16] after 3 weeks,[11] on days 25, 27 and 30,[17] after 6 weeks[12] and on day 139.[17] A further case report documented that the pathogen was detectable via PCR 8 days after starting treatment with doxycycline;[8] in another cat it was detectable even 120 days after the initial treatment period of 28–30 days.[17] A further cat tested positive after 37 days and negative on day 139.[17] In dogs there are several studies providing varying information. A study in Germany described complete pathogen elimination in all 18 infected dogs 2–8 weeks after the initiation of doxycycline treatment;[90] however, another study described recurrence of clinical signs after antimicrobial therapy or poor response to treatment.[118]

Due to the zoonotic potential, clinicians should consider testing potentially exposed animals, as clinical signs are vague and non-specific.

All of this confirms that the required duration of treatment in cats is unknown. In comparison, in dogs infected with *A phagocytophilum*, treatment recommendations are doxycycline 5 mg/kg q12h for 14 days.[72]

Prevention and public health considerations

Humans are also susceptible to infections with *A phagocytophilum*, making this pathogen relevant for both human and veterinary medicine.[119] Prevention in animals therefore plays an essential role, especially in order to avoid the development of reservoirs.

It is important to raise awareness of tick prevention in endemic areas. Also, clinicians should consider testing potentially exposed animals, as clinical signs are vague and non-specific. Feline vector-borne infections should be on the list of differential diagnoses in cases with a history of vector contact and clinical signs suspicious of an infection.

If cats are housed indoors and arthropod control (see box on page 437) is maintained, the risk to people should be minimal.[3] In addition to antiparasitic treatment, regular examinations for ticks should be carried out by owners and veterinarians.[3]

The ACVIM guidelines recommend direct and indirect methods of detection for *A phagocytophilum* in blood donor cats. Only seronegative and PCR negative cats should donate blood. If no other blood donors are available in endemic regions, seropositive and PCR negative cats may also be used as blood donors.[39]

Doxycycline is the antibiotic of choice for treating rickettsial infections in cats, although currently there are only retrospective case reports supporting this recommendation, and the required duration of treatment in cats is unknown.

Antiparasitic treatment

Licensed antiparasitic agents with repellent effects against either all ticks, or specifically *Ixodes* ticks, are recommended for both indoor and outdoor cats (Table 3). There are different terms used for the effects of applied compounds:[120] tick repellency sensu stricto is characterised by an irritant effect causing the tick to move away and fall off soon after contact with the haircoat of the host. Various other terms are as follows:

- Disruption of attachment: interference with the natural process of tick fixation
- Tick expellency: disruption of the mechanisms of attachment or prevention of attachment of new infesting ticks
- Antifeeding effect: interference with the natural process of tick feeding, avoiding any blood meal
- Killing effect (acaricidal effect sensu stricto): ability to induce death of the ticks

Licensed antiparasitic agents with repellent effects against either all ticks, or specifically *Ixodes* ticks, are recommended for both indoor and oudoor cats.

Table 3 Antiparasitic agents against *Ixodes* species ticks licensed for use in cats

Application type	Active ingredient	Registered trademark	Repellent effect (ticks)	Antiparasitic effect	Precautions for use
Collar	Imidacloprid, flumethrin	Seresto (Bayer)	Insecticide, acaricide (killing effect) and repellent effect (antifeeding effect)	*Ixodes hexagonus*, *Amblyomma americanum*: 8 months. Dogs: *I hexagonus, A americanum Ixodes scapularis, Dermacentor variabilis, Ixodes holocyclus.* Indirect protection in dogs against transmission of pathogens vectored by *Dermacentor reticulatus* and *Ixodes Ricinus*	Treatment in kittens over 10 weeks
Spot-on	Selamectin, sarolaner	Stronghold Plus (Zoetis)	Insecticide, acaricide (killing effect), but tick has to be attached and has to take up the active ingredient	*I ricinus, I hexagonus*: 5 weeks *D reticulatus, Rhipicephalus sanguineus*: 4 weeks	Treatment in kittens over 8 weeks with body weight over 1.25 kg
	Fluralaner	Bravecto Spot-on Solution (MSD Animal Health)	Insecticide, acaricide (killing effect), but tick has to be attached and has to take up the active ingredient	*I scapularis, I ricinus*: 12 weeks *D variabilis*: 8 weeks	Not effective for 12 weeks' duration in kittens less than 6 months of age Treatment with body weight over 2.6 kg
	Fluralaner, moxidectin	Bravecto Plus (MSD Animal Health)	Insecticide, acaricide (killing effect), but tick has to be attached and has to take up the active ingredient	*I ricinus*: 12 weeks	Treatment in kittens over 9 weeks with body weight over 1.2 kg
	Fipronil, (s)-methoprene	Frontline Combo (Boehringer Ingelheim)	Insecticide, acaricide (killing effect), but tick has to be attached and has to take up the active ingredient	*I ricinus, D variabilis, R sanguineus*: up to 2 weeks	Treatment in kittens over 8 weeks and body weight over 1 kg
	Fipronil	Frontline (Boehringer Ingelheim)	Insecticide, acaricide (killing effect), but tick has to be attached and has to take up the active ingredient	*I ricinus, D variabilis, R sanguineus*: up to 2 weeks	Treatment in kittens over 12 weeks
Spray	Fipronil	Frontline Spray (Boehringer Ingelheim)	Insecticide, acaricide (killing effect) but tick has to be attached and has to take up the active ingredient	*I ricinus, D variabilis, R sanguineus*: 4 weeks	Treatment in kittens over 12 weeks

Future research needs and conclusions

Feline vector-borne infections are gaining in importance. Further research to investigate the pathogenesis of *A phagocytophilum* infections in cats is required. The spread of potential vectors and pathogens to currently non-endemic regions due to growing tourism, increasing numbers of imported animals, goods traffic and climatic changes makes prophylaxis for companion animals and biological limitation of the tick population even more relevant. As with other vector-borne infections, *A phagocytophilum* is of great importance for public health in human and veterinary medicine due to its zoonotic potential.

The spread of potential vectors and pathogens to currently non-endemic regions makes prophylaxis for companion animals especially more relevant.

Case notes

A 7-year-old male neutered LaPerm longhair cat with outdoor access was presented due to lethargy and lack of appetite.

Case work-up On physical examination, the cat had a rectal temperature of 40.8°C, and an *I ricinus* tick was observed to be attached. Blood samples were collected for haematological and biochemical analysis and a blood smear was prepared. Laboratory abnormalities at initial presentation included thrombocytopenia and hyperproteinaemia with hyperglobulinaemia (see table). During the course of the disease, the cat developed leukopenia, mild anaemia and azotaemia.

Haematological and biochemical analysis

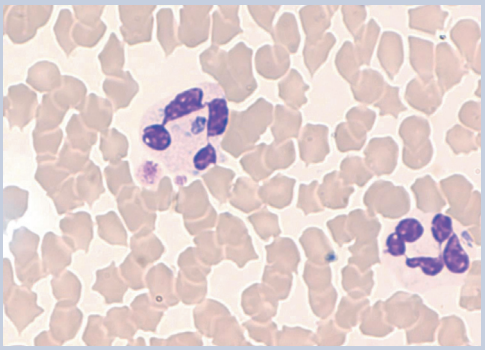
Parameter	Reference interval*	Day 1	Day 2	Day 3	Day 9	Day 21†
WBC (x 10^9/l)	6–11	5.11	3.27	3.9	8.42	6.2 (5–18.9)
Hct (%)	30–44	35	29	35	32	38 (24–45)
Plt (x 10^9/l)	180–550	78‡	88	111§	180	270 (175–500)
Creatinine (µmol/l)	53–168	132	–	144	202	172 (71–212)
Total protein (g/l)	57–78	86	–	78	82	–
Albumin (g/l)	22–40	30	–	27	28	–

WBC = white blood cell count; Hct = haematocrit; Plt = platelet count
*Reference values of the Clinic for Small Animals, Faculty of Veterinary Medicine, Freie Universität Berlin, Germany
†Reference values of the laboratory IDEXX GmbH, Ludwigsburg, Germany
‡Manual count of platelets: 72,000/µl
§Manual count of platelets: 116,000/µl

Information adapted from Schäfer et al (2019)[8]

Diagnosis The diagnosis of an infection with *A phagocytophilum* was established through the microscopic evidence of morulae in the cat's neutrophilic granulocytes (figure), the detection of pathogenic DNA via PCR in EDTA blood and the detection of antibodies using an IFAT (titre 1:40).

Treatment and outcome The cat was treated with intravenous fluids and antipyretic agents for 3 days. Doxycycline 10 mg/kg PO q24h was given over 3 weeks. The rectal temperature, appetite and laboratory abnormalities normalised during the course of treatment. After 9 days of treatment the PCR test was still positive. We were not able to initiate a further PCR or IFAT.



Morulae in neutrophilic granulocytes in a cat infected with *Anaplasma phagocytophilum*

✣ **What this case demonstrates:** *A phagocytophilum* infection and granulocytic anaplasmosis should be on the list of differential diagnoses in cats with outdoor access and/or tick infestation when suspicious clinical signs are present. Cats should be treated with antiectoparasitic agents in order to prevent vector-borne infections.

Conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

KEY POINTS

- Feline vector-borne infections, such as infections with *A phagocytophilum*, should be on the list of differential diagnoses in cases with a history of vector contact and clinical signs suspicious of an infection.
- Clinical signs are often vague and non-specific, which is why diagnosis can be challenging for veterinarians. A thorough history should include information on vector contact and stays abroad.
- Avoidance of vector contact plays an important role both in preventing the development of pathogenic reservoirs and infections with vector-borne pathogens in animals, and in public health in human and veterinary medicine.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

Ethical approval

This work did not involve the use of animals and therefore ethical approval was not necessarily required.

Informed consent

This work did not involve the use of animals and therefore informed consent was not required. No animals or humans are identifiable within this publication, and therefore additional informed consent for publication was not required.

References

1 Cohn LA. **Ehrlichiosis and related infections.** *Vet Clin North Am Small Anim Pract* 2003; 33: 863–884.
2 Dumler JS, Barbet AF, Bekker CP, et al. **Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order *Rickettsiales*: unification of some species of *Ehrlichia* with**

***Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila*.** *Int J Syst Evol Microbiol* 2001; 51: 2145–2165.

3 Lappin MR and Breitschwerdt EB. ***Anaplasma phagocytophilum* infection (feline granulocytotropic anaplasmosis).** In: Greene CE (ed). Infectious diseases of the dog and cat. 4th ed. St Louis, MO: Elsevier, 2012, pp 254–256.

4 Katavolos P, Armstrong PM, Dawson JE, et al. **Duration of tick attachment required for transmission of granulocytic ehrlichiosis.** *J Infect Dis* 1998; 177: 1422–1425.

5 Bergmann M, Englert T, Stuetzer B, et al. **Prevalence of selected rickettsial infections in cats in southern Germany.** *Comp Immunol Microbiol Infect Dis* 2015; 42: 33–36.

6 Morgenthal D, Hamel D, Arndt G, et al. **Prevalence of haemotropic *Mycoplasma* spp., *Bartonella* spp. and *Anaplasma phagocytophilum* in cats in Berlin/Brandenburg (northeast Germany)** [article in German]**.** *Berl Munch Tierarztl Wochenschr* 2012; 125: 418–427.

7 Hamel D, Bondarenko A, Silaghi C, et al. **Seroprevalence and bacteremia [corrected] of *Anaplasma phagocytophilum* in cats from Bavaria and Lower Saxony (Germany)** [article in German]**.** *Berl Munch Tierarztl Wochenschr* 2012; 125: 163–167.

8 Schäfer I, Weingart C and Kohn B. **Infection with *Anaplasma phagocytophilum* in a cat in Germany** [article in German]**.** *Kleintierprax* 2019; 64: 205–215.

9 Kirtz G, Meli ML, Lutz H, et al. ***Anaplasma phagocytophilum* infection in two cats in Austria** [article in German]**.** *Kleintierprax* 2005; 50: 489–494.

10 Adaszek L, Gorna M, Skrzypczak M, et al. **Three clinical cases of *Anaplasma phagocytophilum* infection in cats in Poland.** *J Feline Med Surg* 2013; 15: 333–337.

11 Gorna M, Adaszek L, Policht K, et al. **Detection of *Anaplasma phagocytophilum* in a cat.** *Vet Med-Czech* 2013; 58: 39–43.

12 Schaarschmidt-Kiener D, Graf F, von Loewenich FD, et al. ***Anaplasma phagocytophilum* infection in a cat in Switzerland** [article in German]**.** *Schweiz Arch Tierheilkd* 2009; 151: 336–341.

13 Torina A, Alongi A, Naranjo V, et al. **Characterization of *Anaplasma* infections in Sicily, Italy.** *Ann N Y Acad Sci* 2008; 1149: 90–93.

14 Shaw SE, Binns SH, Birtles RJ, et al. **Molecular evidence of tick-transmitted infections in dogs and cats in the United Kingdom.** *Vet Rec* 2005; 157: 645–648.

15 Heikkilä HM, Bondarenko A, Mihalkov A, et al. ***Anaplasma phagocytophilum* infection in a domestic cat in Finland: case report.** *Acta Vet Scand* 2010; 52: 62. DOI: 10.1189/1751-0147-52-62.

16 Bjoersdorff A, Svendenius L, Owens JH, et al. **Feline granulocytic ehrlichiosis – a report of a new clinical entity and characterisation of the infectious agent.** *J Small Anim Pract* 1999; 40: 20–24.

17 Lappin MR, Breitschwerdt EB, Jensen WA, et al. **Molecular and serologic evidence of *Anaplasma phagocytophilum* infection in cats in North America.** *J Am Vet Med Assoc* 2004; 225: 893–896.

18 Savidge C, Ewing P, Andrews J, et al. ***Anaplasma phagocytophilum* infection of domestic cats: 16 cases from the northeastern USA.** *J Feline Med Surg* 2016; 18: 85–91.

19 Woldehiwet Z and Scott GR. **Tick-borne fever: leucocyte migration inhibition.** *Vet Microbiol* 1982; 7: 437–445.

20 Foggie A. **Studies on the infectious agent of tick-borne fever in sheep.** *J Pathol Bacteriol* 1951; 63: 1–15.

21 Hudson JR. **The recognition of tick-borne fever as a disease of cattle.** *Br Vet J* 1950; 106: 3–17.

22 Foster WNM and Greig JC. **Isolation of tick-borne fever from feral goats in New Galloway.** *Vet Rec* 1969; 85: 585–586.

23 Alberdi MP, Walker AR and Urquhart KA. **Field evidence that roe deer (*Capreolus capreolus*) are a natural host for *Ehrlichia phagocytophila*.** *Epidemiol Infect* 2000; 124: 315–323.

24 Madigan JE and Gribble D. **Equine ehrlichiosis in northern California: 49 cases (1968–1981).** *J Am Vet Med Assoc* 1987; 190: 445–448.

25 Madewell BR and Gribble DH. **Infection in two dogs with an agent resembling *Ehrlichia equi*.** *J Am Vet Med Assoc* 1982; 180: 512–514.

26 Chen SM, Dumler JS, Bakken JS, et al. **Identification of a granulocytotropic *Ehrlichia* species as the etiologic agent of human disease.** *J Clin Microbiol* 1994; 32: 589–595.

27 Richter PJ Jr, Kimsey RB, Madigan JE, et al. ***Ixodes pacificus* (Acari: Ixodidae) as a vector of *Ehrlichia equi* (Rickettsiales: Ehrlichiae).** *J Med Entomol* 1996; 33: 1–5.

28 Telford SR, Dawson JE, Katavolos P, et al. **Perpetuation of the agent of human granulocytic ehrlichiosis in a deer tick-rodent cycle.** *Proc Natl Acad Sci* 1996; 93: 6209–6214.

29 Bown KJ, Lambin X, Telford GR, et al. **Relative importance of *Ixodes ricinus* and *Ixodes trianguliceps* as vectors for *Anaplasma phagocytophilum* and *Babesia microti* in field vole (*Microtus agrestis*) populations.** *Appl Environ Microbiol* 2008; 74: 7118–7125.

30 Santos AS, Santos-Silva MM, Sousa R, et al. **PCR-based survey of *Anaplasma phagocytophilum* in Portuguese ticks (Acari: Ixodidae).** *Vector Borne Zoonotic Dis* 2009; 9: 33–40.

31 Nijhof AM, Bodaan C, Postigo M, et al. **Ticks and associated pathogens collected from domestic animals in the Netherlands.** *Vector Borne Zoonotic Dis* 2007; 7: 585–595.

32 Cao WC, Zhao QM, Zhang PH, et al. **Granulocytic Ehrlichiae in *Ixodes persulcatus* ticks from an area in China where Lyme disease is endemic.** *J Clin Microbiol* 2000; 38: 4208–4210.

33 Zhang L, Liu Y, Ni D, et al. **Nosocomial transmission of human granulocytic anaplasmosis in China.** *JAMA* 2008; 300: 2263–2270.

34 Kemperman M, Neitzel D, Jensen K, et al. ***Anaplasma phagocytophilum* transmitted through blood transfusion – Minnesota, 2007** [reprinted from *MMWR* 2008; 57: 1145–1148]**.** *J Am Med Assoc* 2008; 300: 2718–2720.

35 Horowitz HW, Kilchevsky E, Haber S, et al. **Perinatal transmission of the agent of human granulocytic ehrlichiosis.** *N Engl J Med* 1998; 339: 375–378.

36 Pusterla N, Braun U, Wolfensberger C, et al. **Intrauterine infection with *Ehrlichia phagocytophila* in a cow.** *Vet Rec* 1997; 141: 101–102.

37 Egenvall A, Bjoersdorff A, Lilliehook I, et al. **Early manifestations of granulocytic ehrlichiosis in dogs inoculated experimentally with a Swedish *Ehrlichia* species isolate.** *Vet Rec* 1998; 143: 412–417.

38 Kohn B. **Canine transfusion medicine** [article in German]**.** *Kleintierprax* 2010; 7: 387–395.

39 Wardrop KJ, Birkenheuer A, Blais MC, et al. **Update on canine and feline blood donor screening for blood-borne pathogens.** *J Vet Intern Med* 2016; 30: 15–35.

40 Pennisi MG, Hofmann-Lehmann R, Radford AD, et al. ***Anaplasma*, *Ehrlichia* and *Rickettsia* species infections in cats: European guidelines from the ABCD on prevention and management.** *J Feline Med Surg* 2017; 19: 542–548.

41 Pennisi MG, Hartmann K, Addie DD, et al. **Blood transfusion in cats: ABCD guidelines for minimising risks of infectious iatrogenic complications.** *J Feline Med Surg* 2015; 17: 588–593.

42 Juvet F, Lappin MR, Brennan S, et al. **Prevalence of selected infectious agents in cats in Ireland.** *J Feline Med Surg* 2010; 12: 476–482.

43 Elfving K, Malmsten J, Dalin AM, et al. **Serologic and molecular prevalence of *Rickettsia helvetica* and *Anaplasma phagocytophilum* in wild cervids and domestic mammals in the central parts of Sweden.** *Vector Borne Zoonotic Dis* 2015; 15: 529–534.

44 Diakou A, Di Cesare A, Accettura PM, et al. **Intestinal parasites and vector-borne pathogens in stray and free-roaming cats living in continental and insular Greece.** *PLoS Negl Trop Dis* 2017; 11: e0005335. DOI: 10.1371/journal. pntd.0005335.

45 Mylonakis ME, Schreeg M, Chatzis MK, et al. **Molecular detection of vector-borne pathogens in Greek cats.** *Ticks Tick Borne Dis* 2018; 9: 171–175.

46 Tarello W. **Microscopic and clinical evidence for *Anaplasma (Ehrlichia) phagocytophilum* infection in Italian cats.** *Vet Rec* 2005; 156: 772–774.

47 Ebani VV and Bertelloni F. **Serological evidence of exposure to *Ehrlichia canis* and *Anaplasma phagocytophilum* in central Italian healthy domestic cats.** *Ticks Tick Borne Dis* 2014; 5: 668–671.

48 Spada E, Proverbio D, Galluzzo P, et al. **Molecular study on selected vector-borne infections in urban stray colony cats in northern Italy.** *J Feline Med Surg* 2014; 16: 684–688.

49 Persichetti MF, Solano-Gallego L, Serrano L, et al. **Detection of vector-borne pathogens in cats and their ectoparasites in southern Italy.** *Parasit Vectors* 2016; 9: 247. DOI: 10.1186/ s13071-016-1534-1.

50 Persichetti MF, Pennisi MG, Vullo A, et al. **Clinical evaluation of outdoor cats exposed to ectoparasites and associated risk for vector-borne infections in southern Italy.** *Parasit Vectors* 2018; 11: 136. DOI: 10.1186/s13071-018-2725-8.

51 Alves AS, Milhano N, Santos-Silva M, et al. **Evidence of *Bartonella* spp., *Rickettsia* spp. and *Anaplasma phagocytophilum* in domestic, shelter and stray cat blood and fleas, Portugal.** *Clin Microbiol Infect* 2009; 15 Suppl 2: 1–3. DOI: 10.1111/j.1469-0691.2008.02636.x.

52 Vilhena H, Martinez-Diaz VL, Cardoso L, et al. **Feline vector-borne pathogens in the north and centre of Portugal.** *Parasit Vectors* 2013; 6: 99. DOI: 10.1186/1756-3305-6-99.

53 Maia C, Ramos C, Coimbra M, et al. **Bacterial and protozoal agents of feline vector-borne diseases in domestic and stray cats from southern Portugal.** *Parasit Vectors* 2014; 7: 115. DOI: 10.1186/1756-3305-7-115.

54 Solano-Gallego L, Hegarty B, Espada Y, et al. **Serological and molecular evidence of exposure to arthropod-borne organisms in cats from northeastern Spain.** *Vet Microbiol* 2006; 118: 274–277.

55 Tabar MD, Altet L, Francino O, et al. **Vector-borne infections in cats: molecular study in Barcelona area (Spain).** *Vet Parasitol* 2008; 151: 332–336.

56 Ayllon T, Villaescusa A, Tesouro MA, et al. **Serology, PCR and culture of *Ehrlichia/ Anaplasma* species in asymptomatic and symptomatic cats from central Spain.** *Clin Microbiol Infect* 2009; 15 Suppl 2: 4–5.

57 Ayllon T, Diniz PP, Breitschwerdt EB, et al. **Vector-borne diseases in client-owned and stray cats from Madrid, Spain.** *Vector Borne Zoonotic Dis* 2012; 12: 143–150.

58 Ravicini S, Pastor J, Hawley J, et al. **Prevalence of selected infectious disease agents in stray cats in Catalonia, Spain.** *JFMS Open Rep* 2016; 2. DOI: 10.1177/2055116916634109.

59 Luria BJ, Levy JK, Lappin MR, et al. **Prevalence of infectious diseases in feral cats in northern Florida.** *J Feline Med Surg* 2004; 6: 287–296.

60 Magnarelli LA, Bushmich SL, Ijdo JW, et al. **Seroprevalence of antibodies against *Borrelia burgdorferi* and *Anaplasma phagocytophilum* in cats.** *Am J Vet Res* 2005; 66: 1895–1899.

61 Eberhardt JM, Neal K, Shackelford T, et al. **Prevalence of selected infectious disease agents in cats from Arizona.** *J Feline Med Surg* 2006; 8: 164–168.

62 Hackett TB, Jensen WA, Lehman TL, et al. **Prevalence of DNA of *Mycoplasma haemofelis*, '*Candidatus* Mycoplasma haemominutum', *Anaplasma phagocytophilum*, and species of *Bartonella*, *Neorickettsia*, and *Ehrlichia* in cats used as blood donors in the United States.** *J Am Vet Med Assoc* 2006; 229: 700–705.

63 Lappin MR, Griffin B, Brunt J, et al. **Prevalence of *Bartonella* species, haemoplasma species, *Ehrlichia* species, *Anaplasma phagocytophilum*, and *Neorickettsia risticii* DNA in the blood of cats and their fleas in the United States.** *J Feline Med Surg* 2006; 8: 85–90.

64 Billeter SA, Spencer JA, Griffin B, et al. **Prevalence of *Anaplasma phagocytophilum* in domestic felines in the United States.** *Vet Parasitol* 2007; 147: 194–198.

65 Ishak AM, Radecki S and Lappin MR. **Prevalence of *Mycoplasma haemofelis*, '*Candidatus* Mycoplasma haemominutum', *Bartonella* species, *Ehrlichia* species, and *Anaplasma phagocytophilum* DNA in the blood of cats with anemia.** *J Feline Med Surg* 2007; 9: 1–7.

66 Hoyt K, Chandrashekar R, Beall M, et al. **Evidence for clinical anaplasmosis and borreliosis in cats in Maine.** *Top Companion Anim Med* 2018; 33: 40–44.

67 Saucier J, Leonard B, Hoyt K, et al. **Seroprevalence of antibodies to *Anaplasma phagocytophilum* and *Borrelia burgdorferi* in domestic cats** [abstract]**.** *J Vet Intern Med* 2016; 30: 1479.

68 Shannon AB, Rucinsky R, Gaff HD, et al. ***Borrelia miyamotoi*, other vector-borne agents in cat blood and ticks in eastern Maryland.** *Ecohealth* 2017; 14: 816–820.

69 Galemore ER, Labato MA and O'Neil E. **Prevalence of *Anaplasma phagocytophilum* infection in feral cats in Massachusetts.** *JFMS Open Rep* 2018; 4. DOI: 10.1177/ 2055116917753804.

70 Hegarty BC, Qurollo BA, Thomas B, et al. **Serological and molecular analysis of feline vector-borne anaplasmosis and ehrlichiosis using species-specific peptides and PCR.** *Parasit Vector* 2015; 8. DOI: 10.1186/s13071-015-0929-8.

71 Lee SH, VanBik D, Kim NH, et al. **First molecular detection and genetic analysis of *Anaplasma phagocytophilum* in shelter cats in Seoul, Korea.** *Infect Genet Evol* 2016; 46: 71–73.

72 Carrade DD, Foley JE, Borjesson DL, et al. **Canine granulocytic anaplasmosis: a review.** *J Vet Intern Med* 2009; 23: 1129–1141.

73 Stuen S, Granquist EG, Silaghi C. ***Anaplasma phagocytophilum* – a widespread multi-host pathogen with highly adaptive strategies.** *Front Cell Infect Microbiol* 2013; 3: 31.

74 Chirek A. **Studies on *Anaplasma phagocytophilum* in dogs: clinical presentation of granulocytic anaplasmosis and significance for transfusion medicine** [article in German]**.** Doctoral thesis, Faculty of Verterinary Medicine, Freie Universität Berlin, 2018.

75 des Vignes F, Piesman J, Heffernan R, et al. **Effect of tick removal on transmission of *Borrelia burgdorferi* and *Ehrlichia phagocytophila* by *Ixodes scapularis* nymphs.** *J Infect Dis* 2001; 183: 773–778.

76 Bjoersdorff A. **Ehrlichiosis and anaplasmosis, part 2: *Anaplasma phagocytophilum* comb. nov. infection.** In: Shaw S and Day M (eds). Arthropod-borne infectious diseases of the dog and the cat. London: Manson Publishing, 2005, pp 120–133.

77 Greig B and Armstrong PJ. **Canine granulocytotrophic anaplasmosis (*A. phagocytophilum* infection).** In: Greene CE (ed). Infectious diseases of the dog and the cat. 3rd ed. St Louis, MO: Elsevier, 2006, pp 219–224.

78 Goodman JL, Nelson CM, Klein MB, et al. **Leukocyte infection by the granulocytic ehrlichiosis agent is linked to expression of a selectin ligand.** *J Clin Invest* 1999; 103: 407–412.

79 Herron MJ, Nelson CM, Larson J, et al. **Intracellular parasitism by the human granulocytic ehrlichiosis bacterium through the P-selectin ligand, PSGL-1.** *Science* 2000; 288: 1653–1656.

80 Rikihisa Y. ***Ehrlichia* subversion of host innate responses.** *Curr Opin Microbiol* 2006; 9: 95–101.

81 Popov VL, Han VC, Chen SM, et al. **Ultrastructural differentiation of the genogroups in the genus *Ehrlichia*.** *J Med Microbiol* 1998; 47: 235–251.

82 Ismail N, Bloch KC and McBride JW. **Human ehrlichiosis and anaplasmosis.** *Clin Lab Med* 2010; 30: 261–292.

83 Carlyon JA and Fikrig E. **Mechanism of evasion of neutrophil killing by *Anaplasma phagocytophilum*.** *Curr Opin Hematol* 2006; 13: 28–33.

84 Garcia-Garcia JC, Rennoll-Bankert KE, Pelly S, et al. **Silencing of host cell CYBB gene expression by the nuclear effector AnkA of the intracellular pathogen *Anaplasma phagocytophilum*.** *Infect Immun* 2009; 77: 2385–2391.

85 Garyu JW, Choi KS, Grab DJ, et al. **Defective phagocytosis in *Anaplasma phagocytophilum*-infected neutrophils.** *Infect Immun* 2005; 73: 1187–1190.

86 Dumler JS, Choi KS, Garcia-Garcia JC, et al. **Human granulocytic anaplasmosis and *Anaplasma phagocytophilum*.** *Emerg Infect Dis* 2005; 11: 1828–1834.

87 Sarkar A, Hellberg L, Bhattacharyya A, et al. **Infection with A*naplasma phagocytophilum* activates the phosphatidylinositol 3-Kinase/Akt and NF-kappaB survival pathways in neutrophil granulocytes.** *Infect Immun* 2012; 80: 1615–1623.

88 Foley JE, Leutenegger CM, Dumler JS, et al. **Evidence for modulated immune response to *Anaplasma phagocytophila* sensu lato in cats with FIV-induced immunosuppression.** *Comp Immunol Microbiol Infect Dis* 2003; 26: 103–113.

89 Buoro IB, Atwell RB, Kiptoon JC, et al. **Feline anaemia associated with *Ehrlichia*-like bodies in three domestic short-haired cats.** *Vet Rec* 1989; 125: 434–436.

90 Kohn B, Galke D, Beelitz P, et al. **Clinical features of canine granulocytic anaplasmosis in 18 naturally infected dogs.** *J Vet Intern Med* 2008; 22: 1289–1295.

91 Chirek A, Silaghi C, Pfister K, et al. **Granulocytic anaplasmosis in 63 dogs: clinical signs, laboratory results, therapy and course of disease.** *J Small Anim Pract* 2018; 59: 112–120.

92 Wong SJ and Thomas JA. **Cytoplasmic, nuclear, and platelet autoantibodies in human granulocytic ehrlichiosis patients.** *J Clin Microbiol* 1998; 36: 1959–1963.

93 Lappin MR, Chandrashekar R, Stillman B, et al. **Evidence of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* infection in cats after exposure to wild-caught adult *Ixodes scapularis*.** *J Vet Diagn Invest* 2015; 27: 522–525.

94 Egenvall A, Lilliehook I, Bjoersdorff A, et al. **Detection of granulocytic *Ehrlichia* species DNA by PCR in persistently infected dogs.** *Vet Rec* 2000; 146: 186–190.

95 Franzen P, Aspan A, Egenvall A, et al. **Molecular evidence for persistence of *Anaplasma phagocytophilum* in the absence of clinical abnormalities in horses after recovery from acute experimental infection.** *J Vet Intern Med* 2009; 23: 636–642.

96 Moroff S, Sokolchik I, Woodring T, et al. **Detection of antibodies against *Anaplasma phagocytophilum* in dogs using an automated fluorescence based system.** *Vet J* 2014; 202: 348–352.

97 Stuen S and Bergstrom K. **Persistence of *Ehrlichia phagocytophila* infection in two age groups of lambs.** *Acta Vet Scand* 2001; 42: 453–458.

98 Morgenthal D. **Prevalence of haemotropic *Mycoplasma* spp., *Bartonella* spp. and *Anaplasma phagocytophilum* in cats in Berlin/Brandenburg (northeast Germany)** [article in German]**.** Doctoral thesis, Faculty of Veterinary Medicine, Freie Universität Berlin, 2012.

99 Kohn B, Silaghi C, Galke D, et al. **Infections with *Anaplasma phagocytophilum* in dogs in Germany**. *Res Vet Sci* 2011; 91: 71–76.

100 Jensen J, Simon D, Murua Escobar H, et al. ***Anaplasma phagocytophilum* in dogs in Germany.** *Zoonoses Public Health* 2007; 54: 94–101.

101 Stuen S. ***Anaplasma phagocytophilum* – the most widespread tick-borne infection in animals in Europe.** *Vet Res Commun* 2007; 31 Suppl 1: 79–84.

102 Madigan JE, Richter PJ, Kimsey RB, et al. **Transmission and passage in horses of the agent of human granulocytic ehrlichiosis.** *J Infect Dis* 1995; 172: 1141–1144.

103 Barutzki D, De Nicola A, Zeziola M, et al. **Seroprevalence of *Anaplasma phagocytophilum* infection in dogs in Germany** [article in German]**.** *Berl Munch Tierarztl Wochenschr* 2006; 119: 342–347.

104 Foley JE, Foley P and Madigan JE. **Spatial distribution of seropositivity to the causative agent of granulocytic ehrlichiosis in dogs in California.** *Am J Vet Res* 2001; 62: 1599–1605.

105 Beall MJ, Chandrashekar R, Eberts MD, et al. **Serological and molecular prevalence of *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, and *Ehrlichia* species in dogs from Minnesota.** *Vector Borne Zoonotic Dis* 2008; 8: 455–464.

106 Kohn B and Weingart C. **Störungen der Blutgerinnung (Hämostase), hämorrhagische Diathese** [article in German]**.** In: Forterre F, Kohn B and Lutz H (eds). Krankheiten der Katze, 5th ed. Stuttgart: Enke Verlag, 2015, pp 520–532.

107 Bakken JS and Dumler S. **Human granulocytic anaplasmosis.** *Infect Dis Clin North Am* 2008; 22: 433–448.

108 Diniz PP and Breitschwerdt EB. ***Anaplasma phagocytophilum* infection (canine granulocytotropic anaplasmosis).** In: Greene CE (ed). Infectious diseases of the dog and cat. 4th ed. St Louis, MO: Elsevier, 2012, pp 244–254.

109 Hofmann-Lehmann R, Wagmann N, Meli ML, et al. **Detection of '*Candidatus* Neoehrlichia mikurensis' and other Anaplasmataceae and Rickettsiaceae in Canidae in Switzerland and Mediterranean countries.** *Schweiz Arch Tierheilkd* 2016; 158: 691–700.

110 Klein MB, Nelson CM and Goodman JL. **Antibiotic susceptibility of the newly cultivated agent of human granulocytic ehrlichiosis: promising activity of quinolones and rifamycins.** *Antimicrob Agents Chemother* 1997; 41: 76–79.

111 ESCCAP. **Control of vector-borne diseases in dogs and cats. European Scientific Counsel Companion Animal Parasites.** http://www.esxxap.orguploads/docs/znkh6j1d_0775_ESCCAP_Guideline_GL5_v8_1p.pdf (2019, accessed December 4, 2019).

112 Schäfer I, Volkmann M, Beelitz P, et al. **Retrospective analysis of vector-borne infections in dogs after travelling to endemic areas (2007–2018).** *Vet Parasitol X* 2019; X2: 100015. DOI: 10.1016/j.vpoa.2019.100015.

113 Liu J, Eberts M, Bewsey H, et al. **Sensitivity and specificity levels of two rapid assays for antibodies to *Anaplasma* spp. in dogs.** *J Vet Diagn Invest* 2018; 30: 290–293.

114 Horowitz HW, Hsieh TC, Aguero-Rosenfeld ME, et al. **Antimicrobial susceptibility of *Ehrlichia phagocytophila*.** *Antimicrob Agents Chemother* 2001; 45: 786–788.

115 Maurin M, Bakken JS and Dumler JS. **Antibiotic susceptibilities of *Anaplasma* (*Ehrlichia*) *phagocytophilum* strains from various geographic areas in the United States.** *Antimicrob Agents Chemother* 2003; 47: 413–415.

116 Branger S, Rolain JM and Raoult D. **Evaluation of antibiotic susceptibilities of *Ehrlichia canis*, *Ehrlichia chaffeensis*, and *Anaplasma phagocytophilum* by real-time PCR.** *Antimicrob Agents Chemother* 2004; 48: 4822–4828.

117 McGrotty YL and Knottenbelt CM. **Oesophageal stricture in a cat due to oral administration of tetracyclines.** *J Small Anim Pract* 2002; 43: 221–223.

118 Yancey CB, Diniz P, Breitschwerdt EB, et al. **Doxycycline treatment efficacy in dogs with naturally occurring *Anaplasma phagocytophilum infection*.** *J Small Anim Pract* 2018; 59: 286–293.

119 Sainz A, Roura X, Miro G, et al. **Guideline for veterinary practitioners on canine ehrlichiosis and anaplasmosis in Europe.** *Parasit Vectors* 2015; 8: 75. DOI: 10.1186/s13071-015-0649.0.

120 Halos L, Baneth G, Beugnet F, et al. **Defining the concept of 'tick repellency' in veterinary medicine.** *Parasitology* 2012; 139: 419–423. DOI: 10.1017/50031182011002228.