Epidemiological and Clinicopathological Features of *Anaplasma phagocytophilum* Infection in Dogs: A Systematic Review

Sarah El Hamiani Khatat[1]*, Sylvie Daminet[2], Luc Duchateau[3], Latifa Elhachimi[4], Malika Kachani[5] and Hamid Sahibi[4]

[1] Department of Medicine, Surgery and Reproduction, Hassan II Institute of Agronomy and Veterinary Medicine, Rabat, Morocco, [2] Department of Companion Animals, Faculty of Veterinary Medicine, Ghent University, Ghent, Belgium, [3] Department of Comparative Physiology and Biometrics, Faculty of Veterinary Medicine, Ghent University, Ghent, Belgium, [4] Department of Pathology and Veterinary Public Health, Unit of Parasitology, Hassan II Institute of Agronomy and Veterinary Medicine, Rabat, Morocco, [5] College of Veterinary Medicine, Western University of Health Sciences, Pomona, CA, United States

Anaplasma phagocytophilum is a worldwide emerging zoonotic tick-borne pathogen transmitted by *Ixodid* ticks and naturally maintained in complex and incompletely assessed enzootic cycles. Several studies have demonstrated an extensive genetic variability with variable host tropisms and pathogenicity. However, the relationship between genetic diversity and modified pathogenicity is not yet understood. Because of their proximity to humans, dogs are potential sentinels for the transmission of vector-borne pathogens. Furthermore, the strong molecular similarity between human and canine isolates of *A. phagocytophilum* in Europe and the USA and the positive association in the distribution of human and canine cases in the USA emphasizes the epidemiological role of dogs. *Anaplasma phagocytophilum* infects and survives within neutrophils by disregulating neutrophil functions and evading specific immune responses. Moreover, the complex interaction between the bacterium and the infected host immune system contribute to induce inflammatory injuries. Canine granulocytic anaplasmosis is an acute febrile illness characterized by lethargy, inappetence, weight loss and musculoskeletal pain. Hematological and biochemistry profile modifications associated with this disease are unspecific and include thrombocytopenia, anemia, morulae within neutrophils and increased liver enzymes activity. Coinfections with other tick-borne pathogens (TBPs) may occur, especially with *Borrelia burgdorferi*, complicating the clinical presentation, diagnosis and response to treatment. Although clinical studies have been published in dogs, it remains unclear if several clinical signs and clinicopathological abnormalities can be related to this infection.

Keywords: canine granulocytic anaplasmosis, *Anaplasma phagocytophilum*, dogs, epidemiology, tick-borne disease, zoonosis

INTRODUCTION

Canine granulocytic anaplasmosis (CGA) is an emerging zoonotic tick-borne disease that is distributed worldwide. The causative agent, *Anaplasma phagocytophilum*, is an obligate intracellular gram-negative alpha-proteobacterium that develops within granulocytic cells. It is usually transmitted by ticks belonging to the genus *Ixodes* and it causes disease in several mammalian species (1, 2). In the USA, both canine and human exposures have progressively increased from 2008 to 2010 with the number of reported human cases increasing by 53% during this period (3, 4). Data from the USA Center for Disease Control and Prevention (4) and Morbidity and Mortality Weekly Report (MMWR) reported 36,342 human cases between 2010 and 2018 and almost a 12-fold increase during this same period (4). Currently, human granulocytic anaplasmosis (HGA), is considered amongst the three most important vector-borne disease (VBD) in the USA with Lyme borreliosis and Zika virus (5, 6) and is increasingly being diagnosed in several European and Asian countries (7, 8).

The focus on canine VBDs has increased the past decade as they represent an important threat to both canine and human health (9). Because of their proximity to humans, dogs may serve as reservoirs of vector-borne pathogens, a source of infection for vectors, mechanical transporters of infected vectors, and as sentinel indicators of regional infection risk (2, 3, 10–15). Furthermore, the strong molecular similarity between human and canine isolates of *A. phagocytophilum* in Europe and the USA (16–21) and the positive association in the distributions of human and canine cases in the USA emphasizes the use for dogs as sentinels in epidemiological studies (3, 4, 9, 15, 22, 23).

The lack of specific clinicopathological signs, the frequent rapid evolution and positive prognosis even without treatment, the prompt response to a commonly used antibiotic and the possibility of coinfections (24–28) all make the diagnosis of CGA challenging for veterinarians. Description of signs and laboratory abnormalities associated with *A. phagocytophilum* infection in dogs is mostly available from Europe and North America. Although some studies have described the most common manifestations of CGA (13, 24–35), it remains unclear if some clinical signs and clinicopathological abnormalities are related to this infection. In this paper, we provide an overview of the current knowledge on the worldwide epidemiological features of *A. phagocytophilum* focusing on dogs, and describe the clinicopathological aspects of CGA with an emphasis on missing data.

DESCRIPTION OF *ANAPLASMA PHAGOCYTOPHILUM*

Classification

Anaplasma phagocytophilum is a bacterium belonging to the family of Anaplasmataceae in the order of Rickettsiales (36). The phylogenetic molecular analysis based on the 16S rRNA and the groEL genes sequencing in addition to morphologic and phenotypic characteristics have led to the reorganization of the family of Anaplasmataceae and the reclassification of some agents. Consequently, the name *A. phagocytophilum* was given in 2001 to three previously distinct agents, i.e., the agent that causes equine granulocytic anaplasmosis (*Ehrlichia equi*), the agent that causes tick-borne fever or pasture fever in sheep and cattle (*Ehrlichia phagocytophila*) and the agent that causes HGA [formerly human granulocytic ehrlichiosis (HGE)] (1). The renaming of these three agents as *A. phagocytophilum* has been controversial because of differences in their host tropism and cell target from other *Anaplasma* species, such as *Anaplasma marginale* (37). Additionally, although these three agents share genetic, antigenic and biological characteristics (1), they are considered phenotypic variants due to differences in their distribution, prevalence, virulence and target host species (38, 39).

Morphology and Genome

Anaplasma phagocytophilum typically exhibits coccoid to ellipsoid shapes measuring ∼0.2–2.0 μm in diameter. The bacteria infect myeloid cells primarily neutrophils (and occasionally eosinophils), forming intracytoplasmic inclusions derived from the host cell membrane measuring 1.5–2.5 μm, called "morula" (from Latin "morum": mulberry) (1, 40).

The *A. phagocytophilum* genome is composed of a single circular double-stranded chromosome. The complete genomic sequence is estimated at 1.47 megabases (Mb) and was published on GenBank in 2006 (NC007797) (19, 36). Despite its apparently simple genome, *A. phagocytophilum* exhibits an extensive genomic diversity (19, 41, 42). More than 500 partial *A. phagocytophilum* pseudogene sequences derived from human, ticks and animals from several US, European and Asian regions are available in GenBank (19). Moreover, twenty complete *A. phagocytophilum* genomes have been sequenced including 16 American and four European strains. However, genomes from only a few different strains per host species are available (aside from humans), underscoring the lack of information on strain diversity within different host species (19, 36).

Genetic Variability

Genetic variability between strains may explain the ecological complexity, the host tropism diversity, the differences in incidence and clinical presentation, severity and evolution of the disease documented in different countries (42–47). Many studies demonstrated different virulence and hosts tropism of specific *A. phagocytophilum* strains (17, 41–50). However, the host specificity of strains seems to be restricted and multiple infections with different strains are often observed. Farm and large wild animals, small mammals and ticks were especially prone to carrying multiple genetic variants. In humans and domestic animals double infections are not so frequent (51). The 16S rRNA gene nucleotide sequences analysis discriminated 15 worldwide variants differing in a variable fragment located near the 5' end of the gene. Among them, two are pathogenic for human and abundant all over the world (52). In the USA, several variants have been identified based on the sequencing of the 16S rRNA and the only pathogenic variant to humans (AP-ha) is also able to induce the disease in dogs, horses and mice but not in cattle. In Europe, other variants have been identified in

humans and the AP-ha variant was also detected in wild ruminant species (41–43, 48, 49). Strains infecting domestic ruminants in Europe and white-tailed deer in the USA seem to genetically differ from those infecting humans, horses and dogs (44, 50). In Washington State, five different 16S rRNA variants (named WA1–5) that differed at four nucleotide positions were identified from dogs displaying clinical signs consistent with CGA. All WA variants were distinct from those identified in sheep in Norway and llama-associated ticks but one was identical to equine and human variants (24). In another European study, seven different 16S rRNA variants were identified from dogs, with the two most common variants showing statistically significant differences in the frequency of clinical signs and hematological abnormalities, which suggests possible differences in strain pathogenicity (45). Finally, a recent study showed that dogs can be naturally infected concurrently with *A. phagocytophilum* variant 1, variant 4, and HGE agent (53). The pathogenic role of the classic sheep variant, *A. phagocytophilum* variant 1, in the canine species is uncertain. Previous studies showed that the "HGA agent" appears to be more pathogenic for dogs than other variants (45).

The 16S rRNA gene was considered too conserved for use in the phylogenetic analysis of different strains of *A. phagocytophilum*. It has a poor resolution and failed to discriminate between ecotypes circulating in wild ruminants compared to other animals. Furthermore, the 16S rRNA sequence analysis could not categorize human-infective isolates in order to detect virulent strains and was unable to distinguish variants according to their geographic origin (43, 54–56). As such, other genes have been proposed to study the genetic variability of *A. phagocytophilum* including msp4, ankA, groEL operon, msp2/p44, pfam01617 superfamily, and drhm genes (19–21, 50, 56–59). Sequencing different genes revealed similarities between human and canine isolates, suggesting that dogs and humans may be infected by the same strains (16–21, 24, 45, 53, 60–62).

VECTORS

Although several transmission modes have been reported (mostly in humans) (63–66), *A. phagocytophilum* is commonly transmitted to people and domestic animals through tick bites (67). It is naturally maintained in complex and poorly understood enzootic tick-wild animal cycles (55, 59, 68) and is transmitted most frequently by ticks of the *Ixodes persulcatus* complex. These ticks are commonly found in the northern hemisphere and their occurrence depends on climatic conditions (between 10 and 30°C, and >80% relative humidity) and the availability of hosts (49, 69).

In the USA, several ixodid ticks transmit this pathogen, depending on the geographic location. The main vector in the humid forests of the upper midwestern, north central and northeastern regions is *Ixodes scapularis* whereas *Ixodes pacificus* is located in shrub forests and deserts of the western USA (70–72). The prevalence of *A. phagocytophilum* DNA among ticks varies from <1% up to 50% throughout the country (73–76). Other tick species have been reported to be infected with *A. phagocytophilum*, such as *Amblyomma americanum* and *Dermacentor* spp., and *Ixodes spinipalpis* and *Ixodes dentatus* are

recognized as competent vectors (77–81). Other *Ixodes* species including *Ixodes angustus*, *Ixodes ochotonae*, and *Ixodes woodi* are suggested to act as vectors for the bacterium (82, 83). In central and southern America, very few studies are published on the prevalence of *A. phagocytophilum* among ticks. However, among the three available studies, none have detected the DNA of this bacterium in *Ixodes* spp. ticks. In contrast, its DNA has been amplified from *Rhipicephalus sanguineus*, *Amblyomma cajennense*, *Amblyomma dissimile*, *Amblyomma maculatum*, *Dermacentor variabilis* (84–86). *Amblyomma* spp. and *D. variabilis* were positively correlated with *A. phagocytophilum* infection in Brazil and Mexico (84, 86).

In Europe, the most common vector is *Ixodes ricinus* (69), which is widely distributed from western Europe to central Asia. This tick lives mostly in humid wooded habitats and pastures and is rarely encountered in the Mediterranean region or in mixed or deciduous forests except at high altitudes (67). The prevalence of *A. phagocytophilum* DNA among *I. ricinus* ticks in Europe varies from <1 to 76.7% (87, 88). Other *Ixodes* spp. ticks seem to be involved in epidemiological cycles that are distinct from those involving *I. ricinus* (55, 89, 90). In addition, the DNA of this bacterium has been detected in several other tick species in Europe including *Dermacentor reticulatus*, *Haemaphysalis concinna*, *Hyalomma marginatum*, *Ixodes ventaloi*, and *Ixodes trianguliceps* (58, 91–95). *Rhipicephalus* species were also infected by *A. phagocytophilum* and could act as competent vectors in the eastern Mediterranean area (96–99). *Ixodes persulcatus* is another competent vector of *A. phagocytophilum* in eastern Europe and Asia, with rates of DNA detection up to 16.7 and 21.6%, respectively (100, 101).

Although *I. persulcatus* is considered the primary vector in Asia, *A. phagocytophilum* DNA has been detected in several other tick species including *Ixodes nipponensis*, *Ixodes ovatus*, *Rhipicephalus turanicus*, *Rhipicephalus haemaphysaloides*, *H. marginatum*, *Boophilus kohlsi*, *Dermacentor silvarum*, and several *Haemaphysalis* species (96, 102–106). Molecular investigations indicated that *I. ovatus*, *Dermacentor silvarum*, *Hae. concinna*, *Haemaphysalis longicornis*, *Rhipicephalus microplus*, *R. sanguineus*, and *Dermacentor nuttalli* might be involved in the transmission of *A. phagocytophilum* in China (8, 107–109).

In North Africa, one study in Morocco and Tunisia detected *A. phagocytophilum* DNA in 1 and 3% of *I. ricinus* and *Hyalomma detritum*, respectively (110). Two separate studies detected DNA in *R. sanguineus* from free-roaming dogs in Egypt and *H. marginatum* from horses in Tunisia with prevalence rates of 13.7 and 2.3%, respectively (111, 112). These studies indicate that *A. phagocytophilum* is likely to circulate in a wide variety of ticks, but their involvement in transmitting the bacterium to host has yet to be established (112).

DISTRIBUTION AND PREVALENCE OF *ANAPLASMA PHAGOCYTOPHILUM*

Anaplasma phagocytophilum has a worldwide distribution and endemic areas include some regions of the USA (northeastern and mid-Atlantic, Upper Midwest, and Pacific Northwest states),

Europe and Asia (China, Siberian Russia, and Korea). These regions correspond to occurrence areas of *I. persulcatus* group ticks (12, 13, 24, 29, 113). Several prevalence studies in dogs have been conducted in various American, European, Asian and African countries (**Table 1**). However, data are lacking in large parts of Asia, Africa, South America and Australia. The geographic variation in tick exposure, the differences in inclusion criteria to select dog populations, and the use of different serologic tests [i.e., immunofluorescent antibody test (IFAT), enzyme-linked immunosorbent assay (ELISA) or Western blot] make comparison between studies difficult (234, 235). In addition, cross-reactivity with the most important other *Anaplasma* species infecting dogs, i.e., *Anaplasma platys,* is reported to occur for both IFA and ELISA (1, 9, 120, 121, 236–241). Therefore, in regions where both pathogens could be present (southern USA states, southern Europe, South America, Asia, and Africa), seropositivity may not necessarily reflect exposure to *A. phagocytophilum* and potential overestimation of the true prevalence and distribution can occur (9, 162, 189, 198, 234, 236, 238, 241). As a result, PCR-based assays are necessary to determine which of the two agents is responsible for positive serologic test results in regions where both bacteria are present (241). In areas where the *Ixodes* tick vector is less prevalent or absent, a positive *Anaplasma* spp. serologic result could be the result of *A. platys* exposure (164). Less frequent and minor serological cross-reactions were described at low titers between *A. phagocytophilm* and *Ehrlichia* species (i.e., *Ehrlichia canis, Ehrlichia chaffeensis, Ehrlichia ewingii,* and *Neorickettsis sennetsu formerly Ehrlichia sonnetsu*), especially with hyperimmune sera, when using IFA and immunoblot assay (1, 29, 39, 121, 127, 242, 243). However, it is not clear whether the cross-reactivity with *E. canis* was attributable, in part, to antibodies against *A. platys* because dogs are sometimes exposed to both *E. canis* and *A. platys* (164, 240). In contrast, no cross-reactivity has been documented between *Anaplasma* spp. and *Ehrlichia* spp. when using the point-of-care dot ELISA (234, 240).

The first CGA cases in the USA were detected in California; then, the exposure of dogs to this organism has been recorded in more than 39 USA states and highest rates were noted in the upper Midwestern, northeastern and western states. Serologic surveys revealed prevalence values of *Anaplasma* spp. antibodies ranging from 0.0 to 40.0% (3, 9, 10, 12, 13, 15, 23, 119, 120, 122–126, 236, 242, 244, 245). Five countrywide serologic studies showed an overall prevalence of *Anaplasma* spp. of 1.9 to 4.8% with the highest rates recorded in northeastern regions (3, 9, 15, 23, 119). The study that found a prevalence rate of 1.9%, used species-specific peptides to detect canine antibodies to *A. phagocytophilum* (3). In addition, cases confirmed by PCR were diagnosed in several USA states (12, 13, 24, 26, 27, 29, 124, 245–247). In the USA, over 100,000 and 220,000 dogs were seropositive to *Anaplasma* spp. in 2015 and 2019, respectively (248, 249). Two recent studies analyzing regional trends of *Anaplasma* spp. exposure in dogs showed that seroprevalence increased broadly in the northeastern, upper midwestern states, northern California, mid-atlantic coast and southern Oregon (249, 250). In Canada, six serologic surveys on *Anaplasma* spp. are available (**Table 1**) (3, 114–118), and six cases of CGA from

Vancouver Island (251), Saskatoon (252) and Montreal (253) were confirmed by DNA detection. In Latin America and the Caribbean, the seroprevalence of *Anaplasma* spp. ranges from 1.0 to 53.2% (**Table 1**) (133, 134, 254). In addition, two studies and a case report have detected the DNA of *A. phagocytophilum* in Brazil (**Table 1**) (129, 255).

In Europe, *Anaplasma* spp. seroprevalence has been reported in almost all countries with rates ranging from 1.1 to 56.5% (143, 148, 150, 183, 190). The detection of *A. phagocytophilum* DNA has also been reported mostly from central and northern countries (**Table 1**) with prevalence rates up to 14.2% (174). Additionally, several cases of CGA have been described (25, 28, 30–32, 34, 256–262).

In Asia, *Anaplasma* spp. seroprevalence is available from China, Korea, Malaysia, Taiwan and Israel and range from 1.2 to 24.7% (**Table 1**) (212, 214). *Anaplasma phagocytophilum* DNA has also been detected in dogs with prevalence rates up to 39.5 and 57.3% in Jordan and Iran, respectively (219, 222).

In Africa, only a few prevalence studies have been published on *Anaplasma* spp. in dogs (**Table 1**). Seroprevalence rates recorded in African countries range from 11.8 to 47.7% (**Table 1**) (225, 231). Similarly, very limited studies have investigated *A. phagocytophilum* infection in dogs in this continent. The DNA of this bacterium has been detected in Tunisia, Nigeria, Cape Verde and South Africa (**Table 1**) (224, 228, 229, 232) but not in Algeria and Morocco (225, 227). In addition, an *Anaplasma* species closely related to *A. phagocytophilum* was detected in blood samples from South African dogs based on 16S rRNA gene sequencing (263) whereas all dogs from Algeria, Ghana and Maio Island tested negative by PCR (**Table 1**) (225, 231, 233).

EPIDEMIOLOGICAL ROLE OF DOGS

Several wild and domestic animals are receptive to *A. phagocytophilum* infection. However, the disease has been reported only in a few species including domestic ruminants, horses, cats, dogs and humans (22, 24, 63, 264–269). Although dogs are susceptible to *A. phagocytophilum* infection, they are mostly recognized as incidental hosts and their role as potential reservoirs is still controversial (24, 270). As *A. phagocytophilum* is an obligate intracellular bacterium, its reservoirs should be animal hosts permitting its survival, particularly outside the activity period of its vectors (271). To be considered as a host reservoir, a host must be fed on by an infected vector tick at least occasionally, take up a critical number of the infectious agent during the bite by an infected tick, allow the pathogen to multiply and survive for a period in at least some parts of the body, and allow the pathogen to find its way into other feeding ticks (272, 273). Therefore, the detection of pathogens or their DNA in animal hosts is not enough to consider them as reservoir hosts (274).

Dogs are considered unlikely reservoir hosts due to the probable short duration of bacteremia (<28 days) and uncertainty regarding their ability to host enough nymphal tick stages to contribute to the spread of the bacterium (2, 67). In Austria, no significant difference in the seroprevalence of

TABLE 1 | Prevalence of *Anaplasma* spp. (*A. phagocytophilum* and *A. platys*) antibodies and/or DNA detection of *A. phagocytophilum* in blood samples from dogs in several countries.

Countries	Number of dogs	Type of dog population	Prevalence (%)	Method	References
AMERICA					
Canada	86,251	Sick and healthy dogs from 238 practices	0.2	ELISA	(114)
	115,636	Not stated	0.29	ELISA	(115)
	753,468	Not stated	0.4	ELISA	(116)
7 provinces	285	Not stated	1.1	ELISA	(3)
South Ontario, Quebec	53	Suspected to have TBD	0.0	IFA	(117)
Saskatchewan	515	Sick and healthy client-owned dogs	0.6	ELISA	(118)
USA	3,950,852	Not stated	3.8	ELISA	(15)
	3,588,477	Sick and healthy dogs tested for VBD	4.4	ELISA	(23)
	479,640	Dogs suspected to have a VBD	4.8	ELISA	(9)
	14,496	Dogs suspected to have a VBD	1.9	ELISA	(119)
	6,268	Dogs suspected to have a VBD	1.5–3.5	ELISA	(3)
Oregon, California	2,431	Clinically healthy dogs	2.4	ELISA	(120)
North Carolina, Virginia	1,845	Dogs admitted regardless of the reason for examination to the NCSU-VTH	1.1	IFA	(121)
Maine	1,087	Dogs tested for heartworm or undergoing surgery	7.1	ELISA	(122)
California	1,385	Non-ehrlichial related illnesses or well-animal care	8.7	IFA	(10)
	184	Rural dogs with or without clinical signs	40.0 7.6	IFA PCR	(12)
Minnesota	731	Sick and healthy pet dogs	29.0	ELISA	(13)
	273		9.5	PCR	
Oklahoma	259	Dogs suspected to have a VBD	33.0	IFA	(123)
Northern Arizona	233	Pet and stray dogs	11.6 0.0	ELISA PCR	(124)
New Jersey	202	Healthy dogs	9.4	ELISA	(125)
North Carolina	118	Clinically healthy dogs	0.0	ELISA	(126)
Connecticut, New York	106	Sick client-owned dogs living	9.4	IFA, WB	(127)
Cumberland Gap Region	232	Shelter dogs	0.9	ELISA	(128)
Brazil					
Rio de Janeiro	398	Not stated	6.0	PCR	(84)
	253	Not stated	7.1	PCR	(129)
Southeastern	198	Dogs suspected to have TBD	0.0	PCR	(130)
Southern	196	Companion dogs	9.7	ELISA	(131)
Central-northern Parana	138	Rural and urban dogs	13.8	ELISA	(132)
Puerto Rico	629	Dogs from shelters and a veterinary clinic	1.0	ELISA	(133)
Colombia	498	Not stated (abstract only)	33.0	ELISA	(133)
	218	Working, shelter and client-owned dogs	53.2	ELISA	(134)
Haiti	210	Owned dogs	17.6 0.0	ELISA PCR	(135)
West Indies	157	Not stated	10.8	ICG	(136)
Caribbean region	29	Not stated	10.0	ELISA	(3)
Mexico	1,706	Healthy dogs and dogs with clinical signs compatible with VBD	9.9	ELISA	(137)
Costa Rica	408	Apparently healthy dogs	2.7	IFA	(138)
	374		0.3	PCR	
Ecuador Galapagos Islands	58	Without consideration of the patients' presenting complaint	12.1	ELISA	(139)
Nicaragua	329	Dogs presented at veterinary clinics	28.6 2.2	ELISA PCR	(140)
Chile	905	Urban and rural dogs	44.0	IFA	(141)
EUROPE					
Germany	5,881	Sick dogs suspected to have anaplasmosis	21.5	ELISA	(142)

(Continued)

TABLE 1 | Continued

Countries	Number of dogs	Type of dog population	Prevalence (%)	Method	References
	1,124	Dogs suspected to have anaplasmosis	50.1	IFA	(143)
	522	Healthy dogs and dogs suspected of CGA	43.0	IFA	(144)
			5.7	PCR	
	111	Healthy dogs and dogs suspected of CGA	43.2	IFA	(31)
			6.3	PCR	
	1,862	Traveling dogs to Germany	17.8	IFA	(145)
	792	Retrospective analysis of serum sample	41.9	IFA	(146)
Munich	448	Healthy and sick dogs	19.4	ELISA	(147)
	171	Healthy Bernese Mountain Dogs	50.3	IFA	(148)
	57	Healthy dogs from other breeds	24.6		
Brandenburg	1,023	Blood samples from veterinary clinics or a commercial diagnostic laboratory	1.5	PCR	(149)
Russia					
European part	440	Urban dogs with a history of tick bites	1.1	ELISA	(150)
Voronezh Reserve	82	Dogs owned by Voronezh Reserve staff	34.1	ELISA	
Hungary	1,305	Healthy pet dogs	7.9	ELISA	(151)
Southern Hungary	126	Shepherd, hunting and stray dogs	11.0	PCR	(152)
Slovakia	87	Dogs suspected to have babesiosis	8.0	PCR	(153)
		Dogs randomly selected	11.7	ELISA	(154)
Bulgaria					
Central-southern	167	Dogs presented for various clinical reasons	19.2	IFA	(155)
Austria	1,470		56.5	IFA	(156)
United Kingdom	120	Dogs suspected to have TBD	0.8	PCR	(157)
Sweden	611	Dogs not clinically suspected to be infected by *Ehrlichia* spp or *B. burgdorferi* sensu lato	17.7	IFA	(158)
	100	Not stated	17.0	IFA	(159)
Finland	340	Pet dogs with or without clinical signs of illness	5.3	ELISA	(160)
	50	Healthy hunting dogs	4.0		
Albania	30	Clinically healthy semi-domesticated dogs	40	IFA	(161)
Tirana	602	Client-owned dogs	24.1	IFA	(162)
			1.0	PCR	
Latvia	470	Healthy dogs and dogs suspected to have borreliosis and/or anaplasmosis	11.4	ELISA	(163)
Romania	1,146	Guard, pet, shelter, stray and hunting dogs	5.5	ELISA	(164)
	29	Pet and stray dogs from Romania	7.4	IFA	(165)
	109	Dogs imported from Romania to Germany	2.2	PCR	
Eight counties	357	Not stated	5.3	PCR	(166)
Southeastern	257	Not stated	6.2	PCR	(167)
South Central	149	Asymptomatic shelter dogs	3.3	ELISA	(168)
Serbia					
Vojvodina province	84	Randomly selected dogs	15.5	IFA	(169)
Belgrade municipalities	111	Shelter, free-roaming and hunting dogs	28.8	ELISA	(170)
			0.0	PCR	
Poland	3,094	Healthy dogs with a history of tick bite	12.3	ELISA	(171)
Eastern	400	Healthy dogs	8.0	ELISA	(172)
			2.8	PCR	(173)
Northwestern	192	Dogs from endemic regions of borreliosis	1.0	PCR	(174)
	100	Healthy dogs from a shelter	4.0	PCR	
	92	Dogs suspected to have Lyme disease	14.0	PCR	(175)
	50	Dogs diagnosed with babesiosis	0.0		
	79	Apparently healthy sled dogs	1.3		

(Continued)

TABLE 1 | Continued

Countries	Number of dogs	Type of dog population	Prevalence (%)	Method	References
Czech Republic	296	Healthy dos and dogs suspected to have TBD	3.4	PCR	(176)
			26.0	IFA	
Italy					
Stretto di Messina	249	Outdoor-kennel dogs Not stated	38.0	IFA	(177)
	5,881		32.8	IFA	
Central Italy	1,965	Urban and rural dogs without signs of TBD	4.7	IFA	(178)
	1,232	Not stated	8.8	IFA	(179)
	215	Hunting dogs	14.8	IFA	(180)
			0.9	PCR	
	1,026	Owned dogs	3.3	IFA	(181)
Sicily	344	Pet, pound and hunting dogs	0.0	PCR	(182)
	87	Not stated	44.8	IFA	(183)
			0.0	PCR	
	372	Not stated	4.8	PCR	(184)
Southern	165	Dogs with febrile illness and healthy controls	37.8	IFA	(185)
			0.0	PCR	
Northeastern	488	Privately-owned canine blood donors and free-roaming dogs	3.3	IFA	(186)
			0.0	PCR	
Sardinia	50	Dogs suspected of tick bite–related fever	6.0	PCR	(187)
Portugal	1,185	Healthy dogs and dogs suspected to have VBD	4.5	ELISA	(188)
	55	Dogs suspected to have TBD	54.5	IFA	(189)
			0.0	PCR	
	55	Dogs suspected to have TBD	55.0	IFA	(190)
	100	Apparently healthy military dogs	16.0	IFA	(191)
France	919	Not stated	2.7	ELISA	(192)
Spain	466	Sick and healthy dogs	11.5	IFA	(11)
Nothwestern	1,100	Dogs presented to veterinary clinics	3.1	ELISA	(193)
	479		5.0	IFA	(194)
Northern	556	Healthy dogs and dogs with signs compatible with VBDs	1.26	ELISA	(195)
Central	131	Shelter dogs	19.0	ELISA	(196)
Turkey	757	Stray, shelter and pet dogs	0.5	PCR	(197)
Thrace region	400	Healthy shelter dogs	6.0	PCR	(198)
Croatia	1,080	Apparently healthy dogs	0.3	PCR	(199)
	435	Apparently healthy owned and shelter dogs	6.21	ELISA	(200)
Greece	200	Owned and shelter dogs	1.0	ELISA	(201)
			0.5	PCR	
ASIA					
Japan	154	Sick and healthy dogs	0.0	PCR	(202)
	332	Dogs presented at 6 private veterinary clinics in Ibaraki Prefecture	2.1	IFA	(203)
			0.3	PCR	
China	600	Companion, working and shelter dogs	0.5	ELISA	(204)
	234	Stray and pet dogs	13.2	PCR	(205)
	219	Dogs from rural areas	10.0	IFA	(107)
			10.9	PCR	
	26	Dogs from rural areas	7.7	ELISA	(206)
			50	IFA	
	562	Dogs presented for reasons unrelated to suspicion of VBD	2.7	ELISA	(207)
	637	Apparently healthy indoor and breeding dogs	1.4	ELISA	(208)
	201	Apparently healthy stray dogs	11.9	PCR	(209)
Korea	1,058	Shelter dogs	0.1	PCR	(210)
	532	Outdoor dogs	15.6	ELISA	(211)
			2.3	PCR	
	418	Shelter dogs	1.2	ELISA	(212)

(Continued)

Countries	Number of dogs	Type of dog population	Prevalence (%)	Method	References
	229	Urban shelter dogs and rural hunting dogs	18.8	ELISA	(213)
	245	Blood samples from military dogs	4.4 0.0	IFA PCR	(214)
Malaysia	48	Stray dogs	9.3 4.3	ELISA PCR	(215)
India	191	Pets, stray and working dogs	4.7	ELISA	(216)
	230	stray dogs in Tamil Nadu	0.4	PCR	(217)
Israel	195	Healthy pet dogs, stray and shelter dogs	9.0	IFA	(218)
Jordan	38	Stray dogs	39.5	PCR	(219)
	161	Stray, police, or breeding with tick infestation	9.9	ELISA	(220)
Taiwan	175	Asymptomatic dogs	21.1 0.0	ELISA PCR	(221)
Iran	103	Apparently healthy rural dogs	57.3	PCR	(222)
	150	Owned and stray dogs from Tehran	2.0	PCR	(223)
AFRICA					
Tunisia	286	Healthy and sick pet, kenneled dogs	25.2 0.9	IFA PCR	(224)
Algeria					
Algiers	150	Owned dogs admitted for surgery or vaccination	47.7	IFA	(225)
	63	Stray dogs from a shelter	0.0	PCR	
Morocco	217	Owned urban, rural and military healthy dogs or displaying signs of VBD	16.6	ELISA	(226)
Northwestern	425	Owned urban, rural and military healthy dogs or displaying signs of TBD	21.9 0.0	ELISA PCR	(227)
Nigeria	245	Healthy and sick dogs	0.8	PCR	(228)
South Africa	141	Apparently healthy owned and free roaming dogs	2.1	PCR	(229)
	56	Apparently healthy domestic dogs	0.3	PCR	(230)
Ghana	17	Client-owned dogs presented for a variety of complaints or for vaccination	11.8 0.0	ELISA PCR	(231)
Cape Verde					
Priai	57		1.8	PCR	(232)
Mayo Island	153	Apparently healthy dogs	0.0	PCR	(233)

TBD, tick-borne disease; VBD, vector-borne disease; IFA, immunofluorescence assay; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction, WB, western blot; ICG, immunochromatography.

A. phagocytophilum among owners of seropositive pets and owners without pets was observed, suggesting that pets are not a source of infection for humans (275). However, wild and domestic carnivores are considered the primary source of tick-borne zoonotic agents to humans (276) and contact with pet cats and dogs has been proposed as a risk factor for tick exposure and tick-borne disease among humans (277, 278). Moreover, according to some authors, almost all studies investigating the role of dogs in the transmission of tick-borne diseases (TBDs) focused on companion dogs. These animals are usually treated for ectoparasites, have limited free access to the outdoors and host reservoir habitats, and are less exposed to ticks compared with hunting, stray or shelter dogs. Therefore, these studies may not accurately reflect the public health risk associated with dogs in endemic areas (152). Others suggested that domestic animals including dogs could be considered as reservoir hosts of *A. phagocytophilum* in Europe especially in urban areas (18, 270, 279–282). In a study from Hungary, the prevalence of *A. phagocytophilum* DNA in stray dogs was higher than in several studies from other European countries (152). In addition, two studies reported high prevalence rates of *A. phagocytophilum* DNA in dogs suspected to have Lyme disease and rural dogs from Poland and China, respectively (107, 174). *Anaplasma phagocytophilum* was also the most frequently detected bacterium by PCR in stray dogs that lived in close contact with domestic animals and humans in rural and peri-urban areas of the Mediterranean zone of Jordan (219). In addition, high prevalence rates of *A. phagocytophilum* DNA was found in *I. ricinus* collected from dogs in Belgium and Poland, and *R. sanguineus* (adult and nymphs) from free-roaming dogs in Egypt (111, 280, 283). Moreover, *A. phagocytophilum* DNA was detected in experimentally infected dogs during 60 days without immunosuppressive drug, and the canine immune response seems to have evolved to only partially control

infection, suggesting a longer bacteremia possibly allowing timely transmission to the vector (18, 284). Based on these results, dogs could act as potential reservoir for the bacterium at least in some regions, but further studies are needed.

The geographical distribution of canine infection seems to parallel the distribution of HGA in the USA with a positive association of human and canine cases in many states (3, 23). Indeed, several studies found the highest prevalence rates of *A. phagocytophilum* antibodies in dogs from the upper midwest, northeast, and mid-atlantic, which correlate with areas where the highest incidence of human anaplasmosis were reported (3, 4, 9, 15, 22, 23). In addition, the estimated regression coefficient for the endemic risk factor in the contiguous USA model was positive and significant. This implies a higher prevalence among dogs living in areas where HGA is endemic (15). Furthermore, a study has evaluated regional and local temporal trends of canine *Anaplasma* spp. (*A. phagocytophilum* and *A. platys*) exposure using a Bayesian spatio-temporal binomial regression model for analyzing serologic test results. In this study, similarity was found between temporal trends in canine *Anaplasma* spp. seroprevalence and the reported incidence rate of HGA (249). Finally, human and canine strains of *A. phagocytophilum* were similar according to several gene sequencing studies, and human isolates have been reported to induce clinical disease in dogs in both Europe and the USA (16–21). Therefore, in addition to the possible role of dogs as potential reservoir hosts, the prevalence data of *A. phagocytophilum* infection in dogs provides important information on the incidence, risk factors, exposure sources, and real-time risk of exposure for human infection (3). More generally, several studies have documented the utility of using dogs as sentinels for human vector-borne diseases (VBDs) (14, 17, 18).

PATHOGENESIS OF *ANAPLASMA PHAGOCYTOPHILUM* INFECTION

Anaplasma phagocytophilum is transmitted by ticks to their hosts within 24–48 h of feeding time (285–288) but establishment of infections in dogs is apparently dependent on a minimum inoculation dose (288). Bacteremia, however, develops 4–7 days after the tick bite during natural infection or 3–4 days after experimental blood inoculation, suggesting that the bacterium remains at undetectable levels in the blood or replicates in other cells in the early stages of infection (69). Cell surface analysis suggested that the endothelial cells of the microvasculature provide an excellent site for *A. phagocytophilum* dissemination to peripheral blood granulocytes. Endothelial cells may play a crucial role in the development of persistent infections and are stimulated to express surface molecules and cytokines in a dose-dependent manner that may lead to inflammatory responses at the site of infection (289). After inoculation, *A. phagocytophilum* exhibits a biphasic developmental cycle in which the infectious small dense-cored cells bind to host cellular targets and enter the cytoplasm of neutrophils by endocytosis. After, the non-infectious reticulate cells multiply by binary fission within phagosomes until forming morulae. After 28–32 h, replication

ceases and reticulate cells re-transition to dense-cored cells that are released after cell lysis to initiate the next wave of infection and possibly spread to multiple organs (40, 290, 291).

Anaplasma phagocytophilum has several strategies to dysregulate the bactericidal functions of neutrophils and ensure its survival and replication. This bacterium regulates host defense and antimicrobial mechanisms by a direct interaction with specific gene regulatory regions in the nucleus of the neutrophil, decreasing endothelial adherence, mobility, transmigration, phagocytic activity, and degranulation. It can also alter the respiratory and oxidative burst mechanism of neutrophils, delay apoptosis and increase the inflammatory recruitment of new neutrophils (289, 292–297). In addition, the antigenic variation of the immunodominant surface proteins msp2/p44 enables the bacterium to evade the specific immune response and to subvert the adaptive immune response (297). Neutrophils circulate for 10–12 h before they enter tissues and undergo apoptosis, which may lead to the destruction of the pathogens. Therefore, the decreased endothelial adherence and delayed apoptosis both enhance the bacterial survival and the replication to form morulae in a normally short-lived, terminally differentiated granulocytic cell. Furthermore, the impaired neutrophil function can result in an immune deficiency, predisposing patients to opportunistic infections (293–295). *Anaplasma phagocytophilum* was suggested to possibly manipulate the host endoplasmic reticulum stress signals to facilitate intracellular proliferation and infection of surrounding cells before or after host cell apoptosis (298).

The immune response induced by *A. phagocytophilum* is thought to play an important role in the initial control of the disease but may also induce inflammatory injuries associated with granulocytic anaplasmosis. Indeed, the absence of *A. phagocytophilum* control induces a clear rise in inflammatory lesions, which is considered the major pathogenic effect in humans and murine models (299–301). In dogs, the hematological modifications associated with *A. phagocytophilum* infection are similar to those induced by other members of *Ehrlichia* or *Anaplasma* genera, although they infect different blood cells, suggesting that the major mechanism of cytological injuries is related to an immunological response or to substances secreted from the bacteria (302, 303). *Anaplasma phagocytophilum* induces an upregulation of chemokine and pro-inflammatory cytokine [IL-8, macrophage inflammatory protein (MIP)-1a, MIP-1b, monocyte chemoattractant protein (MCP)-1] expression *in vitro*, which attracts leukocytes and inhibits hematopoiesis leading to myelosuppression (304, 305). In mice, several leukocyte populations expand during infection including NK and NKT cells followed later by CD4 and CD8 T lymphocytes and the immune response proceeds mostly through production of interferon gamma (IFN-γ), commonly produced by T lymphocytes (301–303, 306). In humans, the manifestation of severe disease is associated with hypercytokinemia and macrophage activation or hemophagocytic syndromes (MAS/HPS). The underlying pathogenesis of MAS/HPS is poorly understood; however, it is frequently associated with a defective function or depletion of cytotoxic cells and is driven mostly by the persistent stimulation

of cytokine production, especially the macrophage-activating IFN-γ (307–309). The clear role of IFN-γ in the pathogenesis of the disease is demonstrated by the observation that the lack of this molecule in *A. phagocytophilum*-infected mice resolves inflammatory tissue injury (300).

Several studies have confirmed the role of the IFN-γ in mediating both the pathology and early control of bacteria, although it is not essential for bacterial clearance. Other protective mechanisms might be involved in the control of *A. phagocytophilum* infection, as some infected mice lacking IFN-γ are able to survive (292, 300–303, 306). Data suggest that the humoral immunity may also play an important role in the clearance of ehrlichial infections, as passive immunization has a moderately protective effect. Moreover, severely immunocompromised mice that lack both B and T cells remained persistently infected, as opposed to mice lacking only T cells, which were able to control the infection (301, 310). Experimentally infected dogs develop serologic responses (immunoglobulin G) 7 days after inoculation. However, positive *A. phagocytophilum* PCR assay results persist up to 42 days despite the high antibody response suggesting that the humoral response is not sufficient to clear the infection (284). It appears that the innate immune mediators used to activate phagocytes to kill other intracellular bacteria (reactive nitrogen intermediates, Toll-like receptor 2 and 4, MyD88, phagocyte NADPH oxidase) do not play a crucial role in *A. phagocytophilum* clearance and may contribute to the observed pathology (301, 303, 311).

CANINE GRANULOCYTIC ANAPLASMOSIS
Clinical Signs

The discrepancy between the high seroprevalence and the relatively low number of sick dogs in endemic areas suggests that most infected dogs remain apparently healthy or develop a mild self-limiting illness (9, 10, 13, 25). The severity of the disease varies from mild subclinical to severe acute forms (24, 34), with severe clinical presentation often associated with co-infections, the immune response of the host and the variability of strains pathogenicity (13, 18, 33, 45).

CGA is a multi-systemic unspecific acute illness characterized by many clinicopathological modifications due to the possible involvement of several body systems. After an incubation period of 1–2 weeks, the most frequently observed clinical signs include fever, lethargy, inappetence or anorexia, weight loss and musculoskeletal pain or discomfort (**Table 2**) (24, 25, 29, 30, 35, 53, 60, 312, 314). More than 75% of dogs display lethargy and inappetence or anorexia (24, 26, 29, 30, 33, 35). Lethargy has been reported in almost all infected dogs (13, 24–26, 29–31, 33, 35, 45) and was the most frequent clinical signs in several studies (24–27, 29, 33, 35). It was also reported to be disproportionately severe in comparison with the lack of other clinical abnormalities in a case report (247). Fever is both inconstant and variable with frequencies ranging from 46 to 100% (24, 33, 35) and values from 39.2 to 41.5°C (25–27, 30, 35, 246, 251, 252, 257, 258, 261, 314). Fever generally coincides with the peak of bacteremia and lasts less than a week (33). Musculoskeletal pain or discomfort has been described in more than 50% of

dogs and manifests in reluctance to move, weakness, stiffness, lameness, and myalgia. However, <10% of dogs have overt joint pain (29, 30). Lameness and joint swelling were reported in 11–34% (25, 34, 35) and 6–62% cases (24, 26, 35) respectively. They are more likely related to neutrophilic inflammation (25, 26, 244, 314, 315), but immune-mediated mechanisms also might be involved (244, 315). In a retrospective study, polyarthropathy (50%) was more frequently observed than monoarthropathy (5%) (27). In a report from California investigating the prevalence of tick-borne infections in dogs with polyarthritis and/or thrombocytopenia, *A. phagocytophilum* was the most frequently detected pathogen (244). Lymphadenopathy, splenomegaly and hepatomegaly were frequent findings in CGA (25, 26, 29, 35, 53, 314, 315). Splenomegaly was reported in 12–100% of naturally infected dogs (25, 26, 35). In canine and murine models of *A. phagocytophilum* infection, lymphadenopathy and splenomegaly are due to reactive lymphoid hyperplasia, with concurrent extra-medullary hematopoiesis in the spleen, enlarged activated lymph nodes and increased numbers of macrophages and plasma cells in the red pulp (284, 285, 314). In experimentally infected dogs, non-specific reactive hepatitis and mild periportal inflammatory lesions were also described (284, 314) and lesions tended to be more pronounced in dogs euthanized in the acute stage (314).

Other clinical signs include gastro-intestinal signs, polyuria, polydipsia, respiratory signs, pale mucous membranes, bleeding disorders, uveitis, scleral congestion, polymyositis, and neurological signs (**Table 2**) (13, 24, 26, 29, 30, 32, 33, 35, 53, 60, 256, 257, 312, 314, 316). Gastrointestinal signs include diarrhea, nausea, vomiting, and abdominal pain (25–27, 33–35, 312), but their origin is still unknown. In two cases of CGA displaying gastrointestinal signs, associated pancreatitis was suspected based on biochemistry and abdominal ultrasound abnormalities (246, 252). Respiratory signs include dyspnea, tachypnea, and coughing, which is usually infrequent, soft and non-productive (35, 238, 314). One patient displayed coughing and presented interstitial patterns on thoracic radiographs associated with focal alveolar patterns, and showed morulae within neutrophils upon microscopic examination of tracheal lavage specimen (238). Bleeding disorders including petechiae, gingival bleeding, melena, fresh blood in feces, epistaxis, pulmonary hemorrhage, vaginal hemorrhage or hematoma (35) are infrequent in dogs infected with *A. phagocytophilum*, unlike other rickettsial infections, such as *E. canis*, *A. platys*, and *Rickettsia rickettsii* infections or other infectious diseases, such as aspergillosis, bartonellosis, and leishmaniasis. Indeed, only 3–11% of CGA cases displayed epistaxis (25, 316). In two separate reports, dogs with CGA that presented with epistaxis had mild to moderate thrombocytopenia that could not explain the bleeding disorder. In addition, these dogs were seronegative to *B. burgdorferi*, *E. canis*, and *Dirofilaria immitis*, but other concurrent diseases were not ruled out. Therefore, other factors than thrombocytopenia may cause epistaxis, such as an infection-induced vasculitis (27, 33). Similarly, another report described two dogs with bleeding disorders associated with *A. phagocytophilum* infection that displayed platelet counts within the reference range (35). Although neurological signs were reported to occur

TABLE 2 | Clinical signs associated with canine granulocytic anaplasmosis after natural infection and corresponding frequency recorded in several studies.

Clinical sign	Frequency (%)	Number of dogs included in the study	References
Fever	84	32	(26)
	47	49	(31)
	61	18	(25)
	46	26	(33)
	89	18	(27)
	52	60	(45)
	100	8	(24)
	57	28	(13)
	67	6	(312)
	60	15	(60)
	67	61	(35)
Fever, lethargy	88	17	(29)
Fever, lethargy, depression	93	14	(30)
Fever, lethargy, anorexia	51	107	(34)
Lethargy/depression	94	18	(25)
	88	17	(29)
	74	34	(26)
	72	18	(27)
	67	49	(31)
	81	26	(33)
	67	60	(45)
	26	28	(13)
	50	6	(312)
	83	63	(35)
	73	15	(60)
Inappetence/anorexia	62	34	(26)
	33	49	(31)
	58	26	(33)
	55	60	(45)
	29	51	(13)
	87	8	(24)
	88	17	(29)
	50	6	(312)
	63	63	(35)
	67	15	(60)
Weight loss	25	8	(24)
Pale mucous membrane	28	18	(25)
	12	8	(24)
	50	6	(312)
Dehydratation	37	8	(24)
Musculoskeletal Signs			
Lameness	32	34	(26)
	16	49	(31)
	11	18	(25)
	50	26	(33)
	23	60	(45)
	34	107	(34)
	16	28	(13)
	16	63	(35)
	27	15	(60)
Joint swelling	62	8	(24)
	6	34	(26)
	19	26	(33)
	55	18	(27)
	14	28	(13)
	33	6	(312)

(Continued)

TABLE 2 | Continued

Clinical sign	Frequency (%)	Number of dogs included in the study	References
Digestive signs	23	107	(34)
	13	15	(60)
Vomiting	24	34	(26)
	11	18	(25)
	15	26	(33)
	6	18	(27)
	50	6	(312)
	8	63	(35)
Diarrhea	9	34	(26)
	17	18	(25)
	50	6	(312)
	14	63	(35)
Abdominal pain	9	34	(26)
Tense abdomen	28	18	(25)
	40	63	(35)
Lymphadenopathy	32	34	(26)
	19	26	(33)
	6	18	(27)
	13	17	(29)
	6	63	(35)
Splenomegaly	12	34	(26)
	40	60	(45)
	13	17	(29)
	100	18	(25)
	17	6	(312)
	84	57	(35)
Hepathomegaly	8	26	(33)
	33	6	(312)
Hepatosplenomegaly	7	17	(29)
	12	57	(35)
RESPIRATORY SIGNS			
High respiratory rate	29	34	(26)
	2	63	(35)
Cough	8	26	(33)
	37	8	(24)
	3	63	(35)
Respiratory or urinary tract disease	7	91	(34)
Bleeding disorders	12	66	(34)
	13	63	(35)
Petechiae	11	18	(25)
	3	63	(35)
Epistaxis	6	18	(25)
	8	26	(33)
	6	18	(27)
	4	28	(13)
	2	63	(35)
Melena	6	18	(25)
	17	6	(312)
Gingival bleeding, hematoma, fresh blood in feces, pulmonary and vaginal hemorrage	2	63	(35)
Neurological signs	7	28	(13)
Left cerebral dysfunction	6	18	(27)

(Continued)

Clinical sign	Frequency (%)	Number of dogs included in the study	References
Cervical pain	6	18	(27)
	2	63	(35)
Proprioceptifon deficit	7	17	(29)
Seizures	15	40	(34)
	7	17	(29)
	2	63	(35)
Ataxia	67	6	(312)
	7	15	(60)
Skin disease	10	61	(34)
	12	33	(313)

in CGA (35), no studies investigating this association have confirmed the infection by PCR. Moreover, two studies failed to demonstrate an association between *A. phagocytophilum* infection and neurological signs (317, 318). Consequently, *A. phagocytophilum* seems to be a rare cause of neurological disease in dogs and other potential etiologies or concurrent diseases should be ruled out before a final diagnosis of CGA. *Anaplasma phagocytophilum* infection is also suspected to induce skin lesions in dogs (34, 313). In one study that investigated skin-associated lesions in seropositive dogs, four of 12 showed positive DNA amplification from skin lesions. The most frequent lesions identified in these dogs included erythema, papules and plaques that resolved after doxycycline therapy (239). Cutaneous lesions were also present in seropositive but PCR-negative dogs (313). In a previous case report, one dog positive to *A. phagocytophilum* by serologic tests, PCR from blood and post-mortem spleen samples, was presented first for skin problem including pruritus, hair loss and seborrhea in association with regenerative anemia, leukocytosis and thrombocytopenia. *Ehrlichia canis* and *E. chaffeensis* exposure were serologically excluded (256). The lack of typical clinical signs and thrombocytopenia in dogs with PCR-positive skin lesions could be suggestive of a persistent infection as reported in studies in sheep, suggesting that skin could be a site of persistence of *A. phagocytophilum* (313).

Evolution of the Disease

CGA is currently considered to be an acute disease. Clinical signs usually develop during the bacteremic phase (24, 25, 29, 30) and the duration of the disease is variable. In a retrospective study, the duration of illness ranged from 1 to 14 days with a median duration of 3 days (27). Two studies demonstrated that the majority of dogs were sick for <7 days prior to diagnosis (26, 35). However, the duration of clinical signs ranged from 1 day to 2 months (26). In another report, the duration of illness ranged from 1 to 8 days, but one dog remained infected for a month before the diagnosis was established (30).

Chronic or persistent *A. phagocytophilum* infection has not been demonstrated in naturally infected dogs and is still controversial (2, 24, 53, 319). In contrast, experimental studies showed a persistent infection in dogs for more than several

months to almost a year (18, 284, 320–324). These studies support the findings of another report that demonstrated that dogs could have long-lasting infections with acute flare-up (30) whereas another one failed to demonstrate a chronic infection in experimentally infected dogs (324). The results of the latter study differ from those of three other reports in which repeated amplification of *A. phagocytophilum* DNA occurred in some dogs probably because of the differences in the way of inoculation. Indeed, in contrast to the other experimental studies in which the bacterium had been inoculated intravenously to the dogs (18, 284, 320–322), in Contreras et al. (324), dogs were infected through tick bites after *Ixodes* spp. infestation. A 1 year persistence of *A. phagocytophilum* infection has been described in a naturally infected Rhodesian ridgeback dog (53). In addition, some authors consider the possibility of a chronic phase characterized by more localized clinicopathological signs (such as lameness and proteinuria) that could be associated with immune-mediated mechanisms secondary to persistent antigen stimulation (34). Studies on *E. canis* infection in dogs showed that the spleen is probably the organ that harbors bacteria for the longest period and is the best source for the diagnosis of carrier state by PCR (325). Similarly, the spleen remained PCR-positive in monkeys and mice experimentally infected with human strains of *A. phagocytophilum* (299, 326).

The prognosis of the disease in dogs is usually favorable with a rapid remission after doxycycline therapy (24, 26–28, 35, 324). However, some fatal cases have been reported (33, 35, 256, 257). Among the 12 fatality cases reported, five died of immune-mediated hemolytic anemia (IMHA) complicated by disseminated intravascular coagulation (DIC) (33, 256, 257). Two of these dogs were seropositive for *Neorickettsia risticii, R. rickettsii*, and *B. burgdorferi* (33). One was euthanized after 14 days because of IMHA and another one died because of epileptic seizures after 3 days (35).

Coinfections

Coinfection with multiple VBPs in dogs appears more frequent in endemic areas (9, 13). In a large retrospective serologic study carried out in North America and the Caribbean, exposure to up to five vector-borne pathogens (VBPs) was detected in the same dogs (3). In a kennel of North Carolina, 40% of dogs had serologic evidence of exposure at the same time with *Anaplasma* spp., *Babesia canis, Babesia vinsonii, E. canis*, or *R. rickettsii* (236). In another study, 16.5% of USA dog samples were found to be seropositive for more than one pathogen (119). Two serologic surveys showed that 1.32 and 14.3% of dogs had antibodies against two pathogens in Italy and Morocco, respectively (178, 226). In Tunisia, 22.4% of dogs were seropositive for *E. canis* and *A. phagocytophilum* (224). In Algeria, coinfections by *A. phagocytophilum* and 1–3 other pathogens were higher in stray than client-owned dogs (225). Two studies investigated the association between co-infections with several VBPs and the occurrence on clinical canine leishmaniosis (327, 328) and one reported a statistical association between dogs with clinical leishmaniosis stages III and IV and the seroreactivity to *A. phagocytophilum* in Spain (327). Coinfection with *B. burgdorferi* and *A. phagocytophilum*

is frequently described in dogs, probably because pathogens are transmitted by Ixodid ticks and maintained in sylvatic cycles with the same rodent reservoir (13, 33, 225, 329–331). In the USA, almost 22% of *A. phagocytophilum*-seropositive samples were also seropositive for *B. burgdorferi* (240). The prevalence of seropositive dogs to both pathogens was as high as 45% (12, 33). The ability of co-infected *I. scapularis* ticks to transmit *B. burgdorferi* and *A. phagocytophilum* was lower compared with transmission of either agent by singly infected ticks (331).

Experimental studies in mouse and human case reports of *A. phagocytophilum* and *B. burgdorferi* coinfection have described an enhanced severity and complexity of clinical signs along with an increased likelihood of disease compared with single infections (13, 329, 330, 332). Similarly, dogs seropositive for both agents (43%) were more likely to display clinical signs than those seroreactive to either *A. phagocytophilum* (25%) or *B. burgdorferi* (9%) (13). Experimental studies in rodents have demonstrated that coinfection modulates the host immune response to *A. phagocytophilum* and the production of interleukins (ILs), decreases IFN-γ levels and the number of CD8$^+$ T cells which leads to more severe clinical signs, increases pathogen burdens in blood and tissues, and induces more persistent infections (13, 329, 330, 333). Furthermore, the interaction of both pathogens at the blood-endothelial cell interface seems to be a critical point in pathogenesis (332). Two *in vitro* studies on human blood-brain barrier models showed that *A. phagocytophilum*-infected neutrophils enhanced *B. burgdorferi* migration across both systemic and brain microvascular endothelial cells. Several mechanisms are thought to be involved including impaired phagocytic neutrophil function caused by *A. phagocytophilum*, increased production of vasoactive and pro-inflammatory molecules (IL-6, IL-8, IL-10, tumor necrosis factor alpha, and macrophage inflammatory protein 1α) and the release of matrix metalloproteinases (329, 332). These factors lead to enhanced vascular permeability and inflammatory response in tissues and promote *B. burgdorferi* migration, which results in worsened clinical manifestations (329, 330, 332, 333).

LABORATORY ABNORMALITIES

Hematological Modifications

Hematological modifications associated with CGA include thrombocytopenia, anemia, leukopenia, and lymphopenia, although variable white blood cells count (WBC) modifications have been described (**Table 3**) (13, 24–27, 29–33, 35, 45). In experimentally infected dogs, hematological changes usually occurred during the acute stage of infection and normalized a few days after morulae disappeared from blood (302, 314). Suggested mechanisms of cytopenia include cytokine myelosuppression, autoantibodies formation, infection of hematopoietic precursors, and blood cell consumption (especially platelets) (25, 304, 334). Bone marrow aspirates of infected dogs were hyper- or normocellular, with normal, increased, or decreased iron storage, a slight increase in immature erythroid cells, and megakaryocyte and myeloid hyperplasia associated with relative shift toward immature myeloid cells, suggesting impaired myelopoiesis (312, 314).

Thrombocytopenia is the most common disorder associated with CGA. It has been described in 16.7–95% of natural (13, 24–26, 29, 30, 35) and 100% of experimental infections (302, 314). According to some authors, thrombocytopenia reflects an ongoing immunological response in dogs even when associated with low antibody titers against *A. phagocytophilum* (34). A recent study showed a significant association between thrombocytopenia and high concentrations of circulating immune complexes (CIC), low albumin to globulin (A/G) ratios and an acute phase protein concentration. The importance of thrombocytopenia was emphasized as an indicator of acute anaplasmosis, regardless of antibody titer (28). Therefore, thrombocytopenia is considered the most relevant abnormality in the diagnosis of CGA after morulae detection (13, 24–26, 29, 30). The severity of thrombocytopenia varies from mild to severe and the platelet count has been reported to range from 5,000 to 164,000 cells/μl (24, 25, 29, 30, 35, 314). However, in a report, none of the 12 dogs seropositive to *A. phagocytophilum* had platelet counts lower than 105,000 cells/ml and dogs that were also seropositive to *B. burgdorferi* had a lower median platelet count of 51,000 cells/μl (33). In another study, five of the six CGA cases with significant thrombocytopenia had concurrent diseases (lymphoma and systemic lupus erythematosus) or were serologically positive to *B. burgdorferi* or *E. canis* (29). A prospective study aiming to investigate the presence of bacteria belonging to the genera *Anaplasma* and *Ehrlichia* in 159 blood samples from thrombocytopenic dogs, detected only two *A. phagocytophilum*-PCR positive dogs (335). As it has been described for a wide range of *Ehrlichia* species, CGA-associated thrombocytopenia may be related to platelet consumption due to DIC, immunological destruction, spleen sequestration or production of inhibitory factors (336–338). The organism seems to be able to enter megakaryocytes lineage but without impairment of their ability to produce platelets (339). The mechanism inducing thrombocytopenia seems to be more associated with an inflammatory process rather than with the direct action of *A. phagocytophilum* (34). Destruction of platelets has been suggested as a probable mechanism because of the increased number of both mature and immature megakaryocytes in the bone marrow (302). On the other hand, anti-platelet antibodies have been detected in both human and canine cases, with up to 60 and 80% of patients with CGA and HGA displaying anti-platelet antibodies, respectively (25, 35, 257, 336–338). However, thrombocytopenia usually occurs during the early stages of infection, before antibody detection and has also been described in severely immunocompromised mice due to B or T cell suppression, suggesting that mechanisms other than decreased hematopoietic production or immune-mediated destruction are involved. Increased platelet consumption is also suspected to play an important role (340, 341). *In vitro*, increased production of monocyte tissue pro-coagulant activity in peripheral blood mononuclear cells has been observed, supporting the platelet consumption hypothesis (27, 340).

Anemia is an inconstant hematological finding (34) described in 3–82% of dogs with clinical signs compatible with CGA either

TABLE 3 | Hematological abormalities associated with canine granulocytic anaplasmosis after natural infection and corresponding frequency recorded in several studies.

Hematological abnormalities	Frequency (%)	Number of dogs included in the study	References
Thrombocytes count modifications			
Thrombocytopenia	95	22	(26)
	71	–	(32)
	69	49	(31)
	89	18	(25)
	56	25	(33)
	94	18	(27)
	65	60	(45)
	87	8	(24)
	86	7	(29)
	86	7	(30)
	57	28	(13)
	17	6	(312)
	86	63	(35)
Erythrocytes modifications			
Anemia	47	34	(26)
	17	–	(32)
	57	49	(31)
	82	11	(25)
	24	25	(33)
	42	60	(45)
	3	15	(29)
	24	14	(30)
	70	63	(35)
Non regenerative anemia	67	18	(27)
	37	8	(25)
	50	6	(312)
Regenerative anemia	27	11	(25)
IMHA	12	25	(33)
	24	17	(35)
Leukocytes modifications			
Leukopenia	9	31	(26)
	9	–	(32)
	18	49	(31)
	55	18	(27)
	10	60	(45)
	62	8	(24)
	7	14	(30)
	14	63	(35)
Leukocytosis	19	31	(26)
	21	–	(32)
	61	49	(31)
	28	25	(33)
	7	15	(29)
	7	14	(30)
	33	6	(312)
	27	63	(35)
Lymphopenia	65	31	(26)
	12	25	(33)
	39	18	(27)
	33	60	(45)
	100	8	(24)
	67	15	(29)
	44	63	(35)
Eosinopenia	10	49	(31)
	50	18	(27)
	9	63	(35)

(Continued)

TABLE 3 | Continued

Hematological abnormalities	Frequency (%)	Number of dogs included in the study	References
Neutropenia	37	8	(24)
	7	15	(29)
Neutrophilia	19	31	(26)
	33	6	(312)
	51	63	(35)
Left shift	28	26	(33)
	20	15	(29)
	33	6	(312)
Monocytosis	45	49	(31)
	6	18	(27)
	33	60	(45)
	43	63	(35)
Morulae			
Morulae detection	36		(29)
	56		(24)
	4	49	(31)
	56	18	(25)
	94	18	(27)
Percentage of neutrophils with morulae	10–24	–	(29)
	7–24	5	(30)
	9–32	8	(24)
	1–5	6	(312)
	0–11	35	(316)

seropositive (33), PCR-positive (25, 27, 35, 45), displaying *A. phagocytophilum*-like morulae on fresh blood smear examination (24, 26) or being positive to two (29, 30) or three (31) aforementioned diagnostic methods (**Table 3**). In a retrospective study, no dogs were anemic, even during the bacteremic phase, but the mean values of hematocrit, hemoglobin concentration and red blood cell counts were significantly lower than in the control group (34). In contrast, three different studies described 63, 67, and 70% of anemic dogs (27, 35, 224). CGA-associated anemia is usually mild to moderate non-regenerative normocytic normochromic resembling anemia of inflammation (24, 27, 29, 31, 302, 312, 314). In nine dogs experimentally infected with *A. phagocytophilum* that developed mild normocytic normochromic anemia, decreased serum iron and total iron-binding capacity were recorded during bacteremia, but levels returned to reference ranges 1 week after the disappearance of morulae (302). In a report, most dogs had mild to moderate anemia with hematocrits ranging from 19 to 39%, but two had severe anemia with hematocrit levels <20% and three had signs of regeneration. Five were suspected to have hemolytic anemia based on increased serum levels of bilirubin but all had negative Coombs tests (25). Regenerative anemia has been less frequently reported, and severe IMHA is an unusual disorder associated with CGA (25, 33, 257, 258). One retrospective duty aiming to investigate infectious causes of lethal immune-mediated anemia in Croatian dogs, only two dogs were found positive to *A. phagocytophilum* DNA and one of these two dogs was also co-infected with *B. canis* (342). Six cases of IMHA in dogs with CGA have been reported in the UK, the USA and Denmark (33, 35, 257, 315). Authors

from Germany described the possible occurrence of IMHA in a small number of dogs (25). Others from Belgium described IMHA in a dog with a positive titer to *A. phagocytophilum* and without other concomitant diseases (262). A previous case report described a dog with *A. phagocytophilum* infection (confirmed by positive PCR from blood and post-mortem spleen samples) with regenerative anemia, severe bilirubinuria, and positive test for osmotic resistance of red blood cells. This dog was serologically negative for babesiosis, leptospirosis, *E. canis* and *E. chaffeensis* infections (256). More recently, four dogs had a positive Coombs test among 17 ones that underwent this analysis in a case series on CGA (35). Only one case series has evaluated the prevalence of IMHA associated with CGA. In this study, three dogs had IMHA based on spherocytes in blood smears and/or positive Coombs test, without evidence of abdominal or thoracic neoplasia. However, two dogs had positive antibodies for at least one other TBP including *Neorickettsia risticii* (formerly *Ehrlichia risticii*), *B. burgdorferi*, and *Rickettsia rickettsii*. The authors emphasized that both *R. rickettsii* and *B. burgdorferi* are not commonly associated with IMHA and *N. risticii* is not yet associated with clinical disease in dogs as suggested by experimental studies (33). In addition, anti-erythrocyte antibodies have been detected in three dogs with CGA in the USA (312). Even if CGA has not yet been proven to be a common cause of IMHA, *A. phagocytophilum* should be included in the differential diagnosis, especially in endemic area (33).

The most diagnostically relevant hematological abnormality in CGA is the identification of *A. phagocytophilum* inclusions within neutrophils during blood smear examinations. Morulae appear classically as basophilic inclusions detectable by light microscopy of peripheral blood smears (41, 234). They are usually present transiently during the bacteremic phase (4–14 days after inoculation) and persist for 4–8 days in experimentally infected dogs (302, 314). Morulae can also be identified from cytocentrifuged synovial fluid, bone marrow aspirates, and they were also present in the abdominal fluid of an unusual CGA case and in the tracheal wash from a dog with respiratory signs (40, 238, 247, 302, 312, 315). The proportion of neutrophils containing morulae in blood smears varies from <1 to 34% (24, 29, 30, 312, 314, 316). In an experimentally study, the most severely affected dogs were those with higher percentage of neutrophils containing morulae and the lowest proportion was recorded in non-febrile dogs (314). In endemic areas, 38% of dogs displaying clinical signs compatible with CGA had morulae within neutrophils (13). Three studies reported that 56%, 94% (25, 27), and 88 to 93% (33) of dogs presented morulae while other reports failed to identify these inclusions (246, 257). It is important to mention that *A. phagocytophilum* morulae cannot be distinguished from those of *E. ewingii*, which can lead to misdiagnosis in the regions where both pathogens are present. Therefore, other methods, such as PCR are needed to confirm the diagnosis (2, 24, 302).

Experimentally infected dogs developed moderate leucopenia (314), but WBC count modifications in naturally infected dogs are considered non-specific and variable, and both decreased and increased WBC counts have been reported (24–27, 29–35, 45). Therefore, the use of the WBC count as a marker of

the course of the disease is controversial (34). Lymphopenia is the most frequently reported WBC count abnormality in CGA (24–26, 29, 302, 314). Other reported modifications include leukocytosis, leukopenia, lymphocytosis, eosinopenia, monocytosis, monocytopenia and mild to moderate neutropenia or neutrophilia (24–27, 29–31, 35, 45, 53, 302, 312, 314). Left shift of neutrophils and toxic changes have also been reported to occur with *A. phagocytophilum* infection in dogs (26, 29, 33, 252, 257, 312).

Serum Biochemistry Profile Modification

Serum biochemistry profile modifications documented in CGA include increased liver enzyme activity, hyperbilirubinemia, hypophosphatemia, hyperproteinemia, hyperglobulinemia, and hypoalbuminemia (**Table 4**) (24–27, 29–31, 33, 35, 45, 314, 316). A moderate increase in alkaline phosphatase (ALP) was reported in 7–100% of CGA cases and mild to moderate hypoalbuminemia was present in 17–66% (25, 26, 29, 33). In a retrospective study, 30% of dogs displayed a slightly increased alanine aminotransferase (ALT) activity but concurrent diseases had not been ruled out (26). In another report, the most frequent findings in dogs with CGA were increased in liver enzymes and hyperbilirubinemia (35). According to some authors, hypoalbuminemia and hyperglobulinemia might be due to a decreased production of albumin in the liver associated with a rise in α- and β-globulin production (304). In a study investigating serum protein profiles of seropositive and PCR-positive dogs, the major modification was a low A/G ratio (84.4%), mostly in groups with antibody titers higher than 1:1,024. Hyperglobulinemia was due to an increase in the acute phase proteins (α2-, β1-, and β-2 globulin). In the same study, 62 and 71.8% of dogs in the group with lower A/G ratios had thrombocytopenia and clinical signs compatible with CGA, respectively, suggesting an acute infectious process. However, other diseases had not been excluded; hence dysproteinemia could possibly be the result of concurrent diseases (28). Others reported hypergammaglobulinemia as a prominent modification associated with CGA but without exclusion of concurrent diseases (316). Decreased serum levels of urea and hypokalemia have been recorded in 27% of dogs (25) and 27–37% of dogs were reported to have hyperbilirubinemia (25, 26, 35). An increase in serum amylase activity was described in 50% of CGA cases (29). Two case reports described dogs diagnosed with CGA with suspected pancreatitis on the basis of increased serum level of amylase and lipase and clinical signs suggesting pancreatitis (abdominal pain in the pancreatic region of one dog and abdominal ultrasound modifications in the pancreatic region of the other dog) (246, 252). In another previous report, two of seven dogs had increased serum lipase concentrations (24).

Prolonged prothrombin time (PT) and activated partial thromboplastin time (aPTT), along with increased fibrin-degradation product concentration and fibrinogen concentration have been reported in some CGA cases (**Table 4**) (25, 35, 252, 257, 314). DIC was suspected or diagnosed in four dogs; two of which had IMHA (25, 33, 257). Elevated aPTT was also described in one dog with SIRS secondary to *A. phagocytophilum* infection (26). In a recent study on portal vein thrombosis, four of 29

TABLE 4 | Serum biochemistry abormalities associated with canine granulocytic anaplasmosis after natural infection and corresponding frequencies recorded in several studies.

Serum biochemistry abnormalities	Frequency (%)	Number of dogs	References
Hyperproteinemia	12	49	(31)
	43	62	(35)
Hypoproteinemia	10	49	(31)
	20	11	(29)
	2	62	(35)
Hypoalbuminemia	44	27	(26)
	29	49	(31)
	17	23	(33)
	28	60	(45)
	44	9	(29)
	55	18	(25)
	50	6	(312)
	62	61	(35)
Hyperglobulinemia	50	6	(312)
	38	61	(35)
Serum protein electrophoresis A/G ratio<0.8	21	145	(28)
CIC	80	204	(28)
Hypophosphatemia	62	8	(24)
Increased ALP	52	27	(26)
	59	49	(31)
	26	23	(33)
	43	60	(45)
	75	8	(24)
	100	9	(29)
	7	14	(30)
	67	61	(35)
Increased ALT	30	27	(26)
	35	49	(31)
	18	62	(35)
Increased bilirubin	37	27	(26)
	31	49	(31)
	34	61	(35)
Azotemia	27	49	(31)
	3	62	(35)
Increased aPTT	60	10	(25)
	55	29	(35)
Increased PT	30	10	(25)
	34	29	(35)

A/G ratio, albumin to globulins ratio; ALP, alkaline phosphatase; ALT, alanine aminotransferase; apt, activated partial thromboplastin time; CIC, circulating immune complexes.

dogs had infectious diseases and one had *A. phagocytophilum* infection (343).

Urinalysis Modifications

Acute renal failure (ARF) is a complication described in some HGA cases (344, 345). In a recent study, 30.6% of human patients with confirmed *A. phagocytophilum* infection by PCR had abnormalities on urinalysis including hemoglobinuria or myoglobinuria (not distinguished by further analysis). Hemoglobinuria/myoglobinuria could be the precursor of ARF described in severe human cases (346). Experimental studies

TABLE 5 | Urinary abormalities associated with granulocytic anaplasmosis after natural infection and corresponding frequency recorded in several studies.

Urinary abnormalities	Frequency (%)	Number of dogs	References
Hyposthenuria	12	49	(26)
Proteinuria	15	13	(26)
	87	8	(25)
	27	23	(33)
	38	8	(29)
	50	6	(30)
	58	58	(28)
	40	5	(312)
Glucosuria	12	8	(25)
Bilirubinuria	50	8	(25)
	25	8	(29)
	50	5	(312)
Hematuria	87.5	8	(25)
	40	5	(312)
Hemoglobinuria	60	6	(31)
Urinary sediment			
Casts	50	8	(25)
Epithelial cells	75	8	(25)
Protein electrophoresis			(28)
LMWP	42	36	
MMWP and HMWP	30	36	

LMWP, low molecular weight proteins (<66 kDa); MNWP, middle molecular weight proteins (66–76 kDa); HMWP, high molecular weight proteins (>76 kDa).

revealed evidence of *A. phagocytophilum* DNA in the kidneys of three persistently infected lambs and lesions of vasculitis and thrombosis in the kidney of a horse (347, 348). Similarly, one study amplified *A. phagocytophilum* DNA in the kidney of one dog after necropsy (342). CGA is suspected to induce immune-mediated glomerulonephritis (IMGN) likely by vasculitis (349). In contrast to blood modification, urinary abnormalities have not been fully assessed in dogs and only a few reports have described abnormalities in urinalysis (**Table 5**) (25, 26, 28–30, 33, 312). One such study described the presence of mild to moderate proteinuria, glucosuria, bilirubinuria, hematuria, and epithelial cells in urine sediments. In the same report, only three of eight dogs in which urinalysis was performed were also measured for urine protein to creatinine (UPC) ratios, and one displayed a mild increase (0.88) (25). Another report showed a significant difference in proteinuria between *A. phagocytophilum* seropositive and seronegative dogs (34). In a retrospective study, two dogs displayed proteinuria with UPC ratios of 1.5 and 2.2 (26) and 17% of dogs had proteinuria in another report. In this study the only dog with a UPC ratio higher than one had antibodies against both *A. phagocytophilum* and *B. burgdorferi* (33). More recently, 3% of CGA cases included retrospectively displayed signs of azotemia (35), however other concurrent diseases causing azotemia have not been ruled out. In most studies on CGA, proteinuric dogs were identified mainly on the basis of dipstick and only a few of them underwent UPC measurement. Moreover, urinary tract infection (UTI) was not excluded in all dogs. However, another study demonstrated that

38% of dogs had proteinuria without signs of UTI, which could be compatible with kidney injury (29). Proteinuria due to middle and high molecular weight proteins was found exclusively in 30.5% of *A. phagocytophilum*-seropositive dogs. The authors indicated that proteinuria might be the result of chronic antigenic stimulation and suggested that persistent infection can lead to the development of IMGN (34). In one case of CGA, a persistent proteinuria after 28 days of doxycycline therapy was reported. The dog remained asymptomatic during a 305-day follow up; however, mild proteinuria was still present even with a renin-angiotensin-aldosterone system inhibitor (261). More recently, a case report described a dog with IMGN complicated with systemic hypertension and chronic kidney disease without any identified etiology except an active *A. phagocytophilum* infection on the basis of a very high antibody (1:20,480) titer at first consultation and more than a 4-fold decrease in antibody titer several weeks after (262). Finally, the consensus statement of the American College of Veterinary Internal Medicine (ACVIM) for dogs with suspected glomerular disease recommends serologic screening for anaplasmosis of patients with renal proteinuria in addition to other infectious diseases known to induce proteinuria (350).

CONCLUSION AND FUTURES PERSPECTIVES

Understanding granulocytic anaplasmosis is important due to its zoonotic aspect, potential severe outcomes in both dogs and humans, and the possibility of using epidemiological data in canine species as a good estimation of risk for human exposure. The aims of this review were to summarize the wide epidemiological data published on *A. phagocytophilum* in canine species and to describe the clinicopathological aspects of CGA that are available in the few case series and reports. In this manuscript, the authors wanted to gather together all data on *A. phagocytophilum* in dogs that can be valuable for researchers and to highlight the fields where important information is still missing and toward which future research should be focused. Indeed, information regarding the prevalence of *A. phagocytophilum* in some parts of the world, the potential role of dogs as competent reservoir hosts, the possibility of tick species other than *Ixodes* spp. acting as vectors of *A. phagocytophilum* and the implication of the genetic variability in the pathogenesis of the disease with some strains being potentially more virulent for humans is still incomplete or lacking. The pathogenesis of CGA is not fully elucidated too. Finally, some publications on CGA discussed the possibility of a chronic evolution and the association of this disease with serious clinicopathological manifestations with a crucial impact on the prognosis and management, such as immune-mediated hemolytic anemia, glomerulonephritis, and neurological signs that are still incomplete and thus need further investigations.

AUTHOR CONTRIBUTIONS

SE wrote the manuscript. SD, LD, LE, MK, and HS drafted and revised the manuscript. All authors have made a substantial, intellectual contribution to the work, and read and approved the final manuscript.

ACKNOWLEDGMENTS

REFERENCES

1. Dumler JS, Barbet AF, Bekker CPJ, Dasch GA, Palmer GH, Ray SC, 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 and Evol Microbiol*. (2001) 51:2145–65. doi: 10.1099/00207713-51-6-2145

2. Carrade DD, Foley JE, Borjesson DL, Sykes JE. Canine granulocytic anaplasmosis: a review. *J Vet Intern Med*. (2009) 23:1129–41. doi: 10.1111/j.1939-1676.2009.0384.x

3. Qurollo AB, Chandrashekar R, Hegarty BC, Beall MJ, Stillman BA, Liu J, et al. A serological survey of tick-borne pathogens in dogs in North America and the Caribbean as assessed by *Anaplasma phagocytophilum*, *A. platys*, *Ehrlichia canis*, *E. chaffeensis*, *E. ewingii*, and *Borrelia burgdorferi* species-specific peptides. *Infect Ecol Epidemiol*. (2014) 4:24699. doi: 10.3402/iee.v4.24699

4. Centers for Disease Control and Prevention (CDC). *Statistics and Epidemiology: Annual Cases of Anaplasmosis in the United States*. (2020). Available online at: https://www.cdc.gov/anaplasmosis/stats/index.html (accessed March 10, 2021).

5. Dumler JS. The biological basis of severe outcomes in *Anaplasma phagocytophilum* infection. *FEMS Immunol Med Microbiol*. (2012) 64:13–20. doi: 10.1111/j.1574-695X.2011.00909.x

6. Center for Disease Control and Prevention. *Reportable Vector-Borne Disease Cases by State*. (2016). Available online at: https://www.cdc.gov/ncezid/dvbd/vital-signs/2016-data.html (accessed May 03, 2021).

7. Matei IA, Estrada-Peña A, Cutler SJ, Vayssier-Taussat M, Varela-Castro L, Potkonjak A, et al. A review on the eco-epidemiology and clinical management of human granulocytic anaplasmosis and its agent in Europe. *Parasit Vectors*. (2019) 12:599. doi: 10.1186/s13071-019-3852-6

8. Zhang L, Cui F, Wang L, Zhang L, Zhang J, Wang S, et al. Investigation of anaplasmosis in Yiyuan County, Shandong Province, China. *Asian Pac J Tro Med*. (2011) 4:568–72. doi: 10.1016/S1995-7645(11)60148-X

9. Bowman D, Little SE, Lorentzen L, Shields J, Sullivan MP, Carlin EP. Prevalence and geographic distribution of *Dirofilaria immitis*, *Borrelia burgdorferi*, *Ehrlichia canis*, and *Anaplasma phagocytophilum* in dogs in the United States: results of a national clinic-based serologic survey. *Vet Parasitol*. (2009) 160:138–48. doi: 10.1016/j.vetpar.2008.10.093

10. Foley JE, Foley P, Madigan JE. Spatial distribution of seropositivity to the causative agent of granulocytic ehrlichiosis in dogs in California. *Am J Vet Res*. (2001) 62:1599–605. doi: 10.2460/ajvr.2001.62.1599

11. Solano-Gallego L, Llull J, Osso M, Hegarty B, Breitschwerdt E. A serological study of exposure to arthropod-borne pathogens in dogs from northeastern Spain. *Vet Res*. (2006) 37:231–44. doi: 10.1051/vetres:2005054

12. Henn JB, Gabriel MW, Kasten RW, Brown RN, Theis JH, Foley JE, et al. Gray foxes (*Urocyon cinereoargenteus*) as a potential reservoir of a *Bartonella clarridgeiae*-like bacterium and domestic dogs as sentinels for zoonotic arthropod-borne pathogens in northern California. *J Clin Microbiol*. (2007) 45:2411–8. doi: 10.1128/JCM.02539-06

13. Beall MJ, Chandrashekar R, Eberts MD, Cyr KE, Diniz PPVP, Mainville C, et al. Serological and molecular prevalence of *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, and *Ehrlichia* species in dogs from Minnesota. *Vector Borne Zoonotic Dis.* (2008) 8:455–64. doi: 10.1089/vbz. 2007.0236

14. Hamer SA, Tsao JI, Walker ED, Mansfield L, Foster ES, Graham HJ. Use of tick surveys and serosurveys to evaluate pet dogs as a sentinel species for emerging Lyme disease. *Am J Vet Res.* (2009) 70:49–56. doi: 10.2460/ajvr.70.1.49

15. McMahan CS, Wang D, Beall MJ, Bowman DD, Little SE, Pithua PO, et al. Factors associated with *Anaplasma* spp. seroprevalence among dogs in the United States. *Parasit Vectors.* (2016) 9:169. doi: 10.1186/s13071-016-1431-7

16. Pusterla N, Huder J, Wolfensburger C, Litschi B, Parvis A, Lutz H. Granulocytic ehrlichiosis in two dogs in Switzerland. *J Clin Microbiol.* (1997) 35:2307–9. doi: 10.1128/JCM.35.9.2307-2309.1997

17. Morissette E, Massung RF, Foley JE, Alleman AR, Foley P, Barbet AF. Diversity of *Anaplasma phagocytophilum* strains, USA. *Emerg Infect Dis.* (2009) 15:928–31. doi: 10.3201/eid1506.081610

18. Scorpio DG, Dumler JS, Barat NC, Cook JA, Barat CE, Stillman BA, et al. Comparative strain analysis of *Anaplasma phagocytophilum* infection and clinical outcomes in a canine model of granulocytic anaplasmosis. *Vector Borne Zoonotic Dis.* (2011) 11:223–9. doi: 10.1089/vbz.2009.0262

19. Barbet AF, Al-Khedery B, Stuen S, Granquist EG, Felsheim RF, Munderloh UG. An emerging tick-borne disease of humans is caused by a subset of strains with conserved genome structure. *Pathogens.* (2013) 2:544–55. doi: 10.3390/pathogens2030544

20. Al-Khedery B, Barbet AF. Comparative genomics identifies a potential marker of human-virulent *Anaplasma phagocytophilum*. *Pathogens.* (2014) 3:25–35. doi: 10.3390/pathogens3010025

21. Strasek Smrdel K, von Loewenich FD, Petrovec M, Avsic Zupanc T. Diversity of ankA and msp4 genes of *Anaplasma phagocytophilum* in Slovenia. *Ticks Tick Borne Dis.* (2015) 6:164–6. doi: 10.1016/j.ttbdis.2014.11.008

22. Dahlgren FS, Mandel EJ, Krebs JW, Massung RF, McQuinston JH. Increasing incidence of *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum* in the United States, 2000–2007. *Am J Trop Med Hyg.* (2011) 85:124–31. doi: 10.4269/ajtmh.2011.10-0613

23. Little SE, Beall MJ, Bowman DD, Chandrashekar R, Stamaris J. Canine infection with *Dirofilaria immitis*, *Borrelia burgdorferi*, *Anaplasma* spp., and *Ehrlichia* spp. In the United States, 2010–2012. *Parasit Vectors.* (2014) 7:257. doi: 10.1186/1756-3305-7-257

24. Poitout FM, Shinozaki JK, Stockwell PJ, Holland CJ, Shukla SK. Genetic variants of *Anaplasma phagocytophilum* infecting dogs in Western Washington State. *J Clin Microbiol.* (2005) 43:796–801. doi: 10.1128/JCM.43.2.796-801.2005

25. Kohn B, Galke D, Beelitz P, Pfister K. Clinical features of canine granulocytic anaplasmosis in 18 naturally infected dogs. *J Vet Intern Med.* (2008) 22:1289–95. doi: 10.1111/j.1939-1676.2008.0180.x

26. Granick JL, Armstrong PJ, Bender JB. *Anaplasma phagocytophilum* infection in dogs: 34 cases (2000–2007). *J Am Vet Med Assoc.* (2009) 234:1559–65. doi: 10.2460/javma.234.12.1559

27. Eberts MD, Diniz PP, Beall MJ, Stillman BA, Chandrashekar R, Breitschwerdt EB. Typical and atypical manifestations of *Anaplasma phagocytophilum* in dogs. *J Am Anim Hosp Assoc.* (2011) 47:88–94. doi: 10.5326/JAAHA-MS-5578

28. Ravnik U, Bajuk BP, Lusa L, Tozon N. Serum protein profiles, circulating immune complexes and proteinuria in dogs naturally infected with *Anaplasma phagocytophilum*. *Vet Microbiol.* (2014) 173:160–5. doi: 10.1016/j.vetmic.2014.07.007

29. Greig B, Asanovich KM, Armstrong PJ, Dumler JS. Geographic, clinical, serologic, and molecular evidence of granulocytic ehrlichiosis, a likely zoonotic disease, in Minnesota and Wisconsin dogs. *J Clin Microbiol.* (1996) 34:44–8. doi: 10.1128/JCM.34.1.44-48.1996

30. Egenvall AE, Hedhammar AA, Bjoersdorff AI. Clinical features and serology of 14 dogs affected by granulocytic ehrlichiosis in Sweden. *Vet Rec.* (1997) 140:222–6. doi: 10.1136/vr.140.9.222

31. Jensen J, Simon D, Escobar HM, Soller JT, Bullerdiek J, Beelitz P, et al. Anaplasma phagocytophilum in dogs in Germany. *Zoonoses Public Health.* (2007) 57:94–101. doi: 10.1111/j.1863-2378.2007.01028.x

32. Ravnik U, Tozon N, Strasek K, Avsic Zupanc T. Clinical and haematological features in Anaplasma phagocytophilum seropositive dogs. *Clin Microbiol Infect.* (2009) 15:39–40. doi: 10.1111/j.1469-0691.2008.02167

33. Mazepa AW, Kidd LB, Young KM, Trepanier L. Clinical presentation of 26 *Anaplasma phagocytophilum*-seropositive dogs residing in an endemic area. *J Am Animal Hosp Assoc.* (2010) 46:405–12. doi: 10.5326/0460405

34. Ravnik U, Tozon N, Strasek Smrdel K, Avsic Zupanc T. Anaplasmosis in dogs: the relation of haematological, biochemical and clinical alterations to antibody titre and PCR confirmed infection. *Vet Microbiol.* (2011) 149:172–6. doi: 10.1016/j.vetmic.2010.10.005

35. Chirek A, Silaghi C, Pfister K, Kohn B. Granulocytic anaplasmosis in 63 dogs: clinical signs, laboratory results, therapy and course of disease. *J Small Anim Pract.* (2018) 59:112–20. doi: 10.1111/jsap.12787

36. Dunning Hotopp JC, Lin M, Madupu R, Crabtree J, Angiuoli SV, Eisen JA, et al. Comparative genomics of emerging human ehrlichiosis agents. *PLoS Genet.* (2006) 2:e21. doi: 10.1371/journal.pgen.0020021

37. Uilenberg G, Thiaucourt F, Jongejan F. On molecular taxonomy: What is in a name? *Exp Appl Acarol.* (2004) 32:301–12. doi: 10.1023/b:appa.0000023235.23090.a7

38. Barlough JE, Madigan JE, DeRock E, Dumler JS, Bakken JS. Protection against *Ehrlichia equi* is conferred by prior infection with the human granulocytic Ehrlichia species (HGE agent). *J Clin Microbiol.* (1995) 33:3333–4. doi: 10.1128/JCM.33.12.3333-3334.1995

39. Dumler JS, Asavovich KM, Bakken JS, Richter P, Kimsey R, Madigan JE. Serologic cross-reactions among *Ehrlichia equi*, *Ehrlichia phagocytophila*, and human granulocytic Ehrlichia. *J Clin Microbiol.* (1995) 33:1098–103. doi: 10.1128/JCM.33.5.1098-1103.1995

40. Popov VL, Han VC, Chen SM, Dumler JS, Feng HM, Andreadis TG, et al. Ultrastructural differentiation of the genogroups in the genus *Ehrlichia*. *J Med Microbiol.* (1998) 47:235–51. doi: 10.1099/00222615-47-3-235

41. Massung RF, Mather TN, Priestley RA, Levin ML. Transmission efficiency of the AP-variant 1 strain of *Anaplasma phagocytophila*. *Ann N Y Acad Sci.* (2003) 990:75–9. doi: 10.1111/j.1749-6632.2003.tb07340.x

42. Massung RF, Priestley RA, Miller NJ, Mather TN, Levin ML. Inability of a variant strain of *Anaplasma phagocytophilum* to infect mice. *J Infect Dis.* (2003) 188:1757–63. doi: 10.1086/379725

43. Massung RF, Mauel RJ, Owens JH, Allan N, Courtney JW, Stafford KC III, Mather TN. Genetic variants of *Ehrlichia phagocytophila*, Rhode Island and Connecticut. *Emerg Infect Dis.* (2002) 8:467–72. doi: 10.3201/eid0805.010251

44. Massung RF, Courtney JW, Hiratzka SL, Pitzer VE, Smith G, Dryden RL. *Anaplasma phagocytophilum* in white-tailed deer. *Emerg Infect Dis.* (2005) 11:1604–6. doi: 10.3201/eid1110.041329

45. Silaghi C, Kohn B, Chirek A, Thiel C, Nolte I, Liebisch, et al. Relationship of molecular and clinical findings on *Anaplasma phagocytophilum* involved in natural infections of dogs. *J Clin Microbiol.* (2011) 49:4413–4. doi: 10.1128/JCM.06041-11

46. Foley J, Nieto NC, Madigan J, Sykes J. Possible differential host tropism in *Anaplasma phagocytophilum* strains in the western United States. *Ann NY Acad Sci.* (2008) 1149:94–7. doi: 10.1196/annals.1428.066

47. Hoar BR, Nieto NC, Rhodes DM, Foley JE. Evaluation of sequential coinfection with *Anaplasma phagocytophilum* and *Anaplasma marginale* in cattle. *Am J Vet Res.* (2008) 69:1171–8. doi: 10.2460/ajvr.69.9.1171

48. Massung RF, Lee K, Mauel MJ, Gusa A. Characterization of the rRNA genes of *Ehrlichia chaffeensis* and *Anaplasma phagocytophila*. *DNA Cell Biol.* (2002) 21:587–96. doi: 10.1089/104454902320308960

49. Dugat T, Lagrée AC, Maillard R, Boulouis HJ, Haddad N. Opening the black box of *Anaplasma phagocytophilum* diversity: current situation and future perspectives. *Front Cell Infect Microbiol.* (2015) 5:61. doi: 10.3389/fcimb.2015.00061

50. de la Fuente J, Massung RF, Wong SJ, Chu FK, Lutz H, Meli M, et al. Sequence analysis of the msp4 gene of *Anaplasma phagocytophilum* strains. *J Clin Microbiol.* (2005) 43:1309–17. doi: 10.1128/JCM.43.3.1309-1317.2005

51. Huhn C, Winter C, Wolfsperger T, Wüppenhorst N, Strašek Smrdel K, Skuballa J, et al. Analysis of the population structure of *Anaplasma phagocytophilum* using multilocus sequence typing. *PLoS ONE.* (2014) 9:e93725. doi: 10.1371/journal.pone.0093725

52. Rar V, Golovljova I. *Anaplasma*, *Ehrlichia*, and "*Candidatus Neoehrlichia*" bacteria: pathogenicity, biodiversity, and molecular genetic characteristics, a review. *Infect Genet Evol.* (2011) 11:1842–61. doi: 10.1016/j.meegid.2011.09.019

53. Hovius E, de Bruin A, Schouls L, Hovius J, Dekker N, Sprong H. A lifelong study of a pack Rhodesian ridgeback dogs reveals subclinical and clinical tick-borne *Anaplasma phagocytophilum* infections with possible reinfection or persistence. *Parasit Vectors.* (2018) 11:238. doi: 10.1186/s13071-018-2806-8

54. Bown KJ, Lambin X, Ogden NH, Petrovec M, Shaw SE, Woldehiwet Z, Birtles RJ. High-resolution genetic fingerprinting of European strains of *Anaplasma phagocytophilum* by use of multilocus variable-number tandem-repeat analysis. *J Clin Microbiol.* (2007) 45:1771–6. doi: 10.1128/JCM.00365-07

55. Bown KJ, Lambin X, Ogden NH, Begon M, Telford G, Woldehiwet Z, Birtles RJ. Delineating *Anaplasma phagocytophilum* ecotypes in coexisting discrete enzootic cycles. *Emerg Infect Dis.* (2009) 15:1948–54. doi: 10.3201/eid1512.090178

56. Scharf W, Schauer S, Freyburger F, Petrovec M, Schaarschmidt-Kiener D, Liebisch G, et al. Distinct host species correlate with *Anaplasma phagocytophilum* ankA gene clusters. *J Clin Microbiol.* (2011) 49:790–6. doi: 10.1128/JCM.02051-10

57. Barbet AF, Meeus PFM, Belanger M, Bowie MV, Yi J, Lundgren AM, et al. Expression of multiple outer membrane protein sequence variants from a single genomic locus of *Anaplasma phagocytophilum*. *Infect Immun.* (2003) 71:1706–18. doi: 10.1128/iai.71.4.1706-1718.2003

58. Paulauskas A, Radzijevskaja J, Rosef O. Molecular detection and characterization of *Anaplasma phagocytophilum* strains. *Comp Immunol Microbiol Infect Dis.* (2012) 35:187–95. doi: 10.1016/j.cimid.2012.01.001

59. Jahfari S, Coipan EC, Fonville M, van Leeuwen AD, Hengeveld P, Dieter H, et al. Circulation of four *Anaplasma phagocytophilum* ecotypes in Europe. *Parasit Vectors.* (2014) 7:365. doi: 10.1186/1756-3305-7-365

60. Engvall EO, Pettersson B, Persson M, Artursson K, Johansson KE. A 16S rRNA- based PCR assay for detection and identification of granulocytic *Ehrlichia* species in dogs, horses, and cattle. *J Clin Microbiol.* (1996) 34:2170–4. doi: 10.1128/JCM.34.9.2170-2174.1996

61. von Stedingk LV, Gürtelschmid M, Hanson HS, Gustafson R, Dotevall L, Engvall EO, et al. The human granulocytic ehrlichiosis (HGE) agent in Swedish ticks. *Clin Microbiol Infect.* (1997) 3:573–574. doi: 10.1111/j.1469-0691.1997.tb00311.x

62. Pusterla N, Pusterla JB, Deplazes P, Wolfensberger C, Muller W, Horauf A, et al. Seroprevalence of *Ehrlichia canis* and of canine granulocytic *Ehrlichia* infection in dogs in Switzerland. *J Clin Microbiol.* (1998) 36:3460–2. doi: 10.1128/JCM.36.12.3460-3462.1998

63. Zhang L, Liu Y, Ni D, Li Q, Yu Y, Yu XJ, et al. Nosocomial transmission of human granulocytic anaplasmosis in China. *J Am Med Assoc.* (2008) 300:2263–70. doi: 10.1001/jama.2008.626

64. Horowitz HW, Kilchevsky E, Haber S, Aguero-Rosenfeld M, Kranwinkel R, James EK, et al. Perinatal transmission of the agent of human granulocytic ehrlichiosis. *N Engl J Med.* (1998) 339:375–8. doi: 10.1056/NEJM199808063390604

65. Bakken JS, Krueth JK, Lund T, Malkovitch D, Asanovich K, Dumler JS. Exposure to deer blood may be a cause of human granulocytic ehrlichiosis. *Clin Infect Dis.* (1996) 23:198. doi: 10.1093/clinids/23.1.198

66. Fine AB, Sweeney JD, Nixon CP, Knoll BM. Transfusion-transmitted anaplasmosis from a leukoreduced platelet pool. *Transfusion.* (2016) 56:699–704. doi: 10.1111/trf.13392

67. Beugnet F, Marie JL. Emerging arthropod-borne diseases of companion animals in Europe. *Vet Parasitol.* (2009) 163:298–305. doi: 10.1016/j.vetpar.2009.03.028

68. Stuen S, Granquist EG, Silaghi C. *Anaplasma phagocytophilum* — a widespread multi-host pathogen with highly adaptive strategies. *Front Cell Infect Microbiol.* (2013) 3:31. doi: 10.3389/fcimb.2013.00031

69. Woldehiwet Z. The natural history of *Anaplasma phagocytophilum*. *Vet Parasitol.* (2010) 167:108–22. doi: 10.1016/j.vetpar.2009.09.013

70. Keirans JE, Hutcheson HJ, Durden LA, Klompen JS. *Ixodes scapularis* (*Acari: Ixodidae*): redescription of all active stages, distribution, hosts, geographical variation, and medical and veterinary importance. *J Med Entomol.* (1996) 33:297–318. doi: 10.1093/jmedent/33.3.297

71. Richter PJ Jr, Kimsey RB, Madigan JE, Barlough JE, Dumler JS, Brooks DL. *Ixodes pacificus* (*Acari: Ixodidae*) as a vector of *Ehrlichia equi* (*Rickettsiales: Ehrlichieae*). *J Med Entomol.* (1996) 33:1–5. doi: 10.1093/jmedent/33.1.1

72. des Vignes F, Levin ML, Fish D. Comparative vector competence of *Dermacentor variabilis* and *Ixodes scapularis* (*Acari: Ixodidae*) for the agent of human granulocytic ehrlichiosis. *J Med Entomol.* (1999) 36:182–5. doi: 10.1093/jmedent/36.2.182

73. Magnarelli LA, Stafford KC III, Mather TN, Yeh MT, Horn KD, Dumler JS. Hemocytic rickettsia-like organisms in ticks: serologic reactivity with antisera to Ehrlichiae and detection of DNA of agent of human granulocytic ehrlichiosis by PCR. *J Clin Microbiol.* (1995) 33:2710–4. doi: 10.1128/JCM.33.10.2710-2714.1995

74. Moreno CX, Moy F, Daniels TJ, Godfrey HP, Cabello FC. Molecular analysis of microbial communities identified in different developmental stages of *Ixodes scapularis* ticks from Westchester and Dutchess Counties, New York. *Environ Microbiol.* (2006) 8:761–72. doi: 10.1111/j.1462-2920.2005.00955.x

75. Swanson KI, Norris DE. Co-circulating microorganisms in questing *Ixodes scapularis* nymphs in Maryland. *J Vector Ecol.* (2007) 32:243–51. doi: 10.3376/1081-1710(2007)32[243:cmiqis]2.0.co;2

76. Walk ST, Xu G, Stull JW, Rich SM. Correlation between tick density and pathogen endemicity, New Hampshire. *Emerg Infect Dis.* (2009) 15:585–7. doi: 10.3201/eid1504.080940

77. Goethert HK, Telford SR. Enzootic transmission of the agent of human granulocytic ehrlichiosis among cottontail rabbits. *Am J Trop Med Hyg.* (2003) 68:633–7.

78. Baldridge GD, Scoles GA, Burkhardt NY, Schloeder B, Kurtti TJ, Munderloh UG. Transovarial transmission of *Francisella*-like endosymbionts and *Anaplasma phagocytophilum* variants in *Dermacentor albipictus* (*Acari: Ixodidae*). *J Med Entomol.* (2009) 46:625–32. doi: 10.1603/033.046.0330

79. Zeidner NS, Burkot TR, Massung R, Nicholson WL, Dolan MC, Rutherford JS, et al. Transmission of the agent of human granulocytic ehrlichiosis by Ixodes spinipalpis ticks: evidence of an enzootic cycle of dual infection with *Borrelia burgdorferi* in Northern Colorado. *J Infect Dis.* (2000) 182:616–9. doi: 10.1086/315715

80. Holden K, Boothby JT, Anand S, Massung RF. Detection of *Borrelia burgdorferi*, *Ehrlichia chaffeensis*, and *Anaplasma phagocytophilum* in ticks (Acari: Ixodidae) from a coastal region of California. *J Med Entomol.* (2003) 40:534–9. doi: 10.1603/0022-2585-40.4.534

81. Clark L. *Anaplasma phagocytophilum* in small mammals and ticks in northeast Florida. *J Vector Ecol.* (2012) 37:262–8. doi: 10.1111/j.1948-7134.2012.00226.x

82. Foley J, Rejmanek D, Fleer K, Nieto N. Nidicolous ticks of small mammals in *Anaplasma phagocytophilum*-enzootic sites in northern California. *Ticks Tick Borne Dis.* (2011) 2:75–80. doi: 10.1016/j.ttbdis.2011.03.003

83. Rejmanek D, Freycon P, Bradburd G, Dinstell J, Foley J. Unique strains of *Anaplasma phagocytophilum* segregate among diverse questing and non-questing *Ixodes* tick species in the western United States. *Ticks Tick Borne Dis.* (2013) 4:482–7. doi: 10.1016/j.ttbdis.2013.06.003

84. Santos HA, Thomé SM, Baldani CD, Silva CB, Peixoto MP, Pires MS, et al. Molecular epidemiology of the emerging zoonosis agent *Anaplasma phagocytophilum* (Foggie, 1949) in dogs and Ixodid ticks in Brazil. *Parasit Vectors.* (2013) 6:348. doi: 10.1186/1756-3305-6-348

85. Campos-Calderón L, Ábrego-Sánchez L, Solórzano-Morales A, Alberti A, Tore G, Zobba R, et al. Molecular detection and identification of Rickettsiales pathogens in dog ticks from Costa Rica. *Ticks Tick Borne Dis.* (2016) 7:1198–202. doi: 10.1016/j.ttbdis.2016.07.015

86. Sosa-Gutierrez CG, Vargas-Sandoval M, Torres J, Gordillo-Pérez G. Tick-borne rickettsial pathogens in questing ticks, removed from humans and animals in Mexico. *J Vet Sci.* (2016) 17:353–60. doi: 10.4142/jvs.2016.17.3.353

87. Asman M, Nowak M, Cuber P, Strzelczyk J, Szilman E, Szilman P, et al. The risk of exposure to *Anaplasma phagocytophilum*, *Borrelia burgdorferi* sensu lato, *Babesia sp.* and co-infections in *Ixodes ricinus* ticks on the territory of Niepołomice Forest (southern Poland). *Ann Parasitol.* (2013) 59:13–19.

88. Michelet L, Delannoy S, Devillers E, Umhang G, Aspan A, Juremalm M, et al. High-throughput screening of tick-borne pathogens in Europe. *Front Cell Infect Microbiol.* (2014) 4:103. doi: 10.3389/fcimb.2014.00103

89. Bown KJ, Lambin X, Telford GR, Ogden NH, Telfer S, Woldehiwet Z, 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) 74:7118–25. doi: 10.1128/AEM.00625-08

90. Silaghi C, Skuballa J, Thiel C, Pfister K, Petney T, Pfäffle M, et al. The European hedgehog (*Erinaceus europaeus*) — a suitable reservoir forvariants of *Anaplasma phagocytophilum*? *Ticks Tick Borne Dis.* (2012) 3:49–54. doi: 10.1016/j.ttbdis.2011.11.005

91. Santos AS, Santos-Silva MM, Almeida VC, Bacellar F, Dumler JS. Detection of *Anaplasma phagocytophilum* DNA in *Ixodes* ticks (Acari: *Ixodidae*) from Madeira island and Setubal district, mainland Portugal. *Emerg Infect Dis.* (2004) 10:1643–8. doi: 10.3201/eid1009.040276

92. Tomanovic S, Chochlakis D, Radulovic Z, Milutinovic M, Cakic S, Mihaljica D, et al. Analysis of pathogen co-occurrence in host-seeking adult hard ticks from Serbia. *Exp Appl Acarol.* (2013) 59:367–76. doi: 10.1007/s10493-012-9597-y

93. Bown KJ, Begon M, Bennett M, Woldehiwet Z, Ogden NH. Seasonal dynamics of *Anaplasma phagocytophila* in a rodent-tick (*Ixodes trianguliceps*) system, United Kingdom. *Emerg Infect Dis.* (2003) 9:63–70. doi: 10.3201/eid0901.020169

94. Blanarová L, Stanko M, Carpi G, Miklisová D, Víchová B, Mošanský L, et al. Distinct *Anaplasma phagocytophilum* genotypes associated with *Ixodes trianguliceps* ticks and rodents in Central Europe. *Ticks Tick Borne Dis.* (2014) 5:928–38. doi: 10.1016/j.ttbdis.2014.07.012

95. Cicculli V, DeCarreaux D, Ayhan N, Casabianca F, de Lamballerie X, Charrel R, et al. Molecular screening of Anaplasmataceae in ticks collected from cattle in Corsica, France. *Exp App Acarol.* (2020) 81:561–74. doi: 10.1007/s10493-020-00527-w

96. Keysary A, Massung RF, Inbar M, Wallach AD, Shanas U, Mumcuoglu K, et al. Molecular evidence for *Anaplasma phagocytophilum* in Israel. Emerg Infect Dis. (2007) 13:1411–2. doi: 10.3201/eid1309.070455

97. Psaroulaki A, Chochlakis D, Ioannou I, Florentia A, Gikas A, Tselentis Y. Acute anaplasmosis in human in Cyprus. *Clin Microbiol Infect.* (2008) 15:10–1. doi: 10.1111/j.1469-0691.2008.02646.x

98. Chastagner A, Bailly X, Leblond A, Pradier S, Vourc'h G. Single Genotype of *Anaplasma phagocytophilum* identified from ticks, Camargue, France. *Emerg Infect Dis.* (2013) 19:825–6. doi: 10.3201/eid1905.121003

99. Dugat T, Chastagner A, Lagrée AC, Petit E, Durand B, Thierry S, et al. A new multiple-locus variable-number tandem repeat analysis reveals different clusters for *Anaplasma phagocytophilum* circulating in domestic and wild ruminants. *Parasit Vectors.* (2014) 7:439. doi: 10.1186/1756-3305-7-439

100. Eremeeva ME, Oliveira A, Robinson JB, Ribakova N, Tokarevich NK, Dasch GA. Prevalence of bacterial agents in *Ixodes persulcatus* ticks from the Vologda Province of Russia. *Ann NY Acad Sci.* (2006) 1078:291–8. doi: 10.1196/annals.1374.054

101. Ybañez AP, Matsumoto K, Kishimoto T, Yokoyama N, Inokuma H. Dual presence of *Anaplasma phagocytophilum* and its closely related *Anaplasma* sp. in ixodid ticks in Hokkaido, Japan, and their specific molecular detection. *J Vet Med Sci.* (2012) 74:1551–60. doi: 10.1292/jvms.12-0197

102. Ohashi N, Inayoshi M, Kitamura K, Kawamori F, Kawaguchi D, Nishimura Y, et al. *Anaplasma phagocytophilum*-infected ticks, Japan. *Emerg Infect Dis.* (2005) 11:1780–3. doi: 10.3201/eid1111.050407

103. Cao WC, Zhan L, He J, Foley JE, DE Vlas SJ, Wu XM, et al. Natural *Anaplasma phagocytophilum* infection of ticks and rodents from a forest area of Jilin Province, China. *Am J Trop Med Hyg.* (2006) 75:664–8.

104. Chae JS, Yudo H, Shringi S, Klein TA, Kim HC, Chong ST, et al. Microbial pathogens in ticks, rodents and a shrew in northern Gyeonggi-do near the DMZ, Korea. *J Vet Sci.* (2008) 9:285–93. doi: 10.4142/jvs.2008.9.3.285

105. Yoshimoto K, Matsuyama Y, Matsuda H, Sakamoto L, Matsumoto K, Yokoyama N, et al. Detection of *Anaplasma bovis* and *Anaplasma phagocytophilum* DNA from *Haemaphysalis megaspinosa* in Hokkaido, Japan. *Vet Parasitol.* (2010) 168:170–2. doi: 10.1016/j.vetpar.2009.10.008

106. Kuo CC, Huang JL, Chien CH, Shih HC, Wang HC. First molecular detection of *Anaplasma phagocytophilum* in the hard tick *Rhipicephalus haemaphysaloides* in Taiwan. *Exp Appl Acarol.* (2018) 75:437–43. doi: 10.1007/s10493-018-0283-6

107. Zhang L, Liu H, Xu B, Lu Q, Li L, Chang L, et al. *Anaplasma phagocytophilum* infection in domestic animals in ten provinces/cities of China. *Am J Trop Med Hyg.* (2012) 87:185–9. doi: 10.4269/ajtmh.2012.12-0005

108. Jiang BG, Cao WC, Niu JJ, Wang JX, Li HM, Sun Y, et al. Detection and identification of *Ehrlichia* species in *Rhipicephalus* (*Boophilus*) *microplus* ticks in cattle from Xiamen, China. *Vector Borne Zoonotic Dis.* (2011) 11:325. doi: 10.1089/vbz.2009.0219

109. Wang S, Kou ZQ, Wang M, Ren YY, Hu B, Fang M, Bi ZW. A survey and identification of *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum* in Shandong. *Dis Surv.* (2012) 27:642–3. doi: 10.3784/j.issn.1003-9961.2012.8.018

110. Sarih M, M'Ghirbi Y, Bouattour A, Gern L, Baranton G, Pontic D. Detection and identification of *Ehrlichia* spp. in ticks collected in Tunisia and Morocco. *J Clin Microbiol.* (2005) 43:1127–32. doi: 10.1128/JCM.43.3.1127-1132.2005

111. Ghafar MW, Amer SA. Prevalence and first molecular characterization of *Anaplasma phagocytophilum*, the agent of human granulocytic anaplasmosis, in *Rhipicephalus sanguineus* ticks attached to dogs from Egypt. *J Adv Res.* (2012) 3:189–94. doi: 10.1016/j.jare.2011.08.002

112. M'ghirbi Y, Yaïch H, Ghorbel A, Bouattour A. *Anaplasma phagocytophilum* in horses and ticks in Tunisia. *Parasit Vectors.* (2012) 5:180. doi: 10.1186/1756-3305-5-180

113. Dumler JS, Madigan JE, Pusterla N, Bakken JS. Ehrlichioses in humans: epidemiology, clinical presentation, diagnosis, and treatment. *Clin Infect Dis.* (2007) 45:S45–51. doi: 10.1086/518146

114. Villeneuve A, Goring J, Marcotte L, Overvelde S. Seroprevalence of *Borrelia burgdorferi*, Anaplasma phagocytophilum, *Ehrlichia canis*, and *Dirofilaria immitis* among dogs in Canada. *Can Vet J.* (2011) 52:527–30.

115. Herrin BH, Peregrine AS, Goring J, Beall MJ, Little SE. Canine infection with *Borrelia burgdorferi*, *Dirofilaria immitis*, Anaplasma spp. and *Ehrlichia* spp. in Canada, 2013–2014. *Parasit Vectors.* (2017) 10:244. doi: 10.1186/s13071-017-2184-7

116. Evason M, Stull JW, Pearl DL, Peregrine AS, Jardine C, Buch JS, et al. Prevalence of *Borrelia burgdorferi*, *Anaplasma* spp., *Ehrlichia* spp. and *Dirofilaria immitis* in Canadian dogs, 2008 to 2015: a repeat cross-sectional study. *Parasit Vectors.* (2019) 12:64. doi: 10.1186/s13071-019-3299-9

117. Gary AT, Webb JA, Hegarty BC, Breitschwerdt EB. The low seroprevalence of tick-transmitted agents of disease in dogs from southern Ontario and Quebec. *Can Vet J.* (2006) 47:1194–200.

118. Gaunt MC, Carr AP, Taylor SM. Serological survey of canine vector-borne diseases in Saskatchewan, Canada. *Can Vet J.* (2018) 59:1109–11.

119. Yancey CB, Hegarty BC, Qurollo BA, Levy MG, Birkenheuer AJ, Weber DJ, et al. Regional seroreactivity and vector-borne disease co-exposures in dogs in the United States from 2004–2010: utility of canine surveillance. *Vector Borne Zoonotic Dis.* (2014) 14:724–32. doi: 10.1089/vbz.2014.1592

120. Carrade D, Foley J, Sullivan M, Foley CW, Sykes JE. Spatial distribution of seroprevalence for *Anaplasma phagocytophilum*, *Borrelia burgdorferi*, *Ehrlichia canis*, and *Dirofilaria immitis* in dogs in Washington, Oregon, and California. *Vet Clin Pathol.* (2011) 40:293–302. doi: 10.1111/j.1939-165X.2011.00334.x

121. Suksawat J, Hegarty BC, Breitschwerdt EB. Seroprevalence of *Ehrlichia canis*, *Ehrlichia equi*, and *Ehrlichia risticii* in sick dogs from North Carolina and Virginia. *J Vet Intern Med.* (2000) 14:50–5. doi: 10.1892/0891-6640(2000)014<0050:socear>2.3.co;2

122. Rand PW, Lacombe EH, Elias SP, Cahill BK, Lubelczyk CB, Smith RP Jr. Multitarget test for emerging Lyme disease and anaplasmosis in a serosurvey of dogs, Maine, USA. *Emerg Infect Dis.* (2011) 17:899–902. doi: 10.3201/eid1705.100408

123. Rodgers SJ, Morton RJ, Baldwin CA. A serological survey of *Ehrlichia canis*, *Ehrlichia equi*, *Rickettsia rickettsii*, and *Borrelia burgdorferi* in dogs in Oklahoma. *J Vet Diagn Invest.* (1989) 1:154–9. doi: 10.1177/104063878900100212

124. Diniz P, Beall MJ, Omark K, Chandrashekar R, Baniluk DA, Cyr KE, et al. High prevalence of tick-borne pathogens in dogs from an Indian Reservation in northeastern Arizona. *Vector Borne Zoonotic Dis.* (2010) 10:117–23. doi: 10.1089/vbz.2008.0184

125. Gaito A, Gjivoje V, Lutz S, Baxter B. Comparative analysis of the infectivity rate of both *Borrelia burgdorferi* and *Anaplasma phagocytophilum* in humans

and dogs in a New Jersey community. *Infect Drug Resist.* (2014) 7:199–201. doi: 10.2147/IDR.S68742

126. Balakrishnan N, Musulin S, Varanat M, Bradley JM, Breitschwerdt EB. Serological and molecular prevalence of selected canine vector borne pathogens in blood donor candidates, clinically healthy volunteers, and stray dogs in North Carolina. *Parasit Vectors.* (2014) 7:116. doi: 10.1186/1756-3305-7-116

127. Magnarelli L, Ijdo J, Anderson K, Madigan JE, Dumler JS, Fikrig E. Antibodies to *Ehrlichia equi* in dogs from the northeastern United States. *J Am Vet Med Assoc.* (1997) 211:1134–7.

128. Patterson G, Tanhauser M, Schmidt P, Spangler D, Faulkner C, Faulkner V, et al. Serosurvey of arthropod-borne diseases among shelter dogs in the Cumberland Gap Region of the United States. *BMC Vet Res.* (2020) 16:221. doi: 10.1186/s12917-020-02440-1

129. Santos HA, Pires MS, Vilela JAR, Santos TM, Faccini JLH, Baldani CD, et al. Detection of *Anaplasma phagocytophilum* in Brazilian dogs by real-time polymerase chain reaction. *J Vet Diagn Invest.* (2011) 23:770–4. doi: 10.1177/1040638711406974

130. Diniz PP, Schwartz DS, de Morais HS, Breitschwerdt EB. Surveillance for zoonotic vector-borne infections using sick dogs from southeastern Brazil. *Vector Borne Zoonotic Dis.* (2007) 7:689–97. doi: 10.1089/vbz.2007.0129

131. Lasta CS, dos Santos AP, Messick JB, Oliveira ST, Biondo AW, da Costa Vieira RF, et al. Molecular detection of *Ehrlichia canis* and *Anaplasma platys* in dogs in Southern Brazil. *Rev Bras Parasitol Vet.* (2013) 2:360–6. doi: 10.1590/S1984-29612013000300007

132. Vieira TSWJ, da Costa Vieira RF, Gomes do Nascimento AA, Tamekuni K, Dos Santos Toledo R, Chandrashekar R, et al. Serosurvey of tick-borne pathogens in dogs from urban and rural areas from Parana State, Brazil. *Rev Bras Parasitol Vet.* (2013) 22:104–9. doi: 10.1590/s1984-29612013000100019

133. McCown ME, Opel T, Grzeszak B. Vector-borne disease surveillance in Puerto rico: pathogen prevalence rates in canines? Implications for public health and the US Military? Applying the one health concept. *J Spec Oper Med.* (2013) 13:59–63.

134. McCown ME, Alleman A, Sayler KA, Chandrashekar R, Thatcher B, Tyrrell P, et al. Point prevalence survey for tick-borne pathogens in military working dogs, shelter animals, and pet populations in northern Colombia. *J Spec Oper Med.* (2014) 14:81–5.

135. Starkey LA, Newton K, Brunker J, Crowdis K, Edourad EJP, Meneus P, et al. Prevalence of vector-borne pathogens in dogs from Haiti. *Vet Parasitol.* (2016) 224:7–12. doi: 10.1016/j.vetpar.2016.04.017

136. Kelly PJ, Xu C, Lucas H, Loftis A, Abete J, Zeoli F, et al. Ehrlichiosis, babesiosis, anaplasmosis and hepatozoonosis in dogs from St. Kitts, West Indies. *PLoS ONE.* (2013) 8:e53450. doi: 10.1371/journal.pone.0053450

137. Movilla R, García C, Siebert S, Roura X. Countrywide serological evaluation of canine prevalence for *Anaplasma* spp., *Borrelia burgdorferi* (sensu lato), *Dirofilaria immitis* and *Ehrlichia canis* in Mexico. *Parasit Vectors.* (2016) 9:421. doi: 10.1186/s13071-016-1686-z

138. Bonilla MC, Campos-Calderón L, Jiménez-Rocha AE, Romero-Zúñiga JJ, Alberti A, Zobba R, et al. Characterization of *Anaplasma* spp. infection in dogs from Costa Rica. *Vet Parasitol Reg Stud Rep.* (2017) 8:60–5. doi: 10.1016/j.vprsr.2017.02.003

139. Jimenez IA, Vega Mariño PA, Stapleton GS, Prieto JB, Bowman DD. Canine vector-borne disease in domestic dogs on Isla Santa Cruz, Galápagos. *Vet Parasitol Reg Stud Rep.* (2020) 19:100373. doi: 10.1016/j.vprsr.2020.100373

140. Springer A, Montenegro VM, Schicht S, Pantchev N, Strube C. Seroprevalence and current infections of canine vector-borne diseases in Nicaragua. *Parasit Vectors.* (2018) 11:585. doi: 10.1186/s13071-018-3173-1

141. Acosta-Jamett G, Weitzel T, López J, Alvarado D, Abarca K. Prevalence and risk factors of antibodies to *Anaplasma* spp. in Chile: a household-based cross-sectional study in healthy adults and domestic dogs. *Vector Borne Zoonotic Dis.* (2020) 20:572–9. doi: 10.1089/vbz.2019.2587

142. Krupka I, Pantchev N, Lorentzen L, Weise M, Straubinger RK. Tick-transmitted, bacterial infections in dogs: seroprevalences of *Anaplasma phagocytophilum*, *Borrelia burgdorferi* sensu lato and *Ehrlichia canis* in Germany. *Prakt Tierarzt.* (2007) 88:776–88.

143. Barutzki D, De Nicola A, Zeziola M, Reule M. Seroprevalence of *Anaplasma phagocytophilum* infection in dogs in Germany. *Berl Munch Tierarztl Wochenschr.* (2006) 119:342–7.

144. Kohn B, Silaghi C, Galke D, Arndt G, Pfister K. Infections with *Anaplasma phagocytophilum* in dogs in Germany. *Res Vet Sci.* (2011) 91:71–6. doi: 10.1016/j.rvsc.2010.08.008

145. Menn B, Lorentz S, Naucke TJ. Imported and travelling dogs as carriers of canine vector-borne pathogens in Germany. *Parasit Vectors.* (2010) 3:34. doi: 10.1186/1756-3305-3-34

146. Globokar Vrhovec M, Pantchev N, Failing K, Bauer C, Travers-Martin N, Zahner H. Retrospective analysis of canine vector-borne diseases (CVBD) in Germany with emphasis on the endemicity and risk factors of leishmaniosis. *Parasitol Res.* (2017) 116:S131–44. doi: 10.1007/s00436-017-5499-6

147. Barth C, Straubinger RK, Sauter-Louis C, Hartmann K. Prevalence of antibodies against *Borrelia burgdorferi* sensu lato and *Anaplasma phagocytophilum* and their clinical relevance in dogs in Munich, Germany. *Berl Münch Tierärztl Wochenschr.* (2012) 125:337–44.

148. Preyß-Jägeler C, Müller E, Straubinger RK, Hartmann K. Prevalence of antibodies against *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, and *Leptospira interrogans* serovars in Bernese mountain dogs. *Tierarztl Prax Ausg K Kleintiere Heimtiere.* (2016) 44:77–85. doi: 10.15654/TPK-140962

149. Liesner JM, Krücken J, Schaper R, Pachnicke S, Kohn B, Müller E, et al. Vector-borne pathogens in dogs and red foxes from the federal state of Brandenburg, Germany. *Vet Parasitol.* (2016) 224:44–51. doi: 10.1016/j.vetpar.2016.05.012

150. Volgina NS, Romashov BV, Romashova NB, Shtannikov AV. Prevalence of Borreliosis, Anaplasmosis, Ehrlichiosis and *Dirofilaria immitis* in dogs and vectors in Voronezh Reserve (Russia). *Comp Immunol Microbiol Infect Dis.* (2013) 36:567–74. doi: 10.1016/j.cimid.2013.08.003

151. Farkas R, Gyurkovszky M, Lukàcs Z, Aladics B, Solymosi N. Seroprevalence of some vector-borne infections of dogs in Hungary. *Vector Borne Zoonotic Dis.* (2014) 14:256–60. doi: 10.1089/vbz.2013.1469

152. Hornok S, Dénes B, Meli ML, Tanczos B, Fekete L, Gyuranecz M, et al. Non-pet dogs as sentinels and potential synanthropic reservoirs of tick-borne and zoonotic bacteria. *Vet Microbiol.* (2013) 167:700–3. doi: 10.1016/j.vetmic.2013.08.011

153. Majlathova V, Majlath I, Vichova B, Gul'ová I, Derdáková M, Sesztáková E, et al. Polymerase chain reaction confirmation of *Babesia canis* canis and *Anaplasma phagocytophilum* in dogs suspected of babesiosis in Slovakia. *Vector Borne Zoonotic Dis.* (2011) 11:1447–51. doi: 10.1089/vbz.2010.0276

154. Cabanová V, Pantchev N, Hurníková Z, Miterpáková M. Recent study on canine vector-borne zoonoses in southern Slovakia — serologic survey. *Acta Parasitol.* (2015) 60:749–58. doi: 10.1515/ap-2015-0107

155. Pantchev N, Schnyder M, Globokar Vrhovec M, Schaper R, Tsachev I. *Borrelia burgdorferi*, *Ehrlichia canis*, *Anaplasma phagocytophilum*, *Leishmania infantum*, *Babesia canis*, *Angiostrongylus vasorum* and *Dirofilaria immitis* in dogs in Bulgaria. *Parasitol Res.* (2015) 114:S117–30. doi: 10.1007/s00436-015-4518-8

156. Kirtz B, Czettel B, Thum D, Leidinger E. *Anaplasma phagozytophilum* in einer österreichischen Hundepopulation: eine Prävalenz-Studie (2001–2006). *Kleintierpraxis.* (2007) 52:562–8.

157. Shaw SE, Binns SH, Birtles RJ, Day MJ, Smithson R, Kenny MJ. Molecular evidence of tick-transmitted infections in dogs and cats in the United Kingdom. *Vet Rec.* (2005) 157:645–8. doi: 10.1136/vr.157.21.645

158. Egenvall A, Bonnett BN, Gunnarsson A, Hedhammar A, Shoukri M, Bornstein S, et al. Seroprevalence of granulocytic *Ehrlichia* spp. and *Borrelia burgdorferi* sensu lato in Swedish dogs 1991–1994. *Scand J Infect Dis.* (2000) 32:19–25. doi: 10.1080/00365540050164164

159. Elfving K, Malmsten J, Dalin AM, Nilsson K. 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) 15:529–34. doi: 10.1089/vbz.2015.1768

160. Pérez Vera C, Kapiainen S, Junnikkala S, Aaltonen K, Spillmann T, Vapalahti O. Survey of selected tick-borne diseases in dogs in Finland. *Parasit Vectors.* (2014) 7:285. doi: 10.1186/1756-3305-7-285

161. Hamel D, Silaghi C, Knaus M, Visser M, Kusi I, Rapti D, et al. Detection of Babesia canis subspecies and other arthropod-borne diseases in dogs from Tirana, Albania. *Wien Klin Wochenschr.* (2009) 121:42–5. doi: 10.1007/s00508-009-1234-3

162. Hamel D, Shukullari E, Rapti D, Silaghi C, Pfister K, Rehbein S. Parasites and vector-borne pathogens in client-owned dogs in Albania. Blood pathogens

and seroprevalences of parasitic and other infectious agents. *Parasitol Res.* (2016) 115:489–99. doi: 10.1007/s00436-015-4765-8

163. Berzina I, Capligina V, Bormane A, Pavulina A, Baumanis V, Ranka R, et al. Association between Anaplasma phagocytophilum seroprevalence in dogs and distribution of Ixodes ricinus and Ixodes persulcatus ticks in Latvia. *Ticks Tick Borne Dis.* (2013) 4:83–8. doi: 10.1016/j.ttbdis.2012.08.003

164. Mircean V, Dumitrache MO, Gyöke A, Pantchev N, Jodies R, Mihalca D, et al. Seroprevalence and geographic distribution of *Dirofilaria immitis* and tick-borne infections (*Anaplasma phagocytophilum, Borrelia burgdorferi* sensu lato, and *Ehrlichia canis*) in dogs from Romania. *Vector Borne Zoonotic Dis.* (2012) 12:595–604. doi: 10.1089/vbz.2011.0915

165. Hamel D, Silaghi C, Lescai D, Pfister K. Epidemiological aspects on vector-borne infections in stray and pet dogs from Romania and Hungary with focus on Babesia spp. *Parasitol Res.* (2012) 110:1537–45. doi: 10.1007/s00436-011-2659-y

166. Matei IA, Ionică AM, D'Amico G, Corduneanu A, Daskalaki AA, Lefkaditis M, et al. Altitude-dependent prevalence of canine granulocytic anaplasmosis in Romania. *Vector Borne Zoonotic Dis.* (2017) 17:147–51. doi: 10.1089/vbz.2016.1998

167. Enache D, Imre M, Ilie MS, Coprean D. Seroprevalence of *Anaplasma phagocytophilum* in dogs from Constanta county. *J Biotechnol.* (2015) 208:S95. doi: 10.1016/j.jbiotec.2015.06.298

168. Cazan CD, Ionică AM, Matei IA, D'Amico G, Muñoz C, Berriatua E, et al. Detection of *Leishmania infantum* DNA and antibodies against *Anaplasm*a spp., *Borrelia burgdorferi* s.l. and *Ehrlichia canis* in a dog kennel in South-Central Romania. *Acta Vet Scand.* (2020) 62:42. doi: 10.1186/s13028-020-00540-4

169. Potkonjak A, Gutiérrez R, Savic S, Vračar V, Nachum-Biala Y, Jurišić A, et al. Molecular detection of emerging tick-borne pathogens in Vojvodina, Serbia. *Ticks Tick Borne Dis.* (2016) 7:199–203. doi: 10.1016/j.ttbdis.2015.10.007

170. Kovačević Filipović MM, Beletić AD, Ilić BoŽović AV, Milanović Z, Tyrrell P, Buch J, et al. Molecular and Serological Prevalence of *Anaplasma phagocytophilum, A. platys, Ehrlichia canis, E. chaffeenses, E. ewingii, Borrelia burgdorferi, Babesia canis, B. gibsoni* and *B. vogeli* among clinically healthy outdoor dogs in Serbia. *Vet Parasitol Reg Stud Reports.* (2018) 14:117-122. doi: 10.1016/j.vprsr.2018.10.001

171. Krämer F,, Schaper R, Schunack B, Polozowski A, Piekarska J, Szwedko A, et al. Serological detection of *Anaplasma phagocytophilum, Borrelia burgdorferi* sensu lato and *Ehrlichia canis* antibodies and *Dirofilaria immitis* antigen in a countrywide survey in dogs in Poland. *Parasitol Res.* (2014) 113:3229–39. doi: 10.1007/s00436-014-3985-7

172. Dziegiel B, Adaszek L, Carbonero A, Łyp P, Winiarczyk M, Debiak P, et al. Detection of canine vector-borne diseases in eastern Poland by ELISA and PCR. *Parasitol Res.* (2016) 115:1039–44. doi: 10.1007/s00436-015-4832-1

173. Skotarczak B, Adamska M, Rymaszewska A, Supron M, Sawczuk M, Maciejewska A. *Anaplasma phagocytophila* and protozoans of Babesia genus in dogs from endemic areas of Lyme disease in north-western Poland. *Wiad Parazytol.* (2004) 50:555–61.

174. Rymaszewska A, Adamska M. Molecular evidence of vector-borne pathogens coinfecting dogs from Poland. *Acta Vet Hung.* (2011) 59:215–23. doi: 10.1556/AVet.2011.008

175. Welc-Faleciak R, Rodo A, Siński E, Bajer A. *Babesia canis* and other tick-borne infections in dogs in Central Poland. *Vet Parasitol.* (2009) 166:191–8. doi: 10.1016/j.vetpar.2009.09.038

176. Kybicová K, Schánilec P, Hulínská D, Uherková L, Kurzová Z, Spejchalová S. Detection of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* sensu lato in dogs in the Czech Republic. *Vector Borne Zoonotic Dis.* (2009) 9:655–62. doi: 10.1089/vbz.2008.0127

177. Pennisi MG, Caprì A, Solano-Gallego L, Lombardo G, Torina A, Masucci M. Prevalence of antibodies against *Rickettsia conorii, Babesia canis, Ehrlichia canis*, and *Anaplasma phagocytophilum* antigens in dogs from the Stretto di Messina area (Italy). *Ticks Tick Borne Dis.* (2012) 3:315–8. doi: 10.1016/j.ttbdis.2012.10.026

178. Ebani VV, Bertelloni F, Torracca B, Cerri D. Serological survey of *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*, and *Ehrlichia canis* infections in rural and urban dogs in Central Italy. *Ann Agric Environ Med.* (2014) 21:671–5. doi: 10.5604/12321966.1129912

179. Ebani VV, Cerri D, Fratini F, Ampola M, Andreani E. Seroprevalence of *Anaplasma phagocytophilum* in domestic and wild animals from central Italy. *New Microbiol.* (2008) 31:371–5.

180. Ebani VV, Bertelloni F, Turchi B, Cerri D. Serological and molecular survey of Anaplasma phagocytophilum in Italian hunting dogs. *Ann Agric Environ Med.* (2013) 20:289–92.

181. Ebani VV. Serological survey of *Ehrlichia canis* and *Anaplasma phagocytophilum* in dogs from central Italy: an update (2013–2017). *Pathogens.* (2019) 8:3. doi: 10.3390/pathogens8010003

182. de la Fuente J, Torina A, Naranjo V, Nicosia S, Alongi A, La Mantia F, et al. Molecular characterization of *Anaplasma platys* strains from dogs in Sicily, Italy. *BMC Vet Res.* (2006) 2:24. doi: 10.1186/1746-6148-2-24

183. Torina A, Vicente J, Alongi A, Scimeca S, Turla R, Nicosia S, et al. Observed prevalence of tick-borne pathogens in domestic animals in Sicily, Italy during 2003–2005. *Zoonoses Public Health.* (2007) 54:8–15. doi: 10.1111/j.1863-2378.2007.00989.x

184. Torina A, Alongi A, Naranjo V, Scimeca S, Nicosia S, Di Marco V, et al. Characterization of Anaplasma infections in Sicily, Italy. *Ann N Y Acad Sci.* (2008) 1149:90–3. doi: 10.1196/annals.1428.065

185. Solano-Gallego L, Caprì A, Pennisi MG, Caldin M, Furlanello T, Trotta M. Acute febrile illness is associated with *Rickettsia* spp. infection in dogs. *Parasit Vectors.* (2015) 8:216. doi: 10.1186/s13071-015-0824-3

186. Vascellari M, Ravagnan S, Carminato A, Cazzin S, Carli E, Da Rold G, et al. Exposure to vector-borne pathogens in candidate blood donor and free-roaming dogs of northeast Italy. *Parasit Vectors.* (2016) 9:369. doi: 10.1186/s13071-016-1639-6

187. Alberti A, Addis MF, Sparagano O, Zobba R, Chessa B, Cubeddu T, et al. *Anaplasma phagocytophilum*, Sardinia, Italy. *Emerg Infect Dis.* (2005) 11:1322–4. doi: 10.3201/eid1108.050085

188. Cardoso L, Mendão C, Madeira de Carvalho L. Prevalence of *Dirofilaria immitis, Ehrlichia canis, Borrelia burgdorferi* sensu lato, *Anaplasma* spp. and *Leishmania infantum* in apparently healthy and CVBD-suspect dogs in Portugal — a national serological study. *Parasit Vectors.* (2012) 5:62. doi: 10.1186/1756-3305-5-62

189. Santos AS, Alexandre N, Sousa R, Núncio MS, Bacellar F, Dumler JS. Serological and molecular survey of Anaplasma species infection in dogs with suspected tick-borne disease in Portugal. *Vet Rec.* (2009) 164:168–71. doi: 10.1136/vr.164.6.168

190. Santos AS, Bacellar F, Dumler JS. A 4-year study of *Anaplasma phagocytophilum* in Portugal. *Clin Microbiol Infect.* (2009) 15:46–7. doi: 10.1111/j.1469-0691.2008.02172

191. Alho AM, Pita J, Amaro A, Amaro F, Schnyder M, Grimm F, et al. Seroprevalence of vector-borne pathogens and molecular detection of *Borrelia afzelii* in military dogs from Portugal. *Parasit Vectors.* (2016) 9:225. doi: 10.1186/s13071-016-1509-2

192. Pantchev N, Schaper R, Limousin S, Norden N, Weise M, Lorentzen L. Occurrence of *Dirofilaria immitis* and tick-borne infections caused by *Anaplasma phagocytophilum, Borrelia burgdorferi* sensu lato and *Ehrlichia canis* in domestic dogs in France: results of a countrywide serologic survey. *Parasitol Res.* (2009) 105:S101–14. doi: 10.1007/s00436-009-1501-2

193. Miró G, Montoya A, Roura X, Gálvez R, Sainz A. Seropositivity rates for agents of canine vector-borne diseases in Spain: a multicentre study. *Parasit Vectors.* (2013) 6:117. doi: 10.1186/1756-3305-6-117

194. Amusategui I, Tesouro MA, Kakoma I, Sainz A. Serological reactivity to *Ehrlichia canis, Anaplasma phagocytophilum, Neorickettsia risticii, Borrelia burgdorferi* and *Rickettsia conorii* in dogs from northwestern Spain. *Vector Borne Zoonotic Dis.* (2008) 8:797–803. doi: 10.1089/vbz.2007.0277

195. Díaz-Regañón D, Roura X, Suárez ML, León M, Sainz A. Serological evaluation of selected vector-borne pathogens in owned dogs from northern Spain based on a multicenter study using a commercial test. *Parasit Vectors.* (2020) 13:301. doi: 10.1186/s13071-020-04172-5

196. Couto CC, Lorentzen L, Beall MJ, Shields J, Bertolone N, Couto JI, et al. Serological study of selected vector-borne diseases in shelter dogs in central Spain using point-of-care assays. *Vector Borne Zoonotic Dis.* (2010) 10:885–8. doi: 10.1089/vbz.2009.0063

197. Aktas M, Özübek S, Altay K, Ipek NDS, Balkaya I, Armagan Erdem Utuk AE, et al. Molecular detection of tick-borne rickettsial and protozoan

pathogens in domestic dogs from Turkey. *Parasit Vectors*. (2015) 8:157. doi: 10.1186/s13071-015-0763-z

198. Cetinkaya H, Matur E, Akyazi I, Ekiz E, Aydin L, Toparlak M. Serological and molecular investigation of *Ehrlichia* spp and *Anaplasma* spp in ticks and blood of dogs, in the Thrace Region of Turkey. *Ticks Tick Borne Dis*. (2016) 7:706–14. doi: 10.1016/j.ttbdis.2016.02.021

199. Huber D, Reil I, Duvnjak S, Jurkovic D, Lukacevic D, Pilat M, et al. Molecular detection of *Anaplasma platys*, *Anaplasma phagocytophilum* and *Wolbachia* sp. but not *Ehrlichia canis* in Croatian dogs. *Parasitol Res*. (2017) 116:3019–26. doi: 10.1007/s00436-017-5611-y

200. Mrljak V, Kuleš J, Mihaljević Z, Torti M, Gotić J, Crnogaj M, et al. Prevalence and geographic distribution of vector-borne pathogens in apparently healthy dogs in Croatia. *Vector Borne Zoonotic Dis*. (2017) 17:398–408. doi: 10.1089/vbz.2016.1990

201. Diakou A, Di Cesare A, Morelli S, Colombo M, Halos L, Simonato G, et al. Endoparasites and vector-borne pathogens in dogs from Greek islands: pathogen distribution and zoonotic implications. *PLoS Negl Trop Dis*. (2019) 13:e0007003. doi: 10.1371/journal.pntd.0007003

202. Inokuma H, Ohno K, Onishi T, Raoult D, Brouqui P. Detection of ehrlichial infection by PCR in dogs from Yamaguchi and Okinawa Prefectures, Japan. *J Vet Med Sci*. (2001) 63:815–7. doi: 10.1292/jvms.63.815

203. Fukui Y, Inokuma H. Subclinical infections of *Anaplasma phagocytophilum* and *Anaplasma bovis* in dogs from Ibaraki, Japan. *Jpn J Infect Dis*. (2019) 72:168–72. doi: 10.7883/yoken.JJID.2018.470

204. Xia Z, Yu D, Mao J, Zhang Z, Yu J. The occurrence of *Dirofilaria immitis*, *Borrelia burgdorferi*, *Ehrlichia canis* and *Anaplasma phagocytophium* in dogs in China. *J Helminthol*. (2012) 86:185–9. doi: 10.1017/S0022149X11000198

205. Cui Y, Yan Y, Wang X, Cao S, Zhang Y, Jian F, et al. First molecular evidence of mixed infections of Anaplasma species in dogs in Henan, China. *Ticks Tick Borne Dis*. (2017) 8:283–9. doi: 10.1016/j.ttbdis.2016.12.001

206. Wang S, He J, Zhang L. Serological investigation of vector-borne disease in dogs from rural areas of China. *Asian Pac J Trop Biomed*. (2012) 2:102–3. doi: 10.1016/S2221-1691(11)60201-3

207. Zhang J, Liu Q, Wang D, Li W, Beugnet F, Zhou J. Epidemiological survey of ticks and tick-borne pathogens in pet dogs in south-eastern China. *Parasite*. (2017) 24:35. doi: 10.1051/parasite/2017036

208. Wang J, Kelly P, Zhang J, Shi Z, Song C, ZhengX, et al. Detection of *Dirofilaria immitis* antigen and antibodies against Anaplasma phagocytophilum, *Borrelia burgdorferi* and *Ehrlichia canis* in dogs from ten provinces of China. *Acta Parasitol*. (2018) 63:412–5. doi: 10.1515/ap-2018-0047

209. Yang B, Ye C, Sun E, Wen Y, Qian D, Sun H. First molecular evidence of Anaplasma spp. co-infection in stray dogs from Anhui, China. *Acta Trop*. (2020) 206:105453. doi: 10.1016/j.actatropica.2020.105453

210. Lee S, Lee SH, VanBik D, Kim NH, Kim KT, Goo YK, et al. First molecular detection and phylogenetic analysis of Anaplasma phagocytophilum in shelter dogs in Seoul, Korea. *Ticks Tick Borne Dis*. (2016) 7:945–50. doi: 10.1016/j.ttbdis.2016.04.011

211. Suh GH, Ahn KS, Ahn JH, Kim HJ, Leutenegger C, Shin SS. Serological and molecular prevalence of canine vector-borne diseases (CVBDs) in Korea. *Parasit Vectors*. (2017) 10:146. doi: 10.1186/s13071-017-2076-x

212. Jung BY, Gebeyehu EB, Seo MG, Byun JW, Kim HY, Kwak D. Prevalence of vector-borne diseases in shelter dogs in Korea. *Vet Rec*. (2012) 171:249. doi: 10.1136/vr.100650

213. Lim S, Irwin PJ, Lee S, Oh M, Ahn K, Myung B, Shin S. Comparison of selected canine vector-borne diseases between urban animal shelter and rural hunting dogs in Korea. *Parasit Vectors*. (2010) 3:32. doi: 10.1186/1756-3305-3-32

214. Bell DR, Berghaus RD, Patel S, Beavers S, Fernandez I, Sanchez S. Seroprevalence of tick-borne infections in military working dogs in the Republic of Korea. *Vector Borne Zoonotic Dis*. (2012) 12:1023–30. doi: 10.1089/vbz.2011.0864

215. Koh FX, Panchadcharam C, Tay ST. Vector-borne diseases in stray dogs in Peninsular Malaysia and molecular detection of *Anaplasma* and *Ehrlichia* spp. from *Rhipicephalus sanguineus* (Acari: Ixodidae) ticks. *J Med Entomol*. (2016) 53:183–7. doi: 10.1093/jme/tjv153

216. Borthakur SK, Deka DK, Bhattacharjee K, Sarmah PC. Seroprevalence of canine dirofilariosis, granulocytic anaplasmosis and lyme borreliosis of

public health importance in dogs from India's North East. *Vet World*. (2014) 7:665–7. doi: 10.14202/vetworld.2014.665-667

217. Manoj RRS, Iatta R, Latrofa MS, Capozzi L, Raman M, Colella V, et al. Canine vector-borne pathogens from dogs and ticks from Tamil Nadu, India. *Acta Trop*. (2020) 203:105308. doi: 10.1016/j.actatropica.2019.105308

218. Levi O, Waner T, Baneth G, Keysary A, Bruchim Y, Silverman J, et al. Seroprevalence of *Anaplasma phagocytophilum* among healthy dogs and horses in Israel. *J Vet Med B Infect diseases Vet Public Health*. (2006) 53:78–80. doi: 10.1111/j.1439-0450.2006.00911.x

219. Qablan MA, Kubelová M, Siroký P, Modry D, Amr ZS. Stray dogs of northern Jordan as reservoirs of ticks and tick-borne hemopathogens. *Parasitol Res*. (2012) 111:301–7. doi: 10.1007/s00436-012-2839-4

220. Obaidat MM, Alshehabat MA. Zoonotic Anaplasma phagocytophilum, *Ehrlichia canis*, *Dirofilaria immitis*, *Borrelia burgdorferi*, and spotted fever group rickettsiae (SFGR) in different types of dogs. *Parasitol Res*. (2018) 117:3407–12. doi: 10.1007/s00436-018-6033-1

221. Yuasa Y, Tsai YL, Chang CC, Hsu TH, Chou CC. The prevalence of *Anaplasma platys* and a potential novel Anaplasma species exceed that of *Ehrlichia canis* in asymptomatic dogs and *Rhipicephalus sanguineus* in Taiwan. *J Vet Med Sci*. (2017) 79:1494–502. doi: 10.1292/jvms.17-0224

222. Hamidinejat TH, Bahrami S, Mosalanejad B, Pahlavan S. First molecular survey on *Anaplasma phagocytophilum* revealed high prevalence in rural dogs from Khuzestan Province, Iran. *Iran J Parasitol*. (2019) 14:297–302. doi: 10.18502/ijpa.v14i2.1142

223. Yousefi A, Chaechi Nosrati MR, Golmohammadi A, Azami S. Molecular detection of *Anaplasma phagocytophilum* as a zoonotic agent in owned and stray dogs in Tehran, Iran. *Archiv Razi Instit*. (2019) 74:33–8. doi: 10.22092/ari.2018.114893.1142

224. M'ghirbi Y, Ghorbel A, Amouri M, Nebaoui A, Haddad S, Bouattour A. Clinical, serological, and molecular evidence of ehrlichiosis and anaplasmosis in dogs in Tunisia. *Parasitol Res*. (2009) 104:767–74. doi: 10.1007/s00436-008-1253-4

225. Azzag N, Petit E, Gandoin C, Bouillin C, Ghalmi F, Haddad N, et al. Prevalence of select vector-borne pathogens in stray and client-owned dogs from Algiers. *Comp Immunol Microbiol Infect Dis*. (2015) 38:1–7. doi: 10.1016/j.cimid.2015.01.001

226. Elhamiani Khatat S, Khallaayoune K, Errafyk N, Van Gool F, Duchateau L, Daminet S, et al. Detection of *Anaplasma* spp. and *Ehrlichia* spp. antibodies, and *Dirofilaria immitis* antigens in dogs from seven locations of Morocco. *Vet Parasitol*. (2017) 239:86–9. doi: 10.1016/j.vetpar.2017.04.004

227. Elhamiani Khatat S, Daminet S, Kachani M, Leutenegger C, Duchateau L, El Amri H, et al. *Anaplasma* spp. in dogs and owners in northwestern Morocco. *Parasit Vectors*. (2017) 10:202. doi: 10.1186/s13071-017-2148-y

228. Aquino LC, Kamani J, Haruna JM, Paludo GR, Hicks CA, Helps CR, et al. Analysis of risk factors and prevalence of haemoplasma infection in dogs. *Vet Parasitol*. (2016) 221:111–7. doi: 10.1016/j.vetpar.2016.03.014

229. Kolo AO, Sibeko-Matjila KP, Maina AN, Richards AL, Knobel DL, Majtila PT. Molecular detection of zoonotic Rickettsiae and *Anaplasma* spp. in domestic dogs and their ectoparasites in Bushbuckridge, South Africa. *Vector Borne Zoonotic Dis*. (2016) 16:245–52. doi: 10.1089/vbz.2015.1849

230. Kolo AO, Collins NE, Brayton KE, Chaisi M, Blumberg L, Frean J, et al. *Anaplasma phagocytophilum* and other *Anaplasma* spp. in various hosts in the Mnisi Community, Mpumalanga Province, South Africa. *Microorganisms*. (2020) 8:1812. doi: 10.3390/microorganisms8111812

231. Clarke LL, Ballweber LR, Little SE, Lapplin MR. Prevalence of select vector-borne disease agents in owned dogs of Ghana. *J S Afr Vet Assoc*. (2014) 85:996. doi: 10.4102/jsava.v85i1.996

232. Kirchner M, Brunner A, Edelhofer R, Joachim A. Vector-borne parasites of dogs on the Islands of Cabo Verde. *Wien Klin Wochenschr*. (2008) 120:49–53. doi: 10.1007/s00508-008-1075-5

233. Lauzi S, Maia JP, Epis S, Marcos R, Pereira C, Luzzago C, et al. Molecular detection of *Anaplasma platys*, *Ehrlichia canis*, *Hepatozoon canis* and *Rickettsia monacensis* in dogs from Maio Island of Cape Verde archipelago. *Ticks Tick Borne Dis*. (2016) 7:964–9. doi: 10.1016/j.ttbdis.2016.05.001

234. Diniz PP, Breitschwerdt EB. Anaplasma phagocytophilum infection (canine granulocytic anaplasmosis). In: Greene GE, editor. *Infectious Diseases of the Dog and Cat*. St. Louis, MO: Elsevier Sauders (2012). p. 244–54.

235. Sainz A, Roura X, Miró G, Estrada-Pena A, Kohn B, Harrus S, 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

236. Kordick SK, Breitschwerdt EB, Hegarty BC, Southwick KL, Colitz CM, Hancock SI, et al. Coinfection with multiple tick-borne pathogens in a Walker hound kennel in North Carolina. *J Clin Microbiol.* (1999) 37:2631–8. doi: 10.1128/JCM.37.8.2631-2638.1999

237. Inokuma H, Fujii K, Matsumoto K, Okuda M, Nakagome K, Kosugi R, et al. Demonstration of *Anaplasma* (*Ehrlichia*) *platys* inclusions in peripheral blood platelets of a dog in Japan. *Vet Parasitol.* (2002) 110:145–52. doi: 10.1016/s0304-4017(02)00289-3

238. Plier ML, Breitschwerdt EB, Hegarty BC, Kidd LB. Lack of evidence for perinatal transmission of canine granulocytic anaplasmosis from a bitch to her offspring. *J Am Anim Hosp Assoc.* (2009) 45:232–8. doi: 10.5326/0450232

239. Santos F, Coppede JS, Pereira AL, Oliveira LP, Roberto PG, Benedetti RBR, et al. Molecular evaluation of the incidence of *Ehrlichia canis, Anaplasma platys* and *Babesia* spp. in dogs from Ribeirao Preto, Brazil. *Vet J.* (2009) 179:145–8. doi: 10.1016/j.tvjl.2007.08.017

240. Chandrashekar R, Mainville CA, Beall MJ, O, Connor T, Eberts MD, Alleman AR, et al. Performance of a commercially available in-clinic ELISA for the detection of antibodies against *Anaplasma phagocytophilum, Ehrlichia canis,* and *Borrelia burgdorferi* and *Dirofilaria immitis* antigen in dogs. *Am J Vet Res.* (2010) 71:1443–50. doi: 10.2460/ajvr.71.12.1443

241. Harvey JW. Anaplasma platys infection (thrombocytotropic anaplasmosis). In: Greene GE, editor. *Infectious Diseases of the Dog and Cat.* St. Louis, MO: Elsevier Saunders (2012). p. 241–4.

242. Breitschwerdt EB, Hegarty BC, Hancock SI. Sequential evaluation of dogs naturally infected with *Ehrlichia canis, Ehrlichia chaffeensis, Ehrlichia equi, Ehrlichia ewingii,* or *Bartonella vinsonii. J Clin Microbiol.* (1998) 36:2645–51. doi: 10.1128/JCM.36.9.2645-2651.1998

243. Waner T, Harrus S, Jongejan F, Bark H, Keysary A, Cornelissen AW. Significance of serological testing for ehrlichial diseases in dogs with special emphasis on the diagnosis of canine monocytic ehrlichiosis caused by *Ehrlichia canis. Vet Parasitol.* (2001) 95:1–15. doi: 10.1016/s0304-4017(00)00407-6

244. Foley J, Drazenovich N, Leutenegger CM, Chomel BB. Association between polyarthritis and thrombocytopenia and increased prevalence of vector-borne pathogens in Californian dogs. *Vet Rec.* (2007) 160:159–62. doi: 10.1136/vr.160.5.159

245. Liddell AM, Stockham SL, Scott MA, Summer JW, Paddock CD, Gaudreault-Keener M, et al. Predominance of *Ehrlichia ewingii* in Missouri dogs. *J Clin Microbiol.* (2003) 41:4617–22. doi: 10.1128/jcm.41.10.4617-4622.2003

246. Arsenault WG, Messick JB. Acute granulocytic ehrlichiosis in a Rottweiler. *J Am Anim Hosp Assoc.* (2005) 41:323–6. doi: 10.5326/0410323

247. Kane A, Bloc G, Heeb LA. An unusual presentation of granulocytic anaplasmosis in a young dog. *J Am Anim Hosp Assoc.* (2011) 47:276–9. doi: 10.5326/JAAHA-MS-5652

248. Liu Y, Watson SC, Gettings JR, Lund RB, Nordone SK, Yabsley MJ, et al. A bayesian spatio-temporal model for forecasting *Anaplasma* species seroprevalence in domestic dogs within the contiguous United States. *PLoS ONE.* (2017) 12:e0182028. doi: 10.1371/journal.pone.0182028

249. Gettings JR, Self SCW, McMahan CS, Brown DA, Nordone SK, Yabsley MJ. Regional and local temporal trends of *Borrelia burgdorferi* and *Anaplasma* spp. seroprevalence in domestic dogs: contiguous United States 2013–2019. *Front Vet Sci.* (2020) 7:561592. doi: 10.3389/fvets.2020.561592

250. Dewage BG, Little S, Payton M, Beall M, Braff J, Szlosek D, et al. Trends in canine seroprevalence to *Borrelia burgdorferi* and *Anaplasma* spp. in the eastern USA, 2010–2017. *Parasit Vectors.* (2019) 12:476. doi: 10.1186/s13071-019-3735-x

251. Lester SJ, Breitschwerdt EB, Collis CD, Hegarty BC. *Anaplasma phagocytophilum* infection (granulocytic anaplasmosis) in a dog from Vancouver Island. *Can Vet J.* (2005) 46:825–7.

252. Cockwill KR, Taylor SM, Snead ECR, Dickinson R, Cosford K, Malek S, et al. Granulocytic anaplasmosis in three dogs from Saskatoon, Saskatchewan. *Can Vet J.* (2009) 50:835–40.

253. Elhamiani Khatat S, Culang D, Gara-Boivin C. Granulocytic anaplasmosis in two dogs from Quebec. *Can Vet J.* (2018) 9:663–7.

254. McCown ME, Monterroso VH, Cardona W. Surveillance for *Ehrlichia canis, Anaplasma phagocytophilum, Borrelia burgdorferi,* and *Dirofilaria immitis* in dogs from three cities in Colombia. *J Spec Oper Med.* (2014) 14:86–90.

255. Silveira JA, Valente PC, Paes PR, Vasconcelos AV, Silvestre BT, Ribeiro MFB, et al. The first clinical and laboratory evidence of co-infection by *Anaplasma phagocytophilum* and *Ehrlichia canis* in a Brazilian dog. *Ticks Tick Borne Dis.* (2015) 6:242–5. doi: 10.1016/j.ttbdis.2015.01.003

256. Tozon N, Petrovec M, Avsic-Zupanc T. Clinical and laboratory features of the first detected cases of A. phagocytophila infections in dogs from Slovenia. *Ann N Y Acad Sci.* (2003) 990:424–8. doi: 10.1111/j.1749-6632.2003.tb07405.x

257. Bexfield NH, Villiers EJ, Herrtage ME. Immune-mediated haemolytic anaemia and thrombocytopenia associated with *Anaplasma phagocytophilum* in a dog. *J Small Anim Pract.* (2005) 46:543–8. doi: 10.1111/j.1748-5827.2005.tb00284.x

258. Melter O, Stehlik I, Kinska H, Volfova I. Infection with *Anaplasma phagocytophilum* in a young dog: a case report. *Vet Med.* (2007) 52:207–12. doi: 10.17221/2001-VETMED

259. Domingos MC, Trotta M, Briend-Marchal A, Medaille C. Anaplasmosis in two dogs in France and molecular and phylogenetic characterization of *Anaplasma phagocytophilum. Vet Clin Pathol.* (2011) 40:215–21. doi: 10.1111/j.1939-165X.2011.00321.x

260. Dewree R, Coquereaux G, Heymann P, Cochez C. *Anaplasma phagocytophilum* in a dog in Belgium. *Acta Clin Belg.* (2014) 69:S4–5.

261. Dondi F, Russo S, Agnoli C, Mengoli N, Balboni A, Alberti A, et al. Clinicopathological and molecular findings in a case of canine *Anaplasma phagocytophilum* infection in Northern Italy. *ScientificWorldJournal.* (2014) 2014:810587. doi: 10.1155/2014/810587

262. Elhamiani Khatat S, Defauw P, Marynissen S, Van de Maele I. Exposure to *Anaplasma phagocytophilum* in two dogs in Belgium. *Vlaams Diergen Tijds.* (2015) 84:39–46. doi: 10.21825/vdt.v84i1.16621

263. Inokuma H, Oyamada M, Kelly PJ, Jacobson LA, Fournier PE, Itamoto K, et al. Molecular detection of a new *Anaplasma* species closely related to *Anaplasma phagocytophilum* in canine blood from South Africa. *J Clin Microbiol.* (2005) 43:2934–7. doi: 10.1128/JCM.43.6.2934-2937.2005

264. Barlough JE, Madigan JE, Turoff DR, Clover JR. An *Ehrlichia* strain from a llama (*Lama glama*) and llama-associated ticks (*Ixodes pacificus*). *J Clin Microbiol.* (1997) 35:1005–7. doi: 10.1128/JCM.35.4.1005-1007.1997

265. Burgess H, Chilton NB, Krakowetz CN, Williams C, Lohmann K. Granulocytic anaplasmosis in a horse from Saskatchewan. *Can Vet J.* (2012) 53:886–8.

266. Gordon WS, Brownlee A, Wilson DR, Macleod J. Tick-borne fever (a hitherto undescribed disease of sheep). *J Comp Pathol Ther.* (1932) 65:301–7. doi: 10.1016/S0368-1742(32)80025-1

267. Tinkler SH, Firshman AM, Sharkey LC. Premature parturition, edema, and ascites in an alpaca infected with *Anaplasma phagocytophilum. Can Vet J.* (2012) 53:1199–202.

268. Aktas M, SÖzübek S. Bovine anaplasmosis in Turkey: First laboratory confirmed clinical cases caused by *Anaplasma phagocytophilum. Vet Microbiol.* (2015) 178:246–51. doi: 10.1016/j.vetmic.2015.05.021

269. Savidge C, Ewing P, Andrews J, Aucoin D, Lapplin JR, Moroff S. *Anaplasma phagocytophilum* infection of domestic cats: 16 cases from the northeastern USA. *J Feline Med Surg.* (2016) 18:85–91. doi: 10.1177/1098612X15571148

270. Schorn S, Pfister K, Reulen H, Mahling M, Manitz J, Thiel C, et al. Prevalence of *Anaplasma phagocytophilum* in *Ixodes ricinus* in Bavarian public parks, Germany. *Ticks Tick Borne Dis.* (2011) 2:196–203. doi: 10.1016/j.ttbdis.2011.09.009

271. Nuttall PA, Labuda M. Dynamics of infection in tick vectors and at the tick-host interface. *Adv Virus Res.* (2003) 60:233–72. doi: 10.1016/s0065-3527(03)60007-2

272. Gray J, Kahl O, Lane RS, StanekG. *Lyme Borreliosis Biology, Epidemiology and Control.* Wallingford: CABI (2002).

273. Pichon B, Estrada-Pena A, Kahl, Mannelі A. Detection of animal reservoirs of tick-borne zoonoses in Europe. *Int J Med Microbiol.* (2006) 296:129–30.

274. Rizzoli A, Silaghi C, Obiegala A, Rudolf I, Hhbalek Z, Földvari G, et al. *Ixodes ricinus* and its transmitted pathogens in urban and peri-urban areas in Europe: new hazards and relevance for public health. *Front Public Health.* (2014) 2:251. doi: 10.3389/fpubh.2014.00251

275. Skerget M, Wenisch C, Daxboeck F, Krause R, Haberl R, Stuenzner D. Cat or dog ownership and seroprevalence of ehrlichiosis, Q fever, and cat-scratch disease. *Emerg Infect Dis.* (2003) 9:1337–9. doi: 10.3201/eid0910.030206

276. André MR. Diversity of *Anaplasma* and *Ehrlichia/Neoehrlichia* agents in terrestrial wild carnivores worldwide: implications for human and domestic animal health and wildlife conservation. *Front Vet Sci.* (2020) 5:293. doi: 10.3389/fvets.2018.00293

277. Rabinowitz PM, Gordon Z, Odofin L. Pet-related infections. *Am Fam Physician.* (2007) 76:1314–22.

278. Jones EH, Hinckley AF, Hook SA, Meek JI, Backenson B, Kugeler KJ, et al. Pet ownership increases human risk of encountering ticks. *Zoonoses Public Health.* (2018) 65:74–9. doi: 10.1111/zph.12369

279. Silaghi C, Gilles J, Höhle M, Fingerle V, Just FT, Pfister K. *Anaplasma phagocytophilum* infection in *Ixodes ricinus*, Bavaria, Germany. *Emerg Infect Dis.* (2008) 14:972–4. doi: 10.3201/eid1406.071095

280. Claerebout E, Losson B, Cochez C, Casaert S, Dalemans AC, de Cat A, et al. Ticks and associated pathogens collected from dogs and cats in Belgium. *Parasit Vectors.* (2013) 6:183. doi: 10.1186/1756-3305-6-183

281. Coipan EC, Fonville M, Tijsse-Klasen E, van der Giessen JW, Takken W, Sprong H, et al. Geodemographic analysis of *Borrelia burgdorferi* sensu lato using the 5S-23S rDNA spacer region. *Infect Genet Evol.* (2013) 17:216–22. doi: 10.1016/j.meegid.2013.04.009

282. Overzier E, Pfister K, Thiel C, Herb I, Mahling M, Silaghi C. *Anaplasma phagocytophilum* in questing *Ixodes ricinus* ticks: comparison of prevalences and partial 16S rRNA gene variants in urban, pasture, and natural habitats. *Appl Environ Microbiol.* (2013) 79:1730–4. doi: 10.1128/AEM.03300-12

283. Król N, Obiegala A, Pfeffer M, Lonc E, Kiewra D. Detection of selected pathogens in ticks collected from cats and dogs in the Wrocław Agglomeration, South-West Poland. *Parasit Vectors.* (2016) 9:351. doi: 10.1186/s13071-016-1632-0

284. Nair ADS, Cheng C, Ganta CK, Sanderson MW, Alleman AR, Munderloh UG, et al. Comparative experimental infection study in dogs with *Ehrlichia canis*, *E. chaffeensis*, *Anaplasma platys* and *A. phagocytophilum*. *PLoS ONE.* (2016) 11:e0148239. doi: 10.1371/journal.pone.0148239

285. Hodzic E, Fish D, Maretzki CM, De Silva AM, Feng S, Barthold SW. Acquisition and transmission of the agent of granulocytic ehrlichiosis by *Ixodes scapularis* ticks. *J Clin Microbiol.* (1998) 36:3574–8. doi: 10.1128/JCM.36.12.3574-3578.1998

286. des Vignes F, Piesman J, Heffernan R, Schulze TL, Stafford KC III, Fish D. Effect of tick removal on transmission of *Borrelia burgdorferi* and *Ehrlichia phagocytophila* by *Ixodes scapularis* nymphs. *J Infect Dis.* (2001) 183:773–8. doi: 10.1086/318818

287. Katavolos P, Armstrong PM, Dawson JE, Telford SR III. Duration of tick attachment required for transmission of granulocytic ehrlichiosis. *J Infect Dis.* (1998) 177:1422–5. doi: 10.1086/517829

288. Fourie JJ, Evans A, LabuschagneM, Crafford D, Madder M, Pollmeier M, Schunack B. Transmission of *Anaplasma phagocytophilum* (Foggie, 1949) by *Ixodes ricinus* (Linnaeus, 1758) ticks feeding on dogs and artificial membranes. *Parasit Vectors.* (2019) 12:136. doi: 10.1186/s13071-019-3396-9

289. Wang J, Dyachenko V, Munderloh UG, Straubinger RK. Transmission of *Anaplasma phagocytophilum* from endothelial cells to peripheral granulocytes *in vitro* under shear flow conditions. *Med Microbiol Immunol.* (2015) 204:593–603. doi: 10.1007/s00430-015-0387-0

290. Lai TH, Kumagai Y, Hyodo M, Hayakawa Y, Rikihisa Y. The *Anaplasma phagocytophilum* PleC histidine kinase and PleD diguanylate cyclase two-component system and role of cyclic Di-GMP in host cell infection. *J Bacteriol.* (2009) 191:693–700. doi: 10.1128/JB.01218-08

291. Troese MJ, Kahlon A, Ragland SA, Ottens AK, Ojogun N, Nelson KT, et al. Proteomic analysis of *Anaplasma phagocytophilum* during infection of human myeloid cells identifies a protein that is pronouncedly upregulated on the infectious dense-cored cell. *Infect Immun.* (2011) 79:4696–707. doi: 10.1128/IAI.05658-11

292. Martin ME, Bunnell JE, Dumler JS. Pathology, immunohistology, and cytokine responses in early phases of human granulocytic ehrlichiosis in a murine model. *J Infect Dis.* (2000) 181:374–8. doi: 10.1086/315206

293. Borjesson DL, Kobayashi SD, Whitney AR, Voyich JM, Argue CM, Deleo FR. Insights into pathogen immune evasion mechanisms:

294. Carlyon JA, Fikrig E. Mechanism of evasion of neutrophil killing by *Anaplasma phagocytophilum*. *Curr Opin Hematol.* (2006) 13:28–33. doi: 10.1097/01.moh.0000190109.00532.56

295. Rikihisa Y. *Ehrlichia* subversion of host innate responses. *Curr Opin Microbiol.* (2006) 9:95–101. doi: 10.1016/j.mib.2005.12.003

296. Garcia-Garcia JC, Rennoll-Bankert KE, Pelly S, Milstone AM, Dumler JS. Silencing of host cell CYBB gene expression by the nuclear effector AnkA of the intracellular pathogen *Anaplasma phagocytophilum*. *Infect Immun.* (2009) 77:2385–91. doi: 10.1128/IAI.00023-09

297. Palmer GH, Bankhead T, Lukehart SA. 'Nothing is permanent but change'-antigenic variation in persistent bacterial pathogens. *Cell Microbiol.* (2009) 11:1697–705. doi: 10.1111/j.1462-5822.2009.01366.x

298. Yoshikawa Y, Sugimoto K, Ochiai Y, Ohashi N. Intracellular proliferation of *Anaplasma phagocytophilum* is promoted via modulation of endoplasmic reticulum stress signaling in host cells. *Microbiol Immunol.* (2020) 64:270–9. doi: 10.1111/1348-0421.12770

299. Bunnell JE, Trigiani ER, Srinivas SR, Dumler JS. Development and distribution of pathologic lesions are related to immune status and tissue deposition of human granulocytic ehrlichiosis agent-infected cells in a murine model system. *J Infect Dis.* (1999) 180:546–50. doi: 10.1086/314902

300. Martin ME, Caspersen K, Dumler JS. Immunopathology and ehrlichial propagation are regulated by interferon-gamma and interleukin-10 in a murine model of human granulocytic ehrlichiosis. *Am J Pathol.* (2001) 158:1881–8. doi: 10.1016/s0002-9440(10)64145-4

301. Birkner K, Steiner B, Rinkler C, Kern Y, Aichele P, Bogdan C, et al. The elimination of *Anaplasma phagocytophilum* requires CD41 T cells, but is independent of Th1 cytokines and a wide spectrum of effector mechanisms. *Eur J Immunol.* (2008) 38:3395–410. doi: 10.1002/eji.200838615

302. Lilliehöök I, Egenvall A, Tvedten HW. Hematopathology in dogs experimentally infected with a Swedish granulocytic *Ehrlichia* species. *Vet Clin Pathol.* (1998) 27:116–22. doi: 10.1111/j.1939-165x.1998.tb01030.x

303. Scorpio DG, von Loewenich FD, Goebel H, Bogdan C, Dumler JS. Innate immune response to *Anaplasma phagocytophilum* contributes to hepatic injury. *Clin Vaccine Immunol.* (2006) 13:806–9. doi: 10.1128/CVI.00092-06

304. Eckersall PD. Acute phase proteins as markers of inflammatory lesions. *Comp Hematol Int.* (1995) 5:93–7.

305. Klein MB, Hu S, Chao CC, Goodman JL. The agent of human granulocytic ehrlichiosis induces the production of myelosuppressing chemokines without the induction of proinflammatory cytokines. *J Infect Dis.* (2000) 182:200–5. doi: 10.1086/315641

306. Akkoyunlu M, Fikrig E. Gamma interferon dominates the murine cytokine response to the agent of human granulocytic ehrlichiosis and helps to control the degree of early rickettsemia. *Infect Immun.* (2000) 68:1827–33. doi: 10.1128/iai.68.4.1827-1833.2000

307. Fisman DN. Hemophagocytic syndromes and infection. *Emerg Infect Dis.* (2000) 6:601–8. doi: 10.3201/eid0606.000608

308. Dumler JS, Barat NC, Barat CE, Bakken JS. Human granulocytic anaplasmosis and macrophage activation. *Clin Infect Dis.* (2007) 45:199–204. doi: 10.1086/518834

309. Janka GE, Lehmberg K. Hemophagocytic syndromes — an update. *Blood Rev.* (2014) 28:135–42. doi: 10.1016/j.blre.2014.03.002

310. Sun W, Ijdo JW, Telford SR III, Hodzic E, Zhang Y, Barthold SW, Fikrig E. Immunization against the agent of human granulocytic ehrlichiosis in a murine model. *J Clin Invest.* (1997) 100:3014–8. doi: 10.1172/JCI119855

311. von Loewenich FD, Scorpio DG, Reischl U, Dumler JS, Bogdan C. Frontline: control of *Anaplasma phagocytophilum*, an obligate intracellular pathogen, in the absence of inducible nitric oxide synthase, phagocyte NADPH oxidase, tumor necrosis factor, Toll-like receptor (TLR)2 and TLR4, or the TLR adaptor molecule MyD88. *Eur J Immunol.* (2004) 34:1789–97. doi: 10.1002/eji.200425029

312. Goldman EE, Breitschwerdt EB, Grindem CB, Hegarty BC, Walls JJ, Dumler JS. Granulocytic ehrlichiosis in dogs from North Carolina and Virginia. *J Vet Intern Med.* (1998) 12:61–70. doi: 10.1111/j.1939-1676.1998.tb02096.x

Top of right column continuation:

Anaplasma phagocytophilum fails to induce an apoptosis differentiation program in human neutrophils. *J Immunol.* (2005) 174:6364–72. doi: 10.4049/jimmunol.174.10.6364

313. Berzina I, Krudewig C, Silaghi C, Matise I, Ranka R, Müller N, et al. Anaplasma phagocytophilum DNA amplified from lesional skin of seropositive dogs. *Ticks Tick Borne Dis.* (2014) 5:329–35. doi: 10.1016/j.ttbdis.2013.12.010

314. Egenvall A, Bjoersdorff A, Lilliehook I, Engvall EO, Karlstam E, Artursson K, et al. Early manifestations of granulocytic ehrlichiosis in dogs inoculated experimentally with a Swedish *Ehrlichia* species isolate. *Vet Rec.* (1998) 143:412–7. doi: 10.1136/vr.143.15.412

315. Kjelgaard-Hansen M, Lundorff Jensen A, Houser GA, Jessen LR, Kristensen AT. Use of serum C-reactive protein as an early marker of inflammatory activity in canine type II immune-mediated polyarthritis: case report. *Acta Vet Scand.* (2006) 48:9. doi: 10.1186/1751-0147-48-9

316. Tarello W. Canine granulocytic ehrlichiosis (CGE) in Italy. *Acta Vet Hung.* (2003) 51:73–90. doi: 10.1556/avet.51.2003.1.7

317. Jäderlund KH, Egenvall A, Bergström K, Hedhammar A. Seroprevalence of *Borrelia burgdorferi* sensu lato and *Anaplasma phagocytophilum* in dogs with neurological signs. *Vet Rec.* (2007) 160:825–31. doi: 10.1136/vr.160.24.825

318. Jäderlund KH, Bergström K, Egenvall A, Hedhammar A. Cerebrospinal fluid PCR and antibody concentrations against *Anaplasma phagocytophilum* and *Borrelia burgdorferi* sensu lato in dogs with neurological signs. *J Vet Intern Med.* (2009) 23:669–72. doi: 10.1111/j.1939-1676.2009.0313.x

319. Brown WC. Adaptive immunity to *Anaplasma* pathogens and immune dysregulation: implications for bacterial persistence. *Comp Immunol Microbiol Infect Dis.* (2012) 35:241–52. doi: 10.1016/j.cimid.2011.12.002

320. Egenvall A, Lilliehöök I, Bjöersdorff A, Engvall EO, Karlstam E, Artursson K, et al. Detection of granulocytic Ehrlichia species DNA by PCR in persistently infected dogs. *Vet Rec.* (2000) 146:186–90. doi: 10.1136/vr.146.7.186

321. Alleman A, Chandrashekar R, Beall M, Cyr K, Barbet A, Lundgren A, et al. Experimental inoculation of dogs with human isolates (NY18) of *Anaplasma phagocytophilum* and demonstration of persistent infection following doxycycline therapy. *J Vet Intern Med.* (2006) 20:763.

322. Wamsley, H. *Mammalian host cell reservoirs during Anaplasma infection* (dissertation), University of Florida, Gainesville, FL, United States (2009).

323. Granquist EG, Stuen S, Crosby L, Lundgren AM, AR, Barbet AF. Variant-specific and diminishing immune responses towards the highly variable MSP2 (P44) outer membrane protein of *Anaplasma phagocytophilum* during persistent infection in lambs. *Vet Immunol Immunopathol.* (2010) 133:117–24. doi: 10.1016/j.vetimm.2009.07.009

324. Contreras ET, Dowers KL, Moroff S, Lappin MR. Clinical and laboratory effects of doxycycline and prednisolone in *Ixodes scapularis*-exposed dogs with chronic *Anaplasma phagocytophilum* infection. *Top Companion Anim Med.* (2018) 33:147–9. doi: 10.1053/j.tcam.2018.08.001

325. Harrus S, Waner T, Aizenberg I, Foley JE, Poland AM, Bark H. Amplification of ehrlichial DNA from dogs 34 months after infection with *Ehrlichia canis. J Clin Microbiol.* (1998) 36:73–6. doi: 10.1128/JCM.36.1.73-76.1998

326. Foley JE, Lerche NW, Dumler JS, Madigan JE. A simian model of human granulocytic ehrlichiosis. *Am J Trop Med Hyg.* (1999) 60:987–93. doi: 10.4269/ajtmh.1999.60.987

327. Baxarias M, Álvarez-Fernández A, Martínez-Orellana P, Montserrat-Sangrà S, Ordeix L, Rojas A, et al. Does co-infection with vector-borne pathogens play a role in clinical canine leishmaniosis? *Parasit Vectors.* (2018) 11:135. doi: 10.1186/s13071-018-2724-9

328. Attipa C, Solano-Gallego L, Leutenegger CM, Papasouliotis K, Soutter F, Balzer J, et al. Associations between clinical canine leishmaniosis and multiple vector-borne co-infections: a case-control serological study. *BMC Vet Res.* (2019) 15:331. doi: 10.1186/s12917-019-2083-6

329. Nyarko E, Grab DJ, Dumler JS. Anaplasma phagocytophilum-infected neutrophils enhance transmigration of *Borrelia burgdorferi* across the human blood brain barrier *in vitro. Int J Parasitol.* (2006) 36:601–5. doi: 10.1016/j.ijpara.2006.01.014

330. Nieto NC, Foley JE. Meta-analysis of coinfection and coexposure with Borrelia burgdorferi and Anaplasma phagocytophilum in humans, domestic animals, wildlife, and Ixodes ricinus-complex ticks. *Vector Borne Zoonotic Dis.* (2009) 9:93–102. doi: 10.1089/vbz.2008.0072

331. Levin ML, Fish D. Acquisition of coinfection and simultaneous transmission of *Borrelia burgdorferi* and *Ehrlichia phagocytophila* by Ixodes scapularis ticks. *Infect Immun.* (2000) 68:2183–6. doi: 10.1128/iai.68.4.2183-2186.2000

332. Grab DJ, Nyarko E, Barat NC, Nikolskaia OV, Dumler JS. *Anaplasma phagocytophilum-Borrelia burgdorferi* coinfection enhances chemokine, cytokine, and matrix metalloprotease expression by human brain microvascular endothelial cells. *Clin Vaccine Immunol.* (2007) 14:1420–4. doi: 10.1128/CVI.00308-07

333. Holden H, Hodzic E, Feng S, Freet KJ, Lefebvre RB, Barthold SW. Coinfection with *Anaplasma phagocytophilum* alters *Borrelia burgdorferi* population distribution in C3H/HeN mice. *Infect Immun.* (2005) 73:3440–4. doi: 10.1128/IAI.73.6.3440-3444.2005

334. Klein MB, Miller JS, Nelson CM, Goodman JL. Primary bone marrow progenitors of both granulocytic and monocytic lineages are susceptible to infection with the agent of human granulocytic ehrlichiosis. *J Infect Dis.* (1997) 176:1405–9. doi: 10.1086/517332

335. De Arcangeli S, Balboni A, Serafini F, Battilani M, Dondi F. *Anaplasma phagocytophilum* infection in thrombocytopenic dogs. *Vet Ital.* (2018) 54:73–8. doi: 10.12834/VetIt.1070.5796.2

336. Smith RD, Ristic M, Huxsoll DL, Baylor RA. Platelet kinetics in canine ehrlichiosis: evidence for increased platelet destruction as the cause of thrombocytopenia. *Infect Immun.* (1975) 11:1216–21. doi: 10.1128/IAI.11.6.1216-1221.1975

337. Waner T, Harrus S, Weiss DJ, Bark H, Keysary A. Demonstration of serum antiplatelet antibodies in experimental acute canine ehrlichiosis. *Vet Immunol Immunopathol.* (1995) 48:177–82. doi: 10.1016/0165-2427(95)05420-b

338. Wong SJ, Thomas JA. Cytoplasmic, nuclear, and platelet autoantibodies in human granulocytic anaplasmosis patients. *J Clin Microbiol.* (1998) 36:1959–63. doi: 10.1128/JCM.36.7.1959-1963.1998

339. Granick JL, Reneer DV, Carlyon JA, Borjesson DL. *Anaplasma phagocytophilum* infects cells of the megakaryocyte lineage through sialyated ligands but fails to alter platelet production. *J Med Microbiol.* (2008) 57:416–23. doi: 10.1099/jmm.0.47551-0

340. Behl R, Klein MB, Dandelet L, Bach RR, Goodman JL, Key NS. Induction of tissue factor procoagulant activity in myelomonocytic cells inoculated by the agent of human granulocytic ehrlichiosis. *Thromb Haemost.* (2000) 83:114–8.

341. Borjesson DL, Simon SI, Tablin F, Barthold SW. Thrombocytopenia in a mouse model of human granulocytic ehrlichiosis. *J Infect Dis.* (2001) 184:1475–9. doi: 10.1086/324518

342. Beck A, Huber D, Antolić M, Anzulović Z, Reil I, Polkinghorne A, et al. Retrospective study of canine infectious haemolytic anaemia cases reveals the importance of molecular investigation in accurate postmortal diagnostic protocols. *Comp Immunol Microbiol Infect Dis.* (2019) 65:81–7. doi: 10.1016/j.cimid.2019.05.006

343. Respess M, O'Toole TE, Taeymans O, Rogers CL, Johnston A, Webster CRL. Portal vein thrombosis in 33 dogs: 1998–2011. *J Vet Intern Med.* (2012) 26:230–7. doi: 10.1111/j.1939-1676.2012.00893.x

344. Li H, Zhou Y, Wang W, Guo D, Huang S, Jie S. The clinical characteristics and outcomes of patients with human granulocytic anaplasmosis in China. *Int J Infect Dis.* (2011) 15:e859–66. doi: 10.1016/j.ijid.2011.09.008

345. Zhuo M, Calev H, Saunders SJ, Li J, Stillman IE, Danziger J. Acute kidney injury associated with human granulocytic anaplasmosis: a case report. *Am J Kidney Dis.* (2019) 74:696–9. doi: 10.1053/j.ajkd.2019.03.428

346. Schotthoefer AM, Meece JK, Ivacic LC, Bertz PD, Zhang K, Weiler T, et al. Comparison of a real-time PCR method with serology and blood smear analysis for diagnosis of human anaplasmosis: importance of infection time course for optimal test utilization. *J Clin Microbiol.* (2013) 51:2147–53. doi: 10.1128/JCM.00347-13

347. Stuen S, Casey ANJ, Woldehiwet Z, French NP, Ogden NH. Detection by the polymerase chain reaction of Anaplasma phagocytophilum in tissues of persistently infected sheep. *J Comp Pathol.* (2006) 134:101–4. doi: 10.1016/j.jcpa.2005.06.006

348. Franzen P, Aspan A, Egenvall A, Gunnarsson A, Karlstam E, Pringle J. Molecular evidence for persistence of *Anaplasma phagocytophilum* from acute experimental infection. *J Vet Intern Med.* (2009) 23:636–42. doi: 10.1111/j.1939-1676.2009.0317.x

349. Littman MP. Protein-losing nephropathy in small animals. *Vet Clin North Am Small Anim Pract.* (2011) 41:31–62. doi: 10.1016/j.cvsm.2010.09.006

350. Littman MP, Daminet S, Grauer GF, Lees GE, van Dongen AM. Consensus recommendations for the diagnostic investigation of dogs with suspected glomerular disease. *J Vet Intern Med.* (2013) 27:S19–26. doi: 10.1111/jvim.12223