Review

Epidemiology, Diagnosis, and Control of Canine Infectious Cyclic Thrombocytopenia and Granulocytic Anaplasmosis: Emerging Diseases of Veterinary and Public Health Significance

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Abstract: This review highlights the diagnostic methods used, the control strategies adopted, and the global epidemiological status of canine cyclic thrombocytopenia and granulocytic anaplasmosis at the animal–human interface. Canine anaplasmosis is an important worldwide disease, mainly caused by *Anaplasma platys* and *A. phagocytophilum* with zoonotic implications. *A. platys* chiefly infects platelets in canids, while *A. phagocytophilum* is the most common zoonotic pathogen infecting neutrophils of various vertebrate hosts. Diagnosis is based on the identification of clinical signs, the recognition of intracellular inclusions observed by microscopic observation of stained blood smear, and/or methods detecting antibodies or nucleic acids, although DNA sequencing is usually required to confirm the pathogenic strain. Serological cross-reactivity is the main problem in serodiagnosis. Prevalence varies from area to area depending on tick exposure. Tetracyclines are significant drugs for human and animal anaplasmosis. No universal vaccine is yet available that protects against diverse geographic strains. The control of canine anaplasmosis therefore relies on the detection of vectors/reservoirs, control of tick vectors, and prevention of iatrogenic/mechanical transmission. The control strategies for human anaplasmosis include reducing high-risk tick contact activities (such as gardening and hiking), careful blood transfusion, by passing immunosuppression, recognizing, and control of reservoirs/vectors.

Keywords: canine anaplasmosis; *Anaplasma platys*; *Anaplasma phagocytophilum*; diagnosis; epidemiology; control

1. Introduction

Anaplasmosis is a vector-borne disease that affects animals and humans worldwide [1]. It is a virulent non-contagious disease caused by strictly intracellular Gram-negative bacteria. These pathogens parasitize circulating blood cells (erythrocytes, monocytes, granulocytes, and platelets) [2]. Ticks act as natural vectors for *Anaplasma* species and play a key role in the biological multiplication of these bacteria in salivary glands and guts [3].

The genus *Anaplasma* (*A.*) consists of several classified species that have a valid taxonomic standing, namely, *A. marginale*, *A. centrale*, *A. bovis*, *A. ovis*, *A. caudatum*, and *A. phagocytophilum* [1]. Infectious canine cyclic thrombocytopenia and granulocytic anaplasmosis are two zoonotic diseases caused by *A. platys* and *A. phagocytophilum*, respectively, mainly affecting dogs and wild canids [1,2]. *Anaplasma platys* was first detected in a dog from Florida and frequently infects platelets. The disease is characterized by fever, anorexia, weight loss, lethargy, petechiae, pale mucous membranes, nasal discharge, bilateral uveitis, epistaxis, and lymphadenomegaly [4,5]. However, *A. phagocytophilum* primarily infects canine granulocytes (especially neutrophils) of a wide range of domestic and wild vertebrate hosts, as well as humans [6]. The first case of human granulocytic anaplasmosis was observed in 1994 in tangential blood smears from six human patients of Wisconsin and Minnesota, states of the United States of America [7]. However, the first case of *A. platys* infection in humans was reported in 1992 [8]. After this, the organism was detected in a veterinarian and two family members of dog owners [9,10]. In 2017, there were 5762 cases of human anaplasmosis in the U.S., and now, global distribution of cases has been achieved [11]. In addition, transplacental transmission has also been reported for *A. phagocytophilum* [12].

Infection with *A. phagocytophilum* in dogs is known as canine granulocytic anaplasmosis. The host range of *A. phagocytophilum* includes ruminants, humans, carnivores, reptiles, birds, and rodents [13]. The increased prominence of human infections, alternative treatment options, availability of whole genome data, and alternative/promising preventive measures are all important contributions, and could perhaps be stressed earlier. Therefore, it is imperative to mention the updated global epidemiological status, diagnosis, and control of canine anaplasmosis at the animal–human interface.

2. History

In 1910, Sir Arnold Theiler discovered bacteria of the *Anaplasma* genus. He was the first to observe these "marginal spots" in the red blood cells of South African cattle, which he called *Anaplasma marginale* [14]. He later described *A. centrale* as subspecies of *A. marginale*, which seems to be less pathogenic and localized more frequently in the center of red blood cells than in the margins of erythrocytes [14].

In 1932, Gordon and his colleagues first noticed a disease in sheep in Scotland without identifying the causative agent in louping ill-affected districts transmitted by *Ixodes ricinus*. Further investigation revealed pathogens in the blood, spleen, and central nervous system. Later, the disease was diagnosed as tick fever, and the clinico-pathological aspects of the disease were studied in detail [14–16].

In 1949, Foggie placed this pathogen in the Rickettsial group, since the disease is transmitted by ticks, and named it *Ehrlichia phagocytophila ovis* [17]. The designation Ehrlichiae was chosen in honor of the German microbiologist Paul Ehrlich [18]. In 1969, Gribble discovered, in California (U.S.), a bacterium of the *Ehrlichia* genus causing a fatal disease in horses. He called the disease "equine granulocytic ehrlichiosis" in reference to the location of the morulae in the granulocytes [19,20]. The bacterium was subsequently described and named *Ehrlichia equi* by Lewis and his colleagues in 1975 [20]. In 1994, Chen and his colleagues in the U.S. diagnosed clinical case of ehrlichiosis in a human patient; they named it human granulocytic ehrlichiosis (HGE) [7,21].

3. Etiology

The word *Anaplasma* is derived from the Greek words *an* and *plasma*; the former means "without" and the latter means "molded." *Anaplasma*, *Ehrlichia*, *Wolbachia*, and *Neorickettsia* belong to obligate intracellular bacteria that invade blood cells. *Anaplasma* is an obligate intracellular, Gram-negative alpha-proteobacteria that belongs to the Anaplasmataceae family, order Rickettsiales. Currently, *A. marginale*, *A. bovis*, *A. ovis*, *A. platys*, and *A.phagocytophilum* are important species of the genus *Anaplasma* (Table 1).

Table 1. Classified and unclassified *Anaplasma* species infecting different cells, their vertebrate hosts, and their potential vectors.

Anaplasma Species	Infecting Cells	Vertebrate Hosts	Potential Vectors	References
A. platys	Platelets	Dogs and camels	*Rhipicephalus*	[22,23]
A. phagocytophilum	Granulocytes	Domestic and wild ruminants, horses, dogs, cats, rabbits, rodents, insectivores, wild swine, and humans	*Ixodes*, *Dermacentor*, *Hyalomma*, *Rhipicephalus*	[13]
A. marginale	Erythrocytes	Domestic ruminants	*Rhipicephalus, Ixodes, Dermacentor*	[24]
A. centrale	Erythrocytes	Domestic and wild ruminants	*Rhipicephalus, Ixodes, Haemaphysalis*	[1]
A. ovis	Erythrocytes	Domestic and wild ruminants and humans	*Rhipicephalus, Dermacentor, Hyalomma*	[1]
A. bovis	Monocytes	Domestic and wild ruminants and small mammals	*Haemaphysalis, Rhipicephalus, Amblyomma*	[25]
A. capra	Erythrocytes	Domestic and wild ruminants and humans	*Haemaphysalis*	[26,27]
A. odocoilei	Platelets	Wild ruminants	Not known	[28]
Candidatus A. camelii	Not known	Camels	Not known	[29]
Candidatus A. boleense	Not known	Not known	*Hyalomma*	[30]
Candidatus A. corsicanum	Not known	Domestic ruminants	Not known	[31]
Candidatus A. mediterraneum	Not known	Domestic ruminants	Not known	[31]
Candidatus A. sphenisci	Not known	African penguins	Not known	[32]
Candidatus A. rodmosense	Not known	Rodents	Not known	[33]

The sequencing of the whole genome of *A. phagocytophilum* was evaluated and shown to contain a $1.2–1.5 \times 10^6$ bp circular chromosome [34]. Likewise, *A. platys* has a 1.196×10^6 bp genome size [35]. *Anaplasma platys* frequently infects dogs; however, it has also been reported in cats, camels, and humans. Nonetheless, *A. ovis* has also been described in humans [36]. *Anaplasma* spp. demonstrates some level of host specificity; this attribute is altered due to the detection of *Anaplasma* species in various hosts, which further complicates the pathology and epidemiology of the disease [36].

4. Taxonomical Position of *Anaplasma* Bacteria

4.1. Evolution of Taxonomy

The Anaplasmataceae family is included in the order Rickettsiales, obligate intracellular bacteria that exist in eukaryotic cells. Morphologically (Gram-negative bacteria) and epidemiologically, they have a particular tropism for blood cells, since all of them are mainly transmitted by ticks. This classification of Anaplasmataceae is based on the pathogenic characteristics of these bacteria, which are strictly intracellular [37]. Studies of Weisburg and Sumner and colleagues have revealed that, in reality, *Ehrlichia phagocytophila* and *Ehrlichia equi* are genetically similar to the etiologic agent of human granulocytic anaplasmosis [38,39]. Based on the sequencing and phylogenetic proximity of these bacterial operons/genes (*groESL*, *gltA*, *ankA*, and *16S ribosomal RNA*), Dumler and his collaborators made profound taxonomic changes, leading to the reorganization of most family members, genera, and species of Rickettsiales [40].

4.2. Current Classification

Carrade and his collaborators reorganized the classification on the basis of their nucleic acid sequences, known antigenic properties, ecology, geographical distribution, and their pathogenicity. They mainly used the *16S rRNA* gene and *groESL* operon, and divided this order into two families. One is Rickettsiaceae, which remains free in the cytoplasm, and the second is Anaplasmataceae, which is contained in a vacuole bound to the cytoplasmic membrane derived from the host cell [41]. The present classification is based on the phylogenetic analysis of the *16S rRNA* and *groESL* genes replacing and renaming *Ehrlichia* (*E.*) *bovis*, *E. platys*, and *E. phagocytophilum* as *A. bovis*, *A. platys*, and *A. phagocytophilum*, respectively [40].

5. Epidemiology

Anaplasmosis has a worldwide distribution, and is potentially endemic in forty-three countries of the world [42]. Although, the prevalence varies among area, species, breeds, due to the presence of different ticks, and diagnostic assays involved. *Anaplasma phagocytophilum* and *A. platys* have been identified on all continents. Sufficient scientific data are available for *A. phagocytophilum,* while less epidemiological and risk factor information is available for *A. platys*. Nevertheless, *A. phagocytophilum* can infect a wide range of wild/domestic animals and humans; however, *A. platys* typically infects dogs and rarely cats [13,43]. Animals recover from acute anaplasmosis, develop a lifelong persistent infection with low cyclic rickettsiemia, and act as a reservoir host for further spread [44]. *A. platys* has zoonotic potential and there are reports of human infection to a lesser extent [45].

5.1. Anaplasma platys

Anaplasma platys was first observed in a blood test of a dog in the United States in 1978. It has been detected on almost all continents with worldwide distribution [4,46–51]. *A. platys* widely infects dogs; however, it has also been shown in deer, cats, cattle, and humans [5]. In dogs, severe thrombocytopenia results in recrudescence after two weeks of incomplete recovery. Thrombocytopenia can occur as a result of direct damage to platelets and immune cells caused by immune-mediated mechanisms [4]. In dogs and cats, the serological and molecular prevalence rates range from 0.4% to 87.5% and from 0.6% to 6.6%, respectively, depending on the region, breed, and involved test (Table 2). Regarding the infected host, the overall prevalence and distribution of *A. platys* in domestic canids are shown in Table 2. Interestingly, the camel is an animal species that significantly harbors canine *A. platys* and various *A. platys*-like strains during natural infection [52–54]. It is necessary to determine the pathogenicity and the epidemiological role of camelids in the transmission of this *Anaplasma* species.

5.2. Anaplasma phagocytophilum

Anaplasma phagocytophilum is one of the most diverse pathogens infecting humans, and domestic and wild animals. This species is most widespread in northern Europe. Small mammals play a vital role in disease transmission. *A. phagocytophilum* is mainly transmitted by tick bites [55]. Disease outcome and response to treatment are complex in dogs, co-infected with *H. canis*, *B. vogeli*, and/or *Ehrlichia canis*. After the incidence of animal anaplasmosis in an area, the screening of human anaplasmosis should be considered. Sero-surveillance has shown a prevalence of 15–36% in humans with an annual incidence of approximately 58 cases per 100,000 individuals in the U.S. [18]. The rate of human infection increases with infected vectors. Indeed, there is a high rate of incidence of human granulocytic anaplasmosis (HGA) in the U.S. There were approximately 2782 cases of HGA recorded during the year 2013 [11].

Regarding HGA, patients' clinical signs range from asymptomatic to severe clinical disease, and approximately 40% of patients require hospitalization [18,56,57]. The mortality rate in the U.S. ranges from 7% to 10% [58–60]. The severity of infection depends on the

phase of bacterial growth, the susceptibility of the host, and the pathogenic bacterial strain. A blood test of the infected host reveals that there is a decrease in the number of neutrophils and leukocytes, resulting in immunosuppression and a tendency of opportunistic infection [61]. Approximately30% of the patients required prompt hospitalization due to the development of life-threatening conditions, including severe sepsis, anaphylactic shock, and respiratory syndrome [57]. Death occurs mainly due to a combination of other health problems, including intravascular coagulation, kidney failure, enlarged heart, coma, and seizures.

Table 2. Detection of *A. platys* in domestic canid hosts from different countries *.

Domestic Canid	Countries (Region)	Prevalences (%)	Methods (Target Genes)	References
Dog	Thailand	13.9	PCR[a] (*groEL*)	[62]
	Thailand	29.4	PCR[a] (*16S rRNA*)	[63]
	Thailand	7.0	PCR[a] (*16S rRNA*)/mHRM[b]	[64]
	West Indies (Grenada)	18.7	PCR[a] (*16S rRNA*)	[65]
	West Indies (Grenada)	33.0	PCR[a] (*16S rRNA*)/ELISA[c]	[66]
	West Indies (Grenada)	16.4	RT-PCR[d] (*16S rRNA*)	[67]
	West Indies (Trinidad)	2.3	PCR[a] (*16S rRNA*)/RBL[e]	[68]
	Pakistan	11.34	PCR[a] (*16S rRNA*)	[69]
	Paraguay	10.67	PCR[a] (*16S rRNA*)	[70]
	Colombia	20.2	RT-PCR[d] (*16S rRNA*)	[71]
	Greece	Case report	Blood smear/ELISA[c]	[72]
	Indonesia	11.76	PCR[a] (*groEL*)	[73]
	Cape Verde	7.7	PCR[a] (*16S rRNA*)	[74]
	Italy	70.5	PCR[a] (*groEL*)	[75]
	Italy (Putignano)	52.9	RT-PCR[c] (*16S r RNA*)	[76]
	Italy (Teramo Kennel)	33.0	PCR[a] (*16S rRNA*)/RLB[e]	[48]
	Croatia	Case report	RT-PCR[d] (*groEL*)	[77]
	Australia	51.3	RT-PCR[d] (*16S rRNA*)	[78]
	Australia	23.7	ELISA[c]	[78]
	Australia	32.0	PCR[a] (*16S/18S rRNA*)	[49]
	Australia	3.8	Blood smear/ELISA[c]/PCR[a]	[79]
	Romania	Case report	PCR[a] (*16S rRNA*)	[80]
	Dominican Republic	11	RT-PCR[d] (*16S/18S rRNA*)	[81]
	Nicaragua	13	RT-PCR[d](*16S/18S rRNA*)	[82]
	Caribbean	10.3	ELISA[c]	[83]
	Canada	1.8	ELISA[c]	[83]
	USA (South)	2.0	ELISA[c]	[83]
	USA (Mid Atlantic)	1.1	ELISA[c]	[83]
	USA (Northeast)	1.5	ELISA[c]	[83]
	USA (Midwest)	0.6	ELISA[c]	[83]
	USA (West)	1.0	ELISA[c]	[83]
	Mexico	31.0	PCR[a] (*16S rRNA*)	[84]
	Brazil	7.19	PCR[a] (*16S rRNA*)	[51]
	Turkey	0.5	RLB[d]	[85]
	Costa Rica	1	PCR[a] (*16S rRNA, groEL*)	[86]
	Brazil	16.96	nPCR[f] (*16S rRNA*)	[87]
	Brazil	19.4	PCR[a] (*16S rRNA*)	[88]
	Brazil	14.07	nPCR[f] (*16S rRNA*)/ELISA[c]	[89]
	Colombia	53.0	PCR[a] (*16S rRNA*)/ELISA[c]	[90]
	Palestine	53.0	PCR[a] (*16S rRNA*)	[91]
	China	62.1	RT-LAMP[g]/nPCR[f] (*16S rRNA*)	[92]
	Caribbean	18.7	PCR[a] (*16S rRNA, gltA, groEl*)	[65]
	Argentina	37.5	PCR[a] (*16S rRNA, groESL*)	[93]
	Costa Rica	6.25	nPCR[f] (*16S rRNA*)/ELISA[c]	[94]
	Myanmar	0.25	PCR[a] (*16S rRNA*)	[95]
	Malawi	2.4	PCR[a] (*16S rRNA*)	[96]

Table 2. *Cont.*

Domestic Canid	Countries (Region)	Prevalences (%)	Methods (Target Genes)	References
	Galápagos	6.9	PCR[a] (*16S rRNA*)/ELISA[c]	[97]
	Saudi Arabia	57.1	RT-PCR[c] (*16S rRNA*)	[98]
	Greek islands	18.0	PCR[a] (*16S rRNA*)/IFAT[h]	[99]
	Malta	22.7	PCR[a] (*16S rRNA, cox1*)	[100]
	Haiti	6.3	PCR[a] (*16S/18S rRNA*)	[101]
	Cambodia	32.0	NGS[i] based metabarcoding	[102]
	Uganda	18.9	RT-PCR[d] (*16S rRNA*)/IFAT[h]	[103]
	Albania	3.3	PCR[a] (*16S rRNA*)/ELISA[b]	[104]
	Nigeria	6.6	RT-PCR[d] (*16S rRNA*)	[105]
	Qatar	1.6	PCR[a] (*16S rRNA*)	[106]
	Texas	0.17	RT-PCR[d] (*16S rRNA*)	[107]
	India	22.6	PCR[a] (*16S rRNA*)	[108]
	Japan	32.0	PCR[a] (*16S rRNA*)	[109]

[a] Polymerase chain reaction; [b] multiplex high-resolution melting analysis; [c] enzyme-linked immunosorbent assay; [d] real-time polymerase chain reaction; [e] reverse line blot hybridization; [f] nested polymerization chain reaction; [g] real-time loop-mediated isothermal amplification; [h] indirect fluorescent antibody test; [i] next-generation sequencing based on metabarcoding. * Detection of *A. platys* from 1991 up to date.

The disease is more severe in elderly patients and immunocompromised children [58,59].
Anaplasma phagocytophilum is mainly transmitted by ixodid ticks of the genera *Ixodes*, *Dermacentor*, *Haemaphysalis*, and *Amblyomma* in Europe, the U.S., and Asia [24]. In ticks, transstadial transmission occurs [110,111], while other routes of transmission are less common, such as contact with infected blood and tissues [57,112]. Serological and molecular prevalence rates vary from 0.3% to 55.6% for dogs and 0.9% to 37.6% for cats depending upon the area, breed, and test used (Table 3). The infected host, the global prevalence of infection, and the distribution of *A. phagocytophilum* in domestic canid hosts are listed in Table 3.

Table 3. Detection of *A. phagocytophilum* from the tissue or blood of domestic canids hosts *.

Domestic Canid	Countries (Regions)	Prevalences (%)	Methods(Target Genes)	References
Dog	Iraq	55.6	Blood smear	[113]
	Iran	2.0	PCR [a] (*msp4*)	[114]
	Mexico	27	PCR[a] (16S rRNA)	[115]
	USA (California)	7.6	RT-PCR[b] (*msp2*)	[116]
	Brazil	7.1	RT-PCR[b] (*msp2*)	[117]
	USA (South)	2.1	ELISA[c]	[83]
	USA (Mid-Atlantic)	5.4	ELISA[c]	[83]
	USA (Northeast)	13	ELISA[c]	[83]
	USA (Midwest)	1.9	ELISA[c]	[83]
	USA (West)	2.0	ELISA[c]	[83]
	Canada	1.1	ELISA[c]	[83]
	Caribbean	3.4	ELISA[c]	[83]
	Sweden	17.0	IFAT[d]	[118]
	Colombia	1.1	PCR[a] (16S rRNA)	[119]
	Costa Rica	0.3	PCR[a] (16SrRNA, *groEL*)	[86]
	India	0.4	PCR[a] (16S/18S rRNA)	[108]
	Turkey	4.0	nPCR[e] (16S rRNA)	[120]

[a] Polymerase chain reaction; [b] real-time polymerase chain reaction; [c] enzyme-linked immunosorbent assay; [d] indirect fluorescent antibody test; [e] nested polymerization chain reaction. * Detection of *A. phagocytophilum* from 1998 up to date.

6. Transmission

Ixodidae ticks act as biological vectors and play an essential role in the spread and propagation of *Anaplasma* during various stages of its life cycle [121]. Nonetheless, vertical transmission has also been reported for *A. platys* infection in bitches during early gestation

(25–35 days) and intrauterine transmission for *A. phagocytophilum* as well [122–124]. Vertebrates are definitive hosts and also serve as reservoirs [125]. *Rhipicephalus* (*R.*) *sanguineus* and *I. ricinus* are the major vectors of *A. platys* and *A. phagocytophilum*, respectively [124,126].

7. Life Cycle

The life cycle of all *Anaplasma* species hasnot yet been completely studied. Most studies have been performed on *A. marginale* in cattle in association with *R. microplusticks*. The life cycle begins with the ingestion of *Anaplasma* by tick vectors during a blood meal [127]. *A. phagocytophilum* frequently infects granulocytes, causing leukopenia and thrombocytopenia. This changes the host's immune system and positively regulates cellular cholesterol and several tick genes.

However, *A. platys* primarily infects platelets causing thrombocytopenia, and can also infect megakaryocytes and promegakaryocytes [127]. Transstadial, transovarial, and mechanical propagation, as well as several other host-related factors, make the conditions essential for the maintenance of *Anaplasma* in nature [128].

8. Clinical Findings

In dogs, *A. platys* causes canine cyclic thrombocytopenia with variable signs of fever, anorexia, weakness, anemia, lethargy, eye discharge, spot hemorrhage on the eye, oral mucosa and skin, respiratory distress, lymphadenomegaly, epistaxis, splenomegaly, and muzzle hyperkeratosis [46,129,130]. Thrombocytopenia may occur as a result of direct damage to platelets by the pathogen and immune-mediated systems [4]. Camels infected with *A. platys* generally remain asymptomatic, with some evidence of anorexia, dullness, progressive loss of physical condition, and stamina, as well as neutrophilia and eosinophilia [22]. Canine granulocytic anaplasmosis shows signs of high fever, vomiting, diarrhea, loss of appetite, lameness, polyuria, jaundice, epistaxis, lymphatic adenomegaly, and splenomegaly [131,132]. Cats show no specific clinical signs; however, signs of anorexia, fever, lethargy, and dryness with neutrophilia, lymphopenia, thrombocytopenia, and hyperglycemia can be observed [133–135].

In humans, *A. phagocytophilum* causes human granulocytic anaplasmosis. Patients present with flu-like symptoms ranging from asymptomatic to severe clinical illnesses. High fever, severe headache, stiff neck, myodynia, restlessness, cough, nausea, and vomiting are important clinical signs, and even diarrhea, joint pain, and neurological signs [136,137]. During illness, certain threatening conditions can develop in patients due to opportunistic pathogens. Often, laboratory tests are needed to maintain the diagnosis. Approximately 30% of the patients require hospitalization due to anaphylactic shock, severe sepsis, and respiratory syndrome [57]. The disease mortality rate is 7–10% in the United States [58,60]. Death occurs mainly due to the combination of other health problems related to intravascular coagulation, kidney failure, enlarged heart, coma, and seizures. The disease is more harmful in elderly immunocompromised patients [112].

9. Diagnosis

Diverse conventional, serological, and molecular methods have been validated for causative agent identification and disease diagnosis.

9.1. Direct Detection

Conventional light microscopy of freshly prepared stained blood smears (Giemsa, Diff-Quik) taken from a vein are used for diagnosis in the acute phase of the disease (Figures 1–3). *A. phagocytophilum* leads to the development of "morulae," which are a combination of mulberry-type colonies formed in the neutrophils and eosinophils of infected organisms [61].
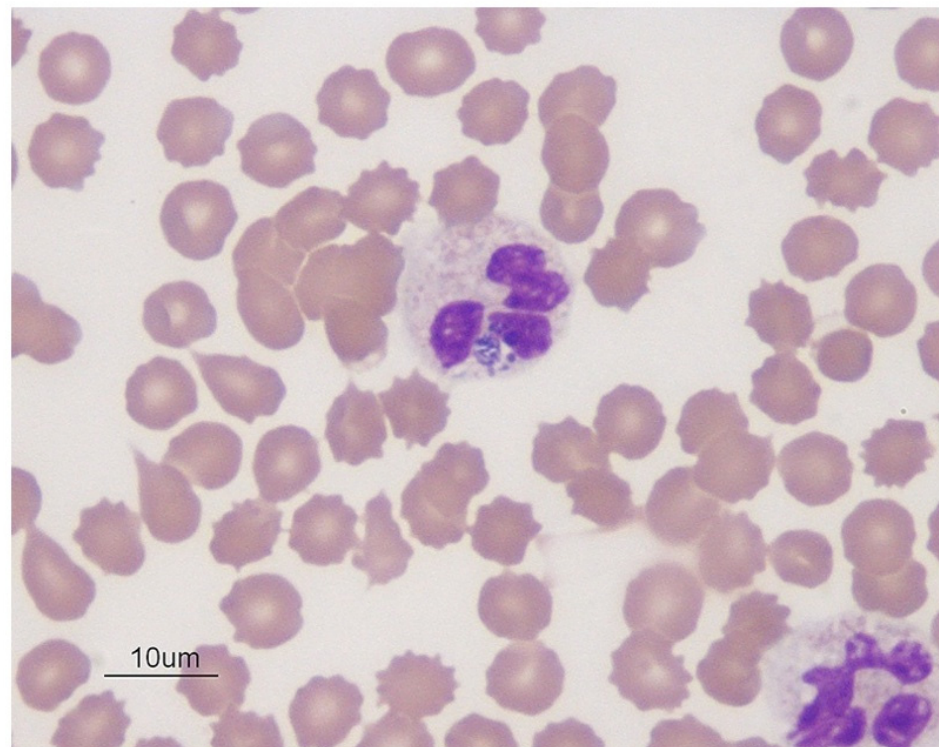
Figure 1. Blue color *Anaplasma phagocytophilum* in the cytoplasm of neutrophils in dog blood; Wright's stain, 1000× (source: https://eclinpath.com; accessed on 11 August 2021; Cornell University College of Veterinary Medicine).
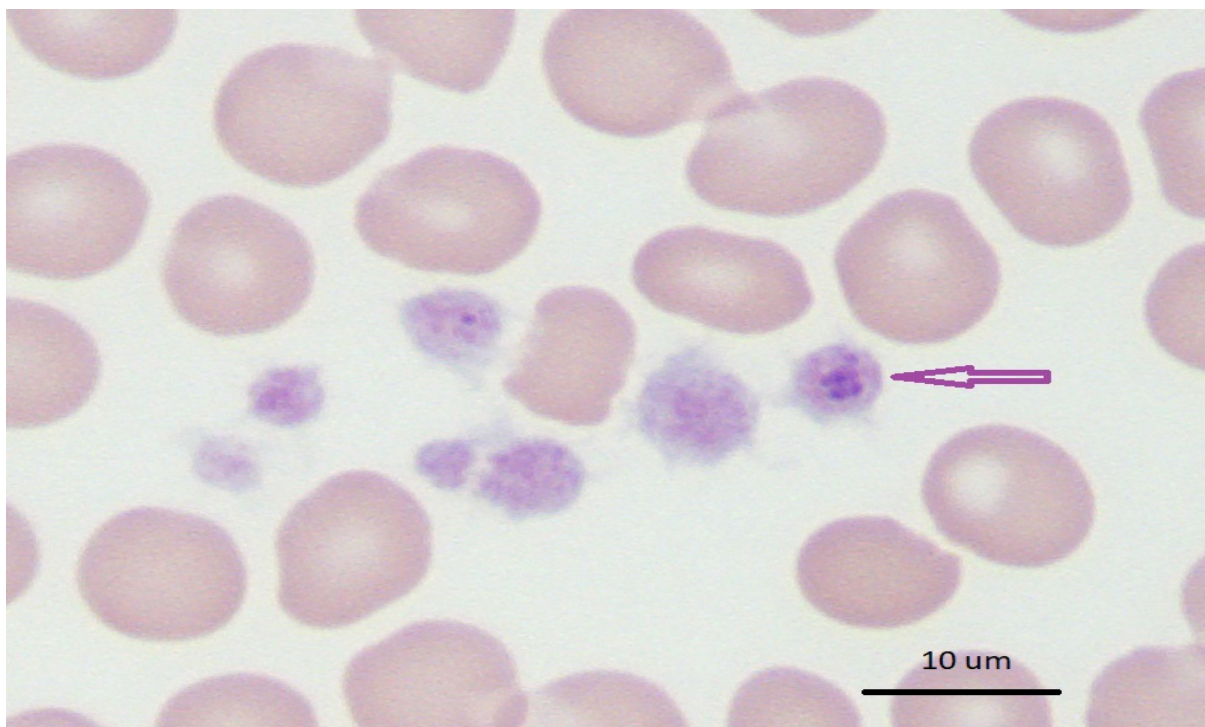

Figure 2. Blue–purple inclusions of *Anaplasma platys* in dog blood with thrombocytopenia; Wright's stain 1000× (source: https://eclinpath.com; accessed on 11 August 2021; Cornell University College of Veterinary Medicine).

Typically, *Anaplasma* morulae resembledark blue to purple inclusion bodies. Conversely, refrigerated samples mixed with anticoagulants can be processed within 24–48 h.

This is a quick, inexpensive, and best way to directly visualize bacteria before the start of antibacterial treatment. However, this method is less sensitive to lower bacteremia during persistent infection with monocytopenia, neutropenia, thrombocytopenia, and anemia [138,139]. The sample collection time is critical for the direct identification of bacteria involving microscopy, *in vitro* culture, and nucleic acid detection in order to detect sufficient number of organisms in the circulating blood [140]. Leukocyte smears would be a good option for *A. platys* and *A. phagocytophilum* morulae compared to whole blood. As these organisms are limited to platelets and leukocytes, this enriched fraction is cogently useful for the identification of cases of leucopenia and thrombocytopenia encountered as clinical sequelae [140].

For specific research, scanning electron microscopy, confocal microscopy, and transmission electron microscopy can be useful for the detection of these bacteria in ticks, mites, lice, and other invertebrates. Smears of tissue impressions from the liver, spleen, heart, lungs, kidneys, and/or blood vessels can be used during necropsy, especially for wild animal species [139,141].
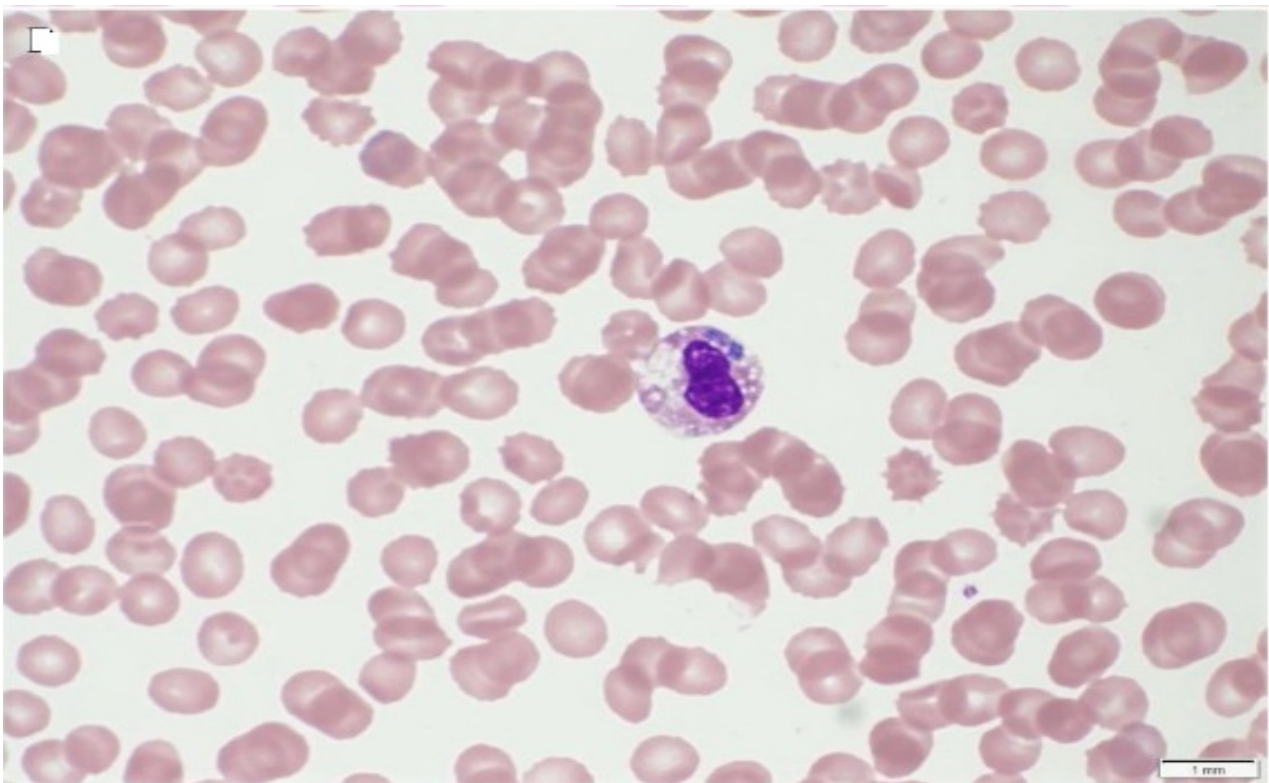

Figure 3. Human *A. phagocytophilum* infection, indicating morulae in infected neutrophils [142].

9.2. Serology

Some companies provide commercial diagnostic kits for the serodiagnosis of anaplasmosis in animals and humans with variable accuracy. The IgG and IgM antibodies for *A. phagocytophilum* can be detected using a commercial kit based on IFAT "Fuller Laboratories" [143]. In addition, "SNAP 4Dx Plus," a commercial test, allows for the detection of antibodies to *A. platys* and *A. phagocytophilum*, as well as other canine pathogens (*Ehrlichia canis* or *Ehrlichia ewingii*, *Borrelia burgdorferi*, and *Dirofilaria immitis*).

Likewise, the "Canine *Anaplasma* Antibody Test Kit" of VetScan® (Abaxis) provides a rapid test thatqualitatively detects *A. platys* and/or *A. phagocytophilum* antibodies in dog serum or plasma. Similarly, "Anti-*Anaplasma phagocytophilum* ELISA Dog (IgG)" from EUROIMMUN (Medizinische Labordiagnostika AG) identifies seropositivity to *A. phagocytophilum* in dogs. Furthermore, the "Rapid *Anaplasma* Ab Test Kit" from the BIONOTE

company is a commercial immunological test kit thatallows the qualitative chromatographic detection of the antibodies of *A. phagocytophilum* and *A. platys*.

Nevertheless, "MegaCorDiagnostik" performs immunochromatographic testing based on the lateral flow method, and "FASTest *Anaplasma*" provides qualitative discovery of *Anaplasma phagocytophilum* antibodies in dog and horse serum/plasma, which are commercially available for the convenient screening of anaplasmosis.

9.3. Molecular Detection

Nucleic acid detection methods, including conventional, nested and semi-nested PCR, real-time PCR, and LAMP (loop-mediated isothermal amplification), have been used for diagnosis. The *16S rRNA*, citrate synthase, heat shock, and major surface proteins (Msp1, Msp2, Msp4, and Msp5) are the most targeted genes for the molecular diagnosis of anaplasmosis [144].

Various high-performance single molecular and multiplex detection techniques with automation potential are in vogue. Whole blood containing ethylenediaminetetraacetic acid/citrate and buffy coat are good samples for diagnosis, while spleen samples are offered for the detection of carrier animals, especially in cases of wild animals [140,145,146]. Other types of samples, including the plasma/serum, lungs, liver, lymph nodes, skin, and bone marrow, have been used for screening [140,147–149]. For molecular diagnosis, multi copy genes are preferred over single copy genes. There is a growing trend to use fast and sensitive real-time assays over nested PCRs. Nonetheless, real-time screening assays yield short DNA products of less than 150 bp, yielding limited phylogenetical data. Sequencing and cross-matching are generally required for confirmation.

Different LAMP protocols have been developed for the identification of several *Anaplasma* species targeting the *msp1b*, *gltA*, *16S rRNA*, and *msp5* genes with variable detection limits. LAMP has the advantage of being a simple, robust, inexpensive, rapid, highly sensitive, and explicit diagnostic tool with low heat requirements, as well as options to use numerous primers [150]. For example, Lee and coworkers developed LAMP for the detection of *A. phagocytophilum* in dogs using the *gltA* gene, and this method was found to be more sensitive than nested PCR [151]. Likewise, Li and his colleagues developed a real-time LAMP for *A. platys* in dogs using citrate synthase gene sequences at 63 °C for 30 min. Uniform results and no cross-reactivity with other *Anaplasma/Ehrlichia* species were observed compared to nested PCR results [152].

Real-time molecular diagnostic methods have been developed for direct detection in blood, tissue, ticks/vectors that target multiple genes, which can be further used for taxonomic and phylogenetic studies. Whole genome sequencing of *A. phagocytophilum* and *A. platys* has been completed [34,35,153]. This will further contribute to the development of vaccines and diagnostic and control approaches for these important bacteria.

9.4. Isolation and In Vitro Cultivation

Isolation and *in vitro* culture are crucial, as all emerging bacteria have been grown on artificial media or cell lines. Indeed, ethical implications for animal use have led researchers to adapt the isolation and propagation of *Anaplasma* in cell culture lines derived from mammals on an uninterrupted basis. The HL-60 and THP-1 cells have been widely used [154,155]. Tick cell lines are alternative options for the in vitro cultivation of *Anaplasma* species [156]. Fresh infected blood from animals or humans is the best inoculum for in vitro propagation. For example, the cell line derived from embryos of *Ixodes scapularis* (IDE8, ISE6), *R. appendiculatus* (RAE25), *Dermacentor variabilis* (DVE1), as well as the cell lines *I. ricinus* IRE/CTVM19, IRE11, L610, and IRE/CTVM20, have been used for the culture and isolation of *A. phagocytophilum* [144,157]. Cells derived from *I. scapularis* (ISE6) are also used for culturing new isolates of *A. platys*-like bacteria [28].

10. Control

Usually, the control of anaplasmosis is difficult due to the existing antigenic/genetic diversity, the involvement of several hosts and multiple arthropod vectors, as well as different transmission potentials (biological, mechanical, and transplacental) [1]. In general, control measures include the control of arthropod vectors, host resistance and vaccination, sanitary/hygienic measures, and rarely chemoprophylaxis. However, the deterrence of tick infestation during periods of active transmission appears to be the best policy for the control of animal and human anaplasmosis [1].

10.1. Vector Control

Prevention strategies for common tick-borne diseases of domestic animals are based on the reduction of tick infestation using chemical acaricides [2]. Acaricidal treatment should be applied especially during the tick season. Biological tick control is gaining in importance as a striking approach to take, but it is generally difficult to achieve, since ticks have few natural enemies. Therefore, studies have focused on bacteria, entomopathogenic nematodes, and fungi [158,159]. However, the major concern is to establish sustainable biological control of ticks in natural habitats.

Tick vaccines are alternative control options against acaricides. The vaccination of animal–human populations at risk and/or the reservoir are important for limiting the distribution of tick-borne pathogens [160,161]. The development of combined vaccines targeting both pathogens transmitted by ticks and ticks themselves would be beneficial at large. There are various candidate proteins for a tick vaccine such as Bm86, Ba86, 64P, and RmAQP1. In addition, the salivary proteins Salp16 and Sialo L2 from *I. scapularis* protect the transmission of *A. phagocytophilum* infection [162].

Similarly, *I. ricinus* heme lipoprotein and uncharacterized secreted protein, as well asfive of the secreted proteins of *D. reticulatus* (glypican-like), which are involved in anion or sulfate exchangers, homophilic cell adhesion, subunit 3 of the signal peptidase complex, and other secreted proteins have been identified as the most effective vaccine candidates [163].

Subolesin is a protein that plays a role in reproduction, blood digestion, and development of ticks [164]. These types of vaccines cause disintegration of reproductive and embryonic tissues, causing sterility in male ticks, as well as degeneration of tick guts and salivary glands [165]. Tick vaccines are possible, cost-effective, and environmentally friendly methods compared to chemical control [44].

10.2. Vaccination against A. phagocytophilum and A. platys

Vaccination is the most effective and cheapest defense against anaplasmosis. It should be mentioned that the complete genome sequence of *A. phagocytophilum* and *A. platys* has been accomplished [34,35,153]. This can help to explore many new genes that could be potential candidates for vaccine manufacturing. There are approximately nine *Anaplasma* proteins that have immunogenic potential, namely, the Asp14, Asp55, Msp5, Msp2, AipA, OmpA, APH 0032, and APH 1384 antigens of the type IV secretion system of *A. phagocytophilum* [153,166–169].

10.3. Chemotherapeutic Use

Anaplasma bacteria are sensitive to antibiotics from the tetracycline group. Doxycycline is effective against human granulocytic anaplasmosis [11]. Similarly, doxycycline is also a useful chemotherapeutic agent for *A. platys* infection in dogs at a dose rate of 10 mg/kg body weight orally with or without dexamethasone (0.3 mg/kg IM daily) for 28 days [148]. In a situation of severe anemia, a blood transfusion is necessary.

Likewise, *A. phagocytophilum* infection in dogs can be treated with orbifloxacin at 5 mg/kg SC on day 1 and then orally on day 2 SID for two weeks. Other options that have proven effective for *A. phagocytophilum* infection in dogs include enrofloxacine (5 mg/kg SC, SID) and prednisolone (SID 1.5 mg/kg SC) on day 1, and orbifloxacin (4 mg/kg SC) and

prednisolone (1 mg/kg SC) from days 2–8 [170]. Animals with severe anemia accompanied by debility should be hospitalized.

Concomitant infection with *A. platys* and/or *A. phagocytophilum* in dogs, as well as with *E. canis*, *Babesia vogeli*, *Borrelia burgdorferi*, *Hepatozoon canis* and/or *Leishmania infantum*, has been reported [5,171]. Concurrent infections may obscure epidemiology, alter treatment, and present an atypical clinical picture. The clinical veterinarian should keep this aspect of coinfection in mind when dealing with anaplasmosis cases in the clinic. A summary of the treatment protocols is presented in Table 4.

Table 4. Summary of the clinical findings, diagnosis, and control of canine cyclic thrombocytopenia, and canine and human granulocytic anaplasmosis.

Disease	Clinical Findings	Diagnosis	Treatment	Control
Canine cyclic thrombocytopenia	Dogs usually remain asymptomatic; however, fever, lethargy, anorexia, weight loss, anemia, icterus, petechiae, nasal discharge, lymphadenopathy, and lymphadenomegaly may beobserved [5]	Stained blood smear, thrombocytopenia, serology, and PCR/DNA sequencing [5]	Doxycycline @5–10 mg kg^{-1} q12–24 h for 8–10 days orenrofloxacin @ 5 mg kg^{-1}, q12 h for 14–21 days [5]	Tick elimination, collar, pour-on or spot-on acaricidal products for *R. sanguineus sensu lato* ticks, knowledge of tick seasonality, andecology [5]
Canine granulocytic anaplasmosis	Non-specific signs, fever, anemia, anorexia, dullness, and thrombocytopenia [5]	Morulae in stained blood smear, thrombocytopenia, leucopenia, elevated liver enzymes, serology, andPCR/DNA sequencing [1,5]	Doxycycline 5 mg/kg bid for 28 days [172]	Vector control, habitat modification, rearing tick-resistant breeds, and chemotherapy [5]
Human granulocyti-canaplasmosis	Fever, headache, myalgias, and chills [123]	Morulae in stained blood smear, thrombocytopenia, leucopenia, elevated liver enzymes, serology/IFA, and PCR/DNA sequencing [95,173]	Doxycycline @ 100 mg, orally, twice dailyfor 10–14 days or rifampicin @ 20 mgkg^{-1} day^{-1} orallyfor children, otherwise 300 mg orally, twice dailyfor 5–7 days [47]	Humans: Minimizing high-risk tick exposure activities (hiking, gardening, etc.), blood transfusion, immune suppression, identificationof reservoirs and vectors, and their control [1]

11. Conclusions

Concretely, the adoption of control strategies varies according to geo-ecological circumstances. Integrated control of major reservoirs/vectors/ticks and hygienic sanitary measures are key elements in reducing disease transmission. As there is greater risk of mortality in older animals than younger ones, thus these individuals/animals should be treated a priority. Doxycycline with or without dexamethasone is a significant treatment for human and animal anaplasmosis. However, reducing high-risk tick contact activities in humans (such as gardening and hiking), careful blood transfusion, circumventing immunosuppression, recognizing reservoirs/vectors, and control ofvectorsare significant defense strategies against human anaplasmosis.

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