Open Veterinary Journal, (2024), Vol. 14(12): 3656-3664
ISSN: 2226-4485 (Print)
ISSN: 2218-6050 (Online)

Case Report
DOI: *10.5455/OVJ.2024.v14.i12.47*

Submitted: 31/08/2024 Accepted: 06/11/2024 Published: 31/12/2024

First molecular evidence of *Anaplasma platys* infection in a dog (Labrador retriever) from Bulgaria

Krasimira Gospodinova[1], Vladimir Petrov[1], Iskren Stanilov[2], Lyuba Miteva[2], Ilia Tsachev[1] and Magdalena Baymakova[3]*

[1]*Department of Veterinary Microbiology, Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria*
[2]*Department of Molecular Biology, Immunology and Medical Genetics, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria*
[3]*Department of Infectious Diseases, Military Medical Academy, Sofia, Bulgaria*

ABSTRACT

Background: In recent years, the One Health approach and vector-borne diseases have become an increasingly topical problem around the world. In addition, climate change has a significant impact on zoonoses and public health. We present a case report of tick-borne disease in a dog.

Case Description: A clinical case of *Anaplasma platys* infection in a 10-year-old female dog (Labrador retriever) is described. Clinical, hematological, biochemical, serological, cytological, and polymerase chain reaction tests supporting the diagnosis have been performed.

Conclusion: To the best of our knowledge, this is the first report of *A. platys* from Bulgaria. This report adds to the overall knowledge of *Anaplasma* spp. in our country and the region of Southeastern Europe.

Keywords: *Anaplasma platys*, Bulgaria, Dog, Molecular evidence.

Introduction

Anaplasma platys (formerly *Ehrlichia platys*), responsible for canine infectious cyclic thrombocytopenia is an example of a tick-borne pathogen with zoonotic potential (Breitschwerdt *et al.*, 2014). These microorganisms belong to the family *Anaplasmataceae*, order *Rickettsiales*, and it is gram-negative, obligate intracellular bacteria whose environmental cycle involves complex interactions between invertebrate vectors and vertebrate hosts (Granick *et al.*, 2023). This bacterium is mainly transmitted by the members of the *Rhipicephalus sanguineus* group (Nava *et al.*, 2018). Ticks serve as essential biological vectors in the spread and reproduction of *A. platys* during different stages of its life cycle (Snellgrove *et al.*, 2020). *Anaplasma platys* infects canine platelets and is the causative agent of the infectious canine cyclic thrombocytopenia (Harvey *et al.*, 1978). It is also important to highlight that this pathogen does not only infect dogs, it also infects other hosts—bactrian camel (Li *et al.*, 2015), cats (Lima *et al.*, 2010), cattle (Dahmani *et al.*, 2015), collared peccary (*Pecari tajacu*) (Rojas-Jaimes and Del Valle-Mendoza, 2023), dromedary camel (Bastos *et al.*, 2015), goats (Ben Said *et al.*, 2017), red fox (Cardoso *et al.*, 2015), roe deer (Remesar *et al.*, 2022), sheep (Ben Said *et al.*, 2017), striped field mouse (*Apodemus agrarius*) (Kim *et al.*, 2006), and water buffalo (Nguyen *et al.*, 2020). Although the role of *A. platys* as a zoonotic agent has not been conclusively proven, it is currently believed that *A. platys* can infect humans (CDC, 2024). There are reports suggesting potential zoonotic transmission of *A. platys* to humans (Maggi *et al.*, 2013; Arraga-Alvarado *et al.*, 2014; Breitschwerdt *et al.*, 2014). This is supported by the fact that *A. platys* DNA was sequenced from two human cases and was identical to that found in their respective dogs (Breitschwerdt *et al.*, 2014).

The infection is typically asymptomatic or mild but can be fatal due to severe thrombocytopenia and subsequent hemorrhage (Diniz and Moura de Aguiar, 2022). Clinical symptoms vary but are mainly animal emaciation, lethargy, anorexia, respiratory distress, fever, increased mucous secretion, purulent discharge from the eyes, uveitis splenomegaly and hyperkeratosis of the muzzle (Sainz *et al.*, 2015; Nimsuphan *et al.*, 2020; Atif *et al.*, 2021).

Anaplasma platys was first discovered in 1978, in the United States (US) by Harvey *et al.* (1978) and his scientific team (Harvey *et al.*, 1978). Since then, it has

***Corresponding Author:** Magdalena Baymakova. Department of Infectious Diseases, Military Medical Academy, Sofia, Bulgaria. Email: *dr.baymakova@gmail.com*

also been identified in Africa, Asia, Australia, Central America, and South America (Eiras *et al.*, 2013; Soares *et al.*, 2017; Atif *et al.*, 2021). In Southern Europe, the presence of *A. platys* has been confirmed in several countries, particularly within the Mediterranean basin and the Balkan peninsula—Albania (Hamel *et al.*, 2016), Bosnia and Herzegovina (Maksimovic *et al.*, 2022), Croatia (Dyachenko *et al.*, 2012), Cyprus (Bouzouraa *et al.*, 2016), Greece (Kontos *et al.*, 1991), Italy (de la Fuente *et al.*, 2006), Portugal (Cardoso *et al.*, 2010), Romania (Andersson *et al.*, 2013), Serbia (Ilic Bozovic *et al.*, 2021), Spain (Perez Perez *et al.*, 2021), and Türkiye (Cetinkaya *et al.*, 2016).

In Bulgaria, several studies have investigated *Anaplasma* spp. in various hosts, including—ticks (Christova *et al.*, 2003; Nader *et al.*, 2018; Stanilov *et al.*, 2023; Polsomboon Nelson *et al.*, 2024), rodents (Christova and Gladnishka, 2005), dogs (Tsachev *et al.*, 2008; Pantchev *et al.*, 2015; Iliev *et al.*, 2020; Manev, 2020; Gospodinova *et al.*, 2024), and horses (Tsachev *et al.*, 2018; Tsachev *et al.*, 2019). While all these articles reported on *Anaplasma phagocytophilum* or *Anaplasma* spp., there has been no research from Bulgaria documenting the presence of *A. platys*. In this study, we present, for the first time, polymerase chain reaction (PCR)-based assays for detecting *A. platys* infection in dogs from Bulgaria.

Case Details

A 10-year-old female dog (Labrador retriever) was admitted for medical examination at the Small Animal Clinic, University Veterinary Hospital, Stara Zagora, Bulgaria, in August, 2022. Owners reported fever, fatigue, weight loss, skin lesions, sores on lower extremities, and tick bites about 2 weeks ago. We did not have access to these ticks.

Written informed consent for participation in the current report was obtained from the dog owner. The present case description was approved by the Local Ethics Committee at Trakia University, Stara Zagora, Bulgaria (FVM-09 / 09 June 2020).

A blood sample was obtained from the *vena cephalica antebrachii externa* using vacuum containers with EDTA. The blood was used for hematological and biochemical tests.

The labrador‘s hematological examinations showed deviations in the values of indicators from the red and white blood count: thrombocytopenia, leukocytosis, erythropenia, neutrophilia, hemoglobinemia, and decreased hematocrit (Table 1). Furthermore, we also observed deviations in the biochemical indicators—increased levels of liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and increased levels of total protein and globulins, without changes in the levels of the albumin fraction.

A blood smear from the buffy coat, stained with commercial kit Hemacolor Stain Set (Sigma-Aldrich, Merck, USA) was made for cytological investigation. On microscopic examination, *A. platys* morulae were not observed in platelets.

The clinical signs and symptoms, along with deviations in hematological and biochemical data, provided justification to perform serological and subsequent molecular genetic assays to confirm infection with bacteria from the family *Anaplasmataceae*.

The blood sample was initially tested for the presence of IgG-specific antibodies against *A. phagocytophilum/A. platys*, and *Borrelia burgdorferi*, as well as for the specific antigen of *Dirofilaria immitis* using a SNAP immunoassay (SNAP 4Dx Plus, IDEXX Laboratories, Inc. USA). The test was positive for antibodies against *Anaplasma* spp. Subsequently, a blood sample was used for molecular analysis for the presence of *A. phagocytophilum*, *A. platys,* and *Ehrlichia canis* DNA.

DNA was extracted from blood samples by the column extraction method using the High Pure PCR Template Preparation Kit (Roche Diagnostic, Mannheim, Germany) following the manufacturer’s instructions. The extracted DNA was stored at −20.0°C until PCR analysis. To minimize the chances of contamination, DNA extraction was performed under a biosafety hood in a dedicated room, using equipment exclusively reserved for DNA extraction.

The gradient PCR was conducted to optimize the annealing temperatures for any PCR (AERIS PCR system, Esco, Singapore). The DNA sample was then tested for the presence of the 16S rRNA gene specific to the family *Anaplasmataceae*, with a conventional PCR as previously described by Gospodinova *et al.* (2024). Further screening was performed, using genus-specific primers (Parola *et al.*, 2000) (Table 2).

A second PCR was performed using the *A. platys* species-specific primers targeting a 678 bp amplicon of the 16S rRNA gene. The *A. platys* specific forward primer (Table 2) was combined with the reverse primer from the first round of PCR (Inokuma *et al.*, 2000). Each PCR run included both a positive and a negative control. DEPC-treated water was used as a template in the negative control, while positive *A. platys* genomic DNA was used for the positive control. The positive *A. platys* genomic DNA was kindly provided by Prof. Handan Cetinkaya from the Department of Parasitology, Faculty of Veterinary Medicine, Istanbul University, Türkiye. The PCR reaction mixture contained 10 µl ready for use buffer mix (KAPA2G Robust Hot Start ReadyMix with dye 2x; Roche Diagnostics, Mannheim, Germany) with 5U Taq DNA polymerase, 3 mM $MgCl_2$, and 1 mM dNTPs, 10 pmol of each primer, 2 µl of DNA template and sterile distilled water to a final volume of 20 µl. The amplification was performed in an Aeris PCR Thermal Cycler (Esco Micro Pte Ltd, Singapore) at the appropriate thermal cycling parameters as follows: initial denaturation for 1 minute at 95.0°C, followed by 40 cycles of 30 seconds

Table 1. Laboratory indicators in 10-year-old female dog (Labrador retriever) from Bulgaria.

Parameters	Results	Reference ranges
Haematological profile		
White blood cells, WBC ($\times10^9$/l)	23.57	6.00–17.00
Neutrophils ($\times10^9$/l)	19.63	3.62–12.30
Neutrophils (%)	83.4	52.0–81.0
Lymphocytes ($\times10^9$/l)	2.93	0.83–4.91
Lymphocytes (%)	12.43	12.0–33.0
Monocytes ($\times10^9$/l)	0.66	0.14–1.97
Monocytes (%)	2.8	2.0–13.0
Eosinophils ($\times10^9$/l)	0.25	0.04–1.62
Eosinophils (%)	1.0	0.5–10.0
Basophils ($\times10^9$/l)	0.1	0.0–0.12
Basophils (%)	0.4	0.0–1.30
Red blood cells, RBC ($\times10^{12}$/l)	3.20	5.50–8.50
Hemoglobin (g/l)	97	120–180
Hematocrit (%)	27.3	37.0–55.0
Mean corpuscular volume, MCV (fl)	66.1	60.0–72.0
Mean corpuscular hemoglobin, MCH (pg)	21.1	19.5–25.5
Mean corpuscular hemoglobin concentration, MCHC (g//l)	355	320–385
Platelet ($\times10^9$/l)	129	200–500
Biochemical profile		
Total protein (g/l)	92.0	54.0–78.0
Albumin (g/l)	28.4	25.0–37.0
Aspartate aminotransferase, AST (U/l)	212	15.0–40.0
Alanine aminotransferase, ALT (U/l)	88.0	15.0–55.0
Urea (mmol/l)	4.5	1.7–7.4
Creatinine (µmol/l)	85	40–120

Table 2. A family-specific primer set for *Anaplasmataceae* and species-specific primer sets for *A. platys*, *E. canis*, and *A. phagocytophilum.*

Pathogens	Target gene	Primer sequence	References
Anaplasmataceae	16S rRNA	EHR 16SD— F- 5`- GGTACCYACAGAAGAAGTCC - 3` EHR 16SR—R - 5`- TAGCACTCATCGTTTACAGC - 3`	Parola *et al.*, 2000
A. platys	16S rRNA	PLATYS F - 5'- GATTTTTGTCGTAGC-TTG -CTATG 3' EHR 16SD—F- 5`- GGTACCYACAGAAGAAGTCC - 3`	Inokuma *et al.*, 2000
E. canis	16S rRNA	CANIS – F 5`-CAATTATTTATAGCCTCTGGCTATAGGA-3` GA1UR—R 5`- GAGTTTGCCGGGACTTCTTCT- 3`	Inokuma *et al.*, 2003
A. phagocytophilum	*ankA*	LA1—F 5`-GAGAGATGCTTATGGTAAGAC-3` LA6 - R 5`- CGTTCAGCCATCATTGTGAC- 3`	Caturegli *et al.*, 2000 Walls *et al.*, 2000

at 94.0°C, 30 seconds at 55.0°C, 30 seconds at 72.0°C,

and a final extension for 5 minutes at 72.0°C.

Subsequently, samples were tested with a third and fourth PCR assay using species-specific primer sets for *E. canis* and *A. phagocytophilum*, as presented in

Table 2 and previously described by Inokuma *et al.* (2003), Caturegli *et al.* (2000), and Walls *et al.* (2000). The same procedure and conditions described by Gospodinova *et al.* (2024) were followed.

Amplicons were detected by electrophoresis on a 1.5% agarose gel stained with ethidium bromide (0.5 mg/ml). A DNA Ladder (by 100 bp, ranging from 100 to 1,000 bp) was included to confirm the expected amplicon size. The results of the PCR amplification were visualised under UV light and documented using the Herolab documentation system and EasyWin32 software (Herolab GmbH Laborgeräte, Germany). To minimize the chances of contamination, all manipulations—DNA extraction, fragment amplification, and agarose gel electrophoresis were performed in separate rooms with equipment used exclusively for each step of molecular genetic investigation.

A 678 bp fragment of 16S rRNA gene sequence characteristic of *A. platys* was only detected in the sample from the presented case report (Fig. 1). However, the differentiation of acute infections with *A. platys* from *A. phagocytophilum* was performed using conventional PCR-based methods with high sensitivity and specificity, the main limitation of our study should be pointed out. We cannot provide the sequence data and sequencing should be considered in future studies of *A. platys*. Additionally, the patient was tested for *E. canis* and *A. phagocytophilum*—the PCR tests were negative.

On the basis of the performed examination, a diagnosis of *A. platys* infection was made. A doxycycline therapy (10 mg/kg) for 28 days was prescribed. In conclusion, we observed a good therapeutic outcome.

Discussion

Tick-borne diseases are of increasing importance in human and veterinary medicine (Vayssier-Taussat *et al.*, 2015). The number of pathogens transmitted by ticks is higher than by any other arthropod. In Europe and other temperate zones, ticks are considered the most serious arthropod zoonotic vectors (Munderloh, 2017; Andersson *et al.*, 2018; Kramer *et al.*, 2020).

This research presents the first molecular evidence of *A. platys* infection in a dog from Bulgaria. *Anaplasma platys* specific conventional PCR revealed a 678 bp fragment of the 16S rRNA gene of *A. platys* in the dog. The use of PCR allows the detection of acute *A. platys* infection earlier than by serology or microscopy (Lara *et al.*, 2020). Molecular tests confirmed the current *A. platys* infection in the dog.

From the hematological characteristics in the present case (increased WBC, neutrophils; decreased RBC, platelet, hemoglobin), only anemia and thrombocytopenia are common, major abnormal clinicopathological findings observed in *A. platys* infected dogs, but the severity of hematological abnormalities can be used to predict disease outcome (Piratae *et al.*, 2019). Thrombocytopenia due to *A. platys* infection is thought to result from platelet destruction by the proliferating pathogen during the initial phase of infection (Dyachenko *et al.*, 2012; Bouzouraa *et al.*, 2016; Ilic Bozovic *et al.*, 2021).

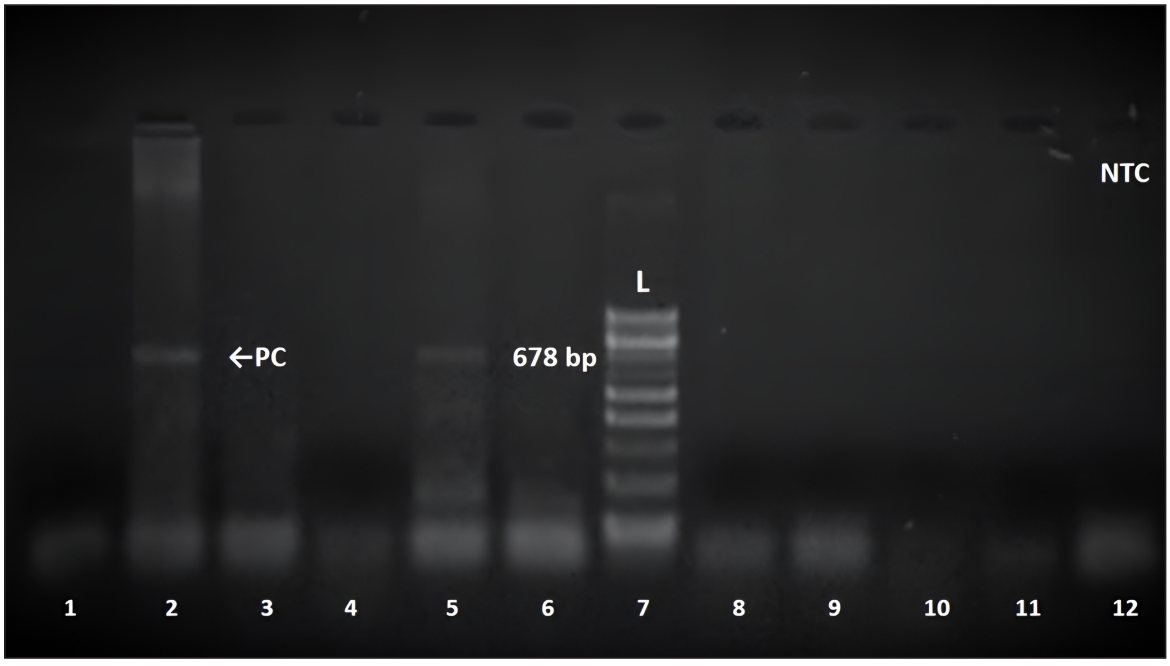

Fig. 1. Amplification of the 16S rRNA gene of *A. platys* with PLATYS F/EHR16SR primers with approximate size 678 bp. Position 2 = positive control (PC); position 12 = negative control (NTC); position 7 = 100 bp DNA ladder; position 5 = positive sample; position 1,3,4,6,8,9,10,11 = negative samples.

Elevated concentration levels of liver transaminases were reported by Bouzouraa *et al.* (2016), who found an increase in the value of alkaline phosphatase (AP) or ALT in 6 of the 10 examined dogs mono- or co-infected with *A. platys* (Bouzouraa *et al.*, 2016). The mechanisms of this phenomenon were not discussed by the authors (Bouzouraa *et al.*, 2016). In our patient, we also found elevated AST and ALT levels.

Commercially available point-of-care serological tests are commonly used in pet clinics as a diagnostic tool. They are used to detect antibodies against rickettsiae of the family *Anaplasmataceae* in clinically suspected dogs (Stillman *et al.*, 2014; Liu *et al.*, 2018). However, they only provide information about pathogen encounters but cannot detect active infection (Nair *et al.*, 2016). Furthermore, SNAP 4Dx Plus Test (IDEXX Laboratories, Inc. USA) detects antibodies against the genus *Anaplasma* without differentiating *A. phagocytophilum* antibodies from those against *A. platys* (Evason *et al.*, 2019). Nucleic acid-based methods allow for the detection and identification of species by using species-specific primers with high sensitivity and specificity (Silvestrini *et al.*, 2023).

Microscopy of stained blood smears, a method also suitable for in-clinic diagnosis, is often unreliable. Direct visualization of *A. platys* in blood smears can be difficult and time-consuming, with low sensitivity, as they are only detectable in the initial phase of the infection (Sykes, 2023). Outcomes are highly dependent on the amount of target cells (degree of thrombocytopenia), levels of bacteremia, and infectious status. For *A. platys,* light microscopic test shows low sensitivity also due to the cyclic nature of thrombocytopenia and the low percentage of infected cells (between 0.5% and 5.0%), recommending to examine between 2,000 and 20,000 platelets (Silaghi *et al.*, 2017). In addition, Lara *et al.* (2020) reported that *A. platys* morula-like structures were occasionally observed in platelets of serologically negative, clinically suspected patients. The authors report that 13 samples showed structures resembling the morula of *A. platys*, with 61% confirmed as positive by PCR (Lara *et al.*, 2020). In this regard, false-negative results from microscopic tests can be detrimental to the patient's health, while false-positive results can lead to unnecessary antibiotic treatment (Lara *et al.*, 2020). The diagnosis of *A. platys*-related diseases in pet clinics can be challenging due to the complex pathogenesis, broad and non-specific clinical symptoms, as well as possible occurrence of co-infection with *A. phagocytophilum* (de Caprariis *et al.*, 2011).

In contrast to serological tests, PCR allows the differentiation of active infections with *A. platys* from those with *A. phagocytophilum*. The conventional PCR protocol, including amplification using a set of *Anaplasmataceae* family-specific primers, followed in a second step by primers specific for a 678 bp fragment of the 16S rRNA gene of *A. platys* should be used (Ferreira *et al.*, 2007). Serological and DNA-based tests have complementary roles in the diagnosis of infectious diseases (Martinescu *et al.*, 2023). Although no data have been published so far, the finding of *A. platys* in Bulgaria is not surprising. This pathogen was discovered in a number of Mediterranean countries (Rymaszewska and Grenda, 2008; Rene-Martellet *et al.*, 2015). Infections of *A. platys* were also confirmed in several countries of the Balkan peninsula—Albania (Hamel *et al.*, 2016), Bosnia and Herzegovina (Maksimovic *et al.*, 2022), Croatia (Dyachenko *et al.*, 2012; Huber *et al.*, 2017), Greece (Kontos *et al.*, 1991), Romania (Andersson *et al.*, 2013), Serbia (Ilic Bozovic *et al.*, 2021), and the European part of Türkiye (Cetinkaya *et al.*, 2016). The vector of this infectious agent *R. sanguineus* is apparently the most prevalent tick species on dogs in Bulgaria (Panayotova-Pencheva *et al.*, 2021).

The present case report and the data by other authors from the Balkan peninsula suggest that *A. platys* is probably a more common pathogen in this part of the world than previously thought. This hypothesis can be confirmed or rejected by conducting large-scale future studies on *A. platys* infection among different animal species from Southeastern Europe.

We identified an *A. platys* infection in a dog in Bulgaria for the first time using PCR assay. The reliable diagnosis provided by molecular methods is imperative for veterinarians in deciding whether to treat a seropositive or clinically suspected dog in disease-endemic areas.

Acknowledgments

The authors thank Prof. Handan Cetinkaya (Istanbul University, Turkey) for their technical support and assistance. Furthermore, the authors thank their families for providing them with the time and support required to complete this report in a timely manner.

Conflict of interest

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Funding

This work was supported and funded by the Bulgarian Ministry of Education and Science in the frames of the Bulgarian National Recovery and Resilience Plan, Component "Innovative Bulgaria", Project No. BG-RRP-2.004- 0006-C02 "Development of research and innovation at Trakia University in service of health and sustainable well-being".

Authors' contributions

KG—conceptualization, methodology, data curation, data interpretation, writing (original draft preparation), and visualization. VP—conceptualization, data collection, and project administration. IS—methodology, data curation, and visualization. LM—methodology, data interpretation, writing (review and editing), and visualization. IT—conceptualization,

data interpretation, writing (review and editing), supervision, and funding acquisition. MB—data interpretation, writing (review and editing), and supervision. All authors have read and agreed to the published version of the manuscript.

Data availability

All data are provided in the manuscript.

References

Andersson, M., Turcitu, M.A., Stefanache, M., Tamba, P., Barbuceanu, F. and Chitimia, L. 2013. First evidence of *Anaplasma platys* and *Hepatozoon canis* co-infection in a dog from Romania—a case report. Ticks Tick Borne Dis. 4, 317–319.

Andersson, M.O., Marga, G., Banu, T., Dobler, G. and Chitimia-Dobler, L. 2018. Tick-borne pathogens in tick species infesting humans in Sibiu County, Central Romania. Parasitol. Res. 117, 1591–1597.

Arraga-Alvarado, C.M., Qurollo, B.A., Parra, O.C., Berrueta, M.A., Hegarty, B.C. and Breitschwerdt, E.B. 2014. Case report: molecular evidence of *Anaplasma platys* infection in two women from Venezuela. Am. J. Trop. Med. Hyg. 91, 1161–1165.

Atif, F.A., Mehnaz, S., Qamar, M.F., Roheen, T., Sajid, M.S., Ehtisham-Ul-Haque, S., Kashif, M. and Ben Said, M. 2021. Epidemiology, diagnosis, and control of canine infectious cyclic thrombocytopenia and granulocytic anaplasmosis: emerging diseases of veterinary and public health significance. Vet. Sci. 8, 312.

Bastos, A.D., Mohammed, O.B., Bennett, N.C., Petevinos, C. and Alagaili, A.N. 2015. Molecular detection of novel *Anaplasmataceae* closely related to *Anaplasma platys* and *Ehrlichia canis* in the dromedary camel (*Camelus dromedarius*). Vet. Microbiol. 179, 310–314.

Ben Said, M., Belkahia, H., El Mabrouk, N., Saidani, M., Alberti, A., Zobba, R., Cherif, A., Mahjoub, T., Bouattour, A. and Messadi, L. 2017. *Anaplasma platys*-like strains in ruminants from Tunisia. Infect. Genet. Evol. 49, 226–233.

Bouzouraa, T., Rene-Martellet, M., Chene, J., Attipa, C., Lebert, I., Chalvet-Monfray, K., Cadore, J.L., Halos, L. and Chabanne, L. 2016. Clinical and laboratory features of canine *Anaplasma platys* infection in 32 naturally infected dogs in the Mediterranean basin. Ticks Tick Borne Dis. 7, 1256–1264.

Breitschwerdt, E.B., Hegarty, B.C., Qurollo, B.A., Saito, T.B., Maggi, R.G., Blanton, L.S. and Bouyer, D.H. 2014. Intravascular persistence of *Anaplasma platys*, *Ehrlichia chaffeensis*, and *Ehrlichia ewingii* DNA in the blood of a dog and two family members. Parasit. Vectors 7, 298.

Cardoso, L., Gilad, M., Cortes, H.C., Nachum-Biala, Y., Lopes, A.P., Vila-Vicosa, M.J., Simoes, M., Rodrigues, P.A. and Baneth, G. 2015. First report of *Anaplasma platys* infection in red foxes (*Vulpes vulpes*) and molecular detection of *Ehrlichia canis* and *Leishmania infantum* in foxes from Portugal. Parasit. Vectors 8, 144.

Cardoso, L., Tuna, J., Vieira, L., Yisaschar-Mekuzas, Y. and Baneth, G. 2010. Molecular detection of *Anaplasma platys* and *Ehrlichia canis* in dogs from the North of Portugal. Vet. J. 183, 232–233.

Caturegli, P., Asanovich, K.M., Walls, J.J., Bakken, J.S., Madigan, J.E., Popov, V.L. and Dumler, J.S. 2000. ankA: An *Ehrlichia phagocytophila* group gene encoding a cytoplasmic protein antigen with ankyrin repeats. Infect. Immun. 68, 5277–5283.

CDC, 2024. Anaplasmosis: epidemiology and statistics (last reported year: 2021). Atlanta, Georgia: CDC. Available via https://www.cdc.gov (Accessed 30 August 2024).

Cetinkaya, H., Matur, E., Akyazi, I., Ekiz, E.E., Aydin, L. and Toparlak, M. 2016. 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. 7, 706–714.

Christova, I. and Gladnishka, T. 2005. Prevalence of infection with *Francisella tularensis*, *Borrelia burgdorferi* sensu lato and *Anaplasma phagocytophilum* in rodents from an endemic focus of tularemia in Bulgaria. Ann. Agric. Environ. Med. 12, 149–152.

Christova, I., Van De Pol, J., Yazar, S., Velo, E. and Schouls, L. 2003. Identification of *Borrelia burgdorferi* sensu lato, *Anaplasma* and *Ehrlichia* species, and spotted fever group Rickettsiae in ticks from Southeastern Europe. Eur. J. Clin. Microbiol. Infect. Dis. 22, 535–542.

Dahmani, M., Davoust, B., Benterki, M.S., Fenollar, F., Raoult, D. and Mediannikov, O. 2015. Development of a new PCR-based assay to detect *Anaplasmataceae* and the first report of *Anaplasma phagocytophilum* and *Anaplasma platys* in cattle from Algeria. Comp. Immunol. Microbiol. Infect. Dis. 39, 39–45.

de Caprariis, D., Dantas-Torres, F., Capelli, G., Mencke, N., Stanneck, D., Breitschwerdt, E.B. and Otranto, D. 2011. Evolution of clinical, haematological and biochemical findings in young dogs naturally infected by vector-borne pathogens. Vet. Microbiol. 149, 206–212.

de la Fuente, J., Torina, A., Naranjo, V., Nicosia, S., Alongi, A., La Mantia, F. and Kocan, K.M. 2006. Molecular characterization of *Anaplasma platys* strains from dogs in Sicily, Italy. BMC Vet. Res. 2, 24.

Diniz, P.P.V.P. and Moura de Aguiar, D. 2022. Ehrlichiosis and Anaplasmosis: an update. Vet. Clin. North Am. Small Anim. Pract. 52, 1225–1266.

Dyachenko, V., Pantchev, N., Balzer, H.J., Meyersen, A. and Straubinger, R.K. 2012. First case of *Anaplasma platys* infection in a dog from Croatia. Parasit. Vectors 5, 49.

Eiras, D.F., Craviotto, M.B., Vezzani, D., Eyal, O. and Baneth, G. 2013. First description of natural *Ehrlichia canis* and *Anaplasma platys* infections in dogs from Argentina. Comp. Immunol. Microbiol. Infect. Dis. 36, 169–173.

Evason, M., Stull, J.W., Pearl, D.L., Peregrine, A.S., Jardine, C., Buch, J.S., Lailer, Z., O'Connor, T., Chandrashekar, R. and Weese, J.S. 2019. Prevalence of *Borrelia burgdorferi*, *Anaplasma* spp., *Ehrlichia* spp. and *Dirofilaria immitis* in Canadian dogs, 2008 to 2015: a repeat cross-sectional study. Parasit. Vectors 12, 64.

Ferreira, R.F., Cerqueira, A.D.M.F., Pereira, A.M., Guimaraes, C.M., de Sa, A.G., Abreu, F.D.S., Massard, C.L. and Almosny, N.R.P. 2007. *Anaplasma platys* diagnosis in dogs: Comparison between morphological and molecular tests. Intern. J. Appl. Res. Vet. Med. 5, 113–119.

Gospodinova, K., Stanilov, I., Miteva, L., Tsachev, I. and Petrov, V. 2024. Molecular detection of *Ehrlichia canis* and *Anaplasma phagocytophilum* in blood samples from dogs in Bulgaria. Bulg. J. Vet. Med. 27, 375–386.

Granick, J., Lappin, M.R., Waner, T., Harrus, S. and Mylonakis, M.E. 2023. Anaplasmosis. 5th ed. In: Sykes JE (ed). Greene's infectious diseases of the dog and cat. Saunders Elsevier, St. Louis, Missouri, USA, 542–554.

Hamel, D., Shukullari, E., Rapti, D., Silaghi, C., Pfister, K. and Rehbein, S. 2016. Parasites and vector-borne pathogens in client-owned dogs in Albania. Blood pathogens and seroprevalences of parasitic and other infectious agents. Parasitol. Res. 115, 489–499.

Harvey, J.W., Simpson, C.F. and Gaskin, J.M. 1978. Cyclic thrombocytopenia induced by a Rickettsia-like agent in dogs. J. Infect. Dis. 137, 182–188.

Huber, D., Reil, I., Duvnjak, S., Jurkovic, D., Lukacevic, D., Pilat, M., Beck, A., Mihaljevic, Z., Vojta, L., Polkinghorne, A. and Beck R. 2017. Molecular detection of *Anaplasma platys*, *Anaplasma phagocytophilum* and *Wolbachia* sp. but not *Ehrlichia canis* in Croatian dogs. Parasitol. Res. 116, 3019–3026.

Ilic Bozovic, A., Radakovic, M., Spariosu, K., Tyrrell, P., Chandrashekar, R., Misic, D. and Kovacevic Filipovic, M. 2021. First confirmed clinical case of *Anaplasma platys* in a dog in Serbia. Acta Vet. 71, 107–112.

Iliev, P.T., Kirkova, Z.T. and Tonev, A.S. 2020. Preliminary study on the prevalence of endoparasite infections and vector-borne diseases in outdoor dogs in Bulgaria. Helminthologia 57, 171–178.

Inokuma, H., Beppu, T., Okuda, M., Shimada, Y. and Sakata, Y. 2003. Epidemiological survey of *Anaplasma platys* and *Ehrlichia canis* using ticks collected from dogs in Japan. Vet. Parasitol. 115, 343–348.

Inokuma, H., Raoult, D. and Brouqui, P. 2000. Detection of *Ehrlichia platys* DNA in brown dog ticks (*Rhipicephalus sanguineus*) in Okinawa Island, Japan. J. Clin. Microbiol. 38, 4219–4221.

Kim, C.M., Yi, Y.H., Yu, D.H., Lee, M.J., Cho, M.R., Desai, A.R., Shringi, S., Klein, T.A., Kim, H.C., Song, J.W., Baek, L.J., Chong, S.T., O'guinn, M.L., Lee, J.S., Lee, I.Y., Park, J.H., Foley, J. and Chae, J.S. 2006. Tick-borne rickettsial pathogens in ticks and small mammals in Korea. Appl. Environ. Microbiol. 72, 5766–5776.

Kontos, V.I., Papadopoulos, O. and French, T.W. 1991. Natural and experimental canine infections with a Greek strain of *Ehrlichia platys*. Vet. Clin. Pathol. 20, 101–105.

Kramer, F., Husken, R., Krudewagen, E.M., Deuster, K., Blagburn, B., Straubinger, R.K., Butler, J., Fingerle, V., Charles, S., Settje, T., Schunack, B. and Stanneck, D. 2020. Prevention of transmission of *Borrelia burgdorferi* sensu lato and *Anaplasma phagocytophilum* by *Ixodes* spp. ticks to dogs treated with the Seresto® collar (imidacloprid 10% + flumethrin 4.5%). Parasitol. Res. 119, 299–315.

Lara, B., Conan, A., Thrall, M.A., Ketzis, J.K., Branford, G.C. and Rajeev, S. 2020. Serologic and molecular diagnosis of *Anaplasma platys* and *Ehrlichia canis* infection in dogs in an endemic region. Pathogens 9, 488.

Li, Y., Yang, J., Chen, Z., Qin, G., Li, Y., Li, Q., Liu, J., Liu, Z., Guan, G., Yin, H., Luo, J. and Zhang, L. 2015. *Anaplasma* infection of bactrian camels (*Camelus bactrianus*) and ticks in Xinjiang, China. Parasit. Vectors 8, 313.

Lima, M.L., Soares, P.T., Ramos, C.A., Araujo, F.R., Ramos, R.A., Souza, I.I., Faustino, M.A. and Alves, L.C. 2010. Molecular detection of *Anaplasma platys* in a naturally-infected cat in Brazil. Braz. J. Microbiol. 41, 381–385.

Liu, J., Eberts, M., Bewsey, H., O'Connor, T.P., Chandrashekar, R. and Breitschwerdt, E.B. 2018. Sensitivity and specificity levels of two rapid assays for antibodies to *Anaplasma* spp. in dogs. J. Vet. Diagn. Invest. 30, 290–293.

Maggi, R.G., Mascarelli, P.E., Havenga, L.N., Naidoo, V. and Breitschwerdt, E.B. 2013. Co-infection with *Anaplasma platys*, *Bartonella henselae* and *Candidatus* Mycoplasma haematoparvum in a veterinarian. Parasit. Vectors 6, 103.

Maksimovic, Z., Dervisevic, M., Zahirovic, A. and Rifatbegovic, M. 2022. Seroprevalence of *Anaplasma* spp. and *Ehrlichia* spp. and molecular detection of *Anaplasma phagocytophilum* and *Anaplasma platys* in stray dogs in Bosnia and Herzegovina. Ticks Tick Borne Dis. 13, 101875.

Manev, I. 2020. Serological survey of vector-borne pathogens in stray dogs from Sofia area, Bulgaria. Vet. Parasitol. Reg. Stud. Reports 21, 100441.

Martinescu, G.V., Ivanescu, L., Stefanescu, R., Andronic, L., Matiut, S., Mindru, R., Solcan, G. and Miron, L. 2023. Strategies for the diagnosis of granulocytic anaplasmosis in two naturally infected dogs. Animals 14, 49.

Munderloh, U. 2017. Comparative studies in tick-borne diseases in animals and humans. Vet. Sci. 4, 32.

Nader, J., Krol, N., Pfeffer, M., Ohlendorf, V., Marklewitz, M., Drosten, C., Junglen, S. and Obiegala, A. 2018. The diversity of tick-borne bacteria and parasites in ticks collected from the Strandja Nature Park in south-eastern Bulgaria. Parasit. Vectors 11, 165.

Nair, A.D., Cheng, C., Ganta, C.K., Sanderson, M.W., Alleman, A.R., Munderloh, U.G. and Ganta, R.R. 2016. Comparative experimental infection study in dogs with *Ehrlichia canis*, *E. chaffeensis*, *Anaplasma platys* and *A. phagocytophilum*. PLoS One 11, e0148239.

Nava, S., Beati, L., Venzal, J.M., Labruna, M.B., Szabo, M.P.J., Petney, T., Saracho-Bottero, M.N., Tarragona, E.L., Dantas-Torres, F., Silva, M.M.S., Mangold, A.J., Guglielmone, A.A. and Estrada-Pena, A. 2018. *Rhipicephalus sanguineus* (Latreille, 1806): neotype designation, morphological re-description of all parasitic stages and molecular characterization. Ticks Tick Borne Dis. 9, 1573–1585.

Nguyen, A.H.L., Tiawsirisup, S. and Kaewthamasorn, M. 2020. Molecular detection and genetic characterization of *Anaplasma marginale* and *Anaplasma platys*-like (*Rickettsiales: Anaplasmataceae*) in water buffalo from eight provinces of Thailand. BMC Vet. Res. 16, 380.

Nimsuphan, B., Prasroedsang, S., Kengradomkij, C., Thayananuphat, A. and Kromkhun, P. 2020. Characterization of serum protein fractions of dogs naturally infected with *Ehrlichia canis* or *Anaplasma platys* associated with uveitis. Trop. Biomed. 37, 551–559.

Panayotova-Pencheva, M.S., Vichova, B., Dakova, V.I. and Salkova, D.S. 2021. Ticks and associated tick-borne pathogens from dogs and red foxes from Bulgaria. Bulg. J. Vet. Med. 24, 608–613.

Pantchev, N., Schnyder, M., Vrhovec, M.G., Schaper, R, and Tsachev, I. 2015. Current surveys of the seroprevalence of *Borrelia burgdorferi*, *Ehrlichia canis*, *Anaplasma phagocytophilum*, *Leishmania infantum*, *Babesia canis*, *Angiostrongylus vasorum* and *Dirofilaria immitis* in dogs in Bulgaria. Parasitol. Res. 114, S117–S130.

Parola, P., Roux, V., Camicas, J.L., Baradji, I., Brouqui, P. and Raoult, D. 2000. Detection of ehrlichiae in African ticks by polymerase chain reaction. Trans. R. Soc. Trop. Med. Hyg. 94, 707–708.

Perez Perez, P., Rodriguez-Escolar, I., Carreton, E., Sanchez Agudo, J.A., Lorenzo-Morales, J., Montoya-Alonso, J.A. and Morchon, R. 2021. Serological survey of canine vector-borne infections in North-Center Spain. Front. Vet. Sci. 8, 784331.

Piratae, S., Senawong, P., Chalermchat, P., Harnarsa, W. and Sae-Chue, B. 2019. Molecular evidence of *Ehrlichia canis* and *Anaplasma platys* and the association of infections with hematological responses in naturally infected dogs in Kalasin, Thailand. Vet. World 12, 131–135.

Polsomboon Nelson, S., Ergunay, K., Bourke, B.P., Reinbold-Wasson, D.D., Caicedo-Quiroga, L., Kirkitadze, G., Chunashvili, T., Tucker, C.L. and Linton, Y.M. 2024. Nanopore-based metagenomics reveal a new *Rickettsia* in Europe. Ticks Tick Borne Dis. 15, 102305.

Remesar, S., Prieto, A., García-Dios, D., Lopez-Lorenzo, G., Martinez-Calabuig, N., Díaz-Cao, J.M., Panadero, R., Lopez, C.M., Fernandez, G., Diez-Banos, P., Morrondo, P. and Díaz, P. 2022. Diversity of *Anaplasma* species and importance of mixed infections in roe deer from Spain. Transbound. Emerg. Dis. 69, e374–e385.

Rene-Martellet, M., Lebert, I., Chene, J., Massot, R., Leon, M., Leal, A., Badavelli, S., Chalvet-Monfray, K., Ducrot, C., Abrial, D., Chabanne, L. and Halos, L. 2015. Diagnosis and incidence risk of clinical canine monocytic ehrlichiosis under field conditions in Southern Europe. Parasit. Vectors 8, 3.

Rojas-Jaimes, J. and Del Valle-Mendoza, J. 2023. Detection of *Bartonella vinsonii*, *Anaplasma platys* and *Bartonella* sp. in *didelphis marsupialis*, *Pecari tajacu* and *Chelonoidis denticulate*: Peru. BMC Res. Notes 16, 150.

Rymaszewska, A. and Grenda, S. 2008. Bacteria of the genus *Anaplasma*—Characteristics of *Anaplasma* and their vectors: a review. Vet. Med. 53, 573–584.

Sainz, A., Roura, X., Miro, G., Estrada-Pena, A., Kohn, B., Harrus, S. and Solano-Gallego, L. 2015. Guideline for veterinary practitioners on canine ehrlichiosis and anaplasmosis in Europe. Parasit. Vectors 8, 75.

Silaghi, C., Santos, A.S., Gomes, J., Christova, I., Matei, I.A., Walder, G., Domingos, A., Bell-Sakyi, L., Sprong, H., von Loewenich, F.D., Oteo, J.A., de la Fuente, J. and Dumler, J.S. 2017. Guidelines for the direct detection of *Anaplasma* spp. in diagnosis and epidemiological studies. Vector Borne Zoonotic Dis. 17, 12–22.

Silvestrini, P., Lloyd-Bradley, B., Glanemann, B., Barker, E.N., Badham, H., Tappin, S., Pascual, M., Haines, A., Mas, A., Roura, X. and Piviani, M. 2023. Clinical presentation, diagnostic investigations, treatment protocols and outcomes of dogs diagnosed with tick-borne diseases living in the United Kingdom: 76 cases (2005-2019). J. Small Anim. Pract. 64, 392–400.

Snellgrove, A.N., Krapiunaya, I., Ford, S.L., Stanley, H.M., Wickson, A.G., Hartzer, K.L. and Levin, M.L. 2020. Vector competence of *Rhipicephalus*

sanguineus sensu stricto for *Anaplasma platys*. Ticks Tick Borne Dis. 11, 101517.

Soares, R., Ramos, C.A., Pedroso, T., Babo-Terra, V., Cleveland, H. and Araujo F. 2017. Molecular survey of *Anaplasma platys* and *Ehrlichia canis* in dogs from Campo Grande, Mato Grosso do Sul, Brazil. An. Acad. Bras. Cienc. 89, 301–306.

Stanilov, I., Blazhev, A. and Miteva, L. 2023. *Anaplasma* and *Ehrlichia* species in *Ixodidae* ticks collected from two regions of Bulgaria. Microorganisms 11, 594.

Stillman, B.A., Monn, M., Liu, J., Thatcher, B., Foster, P., Andrews, B., Little, S., Eberts, M., Breitschwerdt, E.B., Beall, M.J. and Chandrashekar, R. 2014. Performance of a commercially available in-clinic ELISA for detection of antibodies against *Anaplasma phagocytophilum*, *Anaplasma platys*, *Borrelia burgdorferi*, *Ehrlichia canis*, and *Ehrlichia ewingii* and *Dirofilaria immitis* antigen in dogs. J. Am. Vet. Med. Assoc. 245, 80–86.

Sykes, J.E. 2023. Tick-borne diseases. Vet. Clin. North Am. Small Anim. Pract. 53, 141–154.

Tsachev, I., Baymakova, M. and Pantchev, N. 2019. Seroprevalence of *Anaplasma phagocytophilum*, *Ehrlichia* spp. and *Borrelia burgdorferi* infections in horses: first report from Northern Bulgaria—Short communication. Acta Vet. Hung. 67, 197–203.

Tsachev, I., Pantchev, N., Marutsov, P., Petrov, V., Gundasheva, D. and Baymakova, M. 2018. Serological evidence of *Borrelia burgdorferi*, *Anaplasma phagocytophilum* and *Ehrlichia* spp. infections in horses from Southeastern Bulgaria. Vector Borne Zoonotic Dis. 18, 588–594.

Tsachev, I., Petrov, V., Flaming, K. and Brown, C. 2008. First detected case of *Anaplasma phagocytophilum* in a dog in Bulgaria. Revue Med. Vet. 159, 562–564.

Vayssier-Taussat, M., Kazimirova, M., Hubalek, Z., Hornok, S., Farkas, R., Cosson, J.F., Bonnet, S., Vourch, G., Gasqui, P., Mihalca, A.D., Plantard, O., Silaghi, C., Cutler, S. and Rizzoli, A. 2015. Emerging horizons for tick-borne pathogens: from the 'one pathogen-one disease' vision to the pathobiome paradigm. Future Microbiol. 10, 2033–2043.

Walls, J.J., Caturegli, P., Bakken, J.S., Asanovich, K.M. and Dumler, J.S. 2000. Improved sensitivity of PCR for diagnosis of human granulocytic ehrlichiosis using *epank1* genes of *Ehrlichia phagocytophila*-group Ehrlichiae. J. Clin. Microbiol. 38, 354–356.