Natural infection of Anaplasma platys *in dogs from Umbria region (Central Italy)*

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Summary
Anaplasma platys is a tick-borne pathogen causing the Infectious Canine Cyclic Thrombocytopenia. The pathogenesis of this disease is not yet well understood, due to the wide variety of clinico-pathological patterns described worldwide and to the high prevalence of co-infections with other vector-borne pathogens occurring in endemic areas. The present paper reports 3 cases of infection by *A. platys* occurring in dogs native to Central Italy, considered a non-endemic area to date. Infections were initially diagnosed based on clinical data and observation of morulae within platelets and then confirmed by biomolecular techniques. Moreover, two dogs showed an immune-mediated hemolytic anemia, as yet not described in literature in association with *A. platys* infection. The symptoms and the pathological findings observed will be discussed, as well as the importance to include this pathogen in the differential diagnosis of tick-borne diseases even in Central Italy.

Infezione naturale sostenuta da Anaplasma platys *in cani residenti in regione Umbria, Italia Centrale*

Parole chiave
Anaplasma platys,
Anemia emolitica immuno-mediata,
Cane,
Diagnosi,
Italia Centrale,
Trombocitopenia ciclica canina.

Riassunto
Anaplasma platys è un agente patogeno trasmesso da zecche responsabile della trombocitopenia ciclica canina. La patogenesi di questa malattia non è ancora ben conosciuta per la manifestazione di quadri clinico-patologici variabili e la possibile concomitanza del microrganismo con altri agenti patogeni trasmessi da vettori. L'infezione risulta sottostimata. L'articolo descrive tre casi di infezione da *A. platys* in cani stabilmente residenti nella regione Umbria, Italia Centrale, area considerata indenne. Le infezioni sono state diagnosticate sulla base dei rilievi clinici e la presenza, nello striscio ematico, di morule all'interno di piastrine. Successivamente, le infezioni sono state confermate mediante tecniche biomolecolari. In due cani, inoltre, è stata riscontrata contemporaneamente all'infezione anche anemia emolitica immuno-mediata, associazione non riportata in letteratura. L'articolo evidenzia la necessità di dare rilevanza ad *Anaplasma platys* in Italia Centrale e di includere la trombocitopenia ciclica canina nella diagnosi differenziale delle malattie trasmesse da zecche nella specie canina.

Introduction

Anaplasma platys (formerly *Ehrlichia platys*) is a Gram negative, non-mobile, pleomorphic bacterium, belonging to the *Anaplasmataceae* Family, which has been speculated, but not conclusively demonstrated, to be transmitted by *Rhipicephalus sanguineus*, known as the "brown dog tick" (Simpson *et al.* 1991). *Anaplasma platys* is an obligate intracellular microorganism, which appears to parasitise dog platelets exclusively, causing a Canine Vector-Borne Disease (CVBD) named Infectious Canine Cyclic Thrombocytopenia (ICCT) (Cardoso *et al.* 2010) due to the thrombocytopenia that relapses every 7-14 days (Harrus *et al.* 1997).

Since its first identification in Florida (Harvey *et al.* 1978), *A. platys* infection has been reported in several countries around the world, including the United States, China, Thailand, India, Japan, Venezuela, Brazil, Chile, Israel and Australia (Abarca *et al.*, 2007, Abd Rani *et al.* 2011, Brown *et al.* 2001, Cardozo *et al.* 2009, French *et al.* 1983, Hua *et al.* 2000, Inokuma *et al.* 2001, Suksawat *et al.* 2001). With regard to Europe, the presence of *A. platys* has been reported in France, Italy, Spain, Greece, Portugal, and Croatia and in 2 dogs imported in Germany (Beaufils *et al.* 2002, De La Fuente *et al.* 2006, Dyachenko *et al.* 2012, Ferreira *et al.* 2007, Kontos *et al.* 1991).

Despite the increasing interest in Vector Borne Pathogens (VBPs) affecting dogs in Italy (Dantas-Torres *et al.* 2012), the infection by *A. platys* is poorly documented and considered to be sporadic throughout the country. Nonetheless, *A. platys* has been serologically and molecularly detected in dogs from Southern regions (Sicily, Apulia and Abruzzo), mostly in co-infection with other VBPs (16, 17, 35, 37,39). Moreover, the DNA of this pathogen has also been found in *R. sanguineus* ticks by PCR (Sparagano *et al.* 2003).

There is a significant need to improve basic knowledge of the distribution of this VBP affecting autochthonous canine populations and to describe, from a practical standpoint, the potential impact that a single natural *A. platys* infection may have on the patients. This article describes 3 cases of *A. platys* infection occurring in autochthonous dogs from Central Italy.

Materials and methods

History and clinical presentation

Three dogs (dog 1: a 6-year-old female Breton dog; dog 2: a 7-year-old female mongrel dog; dog 3: a 10-year-old female German Shepherd dog) were brought to the Veterinary Teaching Hospital of the University of Perugia (Central Italy) following a few days of depression, anorexia, lethargy, as well as diarrhoea and intermittent nasal and buccal mucosal bleeding (dog 3). The dogs lived outdoors in rural areas of Umbria (region in Central Italy) and, according to the owner's declaration, the dogs had never travelled outside Italy. For each animal, vaccinations and monthly heartworm preventions had been correctly conducted and commercial topical products (spot on formulation of imidacloprid 10 mg/kg and permethrin 50 mg/kg) were used to control tick, flea, mosquito and sand flies. At clinical presentation, all 3 dogs were dehydrated and had pale mucous membranes, enlarged popliteal lymph nodes, splenomegaly, and the dog 3 also had intestinal fluid content at abdominal palpation.

Diagnostic procedures

Venous blood samples were collected for complete blood count (CBC) (Genius Vet hematology analyzer, SEAC-Radim, Calenzano, Italy), serum biochemical profile (Hitachi 904 automatic-analyzer Boehringer Ingelheim, Milan, Italy; Diasys kits, Holzheim, Germany), coagulation panel (Clot 2s, semi-automated coagulation analyzer, SEAC-Radim, Calenzano, Italy), serum protein electrophoresis (Hydrasys LC-SEBIA, Hydragel B1-B2 kits, Florence, Italy), Modified Knott's test for microfilariae detection and 'insaline slide blood autoagglutination test'. Moreover, the direct Coombs' Testwas performed on dogs 2 and 3 in a referral laboratory (Idexx, Vet Med Lab., Giessen, Germany). Blood and buffy coat smears were prepared and stained for microscopic examination using May Grünwald Giemsa (Aerospray slide stainer, Delcon, Wescor, Milan, Italy)

Serum samples were tested by Indirect Immunofluorescence Antibody Tests (IFAT) using commercial antigens (MEGACOR, DIAGNOSTIK Lochauer Straße, Hoerbranz, Austria) to detect IgG and IgM antibodies against the most common VBPs i.e. *Ehrlichia canis* (MegaScreen®, FLUOEHRLICHIA canis), *Anaplasma phagocytophilum* (MegaScreen®, ANAPLASMA ph.), *Leishmania infantum* (MegaScreen®, FLUOLEISH inf.), *Borrelia burgdorferi* (MegaScreen®, FLUOBORRELIA canis), and *Babesia canis* (MegaScreen®, BABESIA c.), following the manufacturer's directions.

Moreover, DNA was extracted from blood samples containing ethylenediaminetetraacetic acid (EDTA) using the QIAamp® DNA Mini Kit (QIAGEN S.p.A., Milan, Italy) according to the manufacturer's instructions, and submitted to PCR assays, previously described in literature: *A. phagocytophilum*, *A. platys* and *E. canis* DNA was searched using a *Ehrlichia genus* set of primers that amplify a 345 bp fragment of the 16S rRNA gene (Martin *et al.* 2005); a specific nested PCR

protocol (Chu *et al.* 2008), amplifying a 226–266 bp fragment (depending on the strains) encompassing the 5S–23S intergenic spacer region of the rRNA, was used for *B. burgdorferi* DNA detection. In order to search for the presence of babesial parasite DNA, PCR was performed using the CRYPTO F (Herwaldt *et al.* 2003) and RLB-R2 (Centeno-Lima *et al.* 2003), which amplifies a fragment of the 18S-rRNA approximately 800 bp in size; *Bartonella* spp. DNA was amplified by conventional PCR targeting a fragment of 16S-23S intergenic transcribed spacer (ITS) as described previously (Diniz *et al.* 2007). PCR assays were run with 50 µl of PCR reaction mixture containing 10 µl of Tfl buffer (Promega, Milan, Italy), 1 µl of 10 mMdNTPs, 1 µl (10 pmol) of each primer, 3 µl of 25 mM MgSO4,1 µl of DNA sample (80 ng/µl), 1 µl of Tfl Polymerase (Promega, Milan, Italy) and 32 µl of nuclease-free water per reaction. Amplification reactions were carried out in a ONE-Personal PCR Thermocycler (EuroClone, Milan, Italy). In order to confirm amplicon identity, all the amplified fragments obtained were purified from excess primers and buffers using the ExoSAP-IT (Affymetrix, Santa Clara, CA, USA) kit and sequenced with a 16-capillary ABI PRISM 3130 Genetic Analyzer (Applied Biosystem, Foster City, CA, USA). The forward and reverse sequences were aligned with ClustalW, validated visually, and consensus sequences were generated using the Applied Biosystems SeqScape Software 1 (version 2.5). The resultant consensus sequences were compared with sequence data in GenBank using the BLAST algorithm[1].

Results

Table I shows the results of the blood analysis. The complete blood count revealed neutrophilic leukocytosis with a left shift, moderate to severe normocytic normochromic anemia, anisocytosis, reticulocytosis, mild to severe thrombocytopenia with increased MPV (Mean Platelet Volume) in all 3 dogs. Lymphopenia was also present in dog 1 and eosinophilia, lymphocytosis and monocytosis in dog 2. The serum biochemical profile, coagulation panel and serum protein electrophoresis revealed an increased concentration of alkaline phosphatase, total and indirect bilirubin, fibrinogen and fibrinogen degradation products (dogs 2 and 3). They also showed an increased concentration of albumin, an elevated albumin to globulin ratio and reduced concentrations of α1, α2 and γ globulins (dog 2), and an increase in prothrombin time, thrombin time and activated partial thromboplastin time (dog 3).

The microscopic examination of the blood and buffy coat smears evidenced the presence of macroplatelets and intracellular basophilic platelet inclusions with 1 or more elements resembling *A. platys* morulae (Figure 1). Spherocytosis was detected in dogs 2 and 3, and therefore the Coombs' Testand the 'insaline slide blood autoagglutination test' returned positive results, suggesting the occurrence of an Immune Mediated Hemolytic Anemia (IMHA). The Modified Knott's test was negative for each dog and all IFAT tests were negative both for IgG and IgM antibodies.

Polymerase chain reaction test for *Anaplasmataceae*-16S rRNA gene was found to be positive in all 3 dogs; the resultant consensus sequences analysis revealed that all the specimens were closely related to *A. platys* (99-100%); the highest identity was obtained with sequences from Croatian (GenBank accession number, JQ396431.1) and Spanish strains (GenBank accession number, AY821826.1). Based on data generated with additional PCR and serology tests, there was no evidence of co-infections with other pathogens, such as *A. phagocytophilum, E. canis, B. canis, B. burgdorferii, Bartonella* spp., *L. infantum, H. canis*, all of which may also occur in Italy and could further influence clinicopathological findings and the clinical course of the disease.

Based on the clinicopathological findings and on the PCR results, all 3 dogs proved to be affected by ICCT and therefore received a compatible fresh blood transfusion and specific therapy with doxycycline (10 mg/kg/day, PO). Dogs 2 and 3 also received prednisone (2mg/kg/day, PO) and ranitidine (2 mg/kg BID, IV) for the IMHA treatment. After 1 week of therapy, dog 1 clinically improved and *A. platys* morulae were no longer detectable by a microscopic evaluation of blood and buffy coat smears; the PCR testing conducted at days 7 and 28 of the therapy were also negative. The dog was still alive 6 months after treatment and has never had recurrence of the infection. On the other hand, dogs 2 and 3 developed worsening signs including lethargy, hypothermia and respiratory failure and died on the 7th and 11th day of therapy, respectively. Post-mortem examination revealed both dogs had not only yellow icteric colouring of the subcutaneous connective tissue and skin, splenomegaly with red pulp hyperplasia and hemosiderosis, pulmonary edema and disseminated pulmonary vascular thrombosis, but also hyperplasia of the erythroid and megakaryocytic lineages in the bone marrow. Dog 3 also showed hepatic nodular hyperplasia with extramedullar diffused hematopoiesis membranous glomerulonephritis and tubular nephrosis. The death of both dogs was attributable to acute cardio-respiratory failure secondary to the development of IMHA and disseminated intravascular coagulation (DIC).

[1] http://blast.ncbi.nlm.nih.gov/ Blast.cgi.

Table I. *Cell Blood Count (CBC), serum biochemical profile, coagulation panel and serum protein electrophoresis results from 3 dogs from Central Italy with* Anaplasma platys *infection.*

	Breton			Mongrel		German Shepherd	Reference intervals
	a	b	c	a	b	a	
CBC							
WBC (x10^9/L)	18.9	5.4	7.0	37.9	42.4	21.5	6.0 - 13.0
Neutrophils (x10^9/L)	16.1	1.3	4.0	18.6	19.8	16.0	3.9 - 12.0
Bands (x10^9/L)	1.1	0.3	0	5.3	6.4	1.7	0 - 0.3
Eosinophils (x10^9/L)	1.0	2.5	1.3	2.7	3.7	0.8	0-1.9
Lymphocytes (x10^9/L)	0.5	1.3	1.6	7.9	8.9	2.2	0.8 - 3.6
Monocytes (x10^9/L)	0.2	0	0.1	3.4	3.6	0.8	0.1 - 1.8
RBC (x10^{12}/L)	1.39	3.32	6.00	1.07	2.74	2.79	5.50- 7.90
Hb (g/dL)	4.0	8.6	12.8	2.6	6.4	7.3	12.0 - 18.0
HCT (%)	11.1	23.4	37.8	7.2	17.9	21.5	37.0 - 55.0
MCV (fL)	77	70	63	67	69	77	60 – 77
MCHC (g/dL)	36.3	36.6	32.5	33.5	36.4	33.9	32.0 - 38.0
Reticulocytes (x10^9/L)	531	151.2	51.0	165.3	127.1	161.4	<150.0
RDW (%)	21.8	18.8	15.4	11.9	15.9	18.6	12.0 - 16.0
PLT (x10^9/L)	163	303	356	164	179	59	200 – 500
MPV (fL)	10.0	9.6	6.5	11.2	11.3	11.9	4.9 - 7.0
BIOCHEMICAL PROFILE							
Urea (mg/dL)	42	41	47	80	86	39	20 – 50
Creatinine (mg/dL)	0.83	0.89	0.90	0.83	0.70	1.0	< 1.8
Total Protein (g/dL)	6.2	6.4	6.6	6.3	6.0	6.0	6 - 7.5
Albumin (g/dL)	2.9	3.3	3.3	3.6	3.2	3.1	2.9 - 3.7
ALT (IU/L)	20	17	21	24	30	75	< 80
ALP (IU/L)	70	74	73	410	670	309	< 100
GGT (IU/L)	6	5	5	3	3	9	< 10
AST (IU/L)	28	22	20	28	34	49	< 60
Total bilirubin	0.22	0.17	0.19	4.55	2.84	3.98	< 0.2
Indirect bilirubin (mg/dL)	0.10	0.09	0.11	3.19	2.00	3.45	< 0.1
Phosphorus (mg/dL)	5	3	3	4	4	5	2.9 - 5.0
COAGULATION PANEL							
Prothrombin Time (sec)	7.3	6.5	8.3	6.2	12.7	72.3	5 – 8
Thrombin Time (sec)	16.4	19.3	28.9	12.2	112.6	99.2	15 – 30
Activated partial thromboplastin time (sec)	17.5	12.4	17.0	15.1	120.1	185.2	9 - 20
Fibrinogen (mg/dL)	53	130	207	980	410	633	100 – 400
FDP (µg/ml)	23	9	3	14	10	6	< 10
SERUM PROTEIN ELECTROPHORESIS							
Albumin (g/dL)	2.7	3.4	3.7	4.8	4.0	3.6	2.2 - 3.8
α1 - globulins (g/dL)	0.2	0.2	0.3	0.1	0.3	0.2	0.2 - 0.5
α2 - globulins (g/dL)	0.5	0.5	0.2	0.1	0.4	0.6	0.5 - 1.0
β1- globulins (g/dL)	0.9	0.6	0.5	0.6	0.3	0.1	0.5 - 1.1
β2 - globulins (g/dL)	0.7	0.5	0.6	0.5	0.5	0.3	0.3 - 0.7
γ - globulins (g/dL)	1.2	1.2	1.3	0.2	0.5	0.9	0.5 - 1.8
Albumin: Globulin ratio	0.79	1.16	1.27	3.40	2.00	1.60	0.80 - 1.65

a = at hospitalization; b = 7 days after treatment; c = 28 days after treatment.
PLT = platelets; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma-glutamyl transferase; FDPs = fibrinogen degradation products.

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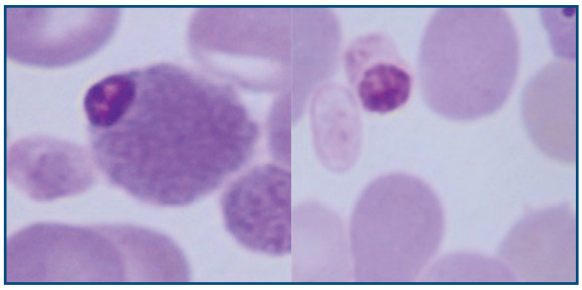

Figure 1. *Anaplasma platys morulae in the platelets (arrow) and giant platelets in blood from 3 dogs with* A. platys *infection in Central Italy.* May Grünwald Giemsa (MGG), X 100 objective.

Discussion

To the authors' knowledge, this report is the first documentation of single natural *A. platys* infections in dogs native of and living permanently in Central Italy.

Among the current available diagnostic methods for detection of *A. platys* infection, the most commonly used include morulae identification in the blood smears, antibody detection and DNA amplification by PCR (Otranto *et al.* 2010). Demonstration of the intra-platelet inclusion bodies of *A. platys* on blood or buffy-coat smears commonly represents the first diagnostic approach in *A. platys* infection, especially during the acute phase of disease. On the basis of the study described in this article, an accurate, light microscopy analysis of the stained blood smears appears to be a reliable method to point the diagnosis in the direction of *A. platys* infection, as it allowed platelet cytoplasmic inclusions resembling *A. platys* morulae to be detected and acute infection to be suspected in all 3 clinical cases. However, a definitive detection of the organisms in blood films may be difficult and cannot be considered a reliable diagnostic method in the chronic phase of the infection due to the cyclic course of bacteremia, the rarely found parasitemia and the fairly frequent presence of a very low number of infected platelets (Harrus *et al.* 1997, Otranto *et al.* 2010). Furthermore, it should be considered that inclusion bodies within platelets may be present and related to platelet activation during inflammation and *E. canis* infection and, thus, misdiagnosed as *A. platys* morulae (Ferreira *et al.* 2007).

Serological methods, such as IFAT, were not taken into account in the diagnostic approach of *A. platys* infection because they are uncommonly applied, due to the difficulty in obtaining *A. platys*-infected platelets to use as antigen (*A. platys* has not yet been cultured) (Lai *et al.* 2011, Martin *et al.* 2005) and the possible false-positive results linked to the serologic cross-reactivity between organisms belonging to the same sero-group (e.g. *A. phagocytophilum*). Recently, a simple qualitative in-clinic Enzyme Linked Immunosorbent Assay (ELISA), the Snap®4Dx Plus (IDEXX Laboratories, Westbrook, ME, USA) was developed in order to identify antibodies against *A. platys*, as well as to detect *Dirofilaria immitis* antigen and antibodies for further VBPs e.g. *A. phagocytophilum, E. canis, E. erwiingi, B. burgdorferi.* This rapid ELISA gained favour among small-animal practitioners due both to its ease of use and its accuracy, however is not able to distinguish between *A. phagocytophilum* and *A. platys*. Moreover, the presence of anti-*A. platys* antibodies does not mean clinical infection, but rather exposure to the infectious agent (Martin *et al.* 2005).

Recently, more specific and sensitive strategies focusing on molecular methods based on PCR approaches were employed (Eddlestone *et al.* 2007, Ferreira *et al.* 2007, Inokuma *et al.* 2002, Lai *et al.* 2011, Martin *et al.* 2005) to enable the diagnosis of active cases of *A. platys* infection, which would otherwise have gone undetected due to low-sensitivity of microscopy and the low-specificity of the serological diagnosis. It has been demonstrated that PCR is positive even in the case of low-level parasitemia (Otranto *et al.* 2010). Several PCR assays were optimized to allow for accurate identification of *A. platys* infection in dogs using different targets (16S rRNA, p44, groESL, gltA). Therefore, the PCR test, confirmed by a sequence analysis of amplicons, is considered to be the most reliable diagnostic test for this pathogen to date (Aguirre *et al.* 2006, De La Fuente *et al.* 2006, Gaunt *et al.* 2010).

Since PCR represents a diagnostic method offering more specific resources to identify and confirm the *A. platys* infections compared to the standard assay, such as microscopic evaluation, a PCR protocol which amplified a target fragment of the 16S rRNA (Martin*et al.* 2010), common to several species of *Anaplasmataceae* pathogens (i.e. *E. canis* and *A. phagocytophilum*) included in the differential diagnosis of the ICCT, was performed for all 3 cases herein. All 3 dogs showed 16S rRNA sequences closely related or identical to other *A. platys* European strains previously reported (Aguirre *et al.* 2006, Dyachenko *et al.* 2012). These results agree with previous reports, in which low, genetic diversity was observed between 16S rRNA sequences of the *A. platys* strains above and beyond their different geographical origins (Aguirre *et al.* 2006, De La Fuente *et al.* 2006, Torina *et al.* 2008, Unver *et al.* 2003), and they confirm that such a genetic target is quite a crucial tool for diagnostic confirmation, even though it does not represent the best choice for further phylogenetic analysis.

The common detection of dogs co-infected by *A. platys* and other infectious agents (*E. canis, Babesia* spp., *A. phagocytophilum,* and *H. canis*), together with the fact that *A. platys* infection develops in a similar way to other tick-borne

infections causing thrombocytopenia, may lead to a misunderstanding of the real pathogenic role of this pathogen (Antognoni *et al.* 2010, De Caprariis *et al.* 2011, Eddlestone *et al.* 2007).

A wide range in the degree of severity of *A. platys* infection has been described in animals from different geographical areas, suggesting that the variety of clinical developments could be strongly related to the origin of the *A. platys* strains. In the United States and in Australia, most reports of experimental and natural *A. platys* infections emphasized the occasional discovery of the parasite and the subclinical or asymptomatic course of disease (Barker *et al.* 2012, Carr *et al.* 2002, Woody and Hopkins 1991), whereas in other countries, especially those of the Mediterranean basin (Israel, France, Greece and Spain), the *A. platys* strains appear to be more virulent (Aguirre *et al.* 2006, Beaufils *et al.* 2002, Cardoso *et al.* 2010, Sparagano *et al.* 2003). At the same time, some findings suggest that the severity and course of disease caused by this agent could be related not only to the strain, but also to conditioning factors (immune status of the animal, stress conditions, breed, age, etc.), as it has also been previously suggested for *E. canis* infection (Aguirre *et al.* 2006, Harrus *et al.* 1997).

At presentation, the 3 dogs herein showed severe, acute disease, which could be explained partly by the occurrence of high virulent *A. platys* strains, as recently reported in cases from Israel, France and Greece (Beaufils at al. 2002, Harrus *et al.* 1997, Kontos *et al.* 1991).

Common haematological abnormalities recorded in the 3 dogs included mild to moderate thrombocytopenia with increased MPV, moderate to severe normocytic normochromic anaemia and evidence of giant platelets on blood films, these findings are consistent with experimental (Baker *et al.* 1988, Gaunt *et al.* 2010) and natural infections (Beaufils *et al.* 2002, De Caprariis *et al.* 2011) of *A. platys*. At the beginning of the disease, the fundamental mechanism of thrombocytopenia and thrombocytopathy, found in both single and co-infections with *A. platys*, may be attributed mainly to the direct action of the parasite (increased platelet consumption, production of inhibitory factors) and, at a later stage, to the hypersplenism or immunologically mediated platelet destruction stimulated by the bacteria (Baker *et al.* 1988, De Caprariis *et al.* 2011, Harvey *et al.* 1978). The immune mediated phenomena and particularly IMHA, have been previously observed in dogs infected by *A. phagocytophilum* and *E. canis* (Bexfield *et al.* 2005, Harrus *et al.* 1999, Mazepa *et al.* 2010), whereas they have never before been described in dogs infected by *A. platys*. Some authors stated that dogs affected with bacterial infection and IMHA are prone to the development of thromboembolic complications with consequent negative course of the disease (Carr *et al.* 2002). The occurrence of the disseminated intravascular coagulation (DIC) observed in dogs 2 and 3 probably exasperated the overall clinical conditions of the animals, bringing to their death in absence of a prompt anticoagulant prophylaxis. In this respect, it is significant that the symptomatology in dog 1, which showed no immune-mediated complication, rapidly regressed after therapy as also observed by other authors (Beaufils *et al.* 2002, Eddlestone *et al.* 2007, Gaunt *et al.* 2010).

In conclusion, the present findings demonstrate that autochthonous infections by *A. platys* can be observed in dogs living in Central Italy and, therefore, *A. platys* infection should be included in the differential diagnosis of CVBDs and IMHA occurring in such areas.

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