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Clinical and laboratory features of canine *Anaplasma platys* infection in 32 naturally infected dogs in the Mediterranean basin

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ABSTRACT

Since the first description of *Anaplasma platys* Infection (ApI), the disease has been sporadically reported worldwide. Whereas it is considered a subclinical disease in the United States or in Australia, severe cases are reported in Europe. Thus far, little information is available regarding the clinical and laboratory findings associated with the disease and the implication of co-infections with other vector-borne pathogens (VBPs) in Southern Europe. The purpose of the study was to describe clinical and laboratory findings in PCR-confirmed naturally infected dogs in the Mediterranean Basin, and to assess the potential impact of co-infections with other VBPs. This is a retrospective analysis of medical records from 32 client-owned dogs diagnosed with ApI using PCR-based assays. Anorexia (62.5%) and weight loss (43.8%) were the major changes, whereas lethargy was less frequent (34.4%). Lymphadenomegaly (43.8%), hyperthermia (40.6%) and hemorrhage (37.5%) were frequent clinical abnormalities, whereas cutaneous signs (31.3%), musculoskeletal disorders (21.9%), splenomegaly (15.6%), dehydration and ocular inflammation (12.5%) were less common. Hematological abnormalities included thrombocytopenia (81.0%), anemia (81.0%), leukocytosis (33.3%) and leucopenia (23.8%). Seven dogs (33.3%) were severely thrombocytopenic. Among the 28 dogs with complete testing, 15 and 13 were mono- and co-infected, respectively. Co-infections included *Ehrlichia canis* (3 dogs), *Leishmania infantum* (4), *Babesia vogeli* (2) and *Hepatozoon canis* (5). One dog was infected concurrently with *Anaplasma platys*, *Ehrlichia canis* and *Babesia vogeli*. The 1-month mortality rate was 23.9% and only 38.1% improved. In the univariate analysis the 15 mono- and the 13 co-infected dogs did not differ regarding the relative frequencies of clinical and laboratory findings. Sequencing and phylogenetic analyses suggested the existence of 2 different groups of strains: one of them might have higher pathogenicity. In all, ApI was associated with a wide variety of non-specific clinical findings. The most common laboratory findings were thrombocytopenia and anemia. Co-infections were frequent but appeared of limited clinical impact. The absence of improvement despite appropriate treatment, high frequency of hemorrhagic disorders, and case fatalities, suggested the existence of pathogenic European strains supported by subsequent molecular analyses.

1. Introduction

Anaplasma platys infection (ApI) is responsible for infectious cyclic thrombocytopenia in dogs. The disease is caused by the infection of canine platelets with *Anaplasma platys*, a Gram-negative, intracellular bacterium that belongs to the *Anaplasmataceae* family (Cardoso et al., 2010). This etiological agent, formerly known as *Ehrlichia platys*, is assumed to be transmitted by the brown dog

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tick, *Rhipicephalus sanguineus* sensus lato (Simpson et al., 1991), and was first identified in 1978 in dogs from Florida (Harvey et al., 1978). Since then, ApI has been reported in many other countries. In Australia and the United States it is described as a subclinical or asymptomatic disease (Barker et al., 2012; Woody and Hopkins, 1991). By contrast, in South America: Argentina (Eiras et al., 2013), Brazil (Lasta et al., 2013) and Chile (Abarka et al., 2007), Southern Europe: Croatia (Dyachenko et al., 2012), France (Beaufils et al., 2002), Greece (Kontos et al., 1991), Italy (Sparagano et al., 2003; De la Fuente et al., 2006; Antognoni et al., 2014), Portugal (Santos et al., 2009; Cardoso et al., 2010), Romania (Andersson et al., 2013), Spain (Sainz et al., 1999; Aguirre et al., 2006; Yabsley et al., 2008), Northern Africa: Algeria (Dahmani et al., 2015), Israel (Mokhtar et al., 2013), and Asia: Turkey (Ulutaş et al., 2007; Çetinkaya et al., 2016) and Malaysia (Mokhtar et al., 2013), ApI seems more severe. Clinical signs include lethargy, fever, anorexia and bleeding disorders. Co-infections with other vector-borne pathogens (VBPs) or intrinsic factors specific to the host (age, breed, physical condition, immune status, or stress) may contribute to a more severe expression of the disease (Sparagano et al., 2003; Aguirre et al., 2006; De la Fuente et al., 2006; Yabsley et al., 2008; Santos et al., 2009; Cardoso et al., 2010; Andersson et al., 2013; Antognoni et al., 2014; Dahmani et al., 2015). Although a few reports are available upon ApI, the hallmarks of the disease have not been clearly documented. Former diagnostic methods consisted mainly of ancillary tests (Harvey et al., 1978; Glaze and Gaunt, 1986; Baker et al., 1987; Kontos et al., 1991; Harrus et al., 1997) whereas current diagnosis relies on the use of more accurate biomolecular tests such as polymerase chain reaction (PCR). The objective of the present study was to describe clinical and laboratory findings in 32 dogs from the Mediterranean basin (Cyprus, Italy, Portugal and Spain) with naturally occurring ApI as confirmed by PCR-based assays, then to assess the potential impact of co-infections with other VBPs on the course of the disease. Short-term prognosis and phylogenetic analysis of *Anaplasma platys* sequences from infected dogs were also provided.

2. Methods

2.1. Selection of dogs

Dogs were included during 2 distinct phases. First, cases were included during a large-scale multicentric epidemiologic survey on canine monocytic ehrlichiosis (CME) conducted in 2011 in 78 veterinary clinics from Italy, Portugal and Spain. These epidemiological findings were recently published (René-Martellet et al., 2015). In this survey, client-owned dogs were initially included when fitting at least 3 clinical and/or biological criteria compatible with ehrlichial disease according to the ACVIM consensus statement (Neer et al., 2002) and summarized in Table 1. Acute phase proteins were not available in any dog and were the only criteria that were not considered for inclusion. The dogs were tested by serology and PCR for the detection of several VBPs, including a specific PCR targeting *A. platys*. Due to the similar clinical presentation, several dogs included for clinical suspicion of CME were finally diagnosed with ApI. An additional set of cases was enrolled in Cyprus between April 2013 and March 2014 using the same inclusion criteria. For the entire study population, dogs were included on a voluntary basis and testing was performed on surplus blood samples. So, no approval by an ethics committee was required for the applied methodology.

Demographic information, signalment (including age, sex, chief complaint), history of previous treatments and vaccinations, administration of chemotherapy or other potential exposure to toxic drugs, and clinical findings were recorded. For each positive dog in Italy, Portugal and Spain, the region of the country was also recorded and indicated on a map. The origin of Cypriot cases was not recorded, owing to the small size of the area studied. When available, a complete blood count (CBC), biochemistry profile, urinalysis and coagulation profiles were recorded.

Table 1
Clinical and biological signs used for dog selection.

Clinical signs	CBC[a] abnormalities	Biochemical abnormalities
Fever	Moderate to severe	Hypoalbuminemia
Depression, lethargy, weakness	Thrombocytopenia	Hyperglobulinemia
Anorexia	Anemia	Increase in
Lymphadenomegaly	Leukopenia	-alanine aminotransferase (ALT)
Splenomegaly	Lymphocytosis	-alkaline phosphatase (ALP)
Hemorrhagic signs (including dermal petechiae and ecchymoses, epistaxis)		-C-reactive protein (CRP)
Pale mucous membranes		-alpha 1-acid glycoprotein (AAG)
Weight loss		
Ophthalmological lesions (including anterior uveitis, chorioretinitis, papilledema, retinal hemorrhage, retinal perivascular infiltrates, bullous retinal detachment)		
Neurological disorders		

[a] Complete blood count.

For all dogs selected, veterinarians were asked to perform a SNAP 4Dx® test (Idexx, Westbrook, USA) on whole blood or sera to detect antibodies directed against *Anaplasma* species, *Ehrlichia canis*, *Borrelia burgdorferi* and antigens of *Dirofilaria immitis*. SNAP Leish® test (Idexx, Westbrook, USA) was used on samples as well to allow for detection of antibodies directed against *Leishmania infantum* or *Leishmania donovani*. Blood was then stored at 5 ± 1 °C until PCR analysis.

Dogs whose clinical, biological, serological and PCR results indicated CME or ApI were treated with doxycycline at 10 mg/kg q24h PO for 28 days. Additional therapies included imidocarb dipropionate (5–8 mg/kg IM every 14 days) for dogs diagnosed with babesiosis or hepatozoonosis, and allopurinol (10 mg/kg q12h PO) for those with leishmaniasis. Meglumine antimoniate was not administered in any of the dogs diagnosed with leishmaniasis. Because of the retrospective design, response to treatment was only assessed on a 1-month follow-up recheck whenever possible and early response was not recorded. The recheck consisted either in a second appointment after the completion of treatment or phone calls.

Only dogs with ApI, as confirmed by specific PCR were used for subsequent analysis of clinical repercussion of ApI. Medical data for all these dogs has not been reported previously elsewhere.

2.2. Molecular detection of VBPs

For the cases enrolled in Cyprus, DNA extraction and initial PCR analysis were done on surplus blood EDTA samples at the Molecular Microbiology Laboratory of Langford Veterinary Services (University of Bristol, UK) and blood DNA samples were analyzed at the Laboratory of Parasitology of VetAgro Sup (Lyon, France) where all the cases enrolled in Italy, Portugal and Spain were also tested as previously described (René-Martellet et al., 2015). Shortly, quality of all the samples was assessed by PCR amplification of mitosin gene specific for dogs to ensure presence of dog DNA and the absence of PCR inhibition (Criado-Fornelio et al., 2003). Two multiplex PCR amplifications were performed to detect DNA of *E. canis*, *A. platys*, *A. phagocytophilum*, *Babesia* and *Thei-*