The Veterinary Journal 246 (2019) 45–53

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Review

An update on the treatment of canine monocytic ehrlichiosis (*Ehrlichia canis*)

Mathios E. Mylonakis[a,*], Shimon Harrus[b], Edward B. Breitschwerdt[c]

[a] Companion Animal Clinic, School of Veterinary Medicine, Aristotle University of Thessaloniki, 11 Stavrou Voutyra st., Thessaloniki 54627, Greece
[b] Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel
[c] Intracellular Pathogens Research Laboratory, Comparative Medicine Institute, College of Veterinary Medicine, North Carolina State University (NCSU-CVM),1060 William Moore Drive, Raleigh, NC 27607 USA

ARTICLE INFO

Article history:
Accepted 30 January 2019

Keywords:
Aplastic pancytopenia
Canine monocytic ehrlichiosis
Dog
Ehrlichia canis
Treatment

ABSTRACT

Canine monocytic ehrlichiosis (CME), caused by *Ehrlichia canis*, a gram-negative, obligate intracellular bacterium, is a tick-borne disease of worldwide distribution. Experimentally, the course of *E. canis* infection can be sequentially divided into acute, subclinical and chronic phases, although distinction of these phases is challenging in the clinical setting. Spontaneous clinical recovery of acutely infected dogs is common; however, dogs at this stage require medical treatment in order to hasten their clinical recovery, and to prevent clinical exacerbation or death. An unpredictable proportion of subclinically infected dogs will eventually develop the chronic, severe form of ehrlichiosis, characterized by aplastic pancytopenia and high mortality. The aims of antimicrobial treatment in CME include the achievement of clinical remission, resolution of the clinicopathologic abnormalities, and eradication of the infection, although the latter is not always feasible or diagnostically confirmable. Treatment of dogs with aplastic pancytopenia should be undertaken with the clear understanding that medical management will require long-term care, will be expensive, and may eventually prove ineffective. This manuscript reviews the current state of knowledge regarding treatment of ehrlichiosis, caused by *E. canis* infection in dogs, provides expert opinion guidelines for the management of the CME-associated aplastic pancytopenia, and outlines methods for evaluation of treatment outcomes.

Introduction

Ehrlichia canis is recognized as the principal cause of canine monocytic ehrlichiosis (CME) (Neer et al., 2002; Sainz et al., 2015). The disease is endemic in every continent except Australia (Sykes, 2014). In North America, and other less well characterized regions of the world, dogs can be infected with other *Ehrlichia* species, particularly *Ehrlichia chaffeensis* (Neer et al., 2002). *E. canis* bacteria are transmitted transstadially and intrastadially by the brown dog tick (*Rhipicephalus sanguineous*) (Bremer et al., 2005). Experimental transmission has also been accomplished with *Dermacentor variabilis* ticks (Johnson et al., 1998), but the biological role of this vector in nature is obscure.

In the experimental setting, after an incubation period of 8–20 days following blood transfusion or tick attachment, the course of *E. canis* infection can be sequentially divided into acute (2–4 weeks duration), subclinical (months to years) and chronic phases, although the distinction among these phases is not straightforward in dogs with naturally-occurring disease (Harrus et al., 2012). Fever (or hypothermia in profoundly pancytopenic dogs), depression or lethargy, anorexia, generalized lymphadenomegaly, splenomegaly, mucosal pallor, bleeding tendency and ocular abnormalities (e.g. anterior or posterior uveitis) are typical clinical manifestations in naturally-occurring CME. Dogs with acute disease are likely to be infested with ticks. Ulcerative stomatitis and necrotic glossitis, hind limb and/or scrotal edema, and central nervous system signs such as seizures, ataxia, vestibular dysfunction and cervical pain, have been more frequently reported in the chronic disease. Bleeding diathesis may occur in both the acute and chronic phases of CME, but is more common and severe in the chronic phase, and is manifested as cutaneous and mucosal petechiae and ecchymoses, epistaxis, hematuria, melena and prolonged bleeding from venipuncture sites. In the subclinical phase of CME, clinical manifestations and/or hematological abnormalities may be absent, or mild (e.g. splenomegaly, intermittent fever, thrombocytopenia, anemia) (Codner and Farris-Smith, 1986; Waner et al., 1997; Harrus et al., 1997: Harrus et al., 1998a; Mylonakis et al., 2011; Harrus & Waner,

* Corresponding author.
E-mail address: mmylonak@vet.auth.gr (M.E. Mylonakis).

https://doi.org/10.1016/j.tvjl.2019.01.015
1090-0233/

2011). Clinicians should consider concurrent infection with other vector-borne pathogens or co-morbidities in dogs with unusual or atypical disease manifestations.

Spontaneous clinical recovery of acutely infected dogs is common; however, dogs at this stage require medical treatment in order to hasten their clinical recovery, to prevent clinical exacerbation or death. The extent to which naturally infected dogs immunologically eliminate the infection or remain subclinically infected following tick transmission remains unclear. Clearly however, during the acute or the subclinical phase of CME, immunocompetent dogs may eliminate the infection, or alternatively, diminish ehrlichiaemia and tissue bacterial loads to levels not amenable to molecular detection by polymerase chain reaction (PCR) amplification (Breitschwerdt et al., 1998; Eddlestone et al., 2007; Theodorou et al., 2013). Also, an unpredictable proportion of *E. canis*-infected dogs will progress to the chronic illness phase, characterized by variable clinical presentations, of which a subset has severe bone marrow (BM) aplasia, profound peripheral blood pancytopenia, and high mortality due to septicemia and/or severe bleeding (Harrus et al., 1997; Mylonakis et al., 2004; Shipov et al., 2008; Mylonakis et al., 2011). Occasionally, myelosuppression may develop without any premonitory signs indicative of the acute and subclinical phases of CME (Mylonakis et al., 2004). Therefore, the terms "non-myelosuppressive" and "myelosuppressive" CME, may better reflect disease severity in a clinical context, regardless of illness onset or the presumed phase of CME. Significantly, in some *E. canis*-endemic regions, such as Greece, Israel and Brazil (Shipov et al., 2008; Girardi et al., 2017; Frezoulis et al., 2017) and anecdotally in some other areas such as Turkey and South East Asia (S. Harrus, personal communication), CME may be one of the major causes of life-threatening pancytopenia in dogs.

This manuscript comprehensively reviews the current state of knowledge pertaining to antibiotic treatment of *E. canis* infection in dogs, provides guidelines for the supportive care for dogs with aplastic pancytopenia, and outlines the methods for the evaluation of treatment response.

Antibiotic treatment for *E. canis* infection

Dogs with clinical and clinicopathologic abnormalities consistent with CME, in conjunction with seroreactivity to *E. canis* and/or molecular or cytological evidence of *E. canis* infection, should receive antimicrobial therapy. The decision whether to treat or not treat a clinically healthy, *E. canis*-seropositive dog that lacks hematological abnormalities remains controversial, especially in endemic areas where exposure is highly prevalent (Neer et al. 2002; Sykes, 2014). To facilitate judicious use of antibiotics and to avoid unnecessarily inducing antimicrobial resistance, it seems advisable to follow these dogs clinically, hematologically and serologically (indirect fluorescent antibody titers), at least bi-annually, rather than administer an antibiotic to a dog that may have immunologically cleared the infection (Neer et al., 2002). Demonstration at any phase of infection of *E. canis*-specific DNA from the blood or other tissues (e.g. BM or splenic aspirates) or a 4-fold increase in antibody titers should be considered as an active infection and justifies antimicrobial treatment regardless of the dog's clinical status because progression or non-progression to disease cannot be predicted. Treatment would also be recommended for clinically healthy, seropositive, PCR-negative dogs with compatible clinicopathologic abnormalities (e.g. anemia, thrombocytopenia, hyperglobulinemia) that lack evidence of other inciting causes for these findings.

The aims of CME antimicrobial treatment include achievement of clinical remission, resolution of clinicopathologic abnormalities, and eradication of the organism. However, in dogs admitted with advanced aplastic pancytopenia (myelosuppressive CME), treatment should be undertaken with the owner's clear understanding that medical management will require long-term care, will be expensive, and may eventually prove ineffective (Mylonakis et al., 2010a). Unlike acute and subclinical *E. canis* infections, for which some evidence-based treatment information is available, substantially less is known regarding optimal treatment recommendations for myelosuppressive CME. This is partially due to the lack of a suitable experimental model for the induction and study of this disease phase (Neer et al., 2002; Mylonakis et al., 2010a; Harrus et al., 2012; Sainz et al., 2015).

Tetracyclines

Historically, tetracyclines were the first-line antibiotics for the treatment of CME (Amyx et al., 1971; Buhles et al., 1974). They are broad-spectrum antibacterial agents that act by inhibiting attachment of aminoacyl-tRNA to the bacterial ribosome during protein synthesis (Chopra and Roberts, 2001). However, only doxycycline, a semi-synthetic tetracycline, has been critically evaluated in vitro or in association with naturally or experimentally-induced *E. canis* infections (Harrus et al., 2012; Sykes, 2014) (Table 1). In vitro studies indicate that doxycycline is very active against the monocytotropic *Ehrlichia* species (i.e. *E. canis* and *E. chaffeensis*), requiring a very low minimum inhibition concentration (MIC) (0.03 μg/ml) (Brouqui and Raoult, 1992; Brouqui and Raoult, 1993; Branger et al., 2004). Importantly, in vitro sensitivity data may not consistently correlate with clinical response, particularly in the context of intracellular bacteria with potential distribution throughout the vasculature (Reardon and Pierce, 1981; Branger et al., 2004; Stratton, 2006; Theodorou et al., 2013).

Several dosing regimens have been used to treat dogs experimentally or naturally-infected with *E. canis* (Table 1). Despite a substantial number of published studies, determining an optimal duration of doxycycline administration for dogs in the various CME phases, which are often not determinable in the clinical setting, remains challenging. A consensus statement from the infectious disease study group of the American College of Veterinary Internal Medicine suggested 10 mg/kg, per os (PO), once daily, for 4 weeks, regardless of the disease phase (Neer et al., 2002). Shorter durations of doxycycline administration have generated mixed outcome results. In acutely infected dogs, doxycycline at 5 mg/kg, PO, twice daily, for 2 or 4 weeks (Breitschwerdt et al., 1998; Shropshire et al., 2018), or at 10 mg/kg, PO, once daily, for 16 days (Harrus et al., 2004), was effective in eliminating the infection, as inferred by the post-treatment application of PCR in peripheral blood and/or splenic aspirates, or culture isolation. However, in other studies, doxycycline given at 10 mg/kg, PO, once daily, for 3–4 weeks, failed to clear *E. canis* (dogs remained PCR positive) in several acutely infected dogs, despite consistently achieving clinical and hematologic recovery (McClure et al., 2010; Fourie et al., 2015). For subclinical CME, doxycycline at 5 mg/kg, PO, twice daily, administered for 3–4 weeks or 10 days (Neer et al., 1999; Eddlestone et al., 2007) or 10 mg/kg, PO, once daily, for 4 weeks (Schaefer et al., 2008; Gaunt et al., 2010; Jenkins et al., 2018) cleared the infection in all experimentally or naturally-infected dogs. In contrast, despite complete resolution of hematological abnormalities in the majority of dogs studied, doxycycline treatment durations of 1-week (Iqbal and Rikihisa 1994), 2-weeks (Schaefer et al., 2007), 4-weeks (McClure et al., 2010) and 6-weeks (Harrus et al., 1998b) at 10 mg/kg, PO, once daily, failed to eradicate *E. canis* infection in 25–100% of the treated dogs, as demonstrated by PCR or xenodiagnosis. Moreover, in one retrospective clinical study, doxycycline (dosing regimens and clinical phase of the disease upon initiation of treatment were not specified) administered for up to 24 consecutive months was apparently ineffective in clearing the infection in approximately

Table 1
Antimicrobial drugs that have been systematically evaluated for the treatment of experimental or naturally-occurring *Ehrlichia canis* infection in dogs.

Drug tested (reference)	Regimen dose, route, frequency, duration	Dogs & infection phase (control group)	Post-treatment outcome	Comments
Doxycycline Iqbal and Rikihisa, (1994)	10 mg/kg, PO, SID, 1w	*n* = 5, subclinical (no)	Clinical signs: NR CBC: recovery in 4/5 dogs IgG titers: reduced >4-fold in 2/5 dogs (these were culture negative) Culture: positive in 3/5 dogs (B, Sp, Ki, LN)	Observation period post-Tx: 8w SOI: cell culture inoculum Other: All culture positive dogs had a normal PLT count post-Tx
Doxycycline Breitschwerdt et al. (1998)	5 mg/kg, PO, BID, 2w	*n* = 8, acute (yes, *n* = 4)	Clinical signs: NR CBC: recovery in all dogs IgG titers: reduced ≥2-fold in 7/8 dogs PCR/culture: negative in all dogs (B)	Observation period post-Tx: 6.5w SOI: infected blood Other: 3/4 control dogs and 6/8 re-infected dogs eliminated the infection spontaneously
Doxycycline Harrus et al., 1998b	10 mg/kg, PO, SID, 6w	*n* = 4, subclinical (no)	Clinical signs: NR CBC: recovery in all dogs IgG titers: not significantly changed PCR: negative in 3/4 dogs (B, BM, Sp)	Observation period post-Tx: 0w SOI: infected blood Other: One infected dog had a normal PLT count post-Tx
Doxycycline Harrus et al. (2004)	10 mg/kg, PO, SID, 60d	*n* = 5, acute (no)	Clinical signs: recovery in all dogs CBC: recovery in all dogs IgG titers: not significantly changed PCR: negative in all dogs (B, Sp)	Observation period post-Tx: 0w SOI: infected blood
Doxycycline Eddlestone et al. (2007)	5 mg/kg, PO, BID, 3-4w	*n* = 9, subclinical (yes, *n* = 5)	Clinical signs: NR CBC: recovery in all dogs IgG titers: significantly reduced PCR: negative in all dogs (B, BM, Sp, Li, Lu)	Observation period post-Tx: 1-2w SOI: cell culture inoculum Other: 2/5 control dogs eliminated infection spontaneously
Doxycycline Schaefer et al. (2007)	10 mg/kg, PO, SID, 2w	*n* = 4, subclinical (no)	Clinical signs: NR CBC: NR IgG titers: NR PCR: 4/4 dogs positive (B), 4/4 dogs positive (xenodiagnosis)	Observation period post-Tx: 10d SOI: infected ticks
Doxycycline Gaunt et al. (2010)	10 mg/kg, PO, SID, 4w	*n* = 12, subclinical (yes, *n* = 12) Nine dogs were concurrently or sequentially infected by *Anaplasma platys*	Clinical signs: NR CBC: recovery in all dogs IgG titers: not significantly changed PCR: negative in all dogs (B, BM, LN)	Observation period post-Tx: 8m SOI: cell culture inoculum Other: All control dogs remained PCR-positive
Doxycycline McClure et al. (2010)	10 mg/kg, PO, SID, 4w	*n* = 4, acute; *n* = 6, subclinical (no)	Clinical signs: acute infection: recovery; subclinical infection: NR CBC: recovery in all dogs IgG titers: NR PCR: acute: 0/4 dogs positive (B), 4/4 dogs positive (xenodiagnosis) subclinical: 3/6 dogs positive (B), 6/6 dogs positive (xenodiagnosis)	Observation period post-Tx: 2w SOI: infected blood Other: Rifampicin (15 mg/kg, PO, BID, 1w) cleared the infection in 1/2 subclinically infected dogs (previous doxycycline failure)
Doxycycline Fourie et al. (2015)	10 mg/kg, PO, SID, 3w	*n* = 6, acute (no)	Clinical signs: recovery for all dogs CBC: recovery in all dogs IgG titers: not significantly changed PCR: all dogs negative on the completion of the Tx; 5/6 dogs positive 4–6 w after completion of Tx (B)	Observation period post-Tx: 6m SOI: infected ticks
Doxycycline vs. rifampicin Schaefer et al. (2008)	10 mg/kg, PO, SID, 4w (doxycycline, *n* = 2); 15 mg/kg, PO, BID, 1w (rifampicin, *n* = 2)	*n* = 4 (*n* = 3 subclinical, *n* = 1 acute), (no)	Clinical signs: NR CBC: recovery in all dogs IgG titers: NR PCR: negative in all dogs (B)	Observation period post-Tx: NR SOI: infected blood
Minocycline vs. doxycycline Jenkins et al. (2018)	10 mg/kg, PO, BID, 4w (minocycline, *n* = 5); 10 mg/kg, PO, SID, 4w (doxycycline, *n* = 5)	*n* = 10, subclinical (no)	Clinical signs: NR CBC: recovery in 3/5 dogs after both minocycline and doxycycline IgG titers: NR PCR: negative in all dogs after both minocycline and doxycycline (B)	Observation period post-Tx: 1w SOI: natural infection (owned dogs)
Doxycycline Shropshire et al. (2018)	5 mg/kg, PO, BID, 4w	*n* = 4, acute (no)	Clinical signs: recovery in all dogs CBC: recovery in all dogs IgG titers: not significantly changed PCR: negative in all dogs (B)	Observation period post-Tx: 1w SOI: infected blood
Rifampicin Theodorou et al. (2013)	10 mg/kg, PO, SID, 3w	*n* = 5, acute (yes, *n* = 9)	Clinical signs: NR CBC: recovery in 4/5 dogs IgG titers: not significantly changed PCR: 3/5 treated dogs positive (B, BM, Sp)	Observation period post-Tx: 8w SOI: infected blood Other: 6/9 control dogs remained PCR positive, 3/9 control dogs eliminated infection spontaneously; 7 PCR-positive dogs (post-Tx or controls) had normal PLT counts

Table 1 (*Continued*)

Drug tested (reference)	Regimen dose, route, frequency, duration	Dogs & infection phase (control group)	Post-treatment outcome	Comments
Enrofloxacin vs. doxycycline Neer et al. (1999)	5 mg/kg, PO, BID, 3w (enrofloxacin, *n* = 7); 10 mg/kg, PO, BID, 3w (enrofloxacin, *n* = 5); 5 mg/kg, PO, BID, 3w (doxycycline, *n* = 2); 5 mg/kg, PO, BID, 10d (doxycycline, *n* = 11)	*n* = 13, acute (no)	Clinical signs: NR CBC: recovery in 2/12 dogs (enrofloxacin); recovery in all dogs (doxycycline) IgG titers: not significantly changed PCR: 5/5 tested dogs positive after enrofloxacin (B); 1/5 tested dogs positive after doxycycline (B) Culture: 11/12 dogs positive after enrofloxacin; negative in all dogs after doxycycline (B)	Observation period post-Tx: 0-8w SOI: cell culture inoculum Other: Two culture-positive dogs had normal PLT counts (after enrofloxacin)
Imidocarb dipropionate Eddlestone et al. (2006)	6.6 mg/kg, IM, twice 2 w apart	*n* = 10, acute (yes, *n* = 5)	Clinical signs: NR CBC: recovery in 2/10 dogs IgG titers: not significantly changed PCR: positive in all treated dogs (B)	Observation period post-Tx: 7w SOI: cell culture inoculum Other: 2/10 of the treated and 3/5 untreated dogs had normal PLT post-Tx; PCR-positive in all control dogs

PO, per os; IM, intramuscularly; SID, once daily; BID, twice daily; d, day; w, week; m, month; NR, not reported; Tx, treatment; CBC, complete blood count; PCR, polymerase chain reaction; SOI, Source of infection; PLT, platelets; B, blood; Sp, spleen; K, kidney; LN, lymph node; BM, bone marrow; Li, liver; Lu, lung.

50% of naturally infected dogs, as demonstrated by PCR (Wen et al., 1997). In the latter study, published two decades ago, reinfection of dogs post-treatment could not be ruled out and the specificity of the reported PCR results was not confirmed by DNA sequencing or by amplification of second *E. canis* gene target. Therefore, the conclusions established in that study should be interpreted cautiously.

Although anecdotal, minocycline has been used extensively as an effective treatment of CME; however, published efficacy was only very recently reported in a small number of dogs (Jenkins et al., 2018). In the Jenkins study, minocycline cleared the infection in 5/5 naturally infected dogs, demonstrating similar efficacy compared with doxycycline (Table 1). Further studies are warranted to more comprehensively evaluate the efficacy of minocycline for treatment of CME.

Collectively, studies of naturally and experimentally infected dogs suggest that doxycycline induces clinical remission and hematological normalization in dogs with acute or subclinical *E. canis* infections, without consistently eliminating the infection. In addition, suboptimal efficacy does not appear to be clearly phase-dependent, suggesting the potential involvement of concurrent infectious or non-infectious illnesses or an inadequate host immune response in a subset of infected dogs (Table 1). Other potential factors that may account for the inconsistency in the clearance of the infection among the different studies may include the following: the different doxycycline dosing regimens; the host's immunological status; the sensitivity of assays to detect infection; and the samples used for testing.

Although the consensus daily dose of doxycycline is 10 mg/kg, the total dose was administered once daily or divided twice daily among studies. Concurrently, the duration of treatment has ranged from 1 to at least 4 weeks (Table 1). Doxycycline, as a time-dependent drug, is typically administered twice daily. However, doxycycline's low MIC for *E. canis* makes it unlikely that the drug level will drop below the MIC threshold, regardless of the dose interval (i.e. once or twice daily) (Riond et al., 1990; Ruiz et al., 2015). In dogs treated with doxycycline once or twice daily, the success/failure ratio in eradicating *E. canis* infection has been found to be 4/5 and 3/1, respectively, in a series of studies (Table 1). With regard to the duration of treatment, failures in eliminating the infection have been reported regardless of the time span of the doxycycline treatment (i.e. 1-week, 2-week, 3–4 weeks, or >4 weeks) (Table 1). The most favourable success/failure ratio (seven studies with infection clearance compared to two studies with infection clearance failure) has been reported in association with treatment durations of 3–4 weeks, when compared with shorter treatment durations (Table 1). These results lend support to the consensus doxycycline dosing recommendation of a 3–4 week treatment duration for CME (Neer et al., 1999; Neer et al., 2002; Harrus et al., 2004; Eddlestone et al., 2007; Schaefer et al., 2008; Gaunt et al., 2010; McClure et al., 2010; Fourie et al., 2015; Jenkins et al., 2018; Shropshire et al., 2018). Currently, there is insufficient information to endorse a shorter treatment duration (e.g. 2-weeks) for acutely infected dogs, as compared to a longer duration of doxycycline treatment (e.g. ≥3–4 weeks) for chronically infected dogs.

When assessing treatment outcomes from experimental studies, it is important to recognize that most of them involved beagles, German shepherds or mixed breed dogs. Immunologically, studies involving other dog breeds could generate different results (Nyindo et al., 1980). Additional studies are clearly needed to assess duration of antibiotic administration, particularly in the clinical setting where there is substantially greater variation in age, breed and sex.

The variable sensitivity among the *Ehrlichia* infection detection methods currently in use for the post-treatment confirmation of *E. canis* clearance (e.g. PCR, culture or xenodiagnosis) will influence perceived success. For example, in an experimental study (McClure et al., 2010), the efficacy of the 4-week, once daily consensus doxycycline regimen (Neer et al., 2002) was investigated during acute, subclinical and chronic (non-myelosuppressive) CME phases. Despite clinical and hematological recovery and negative blood PCR results in the majority of treated dogs, acquisition-fed *R. sanguineus* ticks became *E. canis* PCR-positive for all dogs. Thus, using xenodiagnosis, rather than clinical response, resolution of hematological abnormalities or blood PCR testing, treatment failures were documented during the acute, subclinical and chronic phases of CME. In support of the fact that *E. canis* organisms persisted post-treatment, most naïve dogs inoculated with pooled blood from the doxycycline-treated dogs became PCR-positive (McClure et al., 2010). Based upon this one study, xenodiagnosis was more sensitive than PCR testing when

determining whether *E. canis* infection was eradicated following doxycycline treatment. However, xenodiagnosis is not practical in the clinical setting, where negative PCR results may be overestimating the rate of *E. canis* clearance and should be interpreted as "no *Ehrlichia* DNA was detected in the sample" rather than "no *Ehrlichia* DNA exists in the sample and/or in the dog".

The testing of different tissue specimens (e.g. blood vs splenic aspirates vs BM aspirates), collected at different time points post-treatment for the PCR-based evaluation of *E. canis* clearance, is another factor that complicates comparisons across studies (Harrus et al. 2004; Eddlestone et al., 2007; Gaunt et al., 2010; Theodorou et al., 2013; Lanza-Perea et al., 2014). Several studies have emphasized the importance of assessing CME treatment response by applying molecular testing to a range of tissues rather than solely blood PCR. Authors have also recommended allowing sufficient time (at least 1–2 months) after the cessation of treatment, to avoid false-negative PCR results, potentially associated with organism sequestration in tissues other than the blood or temporary suppression of ehrlichiaemia during or immediately after completion of the treatment. Both suppression of ehrlichiaemia and tissue sequestration could adversely influence successful PCR amplification because organism load may be below the analytical sensitivity of current molecular assays (Harrus et al., 1998b; Neer et al., 1999; Harrus et al., 2004; Gaunt et al., 2010; Theodorou et al., 2013; Fourie et al., 2015).

In addition to challenges in assessing antibiotic elimination of *E. canis* infections in both the experimental and clinical setting, other factors influence antimicrobial selection and duration of treatment. Some dogs may not tolerate doxycycline administration because of anorexia, vomiting, diarrhea, or rapid post-treatment elevations of alanine aminotransferase and alkaline phosphatase activities (Schulz et al., 2011; Villaescusa et al., 2015). There is currently insufficient evidence-based information supporting the causative role of doxycycline in tooth discoloration in puppies (Schulz et al., 2011; Boy et al., 2016), and the same holds true for children treated with doxycycline for suspected Rocky Mountain spotted fever (Todd et al., 2015). Importantly, widespread and frequently inappropriate use of doxycycline in the clinical setting could increase the risk for doxycycline resistance in *E. canis* or other bacteria (Goodman, 1999, Neer et al., 2002), although currently, the authors are not aware of documentation of resistance to tetracycline derivatives for any *Ehrlichia* species. Collectively, these factors justify the further systematic evaluation of alternative drugs for treatment of CME.

Rifampicin

Rifampicin, an inhibitor of the B subunit of DNA-dependent RNA polymerase, has been assessed as a potential alternative drug to doxycycline for treatment of CME. In in vitro studies, rifampicin was found to be as effective as doxycycline and with an equally low MIC (i.e. 0.03 μg/ml) against *E. canis* (Brouqui and Raoult 1993; Branger et al., 2004). When rifampicin was administered (15 mg/kg, PO, twice daily, for 7 days) to two experimentally-infected, moderately pancytopenic dogs, hematological abnormalities resolved and *E. canis* DNA could no longer be PCR amplified from blood (Schaefer et al., 2008). In the study by McClure et al. (2010), two *E. canis*-infected dogs, previously administered an ineffective course of doxycycline several months previously, were treated 700 days post-inoculation with the rifampicin regimen described above. Based upon Xenodiagnosis using ticks, rifampicin administration resulted in eradication of the infection in one of the two dogs (Table 1). A more recent *E. canis* experimental infection study using rifampicin (10 mg/kg, PO, once daily, for 21 days) hastened hematological recovery compared with untreated dogs, but only achieved *E. canis* clearance in 2/5 dogs, as assessed by blood PCP (Table 1) (Theodorou et al., 2013). On a comparative medical basis, isolated human case reports also indicate that rifampicin may be an effective alternative to doxycycline for treatment of human granulocytic and monocytic ehrlichiosis, affording a rapid clinical recovery (Krause et al., 2003; Branger et al., 2004; Dumler et al., 2007; Abusaada et al., 2016). The efficacy of rifampicin has not been reported for the clearance of *E. chaffeensis*, the causative agent of human monocytic ehrlichiosis. Current evidence suggests that the total daily rifampicin dose for dogs should not exceed 10 mg/kg (Frank, 1990; Branger et al., 2004; Anonymous, 2007; Theodorou et al., 2013; De Lucia et al., 2017). Rifampicin is generally well tolerated, although occasionally, yellow/brown discoloration of urine, tears or saliva, gastrointestinal discomfort, and elevation of liver enzyme activities have been reported in dogs, most often in association with higher doses (Bajwa et al., 2013; Theodorou, 2016; De Lucia et al., 2017).

Overall, results from the experimental infection studies in conjunction with limited clinical experience implies that rifampicin may be efficacious for the treatment of CME, although a comprehensive noninferiority trial is clearly warranted. Therefore, provided that some critical features of this drug are further refined, including its safety profile at recommended doses, the optimal duration of treatment, and the potential for the emergence of resistance to rifampicin when used as single therapy in CME (Kadlec et al., 2011), further assessment of rifampicin as an alternative agent to doxycycline in CME is justified.

Other drugs

There is currently limited evidence-based justification for considering other drugs for the treatment of CME. Imidocarb dipropionate has been used for many years in the treatment of CME. Although it was initially thought to be efficacious in achieving clinical remission (Matthewman et al., 1994; Sainz et al., 2000) and was suggested from the infectious disease study group of the American College of Veterinary Internal Medicine as a second line treatment in CME (Neer et al., 2002), more recent data demonstrated that it was ineffective in providing hematological recovery or eliminating natural and acute experimental *E. canis* infections (Table 1) (Kelly et al., 1998; Kleiter et al., 2001; Eddlestone et al., 2006). Therefore, imidocarb dipropionate is no longer indicated in CME, except in co-infections with protozoa such as *Babesia canis* (Sainz et al., 2015).

Enrofloxacin, a DNA gyrase inhibitor, originally appeared as a promising drug for the treatment of *E. canis* infection (Kontos and Athanasiou, 1998), but was subsequently found to be ineffective based upon in vitro *E. canis* testing and also failed to provide hematological recovery or clearing of acute experimental *E. canis* infection (Table 1) (Neer et al., 1999; Branger et al., 2004). There is the possibility of emergence of a DNA gyrase-mediated fluoroquinolone resistance in the *Ehrlichia* spp. genogroup (Maurin et al., 2001); however, natural fluoroquinolone *E. canis* resistance cannot be ruled out. As described below, fluoroquinolones may be useful for treatment of dogs with severe aplastic pancytopenia, when used as an additional antibiotic for the management of secondary bacterial infections (see below).

Several other anti-bacterial drugs have been used or proposed for treatment of CME, including, though not limited to, chloramphenicol and azithromycin; however, there is currently no evidence-based justification for their use (Buckner and Ewing, 1967; Branger et al., 2004; Rudoler et al., 2015).

Conclusions of antibiotic treatment

Based upon the review of the experimental or clinical treatment trials, the authors recommend doxycycline (5 mg/kg, twice daily or

10 mg/kg, once daily, PO, for 3–4 weeks) as the first line treatment in CME. Minocycline (10 mg/kg, PO, twice daily, for 3–4 weeks) or rifampicin (10 mg/kg, PO, once daily, for 3 weeks) are reasonable alternative medical options in cases where doxycycline is contraindicated or poorly tolerated.

How to treat the dog with *E. canis*-associated aplastic pancytopenia

For *E. canis*-infected dogs presenting with aplastic pancytopenia, individualized, comprehensive supportive care is essential to improve the chances for survival (Mylonakis et al., 2010a). Although comparative, worldwide data are not available, the frequency and severity of pancytopenia in *E. canis*-infected dogs may vary among different geographic regions. Based upon the authors experience (EB), pancytopenia appears to be less prevalent in dogs residing in the United States as compared to other regions of the world (e.g. Mediterranean countries; MM and SH). Reasons for these potential differences remain unclear. Persistent and profound pancytopenia (hematocrit < 25%, white blood cells < 4000/μL and platelets < 50,000/μL), severe leucopenia (white blood cells < 1000/μL) or anemia (hematocrit < 12%) and dogs of the German shepherd breed, independently predict a poor prognosis and the risk for mortality (Harrus et al., 1997; Mylonakis et al., 2004; Shipov et al., 2008; Mylonakis et al., 2011). As German shepherd dogs also have a predilection to develop systemic aspergillosis (Schultz et al., 2008), it is possible that a genetically-mediated immunological defect contributes to an increased frequency of severe *E. canis*-associated pancytopenia in this breed (Nyindo et al., 1980).

The rational administration of balanced crystalloid solutions and/or the periodic administration of blood-typed, cross-matched packed red blood cells or whole blood transfusions should be considered to temporarily counteract the systemic consequences of severe anemia (Shipov et al., 2008). If thrombocytopenia is contributing to continued blood loss or life-threatening bleeding, platelet components (platelet-rich plasma or platelet concentrate) should be given, if available, in the clinical setting. Alternatively, a unit of fresh whole blood (standard 450 mL collection bag) increases the platelet count of a 20 kg dog by approximately 20,000–30,000/μL, which can provide temporary, life-saving hemostasis (Lewis, 2000; Hackner and Rousseaux, 2015). Due to the relatively short life-span of platelets (approximately 5–7 days) compared to canine erythrocytes (approximately 110–120 days), repeated platelet-rich plasma transfusions are often required to achieve prolonged hemostasis or to improve the chances of a dog's positive clinical outcome (Shipov et al., 2008). In dogs that are not septicemic, iron sulphate supplements (100–300 mg, PO, once daily, for 3–5 months, administered at least 2 h (h) prior to, or after oral doxycycline), may be indicated, as iron depletion has been documented in a subset of dogs with myelosuppressive CME, presumably due to the chronic, occult hemorrhage into the gastrointestinal tract (Mylonakis et al., 2010b; Xia et al., 2016; Islam et al., 2016).

Dogs with moderate-to-severe, asymptomatic neutropenia (neutrophil count < 1,000/μL) that persists for more than 1–2 weeks may benefit from the administration of complementary prophylactic antibiotics to reduce the risk of the occurrence of life-threatening bacterial infections (Abrams-Ogg, 2012). In CME-associated pancytopenia, antibiotic choice is influenced by the drug's effectiveness to achieve "selective intestinal decontamination" (i.e. reducing Gram-negative and Gram-positive aerobic organisms, while leaving relatively unaffected the anaerobic intestinal microbiome), minimal effect on platelet function, and the lack of toxicity to an already compromised BM (Wilkens et al., 1995; Abrams-Ogg, 2012). In the context of BM toxicity, sulfonamides, chloramphenicol and penicillins, should be avoided (Weiss, 2003; Abrams-Ogg, 2012; Sainz et al., 2015). Of comparative interest, exacerbation of clinical disease has been associated with the administration of sulfa-containing antibiotics in human patients with monocytic ehrlichiosis (i.e. *E. chaffeensis* infection) (Paddock and Childs, 2003; Schutze et al., 2007). The authors suggest treating asymptomatic neutropenic dogs with second generation fluoroquinolones (e.g. enrofloxacin, 10 mg/kg, PO, once daily), in addition to administering doxycycline for the *E. canis* infection, until the neutrophil count exceeds 1,000/μL. We also recommend that the client perform periodic temperature measurements and confine the dog to the home environment to avoid exposure risks. Febrile neutropenia (at the time of first admission or during prophylactic treatment), requires antibiotic selection based on culture isolation (e.g. optimally blood and urine cultures) and antimicrobial sensitivity testing. Alternatively, an empirical antibiotic combination including an intravenous fluoroquinolone and a β-lactam (e.g. cefazolin, 30 mg/kg, intravenously (IV) or intramuscularly, three times daily) can be administered in conjunction with hospital stabilization. If fever does not resolve within 48–72 h, the addition of metronidazole (15 mg/kg, IV, three times daily) is advisable to expand the anaerobic coverage of the antimicrobial regimen (Abrams-Ogg, 2012).

Hematopoietic growth factors (recombinant human granulocyte-colony stimulating factor and recombinant human erythropoietin) have been used with inconsistent efficacy in a small number of CME-associated pancytopenic dogs (Aroch and Harrus, 2001; Shipov et al., 2008; Mylonakis et al., 2010a, Assarasakorn et al., 2008; Palacios et al., 2017). Apart from the high cost and the limited availability of these agents for the veterinary patients, firm scientific evidence justifying their use is currently lacking. Properly designed studies should be undertaken to objectively assess their safety and potential efficacy in dogs with myelosuppressive CME (Suter, 2010; Sainz et al., 2015).

Desmopressin (1-deamino-8-D-arginine vasopressin, DDAVP), an enhancer of platelet function by increasing serum levels of von Willebrand factor, resolved bleeding in three dogs with thrombocytopenia, presumptively-associated with CME (Giudice et al., 2010). In that study, the temporal association between the *E. canis* infection and the thrombocytopenia for which desmopressin was administered was not firmly established. Regardless, the potential benefit of prohemostatic agents in dogs with CME warrants further investigation.

Glucocorticoids have been advocated in CME, as an adjunct to doxycycline, to attenuate the immune-mediated pathogenetic component of the disease when no response to doxycycline treatment solely was achieved. In the acute phase of CME, for which a partial immune-mediated pathogenesis has been established (Waner et al., 2000; Harrus et al., 2001; Waner and Harrus, 2013), glucocorticoids are not generally an essential component of treatment as the administration of doxycycline achieves rapid clinical and/or hematological remission (Breitschwerdt et al., 1998; Harrus et al., 2004; Eddlestone et al., 2007). However, glucocorticoids may occasionally be warranted, if suspected immune-mediated manifestations (e.g. uveitis, thrombocytopenia) fail to respond to antibiotic treatment alone. There is currently no evidence-based justification for the use of anti-inflammatory or immunosuppressive doses of glucocorticoids in the medical management of myelosuppressive CME. Destruction of BM progenitor cells has not been shown to be immune-mediated in nature in dogs with CME, as opposed to humans, in which aplastic pancytopenia is thought commonly to be an immune-mediated process (Erlacher and Strahm, 2015; Boddu and Kadia, 2017). Importantly, in a retrospective study, glucocorticoids did not seem to be beneficial in a cohort of dogs with pancytopenic CME (Shipov

et al., 2008). In addition, administration of immunosuppressive drugs to a dog with pancytopenic CME could further predispose to secondary infections or potentiate the possibility for gastrointestinal bleeding, both of which are costly and potentially life-threatening complications (Reusch, 2015).

Post-treatment monitoring in the clinical setting

Dogs with acute CME experience rapid clinical improvement within 24–48 h of the initiation of doxycycline treatment, whereas resolution of hematological abnormalities generally takes 1–3 weeks (Breitschwerdt et al., 1998; Neer et al., 2002; Harrus et al., 2004; Eddlestone et al., 2007; Theodorou et al., 2013). Failure of the dog to respond within this period should prompt the clinician to pursue diagnostic testing for co-infecting organisms and to consider the possibility of a concurrent disease process (for example a neoplastic disease or immune-mediated thrombocytopenia) in a dog that has a serological or PCR-based diagnosis of CME. Importantly, clinical and hematological improvement may precede the elimination of *E. canis* infection; thus, treatment should not be terminated based on either clinical or hematologic recovery (Iqbal and Rikihisa, 1994; Harrus et al., 1998b; Neer et al., 1999; Harrus et al., 2004). Recurrence of thrombocytopenia 2–4 weeks after the cessation of doxycycline indicates treatment failure, re-infection or concurrent infection with organisms that are partially doxycycline-responsive but not curable (e.g. *Babesia* spp. and *Bartonella* spp.) (Neer et al., 1999). Therefore, to monitor the hematologic response to treatment, we recommend that hematological examination (including blood smear examination) should be performed two weeks after the initiation, at the end, and at four weeks following the completion of treatment course. Occasionally, resolution of thrombocytopenia may be sustained, yet the dog remains infected based upon PCR testing (Table 1) (Eddlestone et al., 2006; Theodorou et al., 2013). Hyperglobulinemia tends to progressively resolve 3-6 months after the initiation of treatment (Sainz et al., 2000). As *E. canis* IFA antibody titers are not linearly correlated with serum globulin levels, the kinetics of IgG antibodies during the disease and following antibiotic treatment is unpredictable. Although most CME dogs with clinical and hematological remission have a progressive decline in *E. canis* IFA antibody titers to undetectable levels, titers can persist for several months to years following presumptive elimination of the infection in a subset of dogs, which minimizes the value of serology as a sole or definitive post-treatment monitoring tool (Table 1) (Perille and Matus, 1991; Bartsch and Greene, 1996; Wen et al., 1997; Theodorou et al., 2013). Western immunoblotting has also been shown to be of limited value for the confirmation of the therapeutic elimination of *E. canis* infection (Breitschwerdt et al., 1998). Currently, PCR ideally applied in a range of tissues including blood, BM aspirates and splenic aspirates, 4-8 weeks after the completion of treatment, is the most reliable and affordable method in the clinical setting to prove the clearance of *E. canis* infection (Neer et al., 1999; Harrus et al., 2004; Theodorou et al., 2013). If PCR results remain positive, an additional 3–4 week treatment should be administered, acaricide administration to prevent re-infection should be emphasized, and the dog be retested. If PCR results persist to be positive after two treatment courses, an alternative drug may be used (e.g. switch from doxycycline or minocycline to rifampicin), considering that clearance of the infection may not be achieved (Neer et al., 2002). In dogs with profound aplastic pancytopenia, a very slow recovery, potentially months in duration should be anticipated. Without intensive monitoring and supportive care, dogs may succumb before BM recovery is achieved (Mylonakis et al., 2004; Shipov et al., 2008).

Conclusions

Historically, several drugs have been used for antibiotic treatment of CME. Current scientific literature supports the efficacy of only three agents, including doxycycline (first line treatment), minocycline and rifampicin (second line treatments). Importantly, although these drugs appear to be effective in achieving clinical and/or clinicopathologic remission in the majority of experimentally or naturally-infected dogs, they have not been invariably effective in eliminating the *E. canis* infection in all treated cases. In the clinical setting, the achievement of clinical and clinicopathologic remission in conjunction with sequential follow-up evaluations, may therefore be a more realistic goal, as opposed to the clearance of the infection. In dogs admitted with severe aplastic pancytopenia, treatment is largely supportive, and prognosis remains grave.

Conflict of interest statement

None of the authors of this manuscript have a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of this review.

References

Abrams-Ogg, A., 2012. Neutropenia, BSAVA Manual of Canine and Feline Haematology and Transfusion Medicine. Second edn. British Small Animal Veterinary Association, Gloucester, UK, pp. 117–125.

Abusaada, K., Ajmal, S., Hughes, L., 2016. Successful treatment of human monocytic ehrlichiosis with rifampin. Cureus 8, e444.

Amyx, H.L., Huxsoll, D.L., Zeiler, D.C., Hildebrandt, P.K., 1971. Therapeutic and prophylactic value of tetracycline in dogs infected with the agent of tropical canine pancytopenia. J. Am. Vet. Med. Assoc. 159, 1428–1432.

Anonymous,, 2007. Rifampin. The United States Pharmacopeial Convention, pp. 1–10.

Aroch, I., Harrus, S., 2001. The use of recombinant human granulocyte colony stimulating factor and recombinant human erythropoietin in the treatment of severe pancytopenia due to canine monocytic ehrlichiosis. Israel J. Vet. Med. 56, 65–69.

Assarasakorn, S., Kaewthamasorn, M., Manachai, N., 2008. A retrospective study of clinical use of recombinant human erythropoietin for treatment in anemic dogs with canine monocytic ehrlichiosis from an animal hospital in Bangkok, Thailand. Comp. Clin. Pathol. 17, 237–243.

Bajwa, J., Charach, M., Duclos, D., 2013. Adverse effects of rifampicin in dogs and serum alanine aminotransferase monitoring recommendations based on a retrospective study of 344 dogs. Vet. Dermatol. 24, 570–575.

Bartsch, R.C., Greene, R.T., 1996. Post-therapy antibody titers in dogs with ehrlichiosis: follow-up study on 68 patients treated primarily with tetracycline and/or doxycycline. J. Vet. Intern. Med. 10, 271–274.

Boddu, P.C., Kadia, T.M., 2017. Updates on the pathophysiology and treatment of aplastic anemia: a comprehensive review. Expert Rev. Hematol. 10, 433–448.

Boy, S., Crossley, D., Steenkamp, G., 2016. Developmental structural tooth defects in dogs-Experience from veterinary dental referral practice and review of the literature. Front. Vet. Sci. 3, 9.

Branger, S., Rolain, J.M., Raoult, D., 2004. Evaluation of antibiotic susceptibilities of *Ehrlichia canis*, *Ehrlichia chaffeensis*, and *Anaplasma phagocytophilum* by real-time PCR. Antimicrob. Agents Chemother. 48, 4822–4828.

Breitschwerdt, E.B., Hegarty, B.C., Hancock, S.I., 1998. Doxycycline hyclate treatment of experimental canine ehrlichiosis followed by challenge inoculation with two *Ehrlichia canis* strains. Antimicrob. Agents Chemother. 42, 362–368.

Bremer, W.G., Schaefer, J.J., Wagner, E.R., Ewing, S.A., Rikihisa, Y., Needham, G.R., Jittapalapong, S., Moore, D.L., Stich, R.W., 2005. Transstadial and intrastadial experimental transmission of *Ehrlichia canis* by male *Rhipicephalus sanguineus*. Vet. Parasitol. 131, 95–105.

Brouqui, P., Raoult, D., 1992. In vitro antibiotic susceptibility of the newly recognized agent of ehrlichiosis in humans, *Ehrlichia chaffeensis*. Antimicrob. Agents Chemother. 36, 2799–2803.

Brouqui, P., Raoult, D., 1993. Susceptibilities of *Ehrlichiae* to antibiotics, Antimicrobial Agents and Intracellular Pathogens. First edn. CRC Press, Boca Raton, FL, USA, pp. 179–199.

Buckner, R.G., Ewing, S.A., 1967. Experimental treatment of canine ehrlichiosis and haemobartonellosis. J. Am. Vet. Med. Assoc. 150, 1524–1529.

Buhles Jr., W.C., Huxsoll, D.L., Ristic, M., 1974. Tropical canine pancytopenia: clinical, hematologic, and serologic response of dogs to *Ehrlichia canis* infection, tetracycline therapy, and challenge inoculation. J. Infect. Dis. 130, 357–367.

Chopra, I., Roberts, M., 2001. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiol. Mol. Biol. Rev. 65, 232–260.

Codner, E.C., Farris-Smith, L.L., 1986. Characterization of the subclinical phase of ehrlichiosis in dogs. J. Am. Vet. Med. Assoc. 189, 47–50.
De Lucia, M., Bardagi, M., Fabbri, E., Ferreira, D., Ferrer, L., Scarampella, F., Zanna, G., Fondati, A., 2017. Rifampicin treatment of canine pyoderma due to multidrug-resistant methicillin-resistant staphylococci: a retrospective study of 32 cases. Vet. Dermatol. 28 171–e36.
Dumler, J.S., Madigan, J.E., Pusterla, N., Bakken, J.S., 2007. Ehrlichioses in humans: epidemiology, clinical presentation, diagnosis, and treatment. Clin. Infect. Dis. 45 (Suppl. 1), S45–S51.
Eddlestone, S.M., Neer, T.M., Gaunt, S.D., Corstvet, R., Gill, A., Hosgood, G., Hegarty, B., Breitschwerdt, E.B., 2006. Failure of imidocarb dipropionate to clear experimentally induced *Ehrlichia canis* infection in dogs. J. Vet. Intern. Med. 20, 840–844.
Eddlestone, S.M., Diniz, P.P., Neer, T.M., Gaunt, S.D., Corstvet, R., Cho, D., Hosgood, G., Hegarty, B., Breitschwerdt, E.B., 2007. Doxycycline clearance of experimentally induced chronic *Ehrlichia canis* infection in dogs. J. Vet. Intern. Med. 21, 1237–1242.
Erlacher, M., Strahm, B., 2015. Missing cells: pathophysiology, diagnosis, and management of (pan)cytopenia in childhood. Front. Pediatr. 3, 64.
Fourie, J.J., Horak, I., Crafford, D., Erasmus, H.L., Botha, O.J., 2015. The efficacy of a generic doxycycline tablet in the treatment of canine monocytic ehrlichiosis. J. S. Afr. Vet. Assoc. 86, 1193.
Frank, L.A., 1990. Clinical pharmacology of rifampin. J. Am. Vet. Med. Assoc. 197, 114–117.
Frezoulis, P.S., Angelidou, E., Karnezi, D., Oikonomidis, I.L., Kritsepi-Konstantinou, M., Kasabalis, D., Mylonakis, M.E., 2017. Canine pancytopoenia in a Mediterranean region: a retrospective study of 119 cases (2005 to 2013). J. Small Anim. Pract. 58, 395–402.
Gaunt, S., Beall, M., Stillman, B., Lorentzen, L., Diniz, P., Chandrashekar, R., Breitschwerdt, E., 2010. Experimental infection and co-infection of dogs with *Anaplasma platys* and *Ehrlichia canis*: hematologic, serologic and molecular findings. Parasit. Vectors 3, 33.
Girardi, A.F., Campos, A.N., Pescador, C.A., de Almeida, A.B.P.F., Mendonça, A.J., Nakazato, N., de Oliveira, A.C.S., Sousa, V.R.F., 2017. Quantitative analysis of bone marrow in pancytopenic dogs. Semin. Cienc. Agrar. 38, 3639–3646.
Giudice, E., Giannetto, C., Gianesella, M., 2010. Effect of desmopressin on immune-mediated haemorrhagic disorders due to canine monocytic ehrlichiosis: a preliminary study. J. Vet. Pharmacol. Ther. 33, 610–614.
Goodman, J.L., 1999. Ehrlichiosis — ticks, dogs, and doxycycline. N. Engl. J. Med. 341, 195–197.
Hackner, S.G., Rousseaux, A., 2015. Bleeding disorders, Small Animal Critical Care Medicine. Second edn. Elsevier Saunders, St. Louis, MI, USA, pp. 554–566.
Harrus, S., Kass, P.H., Klement, E., Waner, T., 1997. Canine monocytic ehrlichiosis: a retrospective study of 100 cases, and an epidemiological investigation of prognostic indicators for the disease. Vet. Rec. 4, 360–363.
Harrus, S., Waner, T., Aizenberg, I., Foley, J.E., Poland, A.M., Bark, H., 1998a. Amplification of ehrlichial DNA from dogs 34 months after infection with *Ehrlichia canis*. J. Clin. Microbiol. 36, 73–76.
Harrus, S., Waner, T., Aizenberg, I., Bark, H., 1998b. Therapeutic effect of doxycycline in experimental subclinical canine monocytic ehrlichiosis: evaluation of a 6-week course. J. Clin. Microbiol. 36, 2140–2142.
Harrus, S., Day, M.J., Waner, T., Bark, H., 2001. Presence of immune-complexes, and absence of antinuclear antibodies, in sera of dogs naturally and experimentally infected with *Ehrlichia canis*. Vet. Microbiol. 83, 343–349.
Harrus, S., Kenny, M., Miara, L., Aizenberg, I., Waner, T., Shaw, S., 2004. Comparison of simultaneous splenic sample PCR with blood sample PCR for diagnosis and treatment of experimental *Ehrlichia canis* infection. Antimicrob. Agents. Chemother. 48, 4488–4490.
Harrus, S., Waner, T., 2011. Diagnosis of canine monocytotropic ehrlichiosis (*Ehrlichia canis*): an overview. Vet. J. 187, 292–296.
Harrus, S., Waner, T., Neer, M., 2012. *Ehrlichia canis* infection, Infectious Diseases of the Dog and Cat. Fourth edn. Elsevier Saunders, St. Louis, MI, USA, pp. 227–238.
Iqbal, Z., Rikihisa, Y., 1994. Reisolation of *Ehrlichia canis* from blood and tissue of dogs after doxycycline treatment. J. Clin. Microbiol. 32, 1644–1649.
Islam, S., Jarosch, S., Zhou, J., Parquet Mdel, C., Toguri, J.T., Colp, P., Holbein, B.E., Lehmann, C., 2016. Anti-inflammatory and anti-bacterial effects of iron chelation in experimental sepsis. J. Surg. Res. 200, 266–273.
Jenkins, S., Ketzis, J.K., Dundas, J., Scorpio, D., 2018. Efficacy of minocycline in naturally occurring nonacute *Ehrlichia canis* infection in dogs. J. Vet. Intern. Med. 32, 217–221.
Johnson, E.M., Ewing, S.A., Barker, R.W., Fox, J.C., Crow, D.W., Kocan, K.M., 1998. Experimental transmission of *Ehrlichia canis* (Rickettsiales: Ehrlichieae) by *Dermacentor variabilis* (Acari: Ixodidae). Vet. Parasitol. 74, 277–288.
Kadlec, K., van Duijkeren, E., Wagenaar, J.A., Schwarz, S., 2011. Molecular basis of rifampicin resistance in methicillin-resistant *Staphylococcus pseudintermedius* isolates from dogs. J. Antimicrob. Chemother. 66, 1236–1242.
Kelly, P.J., Matthewman, L.A., Brougui, P., Raoult, D., 1998. Lack of susceptibility of *Ehrlichia canis* to imidocarb dipropionate *in vitro*. J. S. Afr. Vet. Assoc. 69, 55–56.
Kleiter, M., Luckschander, N., Willmann, M., Kolbl, S., Lutz, H., 2001. Imidocarb dipropionate alone or in combination with immunosuppressive therapy for treatment of canine ehrlichiosis. Proceedings of the 11th Annual Meeting of the European College of Veterinary Internal Medicine, Dublin, Ireland, pp. 162–163.
Kontos, V.I., Athanasiou, L.V., 1998. Use of enrofloxacin in the treatment of acute canine ehrlichiosis. Canine Pract. 23, 10–14.
Krause, P.J., Corrow, C.L., Bakken, J.S., 2003. Successful treatment of human granulocytic ehrlichiosis in children using rifampin. Pediatrics 112, e252–e253.
Lanza-Perea, M., Zieger, U., Qurollo, B.A., Hegarty, B.C., Pultorak, E.L., Kumthekar, S., Bruhl-Day, R., Breitschwerdt, E.B., 2014. Intraoperative bleeding in dogs from Grenada seroreactive to *Anaplasma platys* and *Ehrlichia canis*. J. Vet. Intern. Med. 28, 1702–1707.
Lewis, D.C., 2000. Disorders of platelet number, BSAVA Manual of Canine and Feline Haematology and Transfusion Medicine. First edn. British Small Animal Veterinary Association, Hampshire, UK, pp. 183–195.
Matthewman, L.A., Kelly, P.J., Brougui, P., Raoult, D., 1994. Further evidence for the efficacy of imidocarb dipropionate in the treatment of *Ehrlichia canis* infection. J. S. Afr. Vet. Assoc. 65, 104–107.
Maurin, M., Abergel, C., Raoult, D., 2001. DNA gyrase-mediated natural resistance to fluoroquinolones in *Ehrlichia* spp. Antimicrob. Agents Chemother. 45, 2098–2105.
McClure, J.C., Crothers, M.L., Schaefer, J.J., Stanley, P.D., Needham, G.R., Ewing, S.A., Stich, R.W., 2010. Efficacy of a doxycycline treatment regimen initiated during three different phases of experimental ehrlichiosis. Antimicrob. Agents Chemother. 54, 5012–5020.
Mylonakis, M.E., Koutinas, A.F., Breitscwerdt, E.B., Hegarty, B.C., Billinis, C.D., Leontides, L.S., Kontos, V.S., 2004. Chronic canine ehrlichiosis (*Ehrlichia canis*): a retrospective study of 19 natural cases. J. Am. Anim. Hosp. Assoc. 40, 174–184.
Mylonakis, M.E., Siarkou, V.I., Koutinas, A.F., 2010a. Myelosuppressive canine monocytic ehrlichiosis (*Ehrlichia canis*): an update on the pathogenesis, diagnosis and management. Israel J. Vet. Med. 65, 129–135.
Mylonakis, M.E., Day, M.J., Siarkou, V., Vernau, W., Koutinas, A.F., 2010b. Absence of myelofibrosis in dogs with *Ehrlichia canis*-induced myelosuppression. J. Comp. Pathol. 142, 328–331.
Mylonakis, M.E., Ceron, J.J., Leontides, L., Siarkou, V.I., Martinez, S., Tvarijonaviciute, A., Koutinas, A.F., Harrus, S., 2011. Serum acute phase proteins as clinical phase indicators and outcome predictors in naturally occurring canine monocytic ehrlichiosis. J. Vet. Intern. Med. 25, 811–817.
Neer, T.M., Eddlestone, S.M., Gaunt, S.D., Corstvet, R.E., 1999. Efficacy of enrofloxacin for the treatment of experimentally induced *Ehrlichia canis* infection. J. Vet. Int. Med. 13, 501–504.
Neer, T.M., Breitschwerdt, E.B., Greene, R.T., Lappin, M.R., 2002. Consensus statement on ehrlichial disease of small animals from the infectious disease study group of the ACVIM: American College of Veterinary Internal Medicine. J. Vet. Intern. Med. 16, 309–315.
Nyindo, M., Huxsoll, D.L., Ristic, M., Kakoma, I., Brown, J.L., Carson, C.A., Stephenson, E.H., 1980. Cell-mediated and humoral immune responses of German Shepherd Dogs and Beagles to experimental infection with *Ehrlichia canis*. Am. J. Vet. Res. 41, 250–254.
Paddock, C.D., Childs, J.E., 2003. *Ehrlichia chaffeensis*: a prototypical emerging pathogen. Clin. Microbiol. Rev. 16, 37–64.
Palacios, M., Arteaga, R., Calvo, G., 2017. High-dose filgrastim treatment of nonregenerative pancytopenia associated with chronic canine ehrlichiosis. Top. Companion Anim. Med. 32, 28–30.
Perille, A.L., Matus, R.E., 1991. Canine ehrlichiosis in six dogs with persistently increased antibody titers. J. Vet. Intern. Med. 5, 195–198.
Reardon, M.J., Pierce, K.R., 1981. Acute experimental canine ehrlichiosis: I. Sequential reaction of the hemic and lymphoreticular systems. Vet. Pathol. 18, 48–61.
Reusch, C.E., 2015. Glucocorticoid therapy, Canine and Feline Endocrinology. Fourth edn. Elsevier Saunders, St. Louis, MI, USA, pp. 556–577.
Riond, J.L., Vaden, S.L., Riviere, J.E., 1990. Comparative pharmacokinetics of doxycycline in dogs and cats. J. Vet. Pharmacol. Ther. 13, 415–424.
Rudoler, N., Harrus, S., Martinez-Subiela, S., Tvarijonaviciute, A., van Straten, M., Ceron, J.J., Baneth, G., 2015. Comparison of the acute phase protein and antioxidant responses in dogs vaccinated against canine monocytic ehrlichiosis and naive-challenged dogs. Parasit. Vectors 8, 175.
Ruiz, S.M., Olvera, L.G., Chacón Sdel, C., Estrada, D.V., 2015. Pharmacokinetics of an oral extended-release formulation of doxycycline hyclate containing acrylic acid and polymethacrylate in dogs. Am. J. Vet. Res. 76, 367–372.
Sainz, A., Tesouro, M.A., Amusategui, I., Rodriguez, F., Mazzucchelli, F., Rodriguez, M., 2000. Prospective comparative study of 3 treatment protocols using doxycycline or imidocarb dipropionate in dogs with naturally occurring ehrlichiosis. J. Vet. Intern. Med. 14, 134–139.
Sainz, A., Roura, X., Miro, G., Estrada-Pena, A., Kohn, B., Harrus, S., Solano-Gallego, L., 2015. Guideline for veterinary practitioners on canine ehrlichiosis and anaplasmosis in Europe. Parasit. Vectors 8, 75.
Schaefer, J.J., Needham, G.R., Bremer, W.G., Rikihisa, Y., Ewing, S.A., Stich, R.W., 2007. Tick acquisition of *Ehrlichia canis* from dogs treated with doxycycline hyclate. Antimicrob. Agents Chemother. 51, 3394–3396.
Schaefer, J.J., Kahn, J., Needham, G.R., Rikihisa, Y., Ewing, S.A., Stich, R.W., 2008. Antibiotic clearance of *Ehrlichia canis* from dogs infected by intravenous inoculation of carrier blood. Ann. N. Y. Acad. Sci. 1149, 263–269.
Schultz, R.M., Johnson, E.G., Wisner, E.R., Brown, N.A., Byrne, B.A., Sykes, J.E., 2008. Clinicopathologic and diagnostic imaging characteristics of systemic aspergillosis in 30 dogs. J. Vet. Intern. Med. 22, 851–859.
Schulz, B.S., Hupfauer, S., Ammerm, H., Sauter-Louism, C., Hartmannm, K., 2011. Suspected side effects of doxycycline use in dogs-a retrospective study of 386 cases. Vet. Rec. 169, 229.
Schutze, G.E., Buckingham, S.C., Marshall, G.S., Woods, C.R., Jackson, M.A., Patterson, L.E., Jacobs, R.F., 2007. Tick-borne Infections in Children Study (TICS) Group: Human monocytic ehrlichiosis in children. Pediatr. Infect. Dis. J. 26, 475–479.
Shipov, A., Klement, E., Reuveni-Tager, L., Waner, T., Harrus, S., 2008. Prognostic indicators for canine monocytic ehrlichiosis. Vet. Parasitol. 153, 131–138.

Shropshire, S., Olver, C., Lappin, M., 2018. Characteristics of hemostasis during experimental *Ehrlichia canis* infection. J. Vet. Intern. Med. 32, 1334–1342.
Stratton, C.W., 2006. In vitro susceptibility testing versus in vivo effectiveness. Med. Clin. North Am. 90, 1077–1088.
Suter, S.E., 2010. Clinical use of hematopoietic growth factors, Schalm's Veterinary Hematology. Sixth edn. Wiley-Blackwell, Ames, IA, USA, pp. 790–795.
Sykes, J.E., 2014. Ehrlichiosis, Canine and Feline Infectious Diseases. First edn Elsevier Saunders, St. Louis, Missouri, USA, pp. 278–289.
Theodorou, K., Mylonakis, M.E., Siarkou, V.I., Leontides, L., Koutinas, A.F., Koutinas, C. K., Kritsepi-Konstantinou, M., Batzias, G., Flouraki, E., Eyal, O., et al., 2013. Efficacy of rifampicin in the treatment of experimental acute canine monocytic ehrlichiosis. J. Antimicrob. Chemother. 68, 1619–1626.
Theodorou, K., 2016. Efficacy and safety of rifampin in the treatment of experimentally induced acute *Ehrlichia canis* infection in the dog Doctoral Thesis. School of Veterinary Medicine, Aristotle University of Thessaloniki, Greece.
Todd, S.R., Dahlgren, F.S., Traeger, M.S., Beltran-Aguilar, E.D., Marianos, D.W., Hamilton, C., McQuiston, J.H., Regan, J.J., 2015. No visible dental staining in children treated with doxycycline for suspected Rocky Mountain spotted fever. J. Pediatr. 166, 1246–1251.
Villaescusa, A., García-Sancho, M., Rodríguez-Franco, F., Tesouro, M.Á., Sainz, Á., 2015. Effects of doxycycline on haematology, blood chemistry and peripheral blood lymphocyte subsets of healthy dogs and dogs naturally infected with *Ehrlichia canis*. Vet. J. 204, 263–268.
Waner, T., Harrus, S., Bark, H., Bogin, E., Avidar, Y., Keysary, A., 1997. Characterization of the subclinical phase of canine ehrlichiosis in experimentally infected beagle dogs. Vet. Parasitol. 69, 307–317.
Waner, T., Leykin, I., Shinitsky, M., Sharabani, E., Buch, H., Keysary, A., Bark, H., Harrus, S., 2000. Detection of platelet-bound antibodies in beagle dogs after artificial infection with *Ehrlichia canis*. Vet. Immunol. Immunopathol. 77, 145–150.
Waner, T., Harrus, S., 2013. Canine monocytic ehrlichiosis: from pathology to clinical manifestations. Israel J. Vet. Med. 68, 12–18.
Wen, B., Rikihisa, Y., Mott, J.M., Greene, R., Kim, H.Y., Zhi, N., Couto, G.C., Unver, A., Bartsch, R., 1997. Comparison of nested PCR with immunofluorescent-antibody assay for detection of *Ehrlichia canis* infection in dogs treated with doxycycline. J. Clin. Microbiol. 35, 1852–1855.
Weiss, D.J., 2003. New insights into the physiology and treatment of acquired myelodysplastic syndromes and aplastic pancytopenia. Vet. Clin. North Am. Small Anim. Pract. 33, 1317–1334.
Wilkens, B., Sullivan, P., McDonald, T.P., Krahwinkel, D.J., 1995. Effects of cephalothin, cefazolin, and cefmetazole on the hemostatic mechanism in normal dogs: implications for the surgical patient. Vet. Surg. 24, 25–31.
Xia, Y., Farah, N., Maxan, A., Zhou, J., Lehmann, C., 2016. Therapeutic iron restriction in sepsis. Med. Hypotheses 89, 37–39.