Canine ehrlichioses: an update

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ABSTRACT

The development of molecular biology techniques and methods for the isolation and growth of ehrlichias in tissue culture have greatly facilitated the study of these organisms. The available knowledge on ehrlichias is thus rapidly increasing and in this review recent findings on the epidemiology, transmission, clinical and laboratory signs of infection, diagnosis and treatment of canine ehrlichioses are described.

Key words: dogs, *Ehrlichia*.

Kelly P J **Canine ehrlichioses: an update**. *Journal of the South African Veterinary Association* (2000) 71(2): 77–86 (En.). Biomedical Research and Training Institute, PO Box CY1753, Harare, Zimbabwe.

INTRODUCTION

Ehrlichias are Gram-negative bacteria that live within membrane-bound vacuoles in the cytoplasm of cells[106]. They were originally classified according to the host cells and mammalian species they infected and their geographic location. In the 1990s the development of cell culture systems for most of these strictly intracellular organisms and advances in molecular biology techniques facilitated the serotypic and genotypic characterisation of the ehrlichias, and led to their phylogenetic positions being more clearly defined. The techniques have also greatly facilitated the diagnosis of ehrlichioses, and research on ehrlichias has been stimulated by the finding that they cause disease in people[24]. Currently, the members of the genus *Ehrlichia* are divided into 3 genogroups based on the sequence of their 16S rRNA genes and *groESL* heat shock operons[80,119] (Table 1). Only *Cowdria ruminantium* and *E. canis* are known to occur in southern Africa.

EHRLICHIA SPECIES INFECTING DOGS

It is now known that there are at least 9 *Ehrlichia* species that may infect dogs.

Ehrlichia canis

Background

This is the agent of canine tropical pancytopaenia, or more correctly an agent of canine monocytic ehrlichiosis. The disease was first described in Algeria in 1935[36] and in Southern Africa in 1938[75,90]. It is now known to have a worldwide distribution, apart from Australia and New Zealand, although it is more prevalent in sub-tropical and tropical areas[55]. In Africa, serological surveys have shown that dogs with antibodies reactive with *E. canis* by indirect immunofluorescence assays (IFA) can be found in Tunisia (68 %)[18], Senegal (53 %)[18], Chad (28 %)[18], Egypt (33 %)[13], Zimbabwe[83,85] (43 %) and South Africa (42 %)[98]. Surveys in Israel have shown an overall serological prevalence of 30 % in dogs[10], and an isolate of *E. canis* has been made from a dog in Israel. This strain of *E. canis* has similar morphological, antigenic and genotypic properties to those of isolates of *E. canis* made in the USA[73].

There is, however, growing evidence of strain variation among *E. canis* organisms. There is considerable variability in the type and severity of clinical and laboratory abnormalities in dogs with *E. canis* infections in southern Africa[81,82,90], and elsewhere in the world[60]. Recent studies have indicated geographic antigenic diversity among *E. canis*[60]. In particular, sera from naturally-infected dogs in Zimbabwe have antibodies against proteins not recognised by sera from dogs from other countries[60]. Also, considerable variability in the antibody titres of naturally-infected dogs against 3 strains of *E. canis* has been reported[16].

E. canis is transmitted transstadially but not transovarially in *Rhipicephalus sanguineus*[50], and all feeding stages can transmit the infection to susceptible dogs with adults being able to transmit *E. canis* for at least 155 days after detachment from an infected host. It has now been confirmed that *E. canis* is present in *R. sanguineus* in the USA[88], and it has been shown that *E. canis* can be transmitted transstadially in *Dermacentor variabilis,* with adults also transmitting the infection to dogs[68]. Attempts to transmit *E. canis* transstadially and transovarially in *Otobius megnini* have been unsuccessful[43]. Other canids may be infected with *E. canis* including wolves, foxes, coyotes, jackals and African wild dogs[120]. It would appear unlikely, however, that these species play significant roles in the epidemiology of *E. canis* infections in domestic dogs.

Clinical findings in dogs with E. canis *infections*

Three phases of *E. canis* infection have been described in experimentally infected dogs. After an incubation period of 1–3 weeks, dogs enter the acute phase of infection and may show depression, lethargy, anorexia, mild weight loss, fever, lymphadenomegaly and splenomegaly, although in many cases signs are mild or inapparent[5,15,23,67,123,129]. Platelet-related bleeding may be observed[55] but this is unusual[48,61]. Most dogs survive the acute phase of infection and recover within 1– 4 weeks to enter the subclinical phase of the disease, where they show no clinical signs but remain infected with *E. canis*[23,129]. This subclinical phase may last for as little as 4 months in experimentally-infected dogs[23] but may persist for up to 10 years in naturally-infected dogs[12].

A significant recent finding is that dogs can spontaneously eliminate *E. canis* infections during the subclinical phase of the disease. In 1 study 33 % (2/6) of the dogs experimentally infected with *E. canis* 34 months previously were found to be negative according to the polymerase chain reaction (PCR), for *E. canis* DNA, to be serologically negative and to have no abnormalities according to laboratory tests[57]. In another study[15], 75 % (3/4) of dogs experimentally infected with *E. canis* 5 months previously were found to have normal haematology values and to be culture-negative, and PCR-negative for DNA of *E. canis.* When blood from the only dog that was PCR-positive but culture-negative was inoculated into an uninfected dog, no clinical signs of infection were observed, and the dog did not seroconvert. There were no apparent changes in IFA titres that could be associated with clearance of infection.

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Received: December 1999. Accepted: May 2000.

Table 1: **The genogroups containing bacteria designated as ehrlichias that are discussed in this article.**

Genogroup III	Genogroup II	Genogroup I
E. canis	*E. phagocytophila*	*E. risticii*
E. chaffeensis	*E. equi*	*Neorickettsia helminthoeca*
E. ewingii	Human granulocytic *Ehrlichia*	*N. elokominicia*
Cowdria ruminantium	*E. platys*	

Previously it has been suggested that naturally-infected dogs (68 %; 12/18) in Zimbabwe that were serologically positive by IFA and Western blotting but had no clinical, haematological or biochemical signs of infection may have self-cured[83]. Similarly, although serologically positive dogs are common in Zimbabwe, histopathological changes consistent with *E. canis* infections are seldom seen during *post mortem* examinations. Spontaneous elimination of *E. canis* infections in naturally-infected dogs may therefore not be uncommon.

In some dogs a severe, life-threatening chronic phase of the disease may develop. In this phase dogs exhibit clinical signs including weight loss and emaciation, fever, pallor, weakness, haemorrhage, and peripheral oedema, particularly of the hind limbs and scrotum[23,48,55,61]. Death usually results from extensive haemorrhage, or is due to secondary bacterial infections.

In naturally-infected dogs in which the stage of infection is not readily determined, depression (67 %), weight loss (59 %), anorexia (56 %), hemorrhagic tendencies, in particular epistaxis (46 %), pyrexia (40 %) and lymphadenomegaly (30 %) are the most commonly-reported clinical signs in the USA[133]. Similar signs have been reported in studies on naturally-infected dogs in Africa[82,100,123]. Neuromuscular, reproductive and ocular signs may also occur in naturally-infected dogs. These signs include polymyositis, paresis, signs of meningoencephalitis, cranial nerve deficits, seizures, abortions and infertility, corneal opacitiy, anterior uveitis, hyphema, focal chorioretinal lesions and retinal detachment[55]. Pulmonary signs including coughing and exercise intolerance may also develop as a result of interstitial lung infiltrates[61].

Reasons proposed for the wide variation in clinical signs and the development of the severe life-threatening chronic phase of the disease in only some dogs, include strain variation in *E. canis*, dose of infection, concurrent diseases and immunological status of the host[55,61,114]. German shepherd dogs and their crosses are particularly likely to show more severe signs of disease, and infections in this breed are associated with a poorer prognosis[55,61,114].

Laboratory findings in dogs with E. canis *infections*

In the acute phase of the experimentally-induced disease, the most common laboratory abnormality is thrombocytopaenia (platelet counts down to 20–100 × $10^3/\mu\ell$)[5,15,67,114,129] with an increase in platelet volume suggesting active thrombopoesis[129]. Other abnormalities that are reported less frequently include anaemia and leukopaenia[5,23,123]. The bone marrow is commonly hypercellular in the acute phase of infection[105].

Laboratory abnormalities described for naturally-infected dogs in the subclinical phase of the disease include hyperglobulinaemia (90 %), thrombocytopaenia (50 %), absolute lymphocytosis (40 %) and absolute neutropaenia (30 %)[30]. In experimentally-infected dogs, thrombocytopaenia (13–180 × $10^3/\mu\ell$) was observed in most cases and mean platelet volumes were increased[129]. While leukopaenia and absolute neutropaenia were not observed, there were significant decreases in leukocyte and neutrophil numbers compared with pre-infection values. Similarly, although none of the dogs became anaemic, some dogs had reduced packed-cell volumes, red-cell counts and haemoglobin concentrations compared to pre-infection levels. The dogs also had increased total serum proteins (33 %), hypoalbuminaemia (22 %), hypergammaglobulinaemia (68 %), increased α_1- and α_2- (22 %) and β_1-globulins (44 %) and decreased β_2-globulins (55 %)[129].

In experimental studies on the chronic phase of the disease, laboratory abnormalities included regenerative or non-regenerative anaemia (red cell count 2–5 × $10^6/\mu\ell$), severe leukopaenia (white cell count <3 × $10^3/\mu\ell$) and thrombocytopaenia (platelet count <30 × $10^3/\mu\ell$)[23]. In the early stages, bone marrow hyperplasia occurs, but as the disease progresses, the bone marrow becomes hypoplastic[112].

In naturally-infected dogs in the USA in which the stage of disease could not be determined, laboratory abnormalities included thrombocytopaenia (86 %), non-regenerative anaemia (57 %), hypoalbuminaemia (43 %), hyperglobulinaemia (39 %), hyperproteinaemia (33 %), leukopaenia (31 %), leukocytosis (20 %), pancytopaenia (17 %), and regenerative anaemia (15 %)[133]. Elevated liver enzymes were found in 35 % of dogs, but prior corticosteroid usage could have been responsible for these elevations in some dogs. Similar abnormalities have been reported for dogs in Africa[82,100,123]. Using serum protein electrophoresis, it has been found that most dogs with natural *E. canis* infections have polyclonal gammopathies, although monoclonal gammopathies may occur[14]. Generally, there are significantly decreased α-1 globulins and significantly elevated α-2, β-2 globulins and γ-globulins[48,61,133]. It has also been found that dogs that were pancytopaenic had significantly lower concentrations of total protein, total globulin and γ-globulins, indicating severely compromised immune function[51].

Pathogenesis of E. canis infections

It appears that monocytes attracted to the site of tick attachment become infected with *E. canis* present in the salivary gland secretions of the tick[48]. Infected monocytes enter the blood stream and lymphatics and localise in tissues throughout the body. The persistence of the organism in these cells results in the typical histological findings of plasmacytosis and generalised perivascular lymphocyte and plasma cell accumulation[63]. Ehrlichias appear to survive in macrophages by producing proteins that prevent fusion of the phagosomes in which they occur with lysosomes in the cells[131].

The continued presence of *E. canis* in the body results in the production of reactive IgA, IgM and IgG, and it has been suggested that these antibodies may enhance the uptake of *E. canis* into macrophages[112]. Experimental studies, however, have shown that immune sera from dogs suppress the growth of *E. canis* in normal macrophages, and macrophages from infected dogs are more resistant to the growth of the organism than normal macrophages[77]. Dogs become susceptible to reinfection with *E. canis* only when existing infections are cleared by appropriate therapy[5,15], although high antibody titres may be present. It has yet to be determined whether dogs in which spontaneous elimination of infections occurs are also susceptible to reinfection. Although the immunological mechanisms that may cause elimination of infections from dogs have yet to be determined, cell-mediated immune responses

probably play an important role[112]. German shepherd dogs are known to show more severe disease when infected with *E. canis* and laboratory studies have indicated that infections of these dogs cause specific and non-specific suppression of their cell-mediated immune responses[91].

Anaemia in dogs with *E. canis* infections results from haemorrhage and/or bone marrow suppression. Although erythrophagocytosis is prominent in the lymph nodes, this is not a feature in other organs, and the erythrophagocytosis is thought to result from haemorrhage rather than sensitisation of red blood cells[63]. Positive Coombs' tests, however, have been reported to occur in up to 27 % of dogs with the disease[133]. Even in dogs with anaemia of several months' duration, there is little evidence of bone marrow activity or extra-medullary erythropoesis in the spleen and other organs, suggesting generalised erythropoetic suppression[63].

The hypergammaglobulinaemia that is commonly found in dogs with *E. canis* infections is not due to antibodies against *E. canis,* and infections may result in non-specific antibody production[55,112,133]. While the prolonged antigenic stimulation associated with *E. canis* infections may result in an exaggerated and aberrant humoral immune response[51], it has also been suggested that the hypergammaglobulinaemia may represent the development of a secondary autoimmune response to damaged host cell components[25].

Hypoalbuminaemia appears not to be due to renal losses, as glomerulonephritis is not common in dogs with *E. canis* infections[31,63]. It may, however, result from haemorrhage, vasculitis and oedema, increased catabolism of the protein during pyrexia and/or decreased production to compensate for the oncotic affects of the hyperglobulinaemia[55].

While haemorrhage is not uncommon in dogs with *E. canis* infections, the severity of the haemorrhage does not always correlate with the platelet count in the dog[55,133]. In some infected dogs, low platelet counts can be found with no apparent bleeding tendencies, while in other dogs haemorrhage is seen with normal platelet counts. In both groups of dogs, activated coagulation times, one-step prothrombin times, activated partial thromboplastin times and levels of fibrin degradation products are usually normal[133]. Evidence is now accumulating that haemorrhage in dogs with *E. canis* infections results from platelet dysfunction. Sera of dogs with acute *E. canis* infections contain factors that prolong platelet aggregation[52]. Antiplatelet antibodies, which have been shown to occur in the acute phase of infection, may be at least partly responsible for the decreased platelet aggregation and possibly also platelet attachment[53].

Thrombocytopaenia is the commonest laboratory abnormality in dogs with *E. canis* infections, and there are numerous possible causes for this abnormality. In the chronic phase of infection, thrombocytopaenia is most often due to bone marrow hypoplasia[63]. Consumption of platelets due to vasculitis appears an unlikely cause of thrombocytopaenia, as thrombosis, endothelial cell hypertrophy and vasculitis, as seen in other rickettsial infections, are not commonly observed in *E. canis* infections[63,112]. Another possible cause of thrombocytopaenia is the production of a platelet migration inhibition factor that enhances platelet sequestration, particularly in the spleen[1]. Further, antiplatelet antibodies occur in the acute phase of *E. canis* infection[53,127], and the half-life of platelets is decreased and associated with increased platelet destruction by the macrophages of the spleen[117]. Recent experiments have shown that the spleen plays an important role in the pathogenesis of *E. canis* infections, with splenectomised dogs having less severe clinical signs and laboratory abnormalities than intact dogs[58]. Inflammatory mediators from the spleen and/or other splenic substances have been proposed to play a key role in the pathogenesis of the disease.

Ehrlichia chaffeensis

E. chaffeensis is the aetiological agent of human monocytic ehrlichiosis, first described in 1987[6], and another agent of canine monocytic ehrlichiosis. The organism occurs in the USA and probably Europe[126]. In the USA, deer, dogs and small rodents are the likely reservoir hosts of the organism, which is transmitted mainly by *Amblyomma americanum* and perhaps by *D. variabilis*[35,44]. In humans there is a history of tick-bite, and in many people the disease is mild or subclinical. In patients with more severe disease, fever, headache, myalgia, anorexia, nausea, chills, weight loss, sweating, thrombocytopaenia and elevated serum hepatic transaminase levels occur[126], and, particularly in immunosuppressed people, the disease may be fatal[94].

Experimental infections of dogs result in only mild clinical and haematological abnormalities, although all dogs seroconvert, and the organism can be re-isolated from infected dogs for at least 26 days after infection[34]. Antibody titres against *E. chaffeensis* in experimentally-infected dogs are 2–8-fold higher than against *E. canis.* High prevalences of natural infections of dogs with *E. chaffeensis* have now been reported from the eastern states in the USA[35,74], and severe infections have been described that are clinically and serologically indistinguishable from disease manifestations of *E. canis*[16,74]. Limited data suggest that infection with *E. chaffeensis* does not protect against subsequent infection with *E. canis*[34].

Dogs in South Africa have been found with higher antibody titres to *E. chaffeensis* than to *E. canis*[98], and there is serological evidence that people may be infected with the organism in Burkino Faso and Mozambique[21]. There are now reports of people from South Africa and Mali with serological and clinical evidence of *E. chaffeensis* infections[99].

Ehrlichia ewingii

This recently-named organism is an aetiological agent of canine granulocytic ehrlichiosis. It has been described only in the USA, where it is transmitted by *A. americanum*[7]. It may also be transmitted by *D. variabilis*[8], and has been demonstrated in *R. sanguineus*[88]. In naturally-infected dogs, *E. ewingii* is commonly seen in circulating neutrophils in the 1st week of clinical signs[118]. Signs of infection are generally far milder than those classically associated with *E. canis* infections, and include suppurative polyarthritis in 1 or more limbs, acute lameness, muscular stiffness, lethargy, mild fever and thrombocytopaenia[32,42,47,118]. Although the organism has yet to be grown in tissue culture and serological assays are not readily available, cross-reactivity between antibodies against *E. ewingii* and *E. canis* has been reported[7], and antibodies against *E. ewingii* have been shown to react with high molecular mass proteins (>40 kDa) of *E. canis* in Western blots[109]. Similar reactions have been reported with a serum sample from a dog in Zimbabwe[60].

Cowdria ruminantium

C. ruminantium is the agent of heartwater in domestic ruminants that occurs widely in Africa and is also present in the Caribbean Islands[80]. It is transmitted by *Amblyomma* spp. and causes neurological and respiratory signs associated with peracute mortalities[107]. While natural infections of dogs with *C. ruminantium* have not been reported, experimental infections of dogs result in no clinical or laboratory abnormalities, although dogs remain infected with the organism for up to 3 weeks[71]. There is serological cross-reactivity between *E. canis* and

C. ruminantium, and dogs infected with *C. ruminantium* are positive in IFA and Western blots against *E. canis*[71,85]. Serological differentiation between infection with these 2 organisms in areas where they coexist may therefore not be possible.

Ehrlichia equi, E. phagocytophila and the agent of human granulocytic ehrlichiosis

There is now considerable evidence that these organisms are strains of a single *Ehrlichia* species that have adapted to 1 or more mammalian hosts[39]. There are only few, if any, nucleotide differences in the sequences of the 16S rRNA gene and the *groESL* heat shock operon[119] in organisms isolated from people, dogs and horses around the world[29,39,69,126]. Also, results of cross-infection and cross-protection studies have shown a close relationship between the organisms in the group[11,126]. Serology demonstrates broad cross-reactivity among members of the group, providing further evidence that the members of this group may be identical species[37,102].

E. equi is the agent of equine granulocytic ehrlichiosis, which has been reported from North and South America and Europe[39]. In horses, the disease is thought to be transmitted by *Ixodes* species, and is usually self-limiting. Characteristics of the disease are depression, fever, anorexia, icterus, petechiae, limb oedaema and ataxia. Laboratory abnormalities include thrombocytopaenia, leukopaenia, anaemia, hyperbilirubinaemia and high percentages of parasitised granulocytes[11]. Dogs have been experimentally infected with *E. equi,*[6] and natural infections with this or a very closely related organism have been reported in dogs in the USA[16,78,113]. Dogs experimentally infected with *E. equi* show no clinical signs or mild pyrexia, and there may be transient thrombocytopaenia and mild anaemia. Morulae can be detected in 1–2 % of neutrophils in some dogs for 1–4 days after infection. Experimentally-infected dogs were susceptible to subsequent infections with *E. canis.*

E. phagocytophila is the agent of tick-borne fever in sheep and pasture fever in cattle in Europe[39]. The organism is transmitted by *I. ricinus,* and infections result in depression, fever, weight loss, thrombocytopaenia and leukopaenia[22]. Organisms are readily visible in neutrophils, and infected animals are predisposed to the development of severe concurrent bacterial, fungal and viral infections. The organism has not been reported to infect dogs.

Human granulocytic ehrlichiosis was first described in the USA in 1994[9], and is also present in Europe[97]. It is caused by an unnamed *Ehrlichia* species thought to be transmitted by *I. scapularis* in the USA and *I. ricinus* in Europe[39,103]. It is usually an uncomplicated febrile illness characterised by headache, myalgia, malaise, thrombocytopaenia, leukopaenia, anaemia and elevations of serum hepatic transaminases. Inclusions are not commonly observed in the neutrophils of patients. Dogs are susceptible to experimental[45] and natural[49] infections, with signs being mild and apparently transient. Fever, lethargy, anorexia and thrombocytopaenia are most commonly seen. Morulae can be detected in neutrophils during the acute phase of the infection, and the dogs seroconvert against the antigen. Dogs in Europe[68,101] can be naturally infected with an *Ehrlichia* with an identical 16S rRNA gene sequence to that of the human granulocytic ehrlichiosis agent.

Ehrlichia platys

E. platys is the aetiological agent of infectious canine cyclic thrombocytopaenia[61] which occurs in the USA and the Middle[55] and Far East[28]. Recent studies have shown that *R. sanguineus* is unlikely to be the vector of the *E. platys*[116]. The organism is found in platelets, and high percentages of platelets are infected in the initial parasitaemic episode, which is associated with anorexia, lethargy, lymphadenomegaly and pallor[54,64]. Parasitaemia is associated with a precipitous decline in platelet numbers that is followed by disappearance of the parasites and return of platelet numbers to normal levels within 3–4 days. Parasitaemias and subsequent thrombocytopaenias recur at 1–2-week intervals, but diminish with time, resulting finally in slowly-resolving thrombocytopaenia associated with sporadically-occurring parasites in the blood.

Ehrlichia risticii

Potomac horse fever or equine monocytic ehrlichiosis is caused by *E. risticii,* and has been reported from North America and Europe. Trematodes of *Juga yrekaensis* snails appear to be vectors of the disease[104], and infected horses develop fever, depression, anorexia, diarrhoea and leukopaenia, followed by leukocytosis[40]. Dogs experimentally infected with *E. risticii* showed no clinical signs of infection, but organisms could be re-isolated from some of the dogs, and all dogs seroconverted after infection[111]. Recently, more than 100 cases of naturally-acquired canine ehrlichiosis have been described from the USA with antibodies against *E. risticii* but not against *E. canis* or *E. sennetsu*[70]. Clinical signs reported for 6 of the dogs included fever, lethargy, haematemesis, bleeding tendencies, dependent oedema, neurological dysfunction, polyarthritis, anaemia and thrombocytopaenia. Isolates made from 3 of the dogs had identical 16S rRNA gene sequences to that of *E. risticii.* It has yet to be determined if this organism is in fact *E. risticii* or a caninotropic strain of the organism.

Neorickettsia helminthoeca and *N. elokominicia*

These organisms are responsible for salmon-poisoning disease and fluke fever in dogs, respectively[62]. The diseases occur in the Pacific North West of the USA when dogs eat fish carrying infected metacercaria of the fluke *Nanophyetus salmincola.* Fever, anorexia, vomiting, diarrhoea and weight loss are the main clinical features of the disease, with organisms being detectable in macrophages of most lymph nodes but never in blood smears.

Concurrent infections

It has now been shown that concurrent infections of dogs with *Ehrlichia* species are not uncommon[16,74]. In 1 study in a kennel of 27 dogs that had been chronically infested with ticks, 15 dogs were infected with *E. canis*, 9 with *E. chaffeensis*, 9 with *E. platys*, 8 with *E. ewingii* and 3 with *E. equi.* Two dogs had concurrent infections with 4 *Ehrlichia* species (*E. canis*, *E. chaffeensis*, *E. ewingii* and *E. platys*). Further studies are indicated to determine the relative contributions that *Ehrlichia* species may make to the overall clinical and laboratory abnormalities that may be detected in dogs with concurrent infections. Studies are also indicated to determine the effects of concurrent infections on the diagnosis, treatment and prognosis of affected dogs.

DIAGNOSIS

Accurate diagnosis of canine ehrlichioses is important, as it enables appropriate treatment to be instituted. Further, it may be important to be able to diagnose and treat dogs in the subclinical phase of *E. canis* infections before they develop the severe life-threatening chronic form of the disease. Also, apparently healthy dogs in the subclinical phase of *E. canis* infections should be excluded as blood donors, as they carry organisms in their blood[129] and may serve as sources of infection for blood recipients already compromised by other diseases. Where ehrlichias coexist, it is important to determine the species causing infections, as this may have important therapeutic, prognostic and zoonotic implications[16]. Infections with *E. ewingii*,

E. equi, the agent of human granulocytic ehrlichiosis and *E. risticii* generally result in mild disease, and the organisms appear to be readily eliminated by appropriate therapy. *E. canis* and *E. chaffeensis*, however, cause more severe disease, and may persist in the infected dog despite appropriate therapy[16,76]. In addition *E. chaffeensis*, the agent of human granulocytic ehrlichiosis, *E. ewingii* (Buller) and perhaps *E. canis*[95] are human pathogens, and households with infected dogs may have infected ticks that can transmit the infections to people.

Dogs with ehrlichioses exhibit no pathognomonic clinical or laboratory signs, and further tests are needed for definitive diagnoses. Morulae of *E. canis* and *E. chaffeensis* are indistinguishable and are seldom observed in infected dogs. In a retrospective study, only 4 % of dogs serologically positive for *E. canis* and with clinical and laboratory signs of disease had morulae detectable in blood smears[133]. Although dogs with acute granulocytic ehrlichioses generally have relatively high numbers of neutrophils infected with morulae, differentiating between the infectious agents is not possible by the appearance of the morulae.

Infections with *E. canis* or *E. chaffeensis* can be diagnosed by isolation of organisms from whole blood in tissue culture. While this is a sensitive method of detecting infections, the procedure is time-consuming, costly, and may take as long as 2 months[38,66], which reduces its clinical usefulness. Although a short-term cell-culture isolation technique has been described, using monocyte cultures from the infected dog and which gives results in 4 days[100], the sensitivity and specificity of the test has yet to be determined. Recently, a sandwich enzyme-linked immunosorbent assay has been shown to detect ehrlichial antigens for relatively short and variable periods of time in the plasma of the dogs experimentally infected with *E. canis*[128].

The indirect fluorescent antibody test (IFA) has become the most widely used test for the diagnosis of *E. canis* infections in dogs since it was developed in 1972[110]. In dogs experimentally infected with *E. canis*, reactive antibodies can be detected as early as 2 days after infection[67]. Thereafter, the titres rise and reach peak levels at 2–5 months[15,129], which may persist for long periods. Generally, single titres of 1:20 or above are considered indicative of previous exposure to *E. canis,* while rising antibody titres in consecutive samples indicate a recent infection. Decreasing antibody titres may indicate that the dog has been successfully treated[23,33] or has eliminated the infection[56]. It should be noted that antibody titres against *E. canis* often remain elevated for long periods after the organism has apparently been eliminated from the body[12,15,16,67,96,132] and the test, then, is not reliable in detecting spontaneous elimination or successful treatment of infections.

It has also been shown that antibody titres in sera from naturally-infected dogs can vary considerably depending on the strain of *E. canis* used in the IFA test[16]. Also, antibodies detected in IFAs against *E. canis* are not specific for the organism. Serological cross-reactivity has been described between *E. canis* and other ehrlichias, in particular *E. chaffeensis*[34], *C. ruminantium*[71] and *E. ewingii*[7,118], but also *N. helminthoeca*[108], *E. equi*[68], *E. phagocytophila*[130] and *E. risticii*[111]. It is not possible, therefore, to use IFA results to readily distinguish between infections with ehrlichias, and in particular amongst those of the same genotype.

Similarly, Western blotting does not enable consistent differentiation between infections with *E. canis*, *E. chaffeensis* and granulocytic *Ehrlichia* species[16,37], and has been reported to be most useful for differentiation between acute and chronic *E. canis* infections or in cases where IFA serology is inconclusive[60]. A dot-blot enzyme-linked immunoassay (DBELIA) using purified *E. canis* antigens[26] or a recombinant P30 protein of the organism[93] has been described that is as sensitive as IFA in the detection of antibodies against *E. canis* in experimentally- and naturally-infected dogs. Commercially available DBELIA kits can now be used in-house to detect antibodies reactive with *E. canis.* Also, an enzyme-linked immunosorbent assay (ELISA)[109] has been described that may also become useful in the in-house diagnosis of *E. canis* infections. The DBELIA and ELISA would, however, be expected to have similar limitations to those described above for IFAs.

The diagnosis of canine ehrlichioses by the detection of ehrlichial DNA in blood and tissue samples by PCR amplification is gaining acceptance as an important adjunct to serological testing[16]. There is generally a good correlation between PCR results and those obtained by isolation of organisms into cell culture. In one-step PCRs, primers have been used that can amplify the DNA of all the ehrlichias from blood and tissue samples[66]. Nested PCRs[16,35,57,88,132] improve the sensitivity and specificity of the PCR assay for ehrlichias. In nested PCRs, genus-specific primers are used in the 1st reaction to determine the presence of ehrlichial DNA, while species-specific primers are used in the 2nd reaction to differentiate between the ehrlichias. Further studies have shown that PCR followed by chemiluminescent hybridisation with a complementary internal oligonucleotide probe can detect as little as 30 fg of *E. canis* genomic DNA, the equivalent of approximately 150 *E. canis* organisms[86]. Although PCRs are extremely sensitive and specific in identifying infections with the different *Ehrlichia* species in dogs, their use is currently restricted to research laboratories.

TREATMENT

Treatment of *E. canis* infections is considered to be successful when dogs recover clinically, the haematology and biochemistry values return to normal and the organism can no longer be shown to be present in the body. There are numerous anecdotal reports of the efficacy of antimicrobials in the treatment of *E. canis* infections. Drugs reported to be effective against *E. canis* include doxycycline[124], short and long-acting oxytetracycline[2,123,125], imidocarb dipropionate[3,92], chloramphenicol[42], sulfapyridine[27] and sulfamethazine[81]. Antibiotics reported to be ineffective against *E. canis* include penicillin G[81], streptomycin[125], erythromycin[125] and chloramphenicol[63]. In general, the significance of these reports is difficult to interpret, as in many cases they were based only on clinical improvement of dogs following treatment, and in some cases the disappearance of *E. canis* morulae from blood smears. These changes also occur, however, in dogs that remain infected and progress from the acute to the subclinical phase of the disease.

Tetracyclines

There are now a number of more controlled studies on the efficacy of tetracyclines in the treatment of experimentally- and naturally-acquired *E. canis* infections. Tetracycline therapy has been found to be effective in bringing about the resolution of clinical and laboratory abnormalities and the elimination of *E. canis* in 78 % (36/46) of dogs experimentally infected with the organism and treated under closely controlled experimental conditions[5,15,56,67]. Tetracycline therapy of naturally-infected dogs treated at home was less effective, with only 50 % (207/418) of the dogs responding to treatment[12,16,33,100,121,132]. The efficacy of tetracyclines against *E. canis* is supported by the results of *in vitro* studies, where doxycycline was found to have a rickettsiocidal effect on the organism[20,72]. *In vitro* studies have shown that

rifampacin may also be effective against *E. canis*, although to a lesser extent than doxycycline, while penicillin, gentamycin, co-trimoxazole, chloramphenicol, pefloxacin and erythromycin were found to have no effect on *E. canis*[20].

Tetracyclines have also been reported to be effective against *E. ewingii*[47], the agent of human granulocytic ehrlichiosis[41,49], *E. platys*[28], *E. risticii*[70], *N. helminthoeca*[62] and *N. elokominicia*[62] infections in dogs. Further, they have been reported to be effective in resolving clinical and haematological abnormalities in dogs naturally infected with *E. chaffeensis,* but not to eliminate infections or necessarily lower antibody titres against the organism[16]. Tetracyclines, however, remain the recommended first line of treatment in other animals and people infected with ehrlichias[20,39,107], and have been shown to be effective *in vitro* against *E. chaffeensis*[19], *E. risticii*[107] and *E. sennetsu*[17].

Imidocarb dipropionate

There are conflicting reports on the efficacy of imidocarb dipropionate, a drug used widely in Africa against canine and bovine babesiosis[3,100], in the treatment of *E. canis* infections. Anecdotal reports suggested that the drug is effective against naturally-acquired infections[3,92], but ineffective in experimental infections with *E. canis*[65]. In dogs naturally infected with *E. canis*, 94 % (59/63) were found to be short-term cell-culture-negative 1–2 months after treatment with imidocarb dipropionate[100]. In studies with experimentally-infected dogs imidocarb dipropionate treatment was found to eliminate *E. canis* infections and laboratory signs of infection in one study[84], while in another study treatment with the drug was found to be ineffective in treating experimental infections[122]. Imidocarb dipropionate has also been found to be ineffective in treating natural *E. risticii* and *E. chaffeensis* infections in dogs[74]. *In vitro* studies have also shown that imidocarb dipropionate is ineffective against *E. canis*[72], even when the organism is exposed to very high concentrations of the drug for relatively short periods. It is possible that the successful treatment of *E. canis* with imidocarb dipropionate may require prolonged exposure of the organism to the drug.

Enrofloxacin

In a recent experimental study, oral enrofloxacin at 5 or 10 mg/kg 12-hourly for 21 days was found to be ineffective in eliminating *E. canis* from dogs in the subclinical phase of infection or in correcting thrombocytopaenia in the dogs[89].

Suggestions for the specific treatment of *E. canis* infections

Only tetracyclines and imidocarb dipropionate have proven effective against *E. canis* infections in dogs. Based on the fact that tetracyclines are known to be generally effective against all rickettsias and that they are effective against *E. canis* in most patients, tetracyclines should remain the drug of choice for veterinarians in the treatment of canine ehrlichioses.

Of the tetracyclines, doxycycline is probably the most suitable for use in dogs, as it has higher lipid solubility than the other tetracyclines and it is thus better absorbed from the gastrointestinal tract and penetrates tissues better[115]. Doxycycline, therefore, has a long half-life (12 hours), and can be given at lower doses and less frequently than other tetracyclines, which would be expected to improve owner compliance in administering the drug. Also, doxycycline is less likely to induce vomiting in dogs, which has been reported to be a common side effect of tetracycline HCl therapy[100]. All tetracyclines may stain the dental enamel of young dogs, and the drug should not be given to pregnant bitches or young puppies. Tetracyclines act by inhibiting protein synthesis at the 30S ribosomal subunits of bacteria[59]. For *E. risticii* it has been shown that tetracyclines may act by inhibiting the synthesis of proteins that prevent fusion of the ehrlichia-containing phagosomes with lysosomes[131].

Treatment with doxycycline is recommended at 10 mg/kg orally daily for at least 2–6 weeks. Oxytetracycline and tetracycline HCl are recommended at 22 mg/kg 3 times daily for at least 2–6 weeks. The drugs should be given 2–3 hours before or after feeding.

The efficacy of imidocarb dipropionate in the treatment of *E. canis* infections remains controversial. The drug has, however, been shown to be effective in naturally-infected and experimentally-infected dogs, and is an accepted treatment for cattle infected with *Anaplasma marginale*[87], an organism that is closely related to other ehrlichias infecting dogs. Use of the drug may be most appropriate in dogs that fail to respond to tetracycline therapy or dogs where tetracyclines cannot be used. The available data suggest that for imidocarb dipropionate to be effective there is a need for prolonged exposure of *E. canis* to the drug[72]. Since the drug is known to have a long half-life in animals[4], it is recommended that imidocarb dipropionate be administered at 5–7 mg/kg by intramuscular injection on at least 2 occasions with a 14-day interval. Injection of the drug is painful and results in transient salivation, diarrhea and depression in a large number of dogs[100]. The use of imidocarb dipropionate is less dependent on owner compliance than tetracycline treatment and has the additional advantage that it is also effective against *B. canis*[3,92,100], and concurrent *B. canis* and *E. canis* infections are known to be common in Africa[82]. In *Babesia* infections, imidocarb dipropionate has been reported to act by blocking the entry of inositol, an essential nutrient, into the erythrocytes containing the parasites, apparently resulting in starvation of the parasites[87]. There is no information, however, on how the drug may be effective against *Anaplasma* or other ehrlichias.

Treatment failures

Although there is considerable evidence for the efficacy of tetracyclines in the treatment of *E. canis* infections, veterinarians using the drug will not infrequently be faced with dogs that have persistent clinical or laboratory signs of infection, persistently high antibody titres and/or the persistence of ehrlichial DNA according to PCR. A recent study has shown that an eventual complete response to treatment can be expected in only 45 % of dogs with ehrlichiosis, and treatment failure or incomplete response to treatment may be anticipated in up to 41 % of dogs[46]. There are numerous possible reasons for these treatment failures and incomplete responses including:

- lack of owner compliance in administering the drug at the correct dosage for the correct duration of therapy and not around times of feeding[100];
- dogs vomiting the tetracycline[100];
- continual reinfections of the dogs with *E.* canis in endemic areas[15];
- concurrent diseases may be present that mimic or exacerbate the signs of *E. canis* infections[16];
- dogs being in the chronic phase of *E. canis* infections. Dogs with minimal signs of decreased cellularity of the bone marrow tend to respond to treatment more quickly. Dogs with severely hypoplastic bone marrow have a grave prognosis, as the non-regenerative anaemias, thrombocytopaenias and/or leukopaenias generally take a long time (2–6 months) to resolve, and dogs often succumb to infections or fatal haemorrhage before recovery[23,48,61];
- resistance of ehrlichias to tetracyclines may also play a role, but this has yet to be documented and seems unlikely[20];
- persistence of high antibody titres following the elimination of *E. canis* due to aberrant immune responses[12];
- inefficacy of tetracycline therapy owing to the persistence of *E. canis* in organs

where tetracycline penetration is poor[115];
- concurrent long-term use of immunosuppressive drugs[96];
- persistence of ehrlichial DNA unassociated with viable organisms[15];
- the presence of concurrent infections with other ehrlichia[16,74] unknown factors.

Supportive therapy

Apart from specific therapy against *E. canis*, supportive therapy is also often indicated and is an important factor in the successful treatment of infections. Dehydration should be corrected by the administration of appropriate fluid therapy. In animals with life-threatening, severe anaemia, blood transfusions should be administered. Fresh whole blood or platelet-rich plasma is indicated in dogs with life-threatening haemorrhage. Multiple transfusions may be required before adequate bone-marrow responses occur, and it is important in such cases that crossmatching be performed to prevent transfusion reactions. Vincristine (0.01–0.025 mg/kg) intravenously once a week may be used to increase platelet numbers[61]. In dogs with suppressed bone marrow function, anabolic steroids[61] have been suggested to be of benefit (oral oxymethalone 1 mg/kg 3 times daily or nandralone decanoate 1.0–1.5 mg/kg weekly by intramuscular injection), although such treatments have also been reported ineffective[123]. Iron supplementation may be indicated if blood loss has been chronic and severe and total body iron stores are depleted.

Unless concurrent immune-mediated diseases are confirmed, long-term immunosuppressive drug therapies should be avoided, as they may interfere with complete elimination of *E. canis* infections, exacerbate bleeding and increase the possibility of secondary bacterial infections. Although tetracyclines are broad-spectrum antibiotics, other antimicrobial therapy may be indicated in dogs with secondary bacterial infections. The choice of such antibiotics depends on the results of bacterial culture and sensitivity testing and attention to drug compatibility with tetracyclines.

Short courses of dexamethasone (5–15 mg) or prednisolone (1 mg/kg) have been reported to be successful in controlling initial epistaxis[23,125]. Attempts may also be made to control epistaxis by instilling vasoconstrictive astringents (epinephrine or phenlyephrine) into the nose, applying ice packs to the area and/or light sedation and placing the animal in a cool area. Treatment with vitamin B complex has been reported to have a beneficial effect in overcoming the extreme anorexia seen in dogs with *E. canis* infections[125]. Therapy with levamisole (3.3–10 mg/kg orally once a day for up to 70 days) has been reported to be beneficial in the treatment of dogs with severe pancytopaenia[123]. The rationale for this therapy was the fact that levamisole had been reported to restore polymorphonuclear, macrophage and T-cell functions, especially in hypofunctional cells.

Good supportive care is also indicated in dogs being treated for *E. canis* infections. This includes placing the dog on a high plane of nutrition, avoidance of environmental stress factors and treatment of concurrent diseases.

VACCINE DEVELOPMENT

It has been shown recently that chemically-inactivated *C. ruminantium* organisms derived from tissue culture and used with appropriate adjuvants can provide substantial levels of protection against challenge in cattle, sheep and goats[80]. Preliminary data from trials in Zimbabwe using inactivated *E. canis* organisms indicate that such vaccines may be effective in protecting dogs from infection[79].

CONCLUSIONS

The development of molecular biological techniques and methods for the isolation and growth of ehrlichias in tissue culture has greatly expanded the available knowledge on ehrlichias infecting dogs. This has been the case particularly in the developed countries of the world, and if these techniques could be applied in less developed countries, similar major advances will be made, which will add significantly to the overall understanding of the ehrlichias. Such knowledge will greatly facilitate the diagnosis and effective treatment of canine ehrlichioses until such time as effective vaccines become available.

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