Canine ehrlichioses: an update

P J Kelly

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.


INTRODUCTION
Ehrlichias are Gram-negative bacteria that live within membrane-bound vacuoles in the cytoplasm of cells. 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 shock operons in ruminantium and Ehrlichia.

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 and in Southern Africa in 1938. 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. 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 %), Senegal (53 %), Chad (28 %), Egypt (33 %), Zimbabwe (43 %) and South Africa (42 %). Surveys in Israel have shown an overall serological prevalence of 30 % in dogs, 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.

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 and worldwide. Recent studies have indicated geographic antigenic diversity among E. canis. In particular, sera from naturally-infected dogs in Zimbabwe have antibodies against proteins not recognised by sera from dogs from other countries. Also, considerable variability in the antibody titres of naturally-infected dogs against 3 strains of E. canis has been reported.

E. canis is transmitted transstadially but not transovarially in Rhipicephalus sanguineus, 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, and it has been shown that E. canis can be transmitted transstadially in Dermacentor variabilis, with adults also transmitting the infection to dogs. Attempts to transmit E. canis transstadially and transovarially in Otobius megnini have been unsuccessful. Other canids may be infected with E. canis including wolves, foxes, coyotes, jackals and African wild dogs. 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. Platelet-related bleeding may be observed but this is unusual. 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. This subclinical phase may last for as little as 4 months in experimentally-infected dogs but may persist for up to 10 years in naturally-infected dogs. A significant recent finding is that dogs can spontaneously eliminate E. canis infections during the subclinical phase of the disease. In 1 study 33 % (26) 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. In another study, 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 infected 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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EHRlichia SPECIES INFECTING DOGS
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Previously it has been suggested that naturally-infected dogs (68%; 12/18) in Zimbabwe that were serologically positive byIFA and Western blotting but had no clinical, haematological or biochemical signs of infection may have self-cured. 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. 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%), anaemia (56%), hemorrhagic tendencies, in particular epistaxis (46%), pyrexia (40%) and lymphadenomegaly (30%) are the most commonly-reported clinical signs in the USA. Similar signs have been reported in studies on naturally-infected dogs in Africa. Neuro muscular, reproductive and ocular signs may also occur in naturally-infected dogs. These signs include polyneuropathy, paralytic signs of meningoencephalitis, cranial nerve deficits, seizures, abortions and infertility, corneal opacity, anterior uveitis, hyphema, focal choroidal lesions and retinal detachment. Pulmonary signs including coughing and exercise intolerance may also develop as a result of interstitial lung infiltrates.

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. 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. 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/µl) with an increase in platelet volume suggesting active thrombopoiesis. Other abnormalities that are reported less frequently include anaemia and leucopaenia. The bone marrow is commonly hypercellular in the acute phase of infection.

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%). In experimentally-infected dogs, thrombocytopaenia (13-180 × 10^3/µl) was observed in most cases and mean platelet volumes were increased. While leucopaenia 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%), hypergamaglobulinaemia (68%), increased α1- and α2- (22%) and β-2-globulins (44%) and decreased β-globulins (55%).

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/µl), severe leucopaenia (white cell count <3 × 10^3/µl) and thrombocytopaenia (platelet count <30 × 10^3/µl). In the early stages, bone marrow hyperplasia occurs, but as the disease progresses, the bone marrow becomes hypoplastic.

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%), hyperproteinemia (33%), leucopaenia (31%), leukocytosis (20%), pancytopenia (17%), and regenerative anaemia (15%). 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. Using serum protein electrophoresis, it has been found that most dogs with natural E. canis infections have polyclonal gammapathies, although monoclonal gammapathies may occur. Generally, there are significantly decreased α1-globulins and significantly elevated α2-, β2-globulins and γ-globulins. It has also been found that dogs that were pancytopenic had significantly lower concentrations of total protein, total globulin and γ-globulins, indicating severely compromised immune function.

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. 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 plasma-cytosis and generalised perivascular lymphocyte and plasma cell accumulation. Ehrlichias appear to survive in macrophages by producing proteins that prevent fusion of the phagosomes in which they occur with lysosomes in the cells.

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. 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. Dogs become susceptible to reinfection with E. canis only when existing infections are cleared by appropriate therapy, 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

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

<table>
<thead>
<tr>
<th>Genogroup III</th>
<th>Genogroup II</th>
<th>Genogroup I</th>
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<tbody>
<tr>
<td>E. canis</td>
<td>E. phagocytophila</td>
<td>E. risticii</td>
</tr>
<tr>
<td>E. chaffeensis</td>
<td>E. equi</td>
<td>Neorickettsia helminthoea</td>
</tr>
<tr>
<td>E. ewingii</td>
<td>Human granulocytic Ehrlichia</td>
<td>N. elokominica</td>
</tr>
<tr>
<td>Cowdria ruminantium</td>
<td>E. platis</td>
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</tr>
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</table>

probably play an important role\textsuperscript{112}. German shepherd dogs are known to show more severe disease when infected with \textit{E. canis} and laboratory studies have indicated that infections of these dogs cause specific and non-specific suppression of their cell-mediated immune responses\textsuperscript{25,38}. Anaemia in dogs with \textit{E. canis} infections results from haemorrhage and/or bone marrow suppression. Although erythropagocytosis is prominent in the lymph nodes, this is not a feature in other organs, and the erythropagocytosis is thought to result from haemorrhage rather than sensitisation of red blood cells\textsuperscript{39}. Positive Coombs' tests, however, have been reported to occur in up to 27% of dogs with the disease\textsuperscript{40}. 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\textsuperscript{41}. The hypergammaglobulinaemia that is commonly found in dogs with \textit{E. canis} infections is not due to antibodies against \textit{E. canis}, and infections may result in non-specific antibody production\textsuperscript{57,112,113}. While the prolonged antigenic stimulation associated with \textit{E. canis} infections may result in an exaggerated and aberrant humoral immune response\textsuperscript{42}, it has also been suggested that the hypergammaglobulinaemia may represent the development of a secondary autoimmune response to damaged host cell components\textsuperscript{25}.

Hypoalbuninaemia appears not to be due to renal losses, as glomerulonephritis is not common in dogs with \textit{E. canis} infections\textsuperscript{63,113}. It may, however, result from haemorrhage, vasculitis and oedema, increased catabolism of the protein during pyrexia and/or decreased production to compensate for the oncostic affects of the hypergammaglobulinaemia\textsuperscript{64}.

While haemorrhage is not uncommon in dogs with \textit{E. canis} infections, the severity of the haemorrhage does not always correlate with the platelet count in the dog\textsuperscript{57,113}. 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\textsuperscript{113}. Evidence is now accumulating that haemorrhage in dogs with \textit{E. canis} infections results from platelet dysfunction. Sera of dogs with acute \textit{E. canis} infections contain factors that prolong platelet aggregation\textsuperscript{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\textsuperscript{99}.

Thrombocytopenia is the commonest laboratory abnormality in dogs with \textit{E. canis} infections, and there are numerous possible causes for this abnormality. In the chronic phase of infection, thrombocytopenia is most often due to bone marrow hypoplasia\textsuperscript{63}. Consumption of platelets due to vasculitis appears an unlikely cause of thrombocytopenia, as thrombosis, endothelial cell hypertrophy and vasculitis, as seen in other rickettsial infections, are not commonly observed in \textit{E. canis} infections\textsuperscript{63,112}. Another possible cause of thrombocytopenia is the production of a platelet migration inhibition factor that enhances platelet sequestration, particularly in the spleen\textsuperscript{1}. Further, antiplatelet antibodies occur in the acute phase of \textit{E. canis} infection\textsuperscript{53,127}, and the half-life of platelets is decreased and associated with increased platelet destruction by the macrophages of the spleen\textsuperscript{117}. Recent experiments have shown that the spleen plays an important role in the pathogenesis of \textit{E. canis} infections, with splenectomised dogs having less severe clinical signs and laboratory abnormalities than intact dogs\textsuperscript{44}. In naturally-infected dogs, \textit{E. ewingii} is commonly seen in circulating neutrophils in the 1st week of infection\textsuperscript{118}. Signs of infection are generally far milder than those classically associated with \textit{E. canis} infections, and include suppurative polyarthritis in 1 or more limbs, acute lameness, muscular stiffness, lethargy, mild fever and thrombocytopenia\textsuperscript{55,64,74,119}. Although the organism has yet to be grown in tissue culture and serological assays are not readily available, cross-reactivity between antibodies against \textit{E. ewingii} and \textit{E. canis} has been reported\textsuperscript{1}, and antibodies against \textit{E. ewingii} have been shown to react with high molecular mass proteins (>40 kDa) of \textit{E. canis} in Western blots\textsuperscript{119}. Similar reactions have been reported with a serum sample from a dog in Zimbabwe\textsuperscript{120}.

\textbf{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 \textit{A. americanum}. It may also be transmitted by \textit{D. variabilis}\textsuperscript{145}, and has been demonstrated in \textit{R. sanguineus}\textsuperscript{146}. In naturally-infected dogs, \textit{E. ewingii} is commonly seen in circulating neutrophils in the 1st week of infection\textsuperscript{118}. Signs of infection are generally far milder than those classically associated with \textit{E. canis} infections, and include suppurative polyarthritis in 1 or more limbs, acute lameness, muscular stiffness, lethargy, mild fever and thrombocytopenia\textsuperscript{55,64,74,119}. Although the organism has yet to be grown in tissue culture and serological assays are not readily available, cross-reactivity between antibodies against \textit{E. ewingii} and \textit{E. canis} has been reported\textsuperscript{1}, and antibodies against \textit{E. ewingii} have been shown to react with high molecular mass proteins (>40 kDa) of \textit{E. canis} in Western blots\textsuperscript{119}. Similar reactions have been reported with a serum sample from a dog in Zimbabwe\textsuperscript{120}.

\textbf{Cowdria ruminantium}

\textit{C. ruminantium} is the agent of heart-water in domestic ruminants that occurs widely in Africa and is also present in the Caribbean Islands\textsuperscript{121}. It is transmitted by \textit{A. mylomma} spp. and causes neurological and respiratory signs associated with peracute mortalities\textsuperscript{122}. While natural infections of dogs with \textit{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\textsuperscript{147}. There is serological cross-reactivity between \textit{E. canis} and
C. ruminantium, and dogs infected with C. ruminantium are positive in IFA and Western blots against E. canis. 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. There are only a few, if any, nucleotide differences in the sequences of the 16S rRNA gene and the groEL heat shock operon in organisms isolated from people, dogs and horses around the world. Also, results of cross-infection and cross-protection studies have shown a close relationship between the organisms in the group. Serology demonstrates broad cross-reactivity among members of the group, providing further evidence that the members of this group may be identical species.

*E. equi* is the agent of equine granulocytic ehrlichiosis, which has been reported from North and South America and Europe. 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 oedema, and ataxia. Laboratory abnormalities include thrombocytopenia, leucopaenia, hyperbilirubinaemia and high percentages of parasitised granulocytes. Dogs have been experimentally infected with *E. equi*, and natural infections with this or a very closely related organism have been reported in dogs in the USA and Europe. Dogs experimentally infected with *E. equi* show no clinical signs or mild pyrexia, and there may be transient thrombocytopenia and mild anaemia. Morulae can be detected in neutrophils during the acute phase of the infection, and the dogs seroconvert against the antigen. Dogs in Europe 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 thrombocytopenia, which occurs in the USA and the Middle East and Far East. Recent studies have shown that *R. sanguinis* is unlikely to be the vector of the *E. platys*. The organism is found in platelets, and high percentages of platelets are infected in the initial parasitaemic episode, which is associated with anorexia, leukaemia, lymphadenomegaly and palor. 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 thrombocytopenias recur at 1–2-week intervals, but diminish with time, resulting finally in slowly-resolving thrombocytopenia 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 yrykaensis* snails appear to be vectors of the disease, and infected horses develop fever, depression, anorexia, diarrhoea and leukopaenia, followed by leukocytosis. 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. 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*. Clinical signs reported for 6 of the dogs included fever, leukaemia, haemorrhage, bleeding tendencies, dependent oedema, neurological dysfunction, polyarthritis, anae mia and thrombocytopenia. 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. elokominicola**

These organisms are responsible for salmon-poisoning disease and fluke fever in dogs, respectively. The diseases occur in the Pacific North West of the USA when dogs eat fish carrying infected metacercaria of the fluke *Nauphoetus 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. In 1 study in a kennel of 27 dogs that had been chronically infected 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 and may serve as sources of infection for blood recipients already compromised by other diseases. Where ehrlichiosis coexists, it is important to determine the species causing infections, as this may have important therapeutic, prognostic and zoonotic implications. 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. chaffensis, however, cause more severe disease, and may persist in the infected dog despite appropriate therapy. In addition E. chaffensis, the agent of human granulocytic ehrlichiosis, E. ewingii (Buller) and perhaps E. canis 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. chaffensis 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. 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. chaffensis 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, 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, the sensitivity and specificity of the test has yet to be determined. Recently, a sandwich enzyme-linked immunosorbent assay (DBELIA) 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.

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. In dogs experimentally infected with E. canis, reactive antibodies can be detected as early as 2 days after infection. Thereafter, the titres rise and reach peak levels at 2-5 months, 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 or has eliminated the infection. 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. The reduction in titre of antibodies following therapy may indicate that the organism no longer persists in the dog, or that the test, and the infection was not treated. Decreasing antibody titres may not be 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. Also, antibodies detected in IFAs against E. canis are not specific for the organism. Serological cross-reactivity has been described between B. canis and other ehrlichias, in particular E. chaffensis, C. ruminantium and E. ewingii. 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. chaffensis and granulocytic Ehrlichia species, 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. A dot-blot enzyme-linked immunosorbent assay (DBELIA) using purified E. canis antigens or a recombinant P30 protein of the organism 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) 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. 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. Nested PCRs improve the sensitivity and specificity of the PCR assay for ehrlichias. In nested PCRs, species-specific primers are used in the 1st reaction to detect the presence of ehrlichial DNA, while species-specific

**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 doxycline, short and long-acting oxytetracycline, imidocarb dipropionate, chloramphenicol, sulfapyridine and sulfamethazine. Antibiotics reported to be ineffective against E. canis include penicillin G, streptomycin, erythromycin and chloramphenicol. 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. Tetracycline therapy of naturally-infected dogs treated at home was less effective, with only 50% (20/41) of the dogs responding to treatment. The efficacy of tetracyclines against E. canis is supported by the results of in vitro studies, where doxycycline was found to have a rickettsicidal effect on the organism. In vitro studies have shown that
rifampicin may also be effective against E. canis, although to a lesser extent than doxycycline, while penicillin, gentamicin, clindamycin, pefloxacin and erythromycin were found to have no effect on E. canis.

Tetracyclines have also been reported to be effective against E. chaffeensis, the agent of human granulocytic ehrlichiosis, E. phylloides, E. risticii, N. helminthoeca and N. caninum infections in dogs. Further, they have been reported to be effective in resolving clinical and haematological abnormalities in dogs naturally infected with E. canis, but not to eliminate infections or necessarily lower antibody titres against the organism. Tetramycyclines, however, remain the recommended first line of treatment in other animals and people infected with ehrlichiae, and have been shown to be effective in vitro against E. chaffeensis, E. risticii and E. caninum infections.

**Imidocarb dipropionate**

There are conflicting reports on the efficacy of imidocarb dipropionate, a drug used widely in Africa against canine and bovine babesiosis, in the treatment of E. canis infections. Anecdotal reports suggested that the drug is effective against naturally-acquired infections, but ineffective in experimental infections with E. canis. 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. In studies with experimentally-infected dogs, imidocarb dipropionate treatment was found to eliminate E. canis infections and laboratory signs of infection in one study, while in another study treatment with the drug was found to be ineffective in treating experimental infections. Imidocarb dipropionate has also been found to be ineffective in treating natural E. risticii and E. chaffeensis infections in dogs. In vitro studies have also shown that imidocarb dipropionate is ineffective against E. canis, 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 thrombocytopenia in the dogs.

**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. 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. 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. 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.

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, 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 that are allergic to tetracyclines or have concurrent infections. The available data suggest that for imidocarb dipropionate to be effective there is a need for prolonged exposure of E. canis to the drug.

Since the drug is known to have a long half-life in animals, 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. 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, and concurrent B. canis and E. canis infections are known to be common in Africa. 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. There is no information, however, on how the drug may be effective against A. phagocytophilum 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. 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;
- dogs vomiting the tetracycline;
- continual reinfections of the dogs;
- concurrent diseases may be present that mimic or exacerbate the signs of E. canis infections;
- 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, thrombocytopenias and/or leucopaenias generally take a long time (2-6 months) to resolve, and dogs often succumb to infections or fatal haemorrhage before recovery.
- resistance of ehrlichias to tetracyclines may also play a role, but this has yet to be documented and seems unlikely;
- persistence of high antibody titres following the elimination of E. canis due to aberrant immune responses;
- inefficacy of tetracycline therapy owing to the persistence of E. canis in organs.
where tetracyclines are poorly absorbed,
• concurrent long-term use of immuno-
  suppressive drugs,
• persistence of ehrlichial DNA unassocia-
  ted with viable organisms,
• the presence of concurrent infections with other ehrlichia,

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. Dehy-
dration should be corrected by the admin-
istration of appropriate fluid therapy. In
animals with life-threatening, severe anae-
mia, blood transfusions should be admin-
istered. 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 pre-
vent transfusion reactions. Vincristine
(0.01-0.025 mg/kg) intravenously once a
week may be used to increase platelet
numbers. In dogs with suppressed bone
marrow function, anabolic steroids have
been suggested to be of benefit (oral
oxymethalone 1 mg/kg 3 times daily or
nandralone decanoate 1.0–1.5 mg/kg
3 times daily or oxymethalone 1 mg/kg
3 times daily or 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. The
rationale for this therapy was the fact
that levamisole had been reported to restore
polymorphonuclear, macrophage and
T-cell functions, especially in hypo-
functional 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 treat-
mant of concurrent diseases.

VACCINE DEVELOPMENT
It has been shown recently that chemi-
cally-inactivated C. ruminantium organ-
isms derived from tissue culture and used
with appropriate adjuvants can provide
substantial levels of protection against
challenge in cattle, sheep and goats.

Preliminary data from trials in Zimbabwe
using inactivated C. canis organisms indi-
cated that such vaccines may be effective
in protecting dogs from infection.

CONCLUSIONS
The development of molecular biolog-
ic techniques and methods for the iso-
lation and growth of ehrlichias in tissue
culture has greatly expanded the avail-
able 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 effec-
tive treatment of canine ehrlichioses until
such time as effective vaccines become
available.

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