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PUPS, PCRs AND PLATELETS*: EHRlichia AND anaplasma infECTIONS OF DOGS IN AUSTRALIA AND OVERSEAS
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TAXONOMY AND MOLECULAR PHYLOGENY

The currently accepted taxonomic convention places the ‘rickettsial’ organisms of humans and animals into one of two large families, the Rickettsiaceae and the Anaplasmataceae. Within these families there has been considerable reorganisation and renaming of individual species based on the phylogenetic analysis of the bacterial 16S rRNA and groESL genes (1). The Anaplasmataceae comprises four genera containing many organisms of veterinary significance; Ehrlichia spp., Anaplasma spp., Wolbachia spp. and Neorickettsia spp.; the Rickettsiaceae comprises just two genera, Rickettsia and Orientia, of more clinical significance in humans.

<table>
<thead>
<tr>
<th>Rickettsial Species known to infect Dogs</th>
<th>Family</th>
<th>Genus</th>
<th>Species (*)</th>
<th>Predominant Cell Tropism</th>
<th>Vector Type</th>
<th>Vector Species</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ehrlichia</td>
<td>E. canis (N)</td>
<td>Monocyte</td>
<td>R. sanguineus D. variabilis</td>
<td>Worldwide (not Australia)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>E. chaffeensis (N/R)</td>
<td>Monocyte</td>
<td>Many</td>
<td>United States</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>E. ewingii (N/R)</td>
<td>Neutrophil</td>
<td>A. americanum</td>
<td>US, Africa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaplasma</td>
<td>A. platys (N)</td>
<td>Platelet</td>
<td>R. sanguineus</td>
<td>Worldwide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A. phagocytophilum (I)</td>
<td>Neutrophil &amp; Endothelium</td>
<td>Ixodes spp.</td>
<td>N hemisphere</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Wolbachia</td>
<td>W. pipiens</td>
<td>Arthropod and filarial nematode cells</td>
<td>Wolbachia, transovarially-transmitted endosymbionts in many animals act as parasites (in arthropods) or mutualists (in nematodes). Potentially of veterinary importance, especially the treatment of heartworm.</td>
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<tr>
<td>Neorickettsia</td>
<td>N. helminthoeca (N)</td>
<td>Monocyte</td>
<td>Nanophytes salmincola</td>
<td>United States</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>N. risticii (E)</td>
<td>Monocyte</td>
<td></td>
<td>United States &amp; Canada</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N. sennetsu (E)</td>
<td>Monocyte</td>
<td></td>
<td>Eastern Asia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rickettsia</td>
<td>R. rickettsii (I)</td>
<td>Endothelium</td>
<td>R. sanguineus, Dermacentor &amp; Amblyomma</td>
<td>N &amp; S America</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>R. conorii (R)</td>
<td>Haematophagous arthropods (many)</td>
<td></td>
<td>Europe &amp; Asia</td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td>R. felis (R)</td>
<td></td>
<td></td>
<td>Worldwide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R. japonica (R)</td>
<td></td>
<td></td>
<td>Japan</td>
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<tr>
<td>Orientia</td>
<td>O. tsutsugamushi (I)</td>
<td>Endothelium</td>
<td>Leptotrombidium spp.</td>
<td>Australasia</td>
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</tbody>
</table>

Species highlighted in bold are clinically significant in dogs. [* N= dog is the natural host, R = dog is a reservoir host, I = dog is incidental host, E = infection in dogs established only experimentally]

Although R. rickettsii, the cause of Rocky Mountain Spotted Fever (RMSF) and N. helminthoeca, the cause of Salmon poisoning disease are clearly important pathogens for dogs, this abstract is concerned primarily with the genera Ehrlichia and Anaplasma.

* I am indebted to Dr Graeme Brown for the original title.
OVERVIEW and ZOONOTIC ASPECTS

*Ehrlichia* and *Anaplasma* are gram-negative obligate intracellular bacteria that lack lipopolysaccharide endotoxins, rely on tick vectors for transmission, and show distinct tropism for different blood cell types (predominantly leucocytes and platelets) and endothelial cells. *Ehrlichia canis* was the first recognised in dogs and achieved notoriety between 1965-1975 as a cause of epizootic losses of military dogs imported from the US and Europe into Singapore, Malaysia, Thailand and Vietnam. Since then other species of *Ehrlichia* and *Anaplasma* that cause disease in dogs have been recognised but *E. canis* remains the most studied and arguably the most significant from a worldwide pathogenic perspective. Among those species described more recently are several that are also capable of infecting humans, notably *E. chaffeensis* (human monocytic ehrlichiosis, HME), *E. ewingii* (human granulocytic ehrlichiosis, HGE) and *A. phagocytophilum* (human granulocytic anaplasmosis). The latter two species both infect neutrophils and cannot be differentiated morphologically, requiring molecular techniques for identification. *Ehrlichia canis* is generally considered not to be zoonotic, yet there has been considerable interest in a closely related organism isolated from humans in South America (2). *E. ewingii* has so far been reported from the US and Africa, *E. chaffeensis* from N America and eastern Asia, and *A. phagocytophilum* has been discovered in a wide range of mammalian host species, including humans, and is regarded as an emerging zoonotic pathogen in Europe and Asia.

EHRLICHIAL INFECTION IN DOGS

*Ehrlichia canis* – Canine Monocytic Ehrlichiosis

*Ehrlichia canis* owes its worldwide (tropical and subtropical) distribution to the ubiquity of its vector, the brown dog tick (*R. sanguineus*). For reasons that are unclear however, CME never established in Australia (*R. sanguineus* is widespread throughout northern and central Australia) and its introduction is now prevented by stringent pre-import and quarantine serological testing.

Based upon experimental studies CME has been divided into three phases; acute, subclinical and chronic, although in the clinical setting the stage of infection can rarely be assessed with certainty. Furthermore, the clinical severity is highly variable between individuals due to factors innate to the dog (such as heritable idiosyncratic or breed susceptibilities) and the strain (virulence) of the organism itself. Regardless, acute and chronic infections are usually associated with non-specific illness (lethargy, anorexia, fever and weight loss), lymphadenopathy, oedema uveitis and bleeding tendencies that reflect a primary haemostatic dysfunction (epistaxis, petechial and ecchymotic haemorrhages). In acute disease these signs are self-limiting over several weeks and/or treatment-responsive, but are significantly more debilitating and associated with a terminal bone marrow failure and overwhelming secondary bacterial infections in the chronic phase of disease. Haematological and serum biochemistry abnormalities include thrombocytopenia, anaemia and leucopenia (pancytopenia), hypoalbuminaemia and hyperglobulinaemia (typically a polyclonal gammopathy but occasionally a monoclonal gammopathy has been reported).

The pathogenesis of CME is associated with dysregulation of the immune system rather than overt immunosuppression, as reported in a recent study (3). Immune-mediated vasculopathy, platelet dysfunction and glomerulonephritis underpin the systemic signs; in vitro studies have revealed down regulation of MHC Class II receptors of the mononuclear cells and alteration in the CD4+:CD8+ lymphocyte subset ratios. In addition, an exuberant yet non-protective antibody response is well documented that in itself contributes to the immunopathologic consequences of *E. canis* infection. However immunocompetent dogs, better able to mount an effective cell-mediated immune response,
may eliminate the infection or otherwise may remain subclinical carriers for life. Laboratory diagnosis of CME is confounded by several important aspects. Microscopic identification of bacterial morulae is limited to acute cases, yet at best is a test with a low sensitivity. Positive serological tests suggest exposure but do not necessarily confirm active disease and this is especially relevant in endemic regions since post-exposure antibody response is long lasting and where multiple co-infections (e.g. Babesia spp., Bartonella spp. and other ‘rickettsial’ organisms) occur. In general the commercially available ELISA-based diagnostic tests perform well when the patient’s serum contains high levels of E. canis antibodies, yet their sensitivity and specificity were reported to be low in sera having low (<1:320) IFA titres (4,5). Ehrlichia canis cross reacts with E. chaffeensis and to a lesser extent with E. ewingii, but not with R. rickettsii, Babesia, A. platys or A. phagocytophilum. PCR amplification and gene sequencing can identify the ehrlichial species involved, but its clinical application is still limited at the current time.

Doxycycline (5mg/kg q12h PO) or tetracycline (22mg/kg q8h PO) administered for 4 weeks is effective in eliminating E. canis infection. Dramatic clinical improvements are often noted in dogs with acute infections. Several other antimicrobials have been recommended in the past, including imidocarb dipropionate, enrofloxacin and sulphonamides, but none has been shown to be reliably efficacious.

**Ehrlichia chaffeensis** – Human Monocytic Ehrlichiosis

Prior to the advent of molecular diagnostic techniques the discovery of morulae within the monocytes of humans with non-specific illness led to the opinion that E. canis was zoonotic. The 16S rRNA gene sequence from such isolates was determined to be different (98.2% similarity) to E. canis giving rise to a new species E. chaffeensis (6). Ehrlichia chaffeensis is now recognised to cause a clinical spectrum of illness in humans that varies from mild, ‘flu-like symptoms to a fulminant sepsis syndrome (in immunocompromised individuals), but in most patients is self-limiting and not fatal. Ehrlichia chaffeensis has been recovered from a range of mammalian hosts (deer, dogs, coyotes, goats) and many species of tick in the US, and more recently was isolated from ticks and humans in Korea (7,8), but has not been found in Australia. Dogs with E. chaffeensis show similar clinical signs to CME that may be severe and there is evidence that E. chaffeensis may not respond to doxycycline as well as E. canis does (9). Serological cross-reactions between E. canis and E. chaffeensis mean that differentiation of the two is not possible without molecular techniques; it is possible that E. chaffeensis is more widespread as a cause of canine illness than is currently recognised.

**Ehrlichia ewingii** – Human Granulocytic Ehrlichiosis

As with E. chaffeensis, E. ewingii has been reported mainly in the US. In the most comprehensive study of E. ewingii infection in dogs to date (10) the authors reported that fever and lameness were the most common clinical findings. Thrombocytopenia is the predominant laboratory finding and morulae are occasionally detected within neutrophils (indistinguishable from A. phagocytophilum). Dogs may be asymptomatic and it is currently unclear whether they are the definitive host or a reservoir host for human infection. Ehrlichia ewingii infections respond to doxycycline therapy.
ANAPLASMA INFECTION IN DOGS

*Anaplasma platys* – Canine Infectious Cyclic Thrombocytopenia

*Anaplasma* (formerly *Ehrlichia*) *platys* was first described in the US and is now considered to have a worldwide distribution. It was reported in dogs living in central Australia in 2001 (11) and since then has been isolated from canine blood samples in most Australian states and territories (unpublished data). There is limited epidemiological information available but it is presumed that *R. sanguineus* is the vector for *A. platys*, although mechanical transmission by lice may also occur (12) where dogs have heavy ectoparasite burdens. The pathogenicity of *A. platys* is generally considered to be mild, resulting in a mild-moderate thrombocytopenia and low grade bleeding tendency, noted especially during surgical procedures. Concurrent infection with other vector-borne pathogens is common. In central Australia a recent study reported that 51% *A. platys*-infected dogs and 72% of dogs co-infected with *A. platys* and *Babesia canis vogeli* were thrombocytopenic (13). Furthermore, some clinical reports suggest more virulent strains may exist in Europe and the Middle East. Intra-platelet morulae are extremely difficult to find and there is no specific *A. platys* test commercially available, so diagnosis relies upon PCR with various modifications (14, 15). Treatment is with doxycycline.

*Anaplasma phagocytophilum* – Human Granulocytic Anaplasmosis

*Anaplasma phagocytophilum* has a wide host range, an expanding distribution and is considered to be one of the most significant emerging pathogens of humans and animals in northern Europe and Asia today. Its ex- and current synonyms also form an impressive list; originally named *Ehrlichia equi* and *Ehrlichia phagocytophila* before the taxonomic reclassification (1), the organism was referred to as the cause of equine and canine granulocytic ehrlichiosis, but is now best described as the cause of granulocytic anaplasmosis in these species and humans so as to avoid confusion with *E. ewingii*.

*Anaplasma phagocytophilum* is vectored by *Ixodes* spp. ticks, so its geographical range and epidemiology closely resembles that of *Borrelia* spp. the cause of borreliosis. There are currently no reports of *A. phagocytophilum* from the southern hemisphere. *Anaplasma phagocytophilum* infection is a cause of generalised lethargy and lameness associated with non-specific clinical signs of fever, lymphadenomegaly and stiffness, and has been isolated from the blood in dogs with polyarthritis and meningitis. As with many rickettsial infections, thrombocytopenia with or without bleeding tendencies is the most common laboratory abnormality reported, but more severe haematological derangements including haemolytic anaemia and disseminated intravascular coagulation have been reported (16). Diagnosis may be confirmed in the acute stages by visualisation of morulae within granulocytes, but is usually made in chronic disease in endemic areas on the basis of clinical signs and positive serological results. PCR confirms the diagnosis and allows for speciation as well. Most dogs respond well to doxycycline therapy.

REFERENCES