Ehrlichia ewingii sp. nov., the Etiologic Agent of Canine Granulocytic Ehrlichiosis

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The 16S rRNA gene was amplified, cloned, and sequenced from the blood of two dogs that were experimentally infected with the etiologic agent of canine granulocytic ehrlichiosis. The 16S rRNA sequence was found to be unique when it was compared with the sequences of other members of the genus Ehrlichia. The most closely related species were Ehrlichia canis (98.0% related) and the human ehrlichiosis agent (Ehrlichia chaffeensis) (98.1% related); all other species in the genus were found to be phylogenetically much more distant. Our results, coupled with previous serologic data, provide conclusive evidence that the canine granulocytic ehrlichiosis agent is a new species of the genus Ehrlichia that is related to, but is distinct from, E. canis and all other members of the genus. We propose the name Ehrlichia ewingii sp. nov.; the Stillwater strain is the type strain.

Ehrlichia canis, the type species of the genus Ehrlichia, was first described by Donatién and Lestoquard in 1935 (7). The first case of canine ehrlichiosis in the United States was described by Ewing in 1963 and was complicated by a concurrent Babesia canis infection (8). Although the clinical manifestations of canine ehrlichiosis depend in part on the breed of dog, the illness is considered severe (11). One of the characteristic features of acute canine ehrlichiosis is the presence of Ehrlichia canis in lymphocytes and monocytes. In 1971, Ewing et al. described a new strain of Ehrlichia canis that was found in granulocytes (primarily neutrophils) and produced a mild form of canine ehrlichiosis (10). This organism has subsequently been referred to as the canine granulocytic Ehrlichia (CGE), and the corresponding disease is called canine granulocytic ehrlichiosis (16). Electron microscopy has revealed that the CGE is found in infected cells as inclusions (termed morulae) that are characteristic of the genus Ehrlichia (8a). The morulae consist of small numbers of pleomorphic individual cells surrounded by a host cell membrane. Serologic cross-reactivity between the CGE and Ehrlichia canis has been demonstrated, but the two organisms can be distinguished from each other and from Ehrlichia equi by the results of quantitative immunofluorescent assays (9). In addition, an enzyme-linked immunosorbent assay and an immunoblot analysis have shown that the CGE and Ehrlichia canis are readily differentiated (14). We amplified, cloned, and sequenced the 16S rRNA gene from the blood of two dogs that were infected with the CGE and compared the resulting 16S rRNA sequence with the sequences obtained previously for all other currently recognized species in the genus Ehrlichia (1). We also compared the sequence obtained from CGE-infected dog blood with the sequence of "Ehrlichia platys," an Ehrlichia sp. that is known to infect platelets in dogs (15). The taxonomic relationship between the CGE and the other ehrlichiae, including the recently proposed species thought to be the agent of human ehrlichiosis (Ehrlichia chaffeensis) (1) is discussed below.

The passage history of the CGE used in this study is described below. A field case of canine granulocytic ehrlichiosis was diagnosed at Boren Veterinary Medical Teaching Hospital, Oklahoma State University, Stillwater, on 18 June 1987. Whole blood from this dog was transfused into a susceptible dog, and three additional passages were performed in an identical fashion. In 1988 and 1989 two successive passages were made via tick feeding (2). Specifically, Amblyomma americanum nymphs were exposed to infected dogs, and the resulting adult ticks transmitted the agent to susceptible dogs. Blood from one of these dogs was used for two subsequent independent passages via blood transfusion at two different locations. The first passage line occurred at Oklahoma State University with two dog-to-dog passages. Blood from the second dog that was infected was collected in the presence of EDTA and used as a source of template DNA in the polymerase chain reaction (PCR). The second, independent passage line occurred at the University of Georgia with three successive dog-to-dog transmissions; blood from the third dog that was infected was used as an additional source of template DNA for the PCR.

Blood specimens from a dog infected with "Ehrlichia platys" were kindly provided by Richard Corstvet, Louisiana State University, Baton Rouge. DNA was extracted from each of the blood samples and PCR amplified as previously described (17). Briefly, two pairs of universal primers that are known to amplify more than 90% of the bacterial 16S rRNA gene as two separate products (21) were used together with a third more specific pair of primers (1) to prime amplification. The DNA extracted from the CGE-infected dog blood was amplified for three cycles consisting of 94°C for 1 min, 48°C for 2 min, and 66°C for 1.5 min; this was followed by amplification for 37 cycles consisting of 88°C for 1 min, 52°C for 2 min, and 68°C for 1.5 min. The 733-bp PCR product which was obtained when universal primers EC9 and EC10 were used to amplify DNA extracted
The phylogenetically most closely related species is *Ehrlichia chaffeensis* Arkansas. The 16S rRNA sequence of the CGE falls into a group within the genus *Ehrlichia* that consists of *Ehrlichia canis* (type species) and *Ehrlichia chaffeensis*. This group of three species is phylogenetically very distant from the *Ehrlichia risticii-Ehrlichia sennetsu* group (1) and somewhat more closely related to the *Ehrlichia phagocytophila-Ehrlichia equi* group (1). The 16S rRNA gene sequence of "*Ehrlichia platys" is most closely related to the *Ehrlichia phagocytophila-Ehrlichia equi* group sequences and is clearly distinct from the sequence of the CGE. The 16S rRNA gene sequencing results provide genetic evidence that the CGE is a unique member of the genus *Ehrlichia* which deserves species level recognition.

The International Committee on Systematic Bacteriology suggested the following definition of a species: "a species is any naturally occurring grouping of nucleic acid sequences that is well demarcated and recognizable from other such groupings by the degree of homology among its members.

Species and/or strain | % Relatedness to *Ehrlichia ewingii*
---|---
*Ehrlichia chaffeensis* Arkansas | 98.1
*Ehrlichia canis* Florida | 98.0
*Ehrlichia equi* | 92.4
*Ehrlichia phagocytophila* OS | 92.4
"*Ehrlichia platys" | 91.7
*Ehrlichia sennetsu* Miyayama | 84.5
*Ehrlichia risticii* Illinois | 83.9

* a The strain sources and 16S rRNA sequences have been described previously (1).

b Data from reference 20.

Additional phenotypic evidence of differences between the CGE and other members of the genus *Ehrlichia* has been reported previously (9, 14, 16). The CGE differs from...
Ehrlichia canis in that it exhibits a preference for granulocytes instead of mononuclear cells as the host cells that are most frequently infected (Fig. 2). The initial (acute-phase) disease produced by the CGE in dogs appears to be milder than classic canine ehrlichiosis caused by Ehrlichia canis, and the chronic phase also differs in nature (2-5, 10). Specifically, transient fever, ocular nasal discharges, lymphadenomegaly, and thrombocytopenia are manifestations that are observed with acute CGE infections. With acute Ehrlichia canis infections, the same abnormalities are usually observed but are more severe, and the hematologic finding is pancytopenia. Peripheral edema, lymphadenomegaly, and suppurative polyarthritis are the most consistent clinical findings with chronic CGE infections. Abnormal findings of leukocytosis, lymphopenia, and thrombocytopenia are variable. In contrast, chronic Ehrlichia canis infections are characterized by debilitation, lymphadenomegaly, fever, hemorrhagic diathesis, and signs referred to as meningoencephalomyelitis. Associated abnormal findings include pancytopenia, hyperglobulinemia, bone marrow hypoplasia, and plasmacytosis of many tissues, most commonly involving the lungs, brain, meninges, kidneys, lymph nodes, and spleen. Serologic data have also shown that the CGE is antigenically related but distinct from other ehrlichiae (9, 10). Thus, the phenotypic observations coupled with the unique 16s rRNA gene sequence provide compelling evidence that a separate species is warranted for the CGE.

Previous attempts by workers in our laboratory to cultivate CGE in various cell lines has failed. Among the cell lines which have been used are cell lines DH82 (a canine macrophage cell line), HL-60 (a human promyelocytic leukemia cell line), CLN (a continuous canine lymph node cell line), and primary canine monocytes. Two of these cell types, cell lines DH82 and primary canine monocytes, have been used successfully to cultivate Ehrlichia canis in vitro. Thus, the CGE and other granulocytic ehrlichiae may be difficult or impossible to cultivate in vitro.

Description of Ehrlichia ewingii sp. nov. Ehrlichia ewingii (ew.in’gi.i N.L. gen. n. ewingii, in honor of Sidney A. Ewing for his pioneering work with this agent). Exhibits all of the characteristics of the genus Ehrlichia (i.e., cytoplasmic growth that appears as compact clusters of organisms termed morulac [Fig. 2] and serologic cross-reactivity with Ehrlichia canis). Ehrlichia ewingii is distinct from Ehrlichia canis in that it is found primarily in granulocytes and seems to cause a milder form of acute canine ehrlichiosis and to result in a chronic phase that differs from classic canine ehrlichiosis. Ehrlichia ewingii and Ehrlichia canis are also serologically distinct. Ehrlichia ewingii can be differentiated from all other members of the genus by its unique 16s rRNA gene sequence. The type species is the Stillwater strain of Ehrlichia ewingii, which was originally obtained from the blood of a naturally infected dog. Attempts to cultivate Ehrlichia ewingii in tissue culture have been unsuccessful.

Nucleotide sequence accession numbers. The GenBank accession number for the 16s rRNA gene of the CGE (Ehrlichia ewingii) is M73227, and the accession number for the “Ehrlichia platys” 16s rRNA gene is M82801.

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REFERENCES


