CLINICAL MICROBIOLOGY REVIEWS, July 1991, p. 286–308 Vol. 4, No. 3
0893-8512/91/030286-23$02.00/0
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The Tribe *Ehrlichieae* and Ehrlichial Diseases

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INTRODUCTION

Recently, two new ehrlichial diseases, Potomac horse fever and human ehrlichiosis, were discovered in the United States. The discoveries created an interest in ehrlichial diseases among veterinary and human medical researchers and motivated the development of a method for ehrlichial culture in a continuous cell line. Success in culturing ehrlichial organisms in quantity in turn enabled researchers to apply various molecular, biochemical, and immunologic procedures that have significantly advanced the understanding of ehrlichial organisms. The purpose of this review is to present a detailed discussion of these organisms and the important human and veterinary diseases they cause.

CHARACTERISTICS OF THE TRIBE *EHRLICHIEAE*

Taxonomy

The tribe *Ehrlichieae* contains obligate intracellular bacterial parasites with a tropism for leukocytes and comprises three genera: *Ehrlichia*, *Cowdria*, and *Neorickettsia* (Fig. 1). The genus *Cytoecetes*, which consists of granulocytic microorganisms (173), was eliminated in *Bergey's Manual of Systematic Bacteriology* published in 1984 (156) and included in the genus *Ehrlichia*. The tribe *Ehrlichieae* is included in the family *Rickettsiaceae* (156) along with the tribes *Rickettsieae* and *Wolbachieae*. At the genetic level, recent studies showed strong relatedness (85.7%) in the sequences of the 16S rRNA in *Ehrlichia risticii* and *Rickettsia prowazekii*, which is the etiologic agent of epidemic typhus, a well-known rickettsial disease responsible for the deaths of millions of people during wartime and natural disasters. Both microorganisms belong to the α subdivision of the purple bacteria (180). Another group of obligate intracellular bacteria, the chlamydiae, are genetically quite different from rickettsiae. Although chlamydiae are currently considered eubacteria, they do not resemble any other known eubacteria. According to analysis of the sequence of the 16S rRNA, they are distantly related to the planctomyces (181).

As for phenotypic characteristics, as described later in more detail, purified *E. risticii* and *E. sennetsu* can metabolize L-glutamine (182, 184) and generate ATP as rickettsia can but unlike chlamydiae, which are "energy parasites." Like rickettsiae, at least four species in the tribe *Ehrlichieae* are known to be vector borne (156), which chlamydiae are

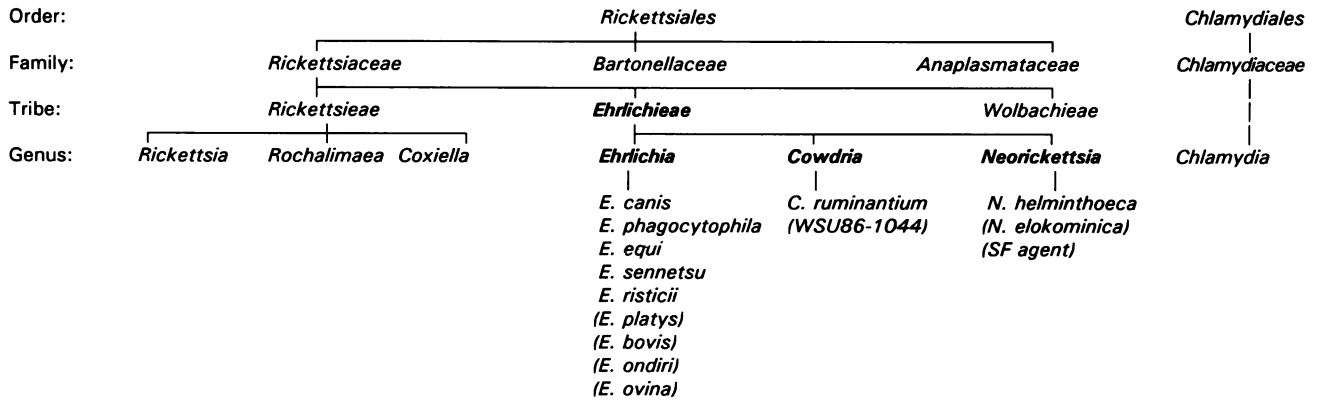

FIG. 1. Rickettsiae and chlamydiae. Parenthesis indicate species not yet recognized in *Bergey's Manual of Systematic Bacteriology* (156) or the *International Journal of Systematic Bacteriology*.

not. However, organisms of the tribe *Ehrlichieae* morphologically resemble chlamydiae in that they are round and grow in the host membrane-lined cytoplasmic vacuoles (133, 156). In the genus *Rickettsia*, the spotted fever group and the typhus group are short rods, and the scrub typhus agent is a coccobacillus. The genus *Rickettsia* grows in the cytosol of the host cells. The tribe *Ehrlichieae* lacks an obvious peptidoglycan layer or thickened outer or inner leaflet of the outer membrane, except for some strains of *Cowdria* (143). *E. risticii* occurs in small electron-dense and large electron-lucent forms (133, 147), but *Ehrlichia* spp. do not show a clear life cycle as *Chlamydia* spp. do. Major characteristics of the genus *Ehrlichia* are compared with those of the genera *Rickettsia* and *Chlamydia* in Table 1.

Four species in the genus *Ehrlichia*, one species in the genus *Cowdria*, and one species in the genus *Neorickettsia* are identified in *Bergey's Manual of Systematic Bacteriology* (156). Since that edition of the manual, one new ehrlichial species, *E. risticii*, has been accepted in the *International Journal of Systematic Bacteriology* (80) (Fig. 1). In addition to these species, there are less well characterized organisms: *E. platys* (69), granulocytic *E. canis* (45), *E. ondiri* (67), *E. bovis* (33, 34, 152), *E. ovina* (33), *Stellantchasmus falcatus* agent (SF agent) isolated from *S. falcatus* fluke (a fish parasite) in Japan (58), *Neorickettsia elokominica* (47), and *E. (Cytoecetes) microti*, an organism found in the polymorphonuclear leukocytes of a vole (173). In 1986, an apparently new agent that shares antigenicity with a strain of *Cowdria ruminantium* was isolated from an aborted bovine fetus (31). The etiologic agent for human ehrlichiosis in the United States (108) has not yet been isolated from human patients. The characteristics of several ehrlichial agents are listed in Table 2. All members of the tribe *Ehrlichieae* with the exception of *E. canis* have a limited geographic distribution.

Host Specificity and In Vitro Culture of the Tribe *Ehrlichieae*

Members of the tribe *Ehrlichieae* infect specific species of animal hosts in nature (Table 3). Experimental injection of cell-cultured *Ehrlichia* spp. or *Ehrlichia*-infected blood has revealed that species other than the usual hosts can be infected with some ehrlichial species (Table 3). Whether this also occurs in nature is, however, unknown. *Ehrlichia* species infect dogs, horses, ruminants, and humans. Although an *Ehrlichia*-like agent associated with anemia in cats has been reported, electron microscopy of the agent is not convincing (18).

Depending on the species, members of the tribe *Ehrlichieae* chiefly infect circulating monocytes, granulocytes, lymphocytes, or platelets and thus can be transmitted by or isolated from blood. Some ehrlichial species, especially those infecting granulocytes and platelets, are easily seen in these cells at acute stages of infection. Monocytic *Ehrlichia* species are less frequently seen in the blood, since the infection level of blood monocytes is low. In addition, in vivo, equine tissue mast cells, macrophages, and intestinal glandular epithelial cells have been found infected with *E. risticii* (147). *Neorickettsia helminthoeca* is seen in macrophages in the canine lymph node (55, 62). *C. ruminantium* is seen in the endothelial cells of small blood vessels of the brains of infected ruminants (25).

None of the tribe *Ehrlichieae* has been cultured outside of eukaryotic cells or in yolk sac. In vitro propagation of the tribe *Ehrlichieae* is a prerequisite for the development of a reliable indirect immunofluorescence test for serodiagnosis of ehrlichial diseases. Successful culture of the tribe *Ehrlichieae* in continuous cell line is essential for achieving the quantities of organisms needed for basic research at the

TABLE 1. Characteristics of the genera *Rickettsia*, *Ehrlichia*, and *Chlamydia*

Genus	Replication site	Developmental cycle	Muramic acid	Glutamine or glutamate oxidation	Genome size (MDa)	Primary host cells	Arthropod association
Rickettsia	Cytoplasm	Simple	Present	Yes	1,100	Endothelial cells	Yes
Ehrlichia	Membrane vacuole	Complex?	Low?	Yes	?	Leukocytes	Yes (some)
Chlamydia	Membrane vacuole	Complex	Absent	No	660	Epithelial cells of mucous membrane	No

TABLE 2. Differential characteristics of species of the tribe *Ehrlichieae*

Species	Natural host	Disease(s)	In vivo-infected cells	Invertebrate vector	Geographic distribution
E. canis	Domestic and wild Canidae	Canine ehrlichiosis, tropical canine pancytopenia	Monocytes-macrophages	*R. sanguineus* (tick), transstadial	Worldwide
E. sennetsu	Human	Sennetsu rickettsiosis	Monocytes-macrophages		Japan, Malaysia
E. risticii	Horse	Equine monocytic ehrlichiosis (Potomac horse fever)	Monocytes-macrophages, intestinal epithelial cells, mast cells		North America, Europe
E. equi	Horse	Equine ehrlichiosis	Granulocytes		United States, Europe
E. phagocytophila	Sheep, cattle, bison	Tick-borne fever	Granulocytes	*I. ricinus* (tick), transstadial	Great Britain, Europe
E. bovis and *E. ovina*	Cattle, sheep	Bovine and ovine ehrlichioses	Monocytes-macrophages	*Hyalomma rhipicephalus*, *H. bursa* (tick)	Middle East, Africa, Sri Lanka
E. ondiri	Cattle, sheep	Bovine petechial fever (Ondiri disease)	Granulocytes		Kenya
E. platys	Canidae	Canine cyclic thrombocytopenia	Platelets		United States
C. ruminantium	Cattle, sheep, goats	Heartwater	Endothelial cells	*Amblyomma* ticks, transovarial	Africa, Caribbean
WSU86-1044	Cattle				Washington
N. helminthoeca	Canidae	Salmon poisoning	Macrophages	*N. salmincola* (fluke), transovarial	Northern California, Washington, Oregon, Idaho
N. elokominica	Canidae, bear, raccoon, ferret	Elokomin fluke fever	Macrophages	*N. salmincola* (fluke), transovarial	Washington
SF agent				*S. falcatus* (fluke)	Japan

molecular and cellular levels and for vaccine development. *E. risticii* has been cultured in vitro in primary canine blood monocytes (78) and in monocyte and macrophage cell lines of murine (39), human (144, 145), and canine (129) origin. *E. risticii* has also been cultured in a human intestinal epithelial cell line (T-84 cells) (132). *E. sennetsu* has been cultured in human (74) and canine (79) primary blood monocytes, human endothelial cells (93), and a continuous murine monocyte cell line (22). Although less efficient than macrophages, fibroblast cell lines have also been used to culture *E. sennetsu* (111, 148, 169). *E. canis* has been propagated in canine primary blood monocytes (114), canine peritoneal macrophages (167), a canine macrophage cell line (29), a human-canine hybrid cell (168), and "spontaneously immortalized" mouse peritoneal macrophage and dog blood monocyte hybrid MDH-SP cells (77). On the other hand, none of the granulocytic *Ehrlichia* species has thus far been cultured in a continuous cell line, and short-term culture is limited to *E. phagocytophila* in the granulocytes from infected sheep (188). *C. ruminantium* has been cultured in irradiated bovine endothelial cell line E5 (13), bovine umbilical endothelial cells (87), and caprine neutrophils (104). Two *Neorickettsia* species have been cultured in canine primary blood monocytes (16, 54), and *N. helminthoeca* has been cultured in a canine macrophage cell line (151). The cells in which these ehrlichial agents have been cultured in vitro are listed in Table 4.

Morphology of the Tribe *Ehrlichieae*

Members of the tribe *Ehrlichieae* are tiny gram-negative cocci and stain dark blue to purple with Romanowsky stain. They stain faintly red by the Macchiavello method and brown-black by silver stain (165). These last two stains, however, are not as distinct for *Ehrlichia* spp. as they are for the tribe *Rickettsieae* and for spirochetes, respectively. Members of the tribe *Ehrlichieae* are generally round but sometimes pleomorphic, especially in tissue culture. The organisms are found in membrane-lined vacuoles within the cytoplasm of infected eukaryotic host cells, primarily leukocytes. Vacuoles containing *E. risticii* have been shown not to fuse with lysosomes (185). The inhibition of lysosomal fusion is not a generalized process but rather is restricted to vesicles that contain *Ehrlichia* spp. (185). Members of the tribe *Ehrlichieae* have distinct ribosomes and DNA strands (133). Clumps of ribosomes are homogenously distributed in the cytoplasm rather than marginated beneath the cytoplasmic membrane. Compared with those of the rickettsiae or ordinary bacteria, the DNA and ribosomes in members of the tribe *Ehrlichieae* are more loosely packed in the cytoplasm (except for the small, dense form of *E. risticii*). Thus, the electron density of ehrlichial organisms is similar to that of the host cell cytoplasmic background, and this low contrast makes it difficult to find *Ehrlichia* cells in infected tissue at low magnification under the electron microscope. Members of the tribe *Ehrlichieae* are surrounded by thin bileaflet outer and inner membranes (Fig. 2). Unlike the rickettisae, ehrlichial organisms show no thickening of either leaflet of the outer membrane (133, 147). The freeze-fracture replica procedure reveals that, like leaflets in the inner membranes of rickettisae (84), the inner (cytoplasmic) leaflet of the inner membrane of *E. risticii* is rich in membrane particles (globular protein molecules), whereas the outer leaflet of the inner membrane has few such particles (Fig. 3). Morphologically, members of the genus *Ehrlichia* do not appear to contain significant amounts of peptidoglycan (133,

TABLE 3. Host animal specificity of members of the tribe *Ehrlichieae*

Species	Primary host	Experimental host (reference)
E. sennetsu	Human	Mouse (57, 113), nonhuman primates (116, 159) but not horse (148)
E. risticii	Horse	Mouse (140), cat (28), dog (154), nonhuman primate (166)
E. canis	Canidae	None; nonhuman primate not infected (99)
E. phagocytophila	Ruminants	Guinea pig, mouse (52)
E. equi	Horse	Cat, dog (101), sheep, goat (164), nonhuman primate (101) but not mouse, rat, guinea pig, rabbit, or cow (164)
E. ondiri	Ruminants	Not in mouse, guinea pig, or hamster (23)
C. ruminantium	Ruminants	Mouse (some strains) (12)
WSU86-1044	Cattle	None
N. helminthoeca	Canidae	None
N. elokominica	Canidae, bear, raccoon, ferret	None
SF agent	Unknown	Mouse (59), dog (60)

147). *C. ruminantium* Mali and Kumm isolates, however, show signs of a peptidoglycanlike substance between the outer and inner membranes (Fig. 4). This substance is unevenly distributed along the circumference of the organism, creating occasional outpouching of the outer membrane where peptidoglycanlike materials are packed (133, 143). A large quantity of capsulelike substance is seen around *E. canis* (29), *E. risticii* in morulae (147), and *C. ruminantium* (143).

E. risticii, *E. sennetsu*, *C. ruminantium*, and *E. canis* tend to occupy one side of the cytoplasm rather than being symmetrically or evenly distributed. *E. risticii* is seen in at least two different forms: multiple dark, small organisms (0.2 to 0.4 μm) (called morulae) enveloped by host membrane (Fig. 5) and relatively light, large forms (0.8 to 1.5 μm) individually tightly wrapped with host membrane (131, 147) (Fig. 2 and 5). Morulae appear to interchange with individually enveloped forms, since an intermediate stage appearing as moderately dense *Ehrlichia* cells tightly enveloped with the host membrane, which is continuous with the membrane surrounding a morula, has been seen (133). Neither extensively condensed elementary bodies nor intermediate stages with condensed structure in the cytoplasm of the microorganism as seen in chlamydiae have been found in any member of the tribe *Ehrlichieae*, with the exception of a recently isolated bovine agent (96).

E. canis cultured in the canine monocyte cell line DH82 is mainly seen as densely packed morulae (Fig. 6) that sometimes contain more than 100 organisms per vacuole. *E. canis* is so tightly packed that individual organisms can hardly be discerned in the morula by light microscopy. On the other hand, *E. risticii* in T-84, P388D$_1$, and U-937 cells, in primary equine monocyte culture and in infected equine tissues are primarily seen as individual forms, especially in intestinal epithelial cells (132, 147). *E. sennetsu* grown in the BS-C-1 line of *Cercopithecus* kidney cells (3), P388D$_1$ cells (22), L929 cells (148), or human endothelial cells (93) is also seen as a single or a few organisms per vacuole. SF agent cultured in the rabbit kidney epithelial cell line RK-13 is ultrastructurally similar to *E. sennetsu* (59) (Fig. 7). *N. helminthoeca* also occurs individually and as tightly enveloped morulae (Fig. 8) and apparently can be found in phagosomes as well (54). These organisms do not make the extremely condensed large morula formed by *E. canis* (151). Human ehrlichiosis agent seen in a patient's leukocytes is morphologically similar to *E. canis*, forming tightly packed morulae (108) (Fig. 9) rather than individual forms like those of *E. sennetsu* and *E. risticii*. *E. equi* and *E. phagocytophila* are seen as loosely packed small morulae (several ehrlichial organisms per vacuole). *C. ruminantium* is seen predominantly as loosely packed morulae of various sizes rather than as individual forms (133).

No plasmid or phage has been detected by agarose gel electrophoresis of DNA fractions from *E. risticii* or *E. sennetsu* cultured in vitro. Lipopolysaccharide is not readily detectable by the conventional extraction method or silver-staining of lipopolysaccharide after polyacrylamide gel electrophoresis of purified *Ehrlichia* spp. (27). No endosporelike structure has been reported, although minicell-like structures resulting from uneven binary fission and apparent outer

TABLE 4. In vitro culture of members of the tribe *Ehrlichieae*

Species	Primary cell (reference)	Continuous cell line (reference)
E. risticii	Canine blood monocytes (78)	P388D$_1$ (39), T-84 (132), U-937 (144, 145)
E. canis	Canine blood monocytes (114), peritoneal macrophages (167)	Human-dog hybrid (168), DH82 (29), mouse-dog hybrid MDH-SP (77)
E. sennetsu	Canine blood monocytes (79), human blood monocytes (74), human vascular endothelial cells (93)	P388D$_1$ (22), L929 (148), FL (human amniotic-membrane derived) (111), HeLa (169)
E. equi	None	None
E. phagocytophila	Ovine blood (188)	None
C. ruminantium	Goat neutrophils (104), bovine umbilical endothelial cells (87)	E5 (bovine endothelial cell) (13)
WSU86-1044	None	BT (bovine turbinate cell) (31), P388D$_1$ (96)
N. helminthoeca	Canine blood monocytes (16, 54)	DH82 (151)
SF agent	None	RK-13 (rabbit kidney epithelial cell) (190)

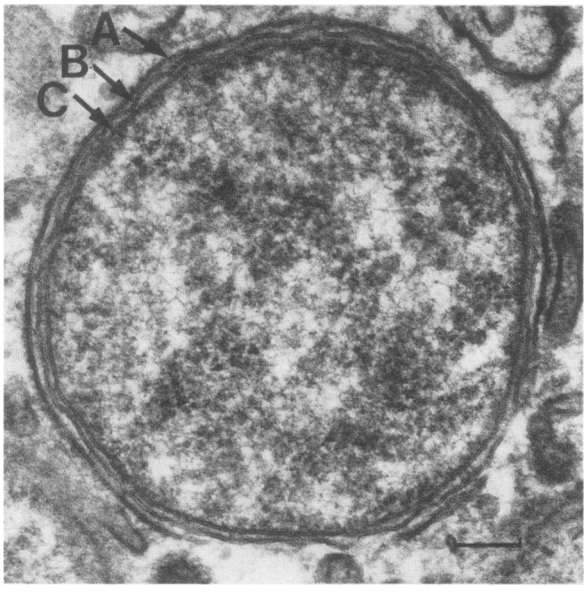

FIG. 2. *E. risticii* in the cytoplasm of a macrophage in the large colon of a horse. *E. risticii* is tightly enveloped by the host membrane (A) and has its own outer (B) and inner (C) membranes. Magnification, ×87,600. Bar = 0.1 μm. Reproduced with permission of Kluwer Academic Publishers (133).

membrane vesicles of various sizes as seen in *Rickettsia tsutsugamushi* (136) have been seen in *E. risticii* (147).

Energy Metabolism of Genus *Ehrlichia*

Members of the genus *Ehrlichia* appear to perform aerobic and asaccharolytic catabolism. The metabolic activities of two species of the genus *Ehrlichia* have been investigated. *Ehrlichia* spp. can utilize glutamine and glutamate and generate ATP (182–184) as rickettsiae do, but unlike rickettsiae, *Ehrlichia* spp. prefer to use glutamine rather than glutamate because they are enveloped by the host membrane and glutamine penetrates phagosomes better than glutamate. Unlike members of the genera *Chlamydia* and *Wolbachia* but like those of the genus *Rickettsia*, members of the genus *Ehrlichia* cannot utilize glucose-6-phosphate or glucose (182). In this sense, the genus *Ehrlichia* is similar to the genus *Rickettsia*, but the rate of ATP synthesis per milligram of protein in vitro is one-fifth that of *Rickettsia typhi* when Percoll density gradient-purified *E. risticii* and *E. sennetsu* are tested (184). The greatest metabolic activity of ehrlichial organisms is observed at pHs 7.2 to 8.0 and rapidly declines at a pH below 7 (182). Thus, members of the genus *Ehrlichia* are not acidophiles, unlike members of the genus *Coxiella*,

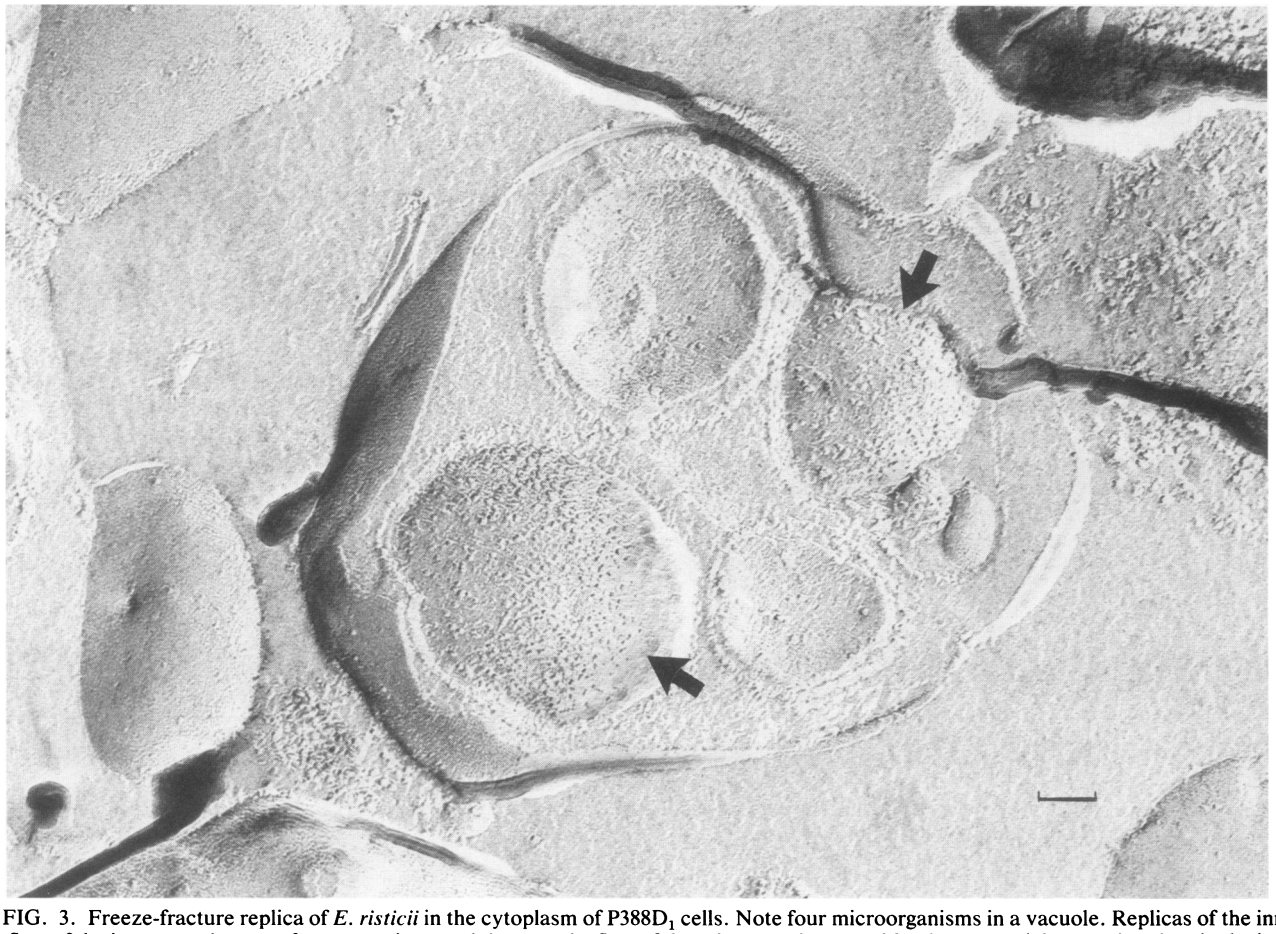

FIG. 3. Freeze-fracture replica of *E. risticii* in the cytoplasm of P388D$_1$ cells. Note four microorganisms in a vacuole. Replicas of the inner leaflets of the inner membranes of two organisms and the outer leaflets of the others can be seen. Membrane particles are abundant in the inner leaflets (arrows) of the inner membranes, but there are very few in the outer leaflets of the inner membranes of *Ehrlichia* spp. Magnification, ×80,000. Bar = 0.1 μm.

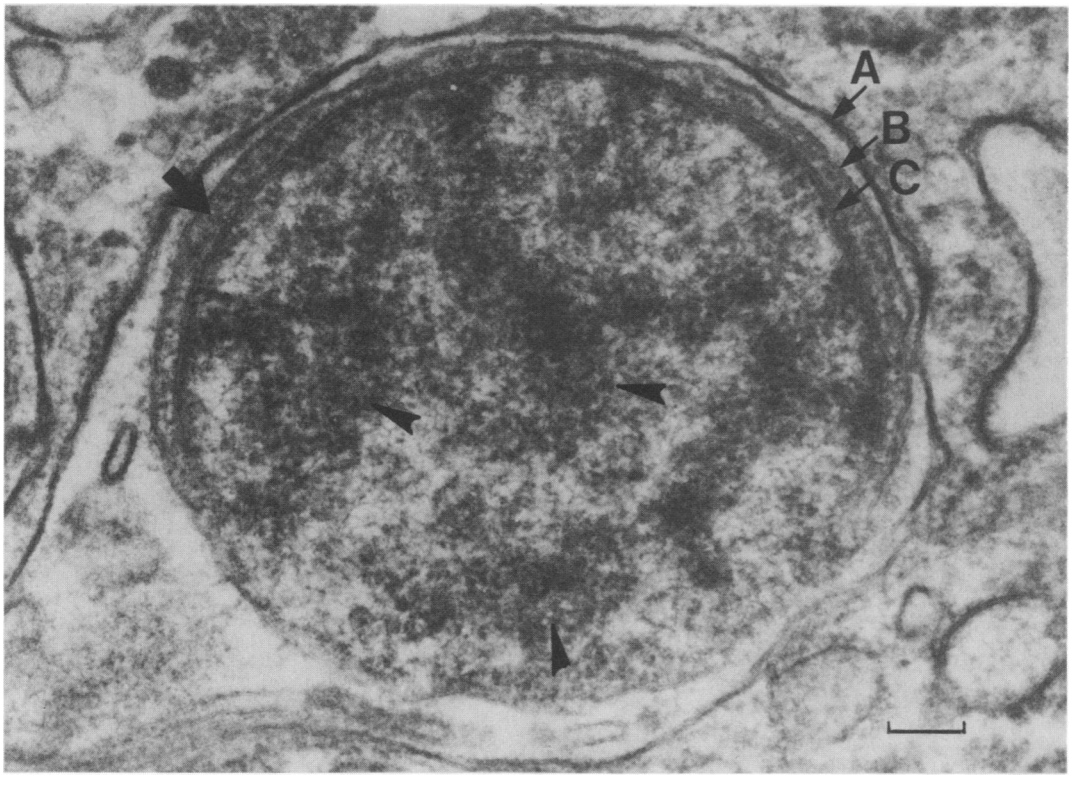

FIG. 4. *C. ruminantium* Mali isolate in the cytoplasm of endothelial cells of a goat brain. The microorganism is loosely enveloped by the host membrane (A). *C. ruminantium* has outer (B) and inner (C) membranes of its own. In between the inner and outer membranes is a peptidoglycanlike substance (left arrow). Fine DNA strands and clusters of ribosomes (arrowheads) are evident. Magnification, ×102,000. Bar = 0.1 μm. Reprinted from reference 133 with permission of Kluwer Academic Publishers.

another bacterium in the family *Rickettsiaceae* that multiplies in the phagolysosome.

Ehrlichial Antigens

There is no serologic cross-reactivity between the tribe *Ehrlichieae* and the tribes *Rickettsieae* and *Chlamydieae* by the indirect fluorescent-antibody (IFA) test (76, 78, 157). Lack of Weil-Felix reaction is reported with *E. canis* (44). *E. risticii* does not appear to have heat-sensitive antigenic determinants like the genus *Rickettsia* does (129). By Western blot (immunoblot) analysis (27), however, 58- to 65-kDa protein antigens of *E. risticii* and *E. sennetsu* react with a monoclonal antibody against *Rochalimaea quintana* 60-kDa antigen, an evolutionally conserved heat shock protein similar to that of several species of the tribe *Rickettsieae*.

Several ehrlichial species antigenically cross-react within the genus when tested by IFA or Western blot analysis (Table 5). Within the genus *Ehrlichia*, *E. risticii* and *E. sennetsu* have the strongest common antigenicity. *E. sennetsu* is nonpathogenic to horses, but it protects them from *E. risticii* infection (148). Human ehrlichiosis patients in the United States develop an antibody that strongly reacts with *E. canis* (108), and this phenomenon is currently used to serologically diagnose human ehrlichiosis in the United States. IFA testing uses *E. canis* as the antigen because the etiologic agent of human ehrlichiosis has not yet been isolated from human patients. A possibly new ehrlichia, WSU86-1044, isolated from an aborted bovine fetus, cross-reacts with a bovine agent, *C. ruminantium*, by IFA (31). The biologically similar *Neorickettsia elokominica* and *N. helminthoeca* are antigenically distinct (54). Although belonging to different genera, *E. equi* and *C. ruminantium* have a strong common antigenicity (76, 103). Low-level serologic cross-reactivity is reported between *C. ruminantium* and *E. phagocytophila* by IFA (89). Contrary to previous reports that *E. sennetsu* and *E. canis* do not cross-react with *N. helminthoeca* by IFA (94, 157), our recent study shows that by both IFA and Western blot analysis (135), *N. helminthoeca* shares antigens with *E. risticii*, *E. sennetsu*, and *E. canis*.

Western blot analysis of ehrlichial antigens has been carried out for *E. risticii*, *E. sennetsu*, *E. canis*, *C. ruminantium*, and *N. helminthoeca*. With sera from horses experimentally infected with *E. risticii*, nine major *E. risticii* proteins of 100, 86, 70, 55, 51, 49, 44, 33, and 28 kDa can be detected (38). Serum from a patient with human sennetsu rickettsiosis reacts with 100-, 80-, 52-, and 46-kDa *E. sennetsu* proteins (170). *E. canis*-infected dog sera reacted with 160-, 100-, 78-, 74-, 64-, 48-, 40-, 30-, 24-, and 21-kDa *E. canis* proteins (134). *N. helminthoeca*-infected dog sera reacted with 80-, 64-, 52-, 36-, 32-, and 26-kDa *N. helminthoeca* proteins (135). Goat and mouse antisera identified a 32-kDa protein as the major common antigen among various *Cowdria* isolates (88). In addition, several other antigenic polypeptides of 200, 75, 54, 44, 40, and 25 kDa were detected in some isolates (88). Dog anti-*N. helminthoeca* serum strongly reacted with the 78- and 64-kDa proteins of *E.*

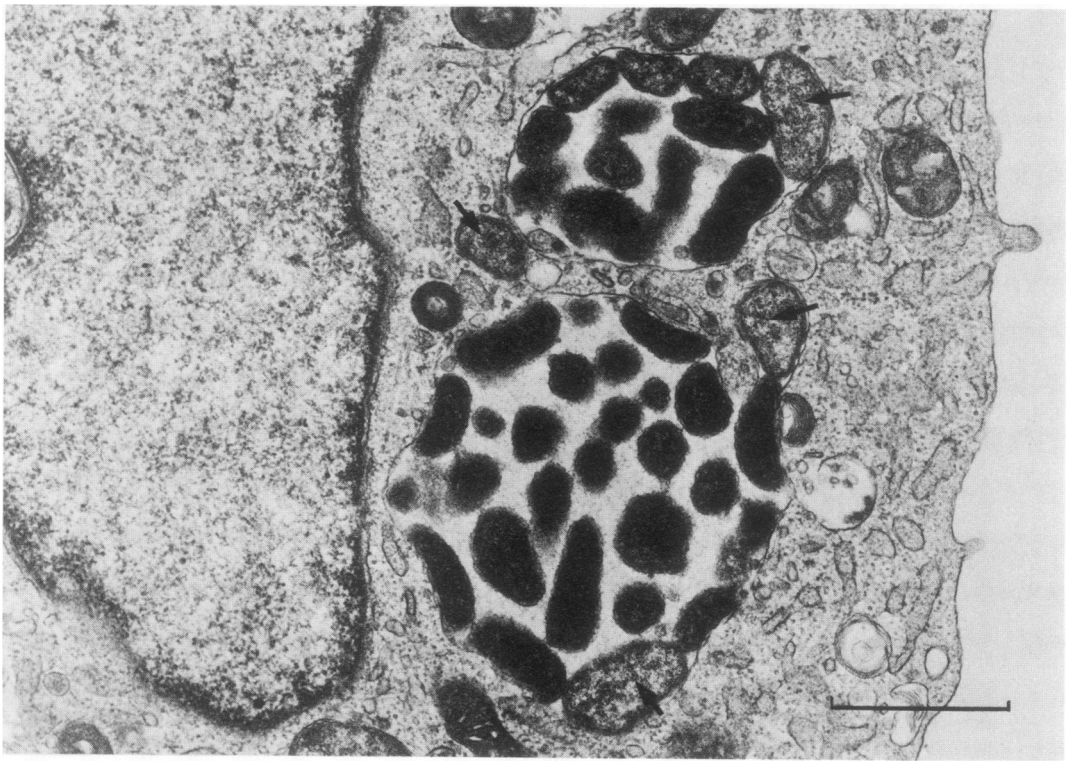

FIG. 5. *E. risticii* in the cytoplasm of U-937 cells in culture. Two vacuoles contain several ehrlichial organisms (morulae). Note the presence of electron-dense smaller organisms and electron-lucent larger organisms (arrows). Magnification, ×23,800. Bar = 1 μm. Reprinted from reference 133 with permission of Kluwer Academic Publishers.

risticii, *E. sennetsu*, and *E. canis* (135). Many *E. risticii* and *E. sennetsu* polypeptides commonly react with equine anti-*E. risticii* and equine anti-*E. sennetsu* sera (135). In contrast, canine anti-*E. canis* sera react poorly with *E. risticii*, *E. sennetsu*, and *N. helminthoeca* (135).

Immune Response

All ehrlichial agents induce a specific antibody response in the natural host or in experimental animals, regardless of the presence of clinical signs. The presence of antibody, however, does not always correlate with clearance of the *Ehrlichia* spp. and the presence of protective immunity. Persistent infection and recurrence or reinfection after recovery from the clinical disease are reported for several *Ehrlichia* species. In *E. canis* infection, parasitemia lasts a long time (years) unless oxytetracycline treatment is given. After tetracycline therapy, clinically recovered dogs with a significantly positive IFA titer against *E. canis* are fully susceptible to challenge with the homologous strain of *E. canis* (17). In chronically infected dogs with demonstrable parasitemia, plasma cell infiltration into various organs (plasmacytosis) occurs, indicating a vigorous humoral immune response (73). Immune canine serum slightly suppresses *E. canis* infection of normal macrophages in vitro (100) but apparently is not effective in vivo. *E. phagocytophila* is also reported to persist in recovered animals (50).

In *E. risticii* infection, parasitemia appears to be limited to the acute febrile stage of infection. Persistence of *E. risticii* antigen, however, was observed in the large colon of a horse for up to 58 days post-experimental infection, that is, 28 days after complete recovery from diarrhea (129). It has been reported that recovered horses are immune to reinfection for at least 20 months (119). Several days after *E. risticii* infection of the horse, tests for immunoglobulin G (IgG) antibody become significantly positive (128). Immune horse serum inhibits *E. risticii* infection of $P388D_1$ cells in vitro and in mice (176). An IgM response occurs a few days after the initial infection and lasts for less than 2 months (128). A challenge injection does not elicit an IgM response (128), but a similar study by Dutta et al. has yielded contradictory results (38).

Animals that recover from *C. ruminantium* infection (heartwater) are immune to reinfection for 6 to 8 months (37). However, protection does not seem to be mediated by antibody, since gamma globulins from heartwater-immune animals fail to protect susceptible animals against heartwater infection (35). In addition, these animals are shown to remain carriers of *C. ruminantium* for long periods after recovery, e.g., 160 to 250 days in the latest tick transmission experiment (4).

Blood collected from ponies at 81 or 114 days after primary infection with *E. equi* induced mild clinical signs (fever, mild thrombocytopenia in susceptible recipient ponies) but did not protect the recipient animals against a second challenge 100 days later (115). It thus appears that *E. equi* may persist in small numbers despite the concomitant presence of antibodies and the demonstrable inhibition of leukocyte migration (115). As in *E. canis* infection, oxytetracycline treatment eliminates *E. equi* from the horses, but rechallenge induces minimal clinical signs, suggesting the development of protective immunity in the recovered horse as opposed to the reaction seen in *E. canis* infection (115).

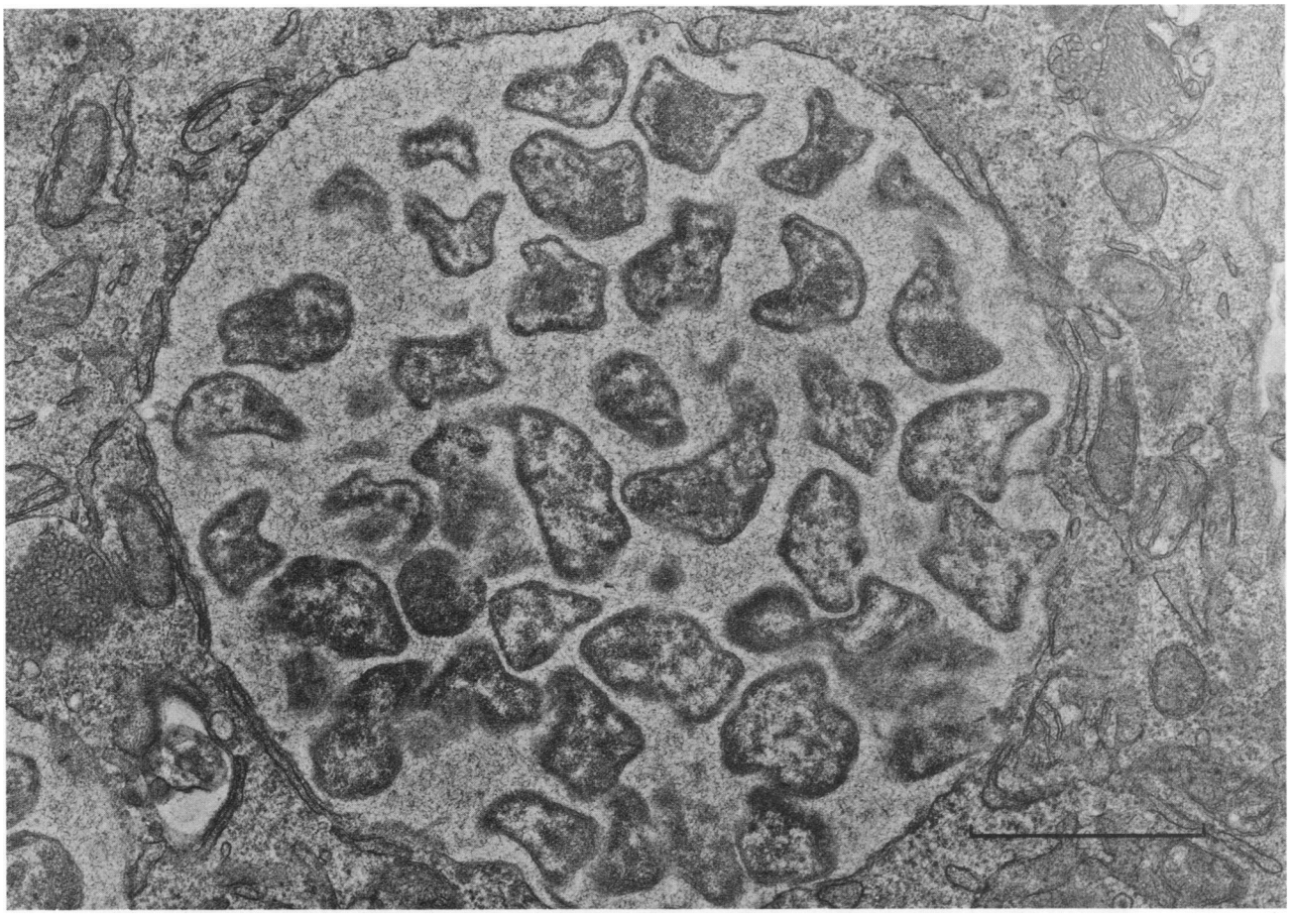

FIG. 6. *E. canis* in the cytoplasm of DH82 cells in culture. Note that the large morula consists of approximately 40 sectioned profiles of microorganisms. *E. canis* cells are embedded in the filamentous ground substance. Presumably this substance is a capsular component of the microorganism. Magnification, ×31,000. Bar = 1 μm. Reprinted from reference 29 with permission of the publisher.

N. elokominica also persists for several months in recovered and clinically normal animals (46). Dogs recovered from *N. helminthoeca* infection develop solid immunity to reinfection (127).

Immunosuppression is associated with infection by two species of *Ehrlichia*. In *E. risticii* infection, when spleen or peripheral blood lymphocyte responses are determined postinfection, there is generalized immunosuppression in infected mice and horses, respectively (140, 141). Class II histicompatibility antigen (Ia antigen) induction on the surface of *E. risticii*-infected macrophages is suppressed in response to gamma interferon used in vitro (110), suggesting inhibition of antigen-specific T-cell activation in *E. risticii* infection. Suppression of the humoral immune response (10) and reduced neutrophil function (187) are documented in cases of *E. phagocytophila* infection.

Pathogenesis

The incubation period for ehrlichial disease (ehrlichiosis) is approximately 1 to 3 weeks, and in general, infection is characterized by fever, depression, and anorexia (Table 6). The disease is apparently dose dependent; i.e., with a lower dosage of *Ehrlichia* spp., the innate defense mechanisms appear to rid the host of *Ehrlichia* cells (140). Only at higher dosages can the organism cause disease. Although *Ehrlichia*

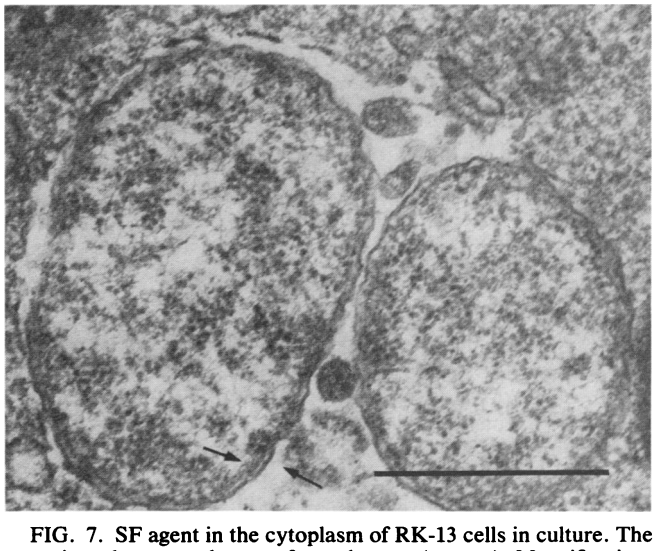

FIG. 7. SF agent in the cytoplasm of RK-13 cells in culture. The organisms have two layers of membranes (arrows). Magnification, ×60,000. Bar = 0.5 μm. Courtesy of S. Yamamoto and T. Fukuda, Miyazaki Department of Health, Miyazaki, Japan.

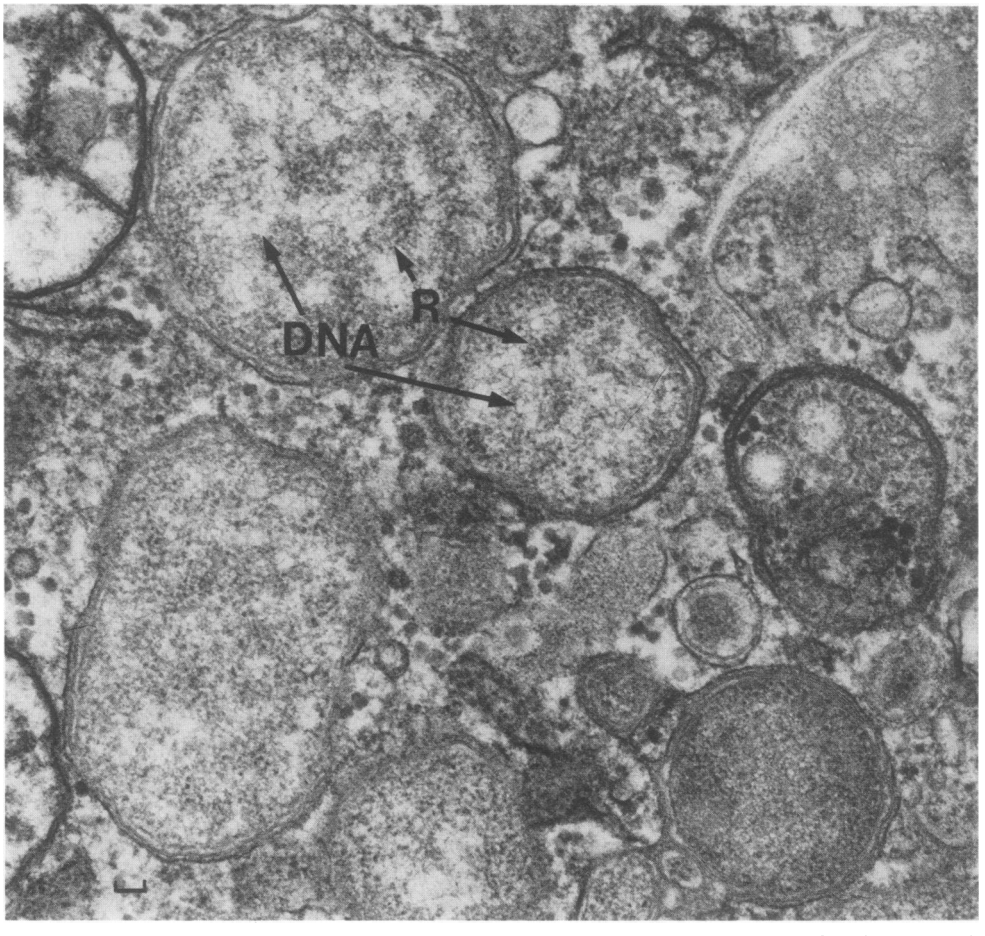

FIG. 8. *N. helminthoeca* isolated from the blood of an experimentally infected dog in the cytoplasm of canine macrophage cell line DH82 cells. Note ribosomes (R) and fine DNA strands. Magnification, ×44,000. Bar = 0.1 μm.

spp. can be isolated from blood at the acute stages of infection, each *Ehrlichia* species seems to have a characteristic tissue tropism that causes a site-specific disease. *E. risticii*-infected cells are found predominantly along the intestinal wall, especially in the equine large colon (147), where they cause watery diarrhea. *E. canis*-infected cells are commonly found in the microvasculature of the canine lungs, kidneys, and meninges (72, 83, 160). Epistaxis is caused by characteristic hemorrhages in the lungs or nasal mucosa. *E. sennetsu* (113) and *Neorickettsiae* spp. (55) are predominantly localized in lymph nodes, where they cause severe lymphadenopathy. *C. ruminantium* commonly localizes in the endothelial cells of the brain tissue (25), where it causes severe neurologic signs.

Generally, patients with ehrlichiosis lack remarkable lesions such as cell lysis, tissue necrosis, abscess formation, or severe inflammatory reactions, especially in the acute stages of the disease. Thrombosis, endothelial-cell hypertrophy/hyperplasia and vasculitis with leukocyte infiltration around blood vessels, all of which generally occur during diseases caused by the rickettsiae, are usually absent during acute ehrlichial infection. Nonfollicular lymphadenopathy is frequently seen in ehrlichial infection. Disappearance of follicles, small lymphocyte depletion and histiocytosis in local lymph nodes are features commonly noted during infection by *E. risticii* (140, 148), *E. sennetsu* (116), *E. canis* (70, 73), *E. phagocytophila* (82), and *N. helminthoeca* (55). In *E. platys* (9) and *N. elokominica* (55) infection, follicles in the lymph nodes remain active. Plasmacytosis in various organs is characteristic during *E. canis* (73) and *E. platys* (9) infection and occurred in a fatal case of human ehrlichiosis in the United States (177). Hemorrhage occurs with *E. canis* (73), *N. helminthoeca* (in the intestinal wall [46]), and *E. ondiri* (163) infection, but this lesion is not conspicuous with other ehrlichial organisms.

In contrast to infection with the tribe *Rickettsieae*, hematologic changes are prominent in ehrlichiosis. These changes are leukopenia and rebound leukocytosis. In addition, thrombocytopenia is predominant with infection by *E. canis* (17, 98), *E. platys* (9), *E. equi* (115), and *E. phagocytophila* (53) and with human ehrlichiosis in the United States (21, 40, 108). Increases in serum hepatic aminotransferase activities are found in patients with human ehrlichiosis in the United States (21, 40) and during *E. canis* (63, 98) and *E. phagocytophila* (179) infection. Hypergammaglobulinemia is noted with *E. canis* infection (19), and an increase in α2-globulin is reported during *E. sennetsu* infection (170).

In contrast to cells infected with virulent strains of the genus *Rickettsia*, host cells infected with *E. risticii*, *E. sennetsu*, *E. canis*, or *C. ruminantium* show little cytolysis in vivo or in vitro until the cytoplasm is completely filled with infecting organisms and the cells burst. Plaque forma-

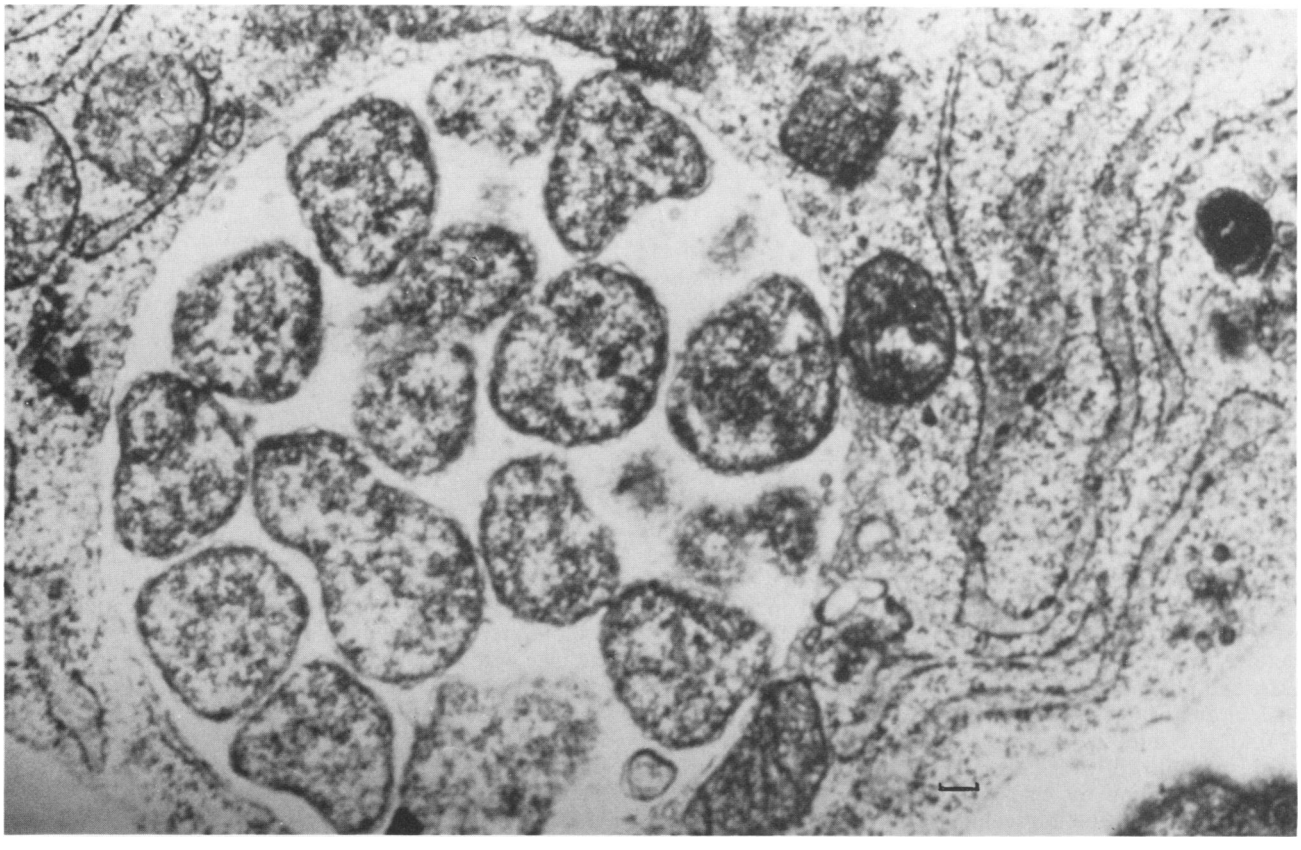

FIG. 9. *Ehrlichia*-like agents in a morula in the cytoplasm of a human patient. Magnification, ×5,000. Bar = 0.1 μm. Reproduced from reference 107 with the permission of the publisher.

tion in a monolayer of host cells is not seen; instead, infected cells dissociate from the bottom of the culture flask and float. This, too, is different from the situation with the genus *Rickettsia*. Ehrlichial release appears to occur not only by cell lysis but also through exocytosis, by fusion of the vacuole membrane with the plasma membrane. In vitro, professional phagocytes-monocytes and macrophages of host animal species have no intrinsic resistance to infection with *E. risticii*, *E. sennetsu*, *E. canis*, and *N. helminthoeca*. In addition, intestinal epithelial cells and mast cells, which are normally not very phagocytic, are induced to take up *E. risticii*. Macrophages phagocytize *Ehrlichia* spp., and *Ehrlichia* spp. inhibit lysosomal fusion with vacuoles, where they reside (185), as do *R. tsutsugamushi* (137) and chlamydiae. Cytochalasin B, which depolymerizes microfilaments of macrophages, inhibits ehrlichial infection (122) as it inhibits *R. tsutsugamushi* (137) and chlamydial infections. It is not known whether the specificity for monocytes and granulocytes is due to a specific receptor for each species of *Ehrlichia* on certain leukocytes or whether *Ehrlichia* spp. simply cannot survive and proliferate in other types of leukocytes.

Entrance of *Ehrlichia* spp. into new host cells is not always preceded by lysis of infected host cells. In the case of intestinal epithelial cells, which make a monolayer tightly connected by circumferential zones of intercellular junc-

TABLE 5. Serologic cross-reactivity among ehrlichial species

Source of antiserum	Antigen response[a]							
	E. risticii	*E. sennetsu*	*E. canis*	*E. equi*	*C. ruminantium*	WSU86-1044	*N. helminthoeca*	SF agent
E. risticii	4 (4)	3 (3)	1 (1)	0	0	—	1 or 0 (2)	—
E. sennetsu	4 (4)	4 (4)	1 (1)	1	0	—	1 (2)	1 or 0
E. canis	1 (1)	1 (1)	4 (4)	2	1	—	2 (1)	—
E. equi	0	1	2	4	3	—	—	—
Human ehrlichiosis agent	1	1	3	2	—	—	—	—
C. ruminantium	0	0	1	3	4	2	—	—
WSU86-1044	—	—	—	—	3	4	—	—
N. helminthoeca	3 (4)	3 (4)	3 (4)	—	—	—	4 (4)	—
SF agent	1	0	—	—	—	—	—	4

[a] IFA (Western blot) reactivity: 4, strong (homologous antigen); 3, moderate; 2, weak; 1, marginal; 0, negative; —, not done.

TABLE 6. Clinical signs and pathology in ehrlichial disease

Organism	Presence of[a]:						
	Incubation period (days[b])	Recurrence or carriage	Fever, depression, and anorexia	Diarrhea	Abortion	Encephalitis	Leukopenia or rebound leukocytosis
E. canis	7–21	+	+	−	−	+/−	+
E. risticii	8–13	−	+	+	+/−	−	+
E. sennetsu	8–14	−	+	−	−	−	+
E. phagocytophila	3–5	+	+	−	+	−	+
E. equi	1–9	−	+	−	−	−	+
E. platys	7–14	+	+/−	−	−	−	−
E. ondiri	4–10	−	+	−	−	−	+/−
C. ruminantium	7–16	+	+	+	−	+	+
N. helminthoeca	6–8	−	+	+	−	+/−	+
Human ehrlichiosis agent	1–21 (after tick exposure)	−	+	+	−	−	+

[a] +, present; +/−, occasionally present; −, absent or not noted.
[b] After experimental transmission.

tions, ehrlichial organisms appear to be transmitted between adjacent cells in vitro by a coupled exocytosis in one cell and endocytosis in an adjacent cell (132). In contrast to ordinary bacteria, *Ehrlichia* spp. do not activate macrophages in vitro (121), and phagocytized *E. risticii* does not induce interleukin-1 or tumor necrosis factor production in macrophages (174). These infected macrophages, however, can be activated and can kill *Ehrlichia* spp., especially in the early stages of infection by certain exogenous stimuli that are known to increase the intracellular Ca^{2+} level in uninfected macrophages. Such stimuli include (in order of decreasing potency) Ca^{2+} ionophore A23187, gamma interferon, and concanavalin A. Although some of these agents are known to increase protein kinase C activity in uninfected macrophages, a direct protein kinase C activator, phorbol myristic acetate, is not effective, which suggests that protein kinase C may not be involved in this process (120). The tribe *Ehrlichieae* in general does not induce a severe inflammatory reaction in the tissues, as noted by Cowdry (25) and Rikihisa et al. (147). In part this may be due to the lack of complete macrophage activation and its attendant cascade of inflammatory reaction.

Thrombocytopenia in *E. canis* infection may be immune mediated (90, 91). A platelet migration inhibitory factor with a molecular weight of 150,000 to 190,000, which is distinct from antiplatelet antibody and is produced by lymphocytes of *E. canis*-infected dogs, is suggested as the cause of thrombocytopenia in cases of tropical canine pancytopenia (1).

HUMAN EHRLICHIOSIS

Sennetsu Ehrlichiosis

Among members of the tribe *Ehrlichieae*, *E. sennetsu*, isolated in mice from a patient's blood, bone marrow, and lymph node in 1953 in Japan (57, 113), is the only species known to cause human disease in nature. Sennetsu ehrlichiosis, characterized by an acute febrile illness with lethargy, prominent hematologic abnormality, lymphocytosis, and postauricular and posterior cervical lymphadenopathy, has existed in the southwestern part of Japan since the late 19th century (112). Seroprevalence was recently recognized in Malaysia among patients suffering from fevers of unknown origin (153); *E. sennetsu* 11908 was isolated from the blood of a human patient in Malaysia (153, 183). Laboratory findings include leukopenia, with an increase in neutrophils at the initial disease stage, and both a relative and an absolute increase in lymphocytes (accompanied by the appearance of atypical cells) in the late febrile to convalescent stages. Erythrocyte and platelet counts are normal. C-reactive protein becomes positive, α2-globulin fraction of serum increases, and serum transaminase values are elevated. Sennetsu ehrlichiosis has never been reported to be fatal. Differential diagnosis of the disease includes infectious mononucleosis and scrub typhus, which occurs in the same part of the world. The mode of transmission of *E. sennetsu* is unknown, although a fish parasite is suspected as a vector because the disease is associated with consumption of a particular raw fish (58). However, intraperitoneal and subcutaneous, but not oral, routes are effective in reproducing the disease in monkeys (116). At necropsy, lymph nodes of experimentally infected monkeys show diffuse proliferation of large basophilic lymphatic cells, disappearance of follicles, and obscure boundaries of the medullary cord and sinus (116).

Mice are highly susceptible to *E. sennetsu* and are used to isolate the organism from human patient specimens (57, 113). An infected mouse shows lymph node enlargement after about 2 weeks. Diarrhea, weakness, and ruffled fur followed by death occur 3 to 4 weeks after inoculation (113). Necropsy of the mouse reveals enlargement of lymph nodes, spleen, and liver but no ascites. When stained by the Romanowsky-type method or IFA labeling, the cytoplasm of peritoneal cells from infected mice contains *E. sennetsu*.

Human Ehrlichiosis in the United States

Recently, a new type of human ehrlichiosis was clinically, serologically, and microscopically (108) recognized in 14 states in the United States (Fig. 10) (for a review, see references 40 and 109). The disease is serologically and clinically distinct from sennetsu ehrlichiosis. Clinical signs of this disease are similar to those of Rocky Mountain spotted fever and include fever, arthralgia, myalgia, headache, anorexia, nausea, vomiting, chills, pneumonia, leukopenia, and, in 20% of the cases, rash (21). More than 100 cases have been confirmed to date (40, 48, 49, 68, 125, 172), and at least three fatalities have been associated with the infection (40, 177). Laboratory test abnormalities such as leukopenia, thrombocytopenia, and elevated levels of he-

TABLE 6—*Continued*

Presence of:								
Thrombocytopenia	Abnormal liver function	Lymphadenopathy	Splenomegaly	Plasmacytosis	Vasculitis	Edema	Hemorrhage	Hydrocardium
+	+	+	+	+	+	+	+	+
+/−	−	−	−	−	−	+/− (Leg)	−	−
−	+	+	+	−	−	−	−	−
+	+	−	−	−	−	−	−	−
+	+	−	−	−	+	+ (Leg)	+	+
+	+/−	+	−	+	−	−	+ (Spleen)	−
−	−	−	−	−	−	−	+	−
−	−	+	+	−	−	+ (Lung)	+	+
−	−	+	+	+	−	−	+ (Intestine)	−
+	+	+	+	+	−	−	−	−

patic aminotransferases are common (21, 40, 109). These laboratory findings for human ehrlichiosis are remarkably similar to those for canine ehrlichiosis (63, 98). In one case, autopsy revealed alveoli containing many erythrocytes and macrophages, hepatic focal acidophilic and centrilobular necrosis, Kupffer cell and bone marrow erythrophagocytosis, splenomegaly, and multifocal perivascular mononuclear infiltrates, including many plasma cells in the kidneys, brain, spinal cord, and heart (177). The pathologic findings closely resemble those for canine ehrlichiosis (73). The patient's serum reacted most strongly with *E. canis* and at a low dilution (1:20) with *E. sennetsu* (108). *E. sennetsu* infects blood monocytes, while this new agent appears to infect lymphocytes, neutrophils, and monocytes (108). The etiologic agent, which is speculated to be a new ehrlichial species or a new strain of *E. canis*, has been isolated and cultured in DH82 cells from a patient with human ehrlichiosis (27a). Approximately 83% of patients had a history of tick exposure in the month before onset of illness (21, 40). Differential characteristics of two human ehrlichioses are shown in Table 7.

VETERINARY IMPORTANCE OF THE TRIBE *EHRLICHIEAE*

E. canis (Type Species)

E. canis is the causative agent of canine ehrlichiosis (tropical canine pancytopenia), a tick-borne disease of dogs described originally in Algeria in 1935 (32) and soon after in the Middle East and Orient. *E. canis* was first recognized in the United States in 1962 (41). The disease is now distributed throughout the world and was especially noted during the Vietnam War, since hundreds of U.S. military dogs in Vietnam died from *E. canis* infection. Clinical signs can be acute, subclinical, or chronic. The acute phase is characterized by mild clinical signs such as fever, depression, dyspnea, and anorexia with lymphadenopathy and mild weight

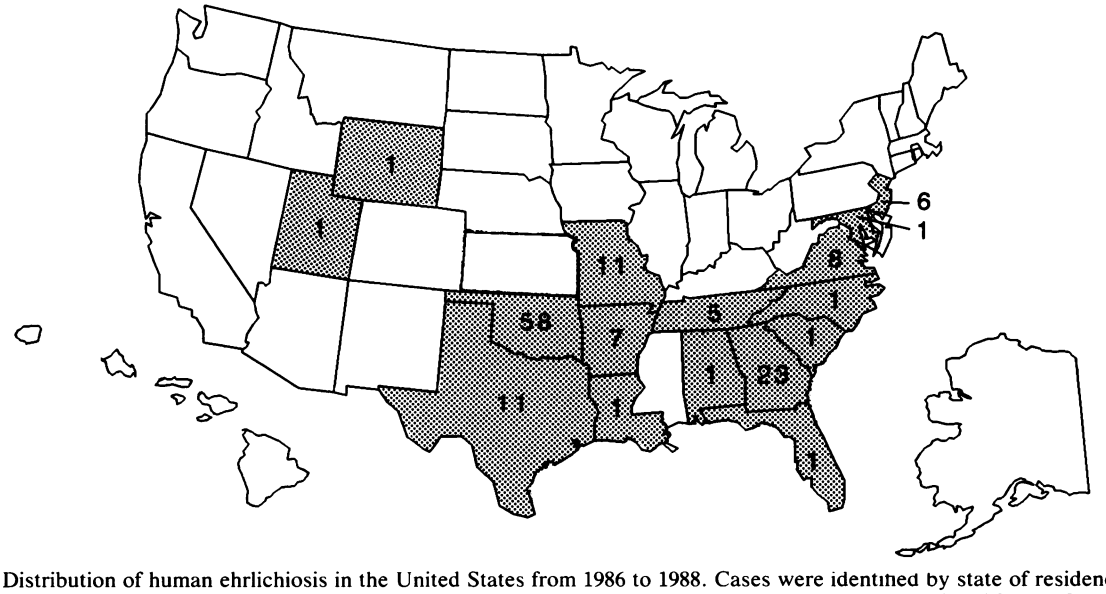

FIG. 10. Distribution of human ehrlichiosis in the United States from 1986 to 1988. Cases were identified by state of residence or where tick exposure occurred. Shaded areas represent states from which cases of ehrlichiosis have been reported. Reprinted from reference 109 with the permission of the publisher.

TABLE 7. Characteristics of human ehrlichiosis

Parameter	Sennetsu ehrlichiosis	Ehrlichiosis in United States
Etiology	*E. sennetsu*	Not yet isolated
Clinical signs	Fever, lymphadenopathy	Rocky Mountain spotted fever-like
IFA antigen	*E. sennetsu*	*E. canis*
Distribution	Japan, Malaysia	United States
Vector	Unknown	Tick
Host	Monocytes	Lymphocytes

loss (17, 63, 178). The chronic phase is characterized by hemorrhages, epistaxis, peripheral edema, emaciation, and hypotensive shock leading to death (17, 63). Laboratory findings are thrombocytopenia, leukopenia, and hypergammaglobulinemia (17, 63, 98). The severity of the disease is related to the breed of dog infected. German shepherd dogs more frequently develop a chronic hemorrhagic syndrome (73). Concomitant disease (e.g., babesiosis or hemobartonellosis) may intensify the clinical signs (41). *E. canis* grows in the cytoplasm of blood monocytes of infected dogs and can be isolated from the peripheral blood monocytes (114).

In addition, Ewing et al. reported granulocytic canine ehrlichiosis, which is a milder disease than monocytic canine ehrlichiosis and is sometimes associated with polyarthritis (42, 45), which monocytic *E. canis* is not. Granulocytic and monocytic *E. canis* strains are antigenically cross-reactive but apparently not cross-protective (42); they primarily infect granulocytes and monocytes, respectively. Although *E. equi* can infect canine granulocytes (101), dogs infected with granulocytic *E. canis* do not develop a higher antibody titer to *E. equi* than to monocytic *E. canis* (42). Granulocytic *E. canis* does not cause parasitemia in horses and does not protect horses from *E. equi* challenge (42). Thus, Ewing et al. believe that granulocytic *E. canis* is more closely related to *E. canis* than to *E. equi* (42). Our Western blot analysis study also supports this observation (134).

At necropsy, splenomegaly, lymphadenopathy, and plasmacytosis in various organs are found in animals infected with *E. canis* (73). The largest numbers of morulae are seen in the lungs, spleens, and mesenteric lymph nodes from experimentally infected dogs (8). By electron microscopy, monocytic *E. canis* is seen in the alveolar macrophages of the lungs of experimentally infected dogs (160).

The disease is primarily transmitted by the larvae, nymphs, and adults of the brown dog tick, *Rhipicephalus sanguineus* (65). Experimental canine transmission of granulocytic *E. canis* by *Amblyomma americanum* has been reported (7), and *Octobius magnini* was also suggested as a vector (43). The attraction of mononuclear cells to the inflamed site of tick attachment and to tick salivary secretions may aid in the infection process of monocytes with *E. canis*. In infected ticks, *E. canis* multiples within hemocytes and salivary gland cells, eventually enters the digestive tract, and then infects the midgut epithelium (161). Ticks transmit the disease transstadially but not transovarially (65). An adult *R. sanguineus* can transmit *E. canis* for a maximum of 155 days after detaching from the host (102). No species other than the Canidae are susceptible to *E. canis* infection. Dogs can be chronically infected without apparent clinical signs for more than 5 years. Thus, wild and domestic canids are considered the natural reservoir of *E. canis* (65).

Antibody formation is prompt with this organism. By 20 days postinfection, 100% of dogs are seropositive, and IFA titers in untreated dogs peak at 80 days postinfection (17).

E. risticii

E. risticii is the causative agent of Potomac horse fever (equine monocytic ehrlichiosis, equine ehrlichial colitis, acute equine diarrhea syndrome). The disease was originally recognized in 1979 (95) along the Potomac River in Maryland and Virginia. It is now known to occur throughout North America, France, and possibly India (153). The disease is seen during the summer, mostly from June through September. In 1984, the ehrlichial organism was seen for the first time in the intestinal walls of affected horses (146), serologic cross-reactivity with *E. sennetsu* was noted in infected horses (78), and the etiologic agent was isolated in human histiocytic lymphoma U-937 cells (144, 145), canine primary monocytes (78), and murine $P388D_1$ cells (39). Clinical signs of the disease are fever, anorexia, depression, watery diarrhea, colic, leukopenia, and laminitis. The case fatality rate varies from 5 to 30%. Transplacental transmission of *E. risticii* has been reported (30), and the organism may induce abortion or resorption of the fetus.

There are few gross pathologic changes in horses with Potomac horse fever except for patchy hyperemia along the wall of the large intestine (147). There is no destruction, foul odor, or significant inflammatory infiltration such as occurs with enterocolitis caused by salmonellae, which may cause similar clinical signs or may coinfect with *E. risticii* in nature (147). However, remarkable goblet cell mucous depletion and reduced height and increased basophilia of mucosal epithelial cells, dilation of intestinal glands, and entrapment of cell debris in the gland lumens are seen during experimental *E. risticii* infection (141, 148). Mesenteric lymph nodes are relatively small and composed of prominent sheets of histiocytes, macrophages, occasional giant cells, and severely depleted inactive lymphoid cells (148).

E. risticii has a predilection for the intestinal wall, especially that of the large colon (146, 147), and infects blood monocytes, tissue macrophages, intestinal glandular (crypt) epithelial cells, and mast cells (131, 147). Watery diarrhea is caused by a reduction in electrolyte transport (Na^+, Cl^-); thus, there is lack of water resorption, mainly in the large and small colons (142). Infected intestinal epithelial cells lose microvilli (132, 142), which may contribute to reduced electrolyte transport and reduced water resorption. An increase in intracellular cyclic AMP is found in both infected mouse macrophages (129) and infected mouse and horse intestinal tissues (130, 142). This change in cyclic-AMP content may also contribute to the reduced luminal absorption of Na^+ and Cl^- in the colon and thus to the lack of water adsorption and the consequent diarrhea.

Prompt IgM and IgG antibody development in *E. risticii* infection has been documented (128). The cell-mediated immune response in infected mice is generally severely depressed in a time- and dose-related manner (140).

The mode of transmission of Potomac horse fever is unknown. The seasonal nature, sporadic existence (95), and experimental transmission by the intradermal route (124) suggest a blood-sucking arthropod as the vector. *Dermacentor variabilis*, blackflies, eye gnats, biting midges, and mosquitoes have been found to be negative for *E. risticii* transmission (158). Experimental oral transmission of the disease has been demonstrated (118), although the feco-oral route is not the natural mode of transmission.

In vitro doxycycline, demeclocycline, and oxytetracycline

are effective in eliminating the organism in P388D_1 cells (138). Although oxytetracycline by itself is bacteriostatic, it was found to induce lysosomal fusion with ehrlichia-containing vacuoles in P388D_1 cells (185), thus becoming bactericidal. In vivo efficacy of antibiotic treatment is discussed below in Treatment and Prevention of Ehrlichial Disease.

E. equi

Equine ehrlichiosis and *E. equi* in the cytoplasm of neutrophils in affected horses were initially reported in 1969 (64). Clinical signs of the disease are fever, depression, partial anorexia, limb edema, petechiation, icterus, ataxia, and reluctance to move (164). In contrast to *E. risticii* infection, laminitis does not develop in equine ehrlichiosis (106). Hematologic changes observed are thrombocytopenia, elevated plasma icterus index, decreased packed-cell volume, and marked leukopenia involving first lymphocytes and then granulocytes. The disease is inapparent to mild and is usually not fatal except for injury resulting from ataxia. In contrast to Potomac horse fever, the disease is found during late fall, winter, and spring. The disease has been observed chiefly in California. In addition, sporadic cases have been reported in Colorado, Illinois, Florida, Washington, New Jersey, Germany (20), Switzerland (71), Sweden (14), and Israel (66). In the United States, the disease occurs much less frequently than Potomac horse fever.

E. equi morulae are found in the cytoplasm of neutrophils. The infection rate of peripheral blood neutrophils varies from 0.5 to 73% (64). The characteristic gross lesions are petechial hemorrhages and edema accompanied by proliferative and necrotizing vasculitis of small arteries and veins in the legs (64). *E. equi* can cause parasitemia in dogs, cats, and nonhuman primates, with mild to no clinical signs (101). Infected animals develop antibody detectable by IFA, and leukocytes from infected animals show inhibition of migration when mixed with *E. equi* antigen (115). The mode of transmission of the disease is unknown, but ticks are the suspected vector. The blood of recovered horses is reported not to be infectious (106).

E. phagocytophila

E. phagocytophila was first described by Gordon et al. in 1940 (61) as the infective agent of tick-borne fever, which was originally described by MacLeod in 1932 (105). It is found in England and other European countries and is transmitted by the sheep tick *Ixodes ricinus*. Tick-borne fever is an acute disease from which most animals recover within 2 weeks after the onset of illness, although spontaneous relapse may occur after splenectomy, other infectious disease, or injury (50). The disease is characterized by fever, reduced milk production (26), reduced growth rate, and leukopenia, due first to a profound decrease in the number of circulating lymphocytes and later to neutropenia and transient but marked thrombocytopenia (52, 171). Abortion and stillbirths occur in infected pregnant heifers (186). Mortality is generally low, although 23.75% mortality was reported by Jamieson (85).

E. phagocytophila is pleomorphic and consists of small "elementary bodies" that develop into larger homogeneous masses called "initial bodies" (189). *E. phagocytophila* primarily infects neutrophils and other granulocytes of sheep, goats, cattle, and deer. At the height of fever, 95% of neutrophils may be infected (50). Lymph nodes devoid of germinal centers and cortical regions replaced by proliferating reticulocytes are the characteristic lesions in this disease (61). Reduced capacity of neutrophils to phagocytize and kill bacteria (187), B-cell depletion (11), and inhibition of humoral immunity but not delayed skin hypersensitivity reaction (10) have been reported. These immunosuppressions make *E. phagocytophila*-infected sheep susceptible to other diseases such as louping ill (ovine encephalomyelitis) (105) and tick pyemia (staphylococcal infection) (51). After an animal recovers from the clinical illness, the organism may persist for up to 25 months and the blood remains infectious (50).

E. platys

E. platys, originally described by Harvey et al. (69) in 1978, is found in the platelets of dogs suffering from infectious canine cyclic thrombocytopenia. Clinical signs of the disease are fever, depression, and anorexia (9). Parasitemia and thrombocytopenia occur in cycles at approximately 10- to 14-day intervals (9). Pathologic changes are generalized lymph node enlargement, follicular hyperplasia in the spleen and lymph nodes, and increased cellularity in the bone marrow (9). Megakaryocytes in bone marrow have not been observed to contain organisms.

Seropositive dogs have been found in Florida, Pennsylvania, Texas, Louisiana, Illinois, California, Arkansas, Mississippi, Idaho, and North Carolina (56). *E. canis* and *E. platys* infections may occur simultaneously (81). Minimal serologic cross-reaction is thought to occur between *E. canis* and *E. platys* (9, 56). Antigenic relationships with other species of *Ehrlichia* are unknown. Transmission is thought to occur by ticks but has not been experimentally proven.

E. (Cytoecetes) ondiri

E. ondiri was identified as the cause of bovine petechial fever (Ondiri disease) in Kenya in 1962 (67). According to Haig and Danskin (67), this disease was first described in 1933 and is characterized by fever, petechiae, and depression. Clinical signs are more severe than those of *E. phagocytophila* infection. Leukopenia is prominent, and *E. ondiri* is found in neutrophils, large lymphocytes, monocytes, and eosinophils of the circulating blood and in the large lymphocytes of the spleen (97). There is no cross-immunity between *E. ondiri* and *C. ruminantium* (67), which also occurs in Kenya. Both cattle and sheep are susceptible. Recovered animals are immune to reinfection (162), which is not the case with *E. phagocytophila* infection. No antigenic or other relationship of *E. ondiri* with *E. bovis*, *E. ovina*, or *E. phagocytophila* is known. The disease is thought to be transmitted by ticks, but this has not yet been proven.

C. ruminantium

Heartwater was first noticed in South Africa in 1838. The disease is characterized by fever of up to 107°F (ca. 42°C), depression, excessive salivation, and neurologic signs—such as muscular twitching, squinting, blind charge, circling, and tetanic seizure—but no rash (25). Peracute or acute disease is usually fatal within a week of the onset of symptoms. Exotic breeds of cattle, sheep, and goats, when introduced into an area where heartwater is endemic, often develop a peracute form of the disease. A subacute or mild form of the disease is seen in antelope and some indigenous breeds of sheep and cattle with high resistance to the disease. Death is rare in this case, and most animals recover after mild clinical

illness. The gross changes observed at necropsy are hydropericardium (thus the name heartwater), ascites, hydrothorax, mediastinal edema, and edema of the lungs. Subendocardial petechial hemorrhages are usually seen, and submucosal and subserosal hemorrhages may occur elsewhere in the body. Degeneration of the myocardium and liver parenchyma, splenomegaly, edema of the lymph nodes, catarrhal and hemorrhagic abomasitis, and enteritis are all commonly encountered. Brain congestion may occur, but brain lesions are remarkably few considering the severity of the nervous system symptoms observed in this disease. Although *C. ruminantium* infects endothelial cells, necrosis, endothelial proliferation, and thrombosis are not found (25). Leukocytosis is the characteristic histopathologic lesion (36). *C. ruminantium* is detectable in the spleen and lymph nodes at the onset of the disease and is readily detectable in endothelial cells of the capillaries of the renal glomeruli and cerebral cortex of sheep, goats, and cattle as the disease progresses (25). Small clusters of extracellular organisms have been noted in the lumina of blood vessels in the brain and lymph node sinuses (36).

Recently, *C. ruminantium* was cultured in bovine endothelial cells (13, 87) and goat neutrophils (104). *C. ruminantium* is antigenically strongly cross-reactive with *E. equi*, only weakly cross-reactive with *E. canis*, and nonreactive with *E. risticii*, *E. sennetsu*, *Rickettsia* spp., *Coxiella burnetii*, and *Anaplasma marginale* (103). Electron microscopy reveals that two isolates of *C. ruminantium* appear to possess large quantities of peptidoglycanlike materials (133, 143). The enzyme-linked immunosorbent assay for IgG antibody becomes positive when animals become febrile (175). Recent research shows that sheep, cattle, and the African buffalo remain carriers of heartwater for long periods after recovery, i.e., 223, 246, and 161 days, respectively (4). So far, the disease is limited to sub-Saharan Africa and the Caribbean region, where it is transmitted by *Amblyomma* ticks. The wide range of antigenic variation among *Cowdria* stocks makes effective vaccine development difficult (12).

WSU86-1044

WSU86-1044 agent was isolated in 1986 in cultured bovine turbinate cells from the liver and lungs of an aborted bovine fetus in the state of Washington (31). The organism antigenically cross-reacts with the Kiswani isolate of *C. ruminantium* but not with several *Ehrlichia* spp., three species of *Chlamydia*, the tribe *Rickettsieae*, or *Anaplasma marginale* (31). The organism has an apparent developmental cycle similar to that of chlamydiae when it is cultured in BT or P388D_1 cells (96). Its antigenic relationship to *E. phagocytophila*, *E. ondiri*, *E. bovis*, or other ehrlichial organisms is unknown. The organism is suspected to cause bovine abortion, but this has not been proven.

N. helminthoeca

N. helminthoeca in dogs was discovered in 1950 (24, 126). *N. helminthoeca* causes salmon poisoning disease, which was recognized by settlers in the early 19th century (127). The disease is characterized by fever, anorexia, depression, dehydration, vomiting, weight loss, and watery and often bloody diarrhea. Mortality exceeds 90% in untreated cases. Intestinal hemorrhage and inflammation are the most characteristic lesions. Generalized lymph node enlargement due to marked infiltration of macrophages is accompanied by severe depletion of small lymphocytes and loss of germinal centers (55). Splenic follicular central hemorrhage and necrosis and obliteration of thymic architecture by macrophage infiltration are common (55). *N. helminthoeca* is found in macrophages in most of the lymph nodes and in histiocytes of the intestinal villi but never in blood (127).

N. elokominica was first isolated in the 1960s as the cause of Elokomin fluke fever along the Elokomin river in the state of Washington (47). By immunofluorescence, *N. helminthoeca* and *N. elokominica* cross-react weakly (94) or not at all (54), and they are not cross-protective of each other (55). Clinical signs of Elokomin fluke fever are milder than those of salmon poisoning disease. Hemorrhagic enteritis is uncommon in Elokomin fluke fever. Without more data to judge by, some researchers believe that *N. elokominica* is an isolate or a strain of *N. helminthoeca* (62). Dogs may be naturally infected with the two species of *Neorickettsia* (46).

N. helminthoeca is a parasite of a fluke *Nanophyetus salmincola*, which has a complex life cycle involving both snails (*Oxytrema silicula*) and salmonid fish as intermediate hosts and carnivores as the final host. Infection of dogs with *N. helminthoeca* occurs following ingestion of salmonid fish that harbor metacercariea containing *Neorickettsia* spp. (62, 126, 127). *N. helminthoeca* is the first and only obligatory helminth-borne pathogen that has been discovered in the bacterial field. *N. salmincola* harbors *Neorickettsia* spp. throughout its life cycle, from egg to adult (126, 127).

Salmon poisoning disease occurs predominantly on the western slopes of the Cascade Mountains from northwestern California to southwestern Washington, corresponding to the distribution of the snail *O. silicula*. The evidence for residence of the pathogen in *N. salmincola* is, however, based entirely on transmission experiments, and the organism has not been seen in any of the developmental stages of *N. salmincola* (neither in the fish nor in the snail). Dogs, foxes, and coyotes are susceptible to the disease. House cats, minks, raccoons, bears, swine, and birds apparently are resistant to neorickettsial infection, although they can be definitive hosts of the trematode vector. *N. elokominica* can infect bears, ferrets, and raccoons in addition to canids (46). Recovered dogs are solidly immune for up to 56 days (127).

SF Agent

In the area where sennetsu fever exists in Japan, what is apparently another species, named SF agent, was isolated in 1973 from the fluke *S. falcatus* by intraperitoneal or oral administration of metacercariae into mice and was maintained by mouse passage of spleen cells of infected mice (58). SF agent antigenically cross-reacts with *E. sennetsu* weakly (59) or not at all (190). SF agent causes a mild clinical sign (fever) in dogs and can be reisolated from the dog (60). It causes severe splenomegaly and lymphadenopathy in mice but does not kill them, unlike *E. sennetsu* (58), which does. In contrast, *N. helminthoeca* and *N. elokominica* are weakly pathogenic to mice but cannot be maintained by mouse passage (127). The antigenic relationship between SF agent and *N. helminthoeca* is unknown. It is not known if SF agent causes illness in animals or humans in nature.

LABORATORY DIAGNOSIS OF EHRLICHIAL DISEASES

The laboratory diagnosis of ehrlichiosis is effected by (i) direct examination by Romanowsky-stained peripheral blood smears (*E. equi*, *E. phagocytophila*) or lymph node

TABLE 8. Laboratory diagnostic procedures for members of the tribe *Ehrlichieae*

Organism	Usefulness of procedure[a]				
	Blood smear	Serology	Isolation in cell culture	Postmortem tissue	
				Immunoperoxidase labeling	Giemsa stain
E. risticii	–	D	R	R	–
E. sennetsu	–	D	D (mouse)	ND	ND
E. canis	R	D	R	R	R
E. equi	D	R	ND	ND	ND
E. phagocytophila	D	R	R	ND	ND
E. platys	D	R	ND	ND	ND
E. ondiri	D	ND	ND	ND	ND
C. ruminantium	–	R	R	ND	D
N. helminthoeca	(Lymphnode aspirate) D	R	R	R	R

[a] D, used for clinical diagnosis; R, used primarily for research; –, not useful; ND, not done.

aspirate (*N. helminthoeca*) for the presence of ehrlichial inclusions, (ii) detection of specific antibodies (IgG or IgM or both) against *Ehrlichia* species in patient serum (*E. canis*, *E. risticii*, *E. sennetsu*), (iii) isolation of *Ehrlichia* spp. from patient blood by using mice (*E. sennetsu*) or cell cultures (*E. risticii*, *N. helminthoeca*), and (iv) postmortem examination of stained brain smears (*C. ruminantium*), lung smears (*E. canis*), or paraffin-embedded colon tissue specimens (*E. risticii*) (Table 8). The best diagnostic procedure varies depending on the ehrlichial species involved. Practical diagnostic methods must therefore be carefully evaluated before they are implemented.

Direct Microscopic Examination

Direct microscopic examination of Romanowsky (we prefer Giemsa or Diff-Quik)-stained peripheral-blood buffy coat smears is simple and inexpensive and provides a permanent record. *Ehrlichia* spp. appear as clusters of round, dark purple-stained, small dots or clusters of dots which appear like mulberry blotches (morulae) in the cytoplasm of leukocytes. This type of examination is useful only during the acute phases of the diseases caused by *E. equi* (Fig. 11), *E. phagocytophila*, and the recently recognized human ehrlichiosis agent (Fig. 12), all of which infect primarily granulocytes or lymphocytes. This method is of limited value for diagnosing *E. sennetsu*, *E. risticii*, and *N. helminthoeca*, which primarily infect blood monocytes, because only a few blood monocytes are infected with very few organisms even at the acute stage of infection. *E. canis* is more frequently found in blood monocytes than *E. sennetsu* or *E. risticii*, and a positive blood smear provides a definitive diagnosis. It is difficult, however, to find *E. canis* in the blood, and we have found that false-negative smears are common. Lung, lymph node, and spleen (frequency of finding in this order) aspirates or biopsy specimens from dogs with canine ehrlichiosis (63) or lymph node aspirates from dogs with salmon poisoning disease reveal intracytoplasmic inclusions (62, 126).

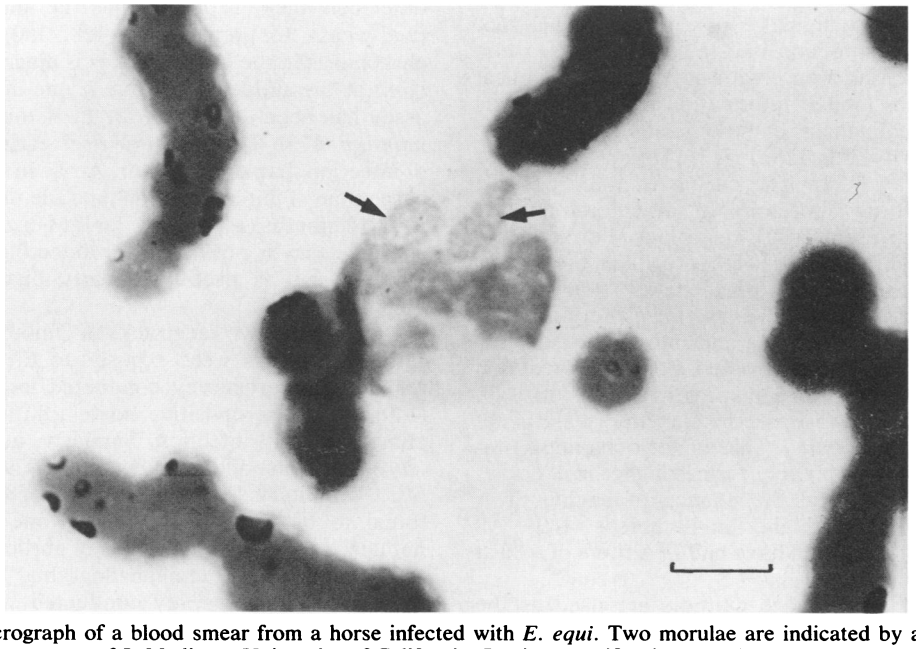

FIG. 11. Light micrograph of a blood smear from a horse infected with *E. equi*. Two morulae are indicated by arrows. Giemsa stain. Original blood smear, courtesy of J. Madigan, University of California, Davis. Magnification, ×1,500. Bar = 10 μm.

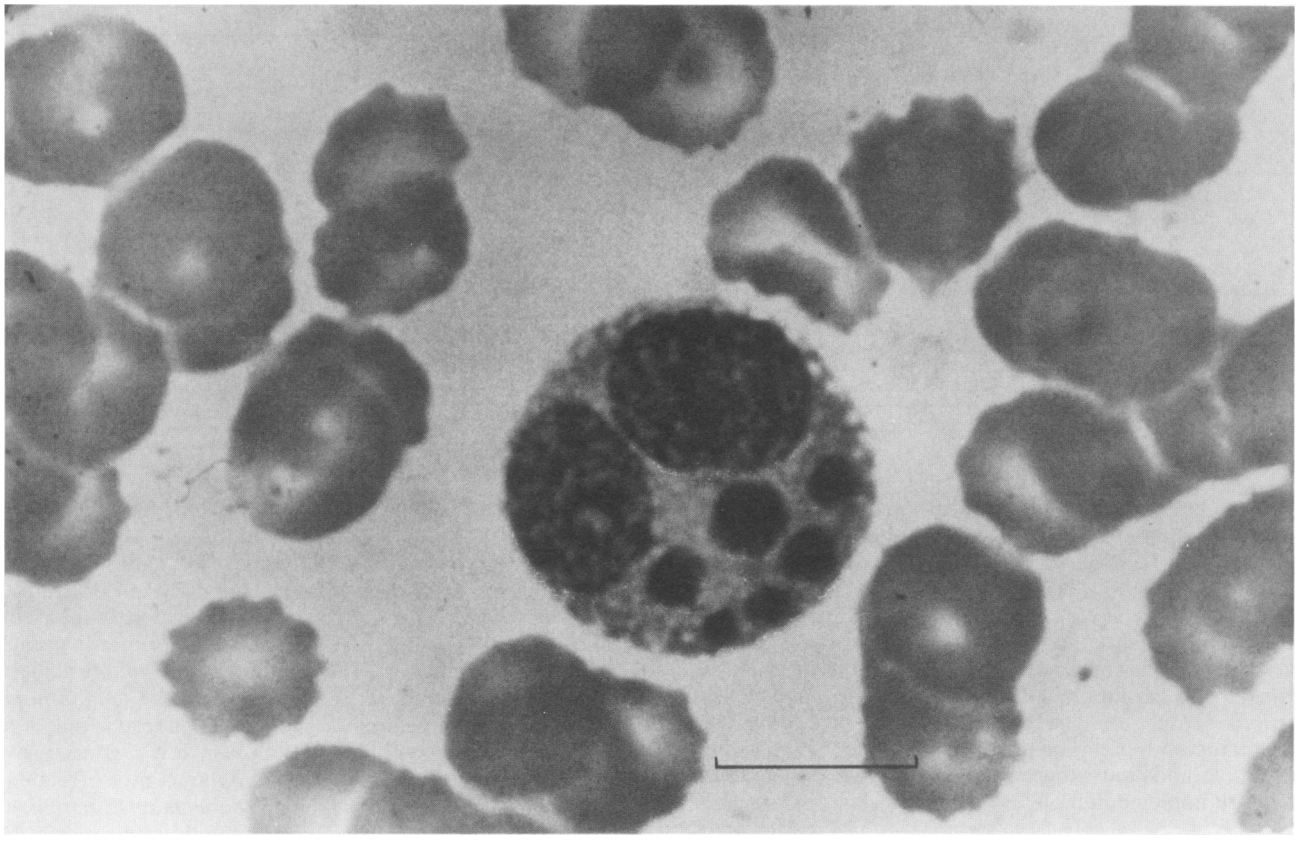

FIG. 12. Light micrograph of a blood smear from a patient suffering from human ehrlichiosis. Six morulae appear in the cytoplasm of a blood monocyte. Leishman stain. Magnification, ×2,700. Bar = 10 μm. Reprinted from reference 107 with the permission of the publisher.

Serologic Diagnosis

All ehrlichial agents induce specific humoral immune responses. These responses are the basis for serologic diagnosis of ehrlichial diseases. However, since a serologic response occurs in every animal exposed to *Ehrlichia* spp. regardless of whether infection is established or disease is present, serologic testing alone, with no supporting clinical information, provides limited information for the diagnosis of disease. Serologic diagnosis of ehrlichial infections can be accomplished either by IFA (128, 155, 157) or enzyme-linked immunosorbent assay (128). The cut-off titer for positive serology varies with the laboratory. In my laboratory, IgG IFA titers of ≥20 represent positive serology. For serologic testing for *E. risticii* infection, the higher the titer, the greater the correlation with clinical Potomac horse fever disease (149). The chance of healthy horses having a titer of ≤40 is similar to that of ill horses (early and late stages and low level of infection with *E. risticii* or possible cross-reaction with other microorganisms). Thus, with a titer of ≤40, although the horse is seropositive, the current disease is not likely to be caused by *E. risticii*. The chance of having a titer of ≥80 and ≤640 is approximately 4 times higher in ill horses than in healthy horses, and the chance of having titers >1,280 is 12 to 26 times higher in ill horses (149). All experimentally infected horses have had IFA titers of ≥80 at the onset of clinical disease.

Ehrlichia-infected macrophage cultures are used as the antigen for *E. sennetsu*, *E. risticii*, and *E. canis* IFA; an experimentally infected animal blood leukocyte smear is used for *E. equi* (75) and *E. phagocytophila* (123); and experimentally infected dog platelets are used for *E. platys* (56). *E. risticii*, *E. sennetsu*, and *E. canis* have been adapted to grow in continuous murine monocyte-macrophage and other cell lines (Table 4); thus the antigen slides are relatively easy to prepare (29, 128, 150). Currently, human ehrlichiosis in the United States is diagnosed by IFA with *E. canis* as the antigen (29). An enzyme-linked immunosorbent assay has been developed for *E. risticii* (128) and *C. ruminantium* (175). The IgM titer rises earlier during the course of infection than the IgG titer. A rise in IgM titer occurs only at the time of initial infection, and the titer becomes negative in 1 to 2 months (128). Thus, an IgM enzyme-linked immunosorbent assay is not useful for detecting chronic or multiple infection but is useful for early diagnosis of a primary infection.

Of horses at two racetracks in Ohio, 138 (13%) of 840 and 116 (20%) of 574 were exposed to *E. risticii*, according to results of a serosurvey conducted in the summer of 1986 (149). Of the seropositive horses, 80 to 90% did not show clinical signs of infection. Similarly, in a serosurvey for *E. canis* infection conducted between 1979 and 1980, 233 (11%) of 2,077 military dogs and 535 (57%) of 938 civilian dogs were found to be seropositive (92). None of the seropositive military dogs had clinical signs of ehrlichiosis, but 216 (23%) of the seropositive civilian dogs had various signs of the disease. In a serosurvey conducted with 74 reservist humans, 9 (12%) were seropositive to *E. canis*, 2 of these 9 were symptomatic, and the remainder had mild clinical signs

(49). Thus, subclinical infection appears to take place with Potomac horse fever, canine ehrlichiosis, and human ehrlichiosis in the United States. An extensive serosurvey has not been conducted for other ehrlichial diseases.

In summary, recommendations for the serologic confirmation of clinical ehrlichial disease include demonstration of seroconversion, a fourfold rise or fall in titer, or the presence of specific IgM. It should be emphasized, however, that the initial diagnosis and treatment of Potomac horse fever should be based on clinical signs, negative test results for other pathogens such as salmonellae for which the use of tetracyclines may be contraindicated, and initial IFA test results if available within 1 to 2 days. Treatment should not be delayed until laboratory confirmation is obtained from a second IFA test. With the recent advent of a commercially available vaccine, however, it has become difficult to serodiagnose vaccinated horses when they develop clinical signs of Potomac horse fever. Immunologic cross-reactivity among members of the tribe *Ehrlichieae* (Table 5) does not create a serious problem because of the limited geographic distribution, host animal specificity of *Ehrlichia* spp. in nature, different clinical signs, and effectiveness of oxytetracycline for all of the ehrlichial diseases, especially at early stages of infection.

Isolation of Members of the Tribe *Ehrlichieae*

The isolation of *E. risticii* (145), *E. canis* (114), and *N. helminthoeca* (151) is accomplished by inoculating buffy coat or mononuclear cell fractions of peripheral blood of affected animals into cell culture. *E. sennetsu* has been isolated by intraperitoneal injection of 0.5 ml of blood from human patients into mice (113). Murine monocyte-macrophage $P388D_1$ cells, human histiocytic lymphoma U-937 cells, or canine primary monocytes are used for isolating *E. risticii*, *E. canis*, and *N. helminthoeca* (Table 4). The procedure is more sensitive than direct observation. Even if a single organism cannot be seen in the original buffy coat smear, the organisms can be isolated by this procedure.

Isolation procedures for diagnosing infection are impractical for clinical laboratories because they take too long (3 days to 1 month) relative to the rapid course of ehrlichial disease and require a good cell culture facility. Although positive isolation provides a definitive diagnosis, negative results do not necessarily indicate the absence of infection. For *E. equi* and *E. phagocytophila*, since organisms can be easily seen in granulocytes, cultivation is not required, and a reliable isolation method has not been developed. If ovine blood is incubated, however, multiplication of *E. phagocytophila* is induced, thereby making diagnosis easier (188). *C. ruminantium* has been cultured, but the method does not appear to be used frequently as a diagnostic procedure.

Postmortem Identification of Ehrlichial Organisms

E. risticii can be demonstrated in intestinal epithelial cells and macrophages in paraffin-embedded tissue specimens with a silver stain or an immunoperoxidase procedure using a specific antibody to *E. risticii* (165) (Fig. 13). *E. canis* has been demonstrated in the impression smears of the canine lung (72, 83) by using Romanowsky staining and in canine tissues by using an immunoperoxidase procedure (8). *C. ruminantium* is routinely identified in brain tissue after similar Romanowsky staining (25). *N. helminthoeca* may be demonstrated in the lymph nodes by Romanowsky staining or IFA (55).

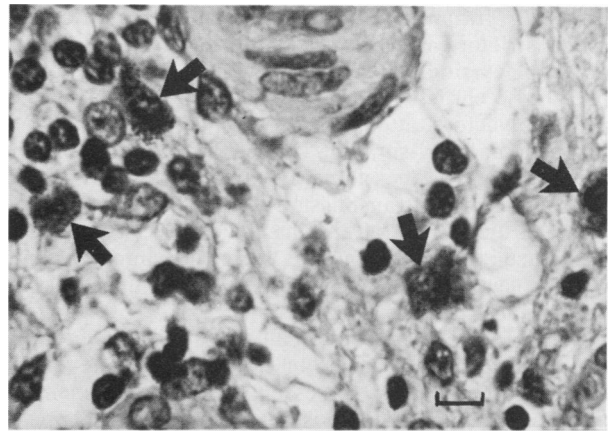

FIG. 13. *E. risticii* in paraffin-embedded equine intestinal tissue stained by immunoperoxidase stain. Note the numerous microorganisms in macrophages (arrows). Magnification, ×616. Bar = 10 μm.

TREATMENT AND PREVENTION OF EHRLICHIAL DISEASES

In vitro *E. risticii* is susceptible to tetracyclines but resistant to erythromycin and nalidixic acid (138). *E. sennetsu* is susceptible to doxycycline and ciprofloxacin but resistant to erythromycin, penicillin, and chloramphenicol (15). As with other rickettsiae, *Ehrlichia* spp. are resistant to aminoglycosides. Oxytetracycline and doxycycline frequently correct the pyrexia and other clinical signs associated with canine ehrlichiosis (17) and human ehrlichiosis in the United States (21, 40, 108) within 24 to 48 h. Importantly, oxytetracycline eliminates carrier status in *E. canis* infection (17). Although doxycycline is more effective than oxytetracycline in vitro (138) and is highly effective in mice inoculated with *E. risticii* (139), oxytetracycline is preferred for the equine species because intravenous administration of doxycycline has an acute toxic effect. In our experience, intravenous oxytetracycline at 6.6 mg/kg of body weight twice a day for 7 days was effective in 71% of cases (five of seven) of experimentally infected ponies when the ponies were treated after the development of severe clinical signs (86). Oxytetracycline is also reported to be effective when given immediately after the development of fever but before the development of diarrhea (120). Tetracyclines are reported to be effective for treating *E. equi* (106), *E. phagocytophila* (5), *E. ondiri* (67), *C. ruminantium* (12), and *N. helminthoeca* (46, 62) at the early stage of infection. A combination of trimethoprim, sulfadimidine-sulfamethylphenazole (5), or chloramphenicol (6) was also reported to be effective for *E. phagocytophila* infection. Dithiosemicarbazone is more efficacious than pyrolidino-methyl-tetracycline or oxytetracycline for *E. ondiri* (163). Intramuscular injection of imidocarb dipropionate has been shown to be effective in treating canine ehrlichiosis, especially when there is coinfection with babesia (2).

Other supportive therapy is frequently required for acutely ill animals or humans if they are to show clinical improvement. Dehydration is corrected by the oral or intravenous administration of polyionic isotonic fluids. Brief therapy (2 to 7 days) with nonsteroidal anti-inflammatory agents (phenylbutazone, flunixin meglumine, aspirin) for horses may be valuable early in the course of treatment to help in the management of clinical signs of Potomac horse

fever (150). Intranasal epinephrine, phenylephrine, or other vasoconstrictors may help to control intranasal hemorrhage in emergency situations in animals with canine ehrlichiosis.

For those ehrlichial diseases known to be transmitted by arthropod vectors, attempts to control the vector population should be made by spraying kennels and barns and dipping animals at 1- to 2-week intervals in areas where the disease is endemic. Vector transmission is probably the only means of spread under natural circumstances, as susceptible animals housed for several months adjacent to infected animals do not have a higher incidence of the disease than those kept far from infected animals (123).

Epizootics of canine ehrlichiosis and heartwater have been successfully controlled or contained by long-term tetracycline prophylaxis. It is essential to use this method of control whenever tick exposure is impossible to prevent. Two vaccines prepared from inactivated cell-cultured *E. risticii* are commercially available for Potomac horse fever, but none have been developed for large-scale prevention of the other ehrlichial diseases (except for an infected-blood vaccine for heartwater). Disease prevention by one of the vaccines for *E. risticii* was recently reviewed (117). For *C. ruminantium*, virulent sheep blood vaccine or *Amblyomma hebraeum* nymphal suspension infected with Ball 3 stock followed by treatment with tetracycline is effective in preventing a more serious course of the disease (12). However, these live, virulent vaccines are not ideal. Recently, an experimental live *C. ruminantium* vaccine attenuated by serial passages in bovine umbilical endothelial cells in culture was reported to be effective in protecting two goats and two sheep against homologous challenge (87).

CONCLUSION

Advances in diagnostic procedures now permit more effective identification and control of certain bacterial diseases. Therefore, members of the tribe *Ehrlichieae* have become increasingly recognized as common disease agents with wide endemicity. New information on these clinically important entities is becoming available; however, additional data are needed in the areas of antigenic comparison (especially among granulocytic ehrlichial organisms), in vitro propagation of thus-far-uncultivable ehrlichial organisms, biochemical and genetic characterization, analysis of host and ehrlichial interaction in the context of pathogenesis and immune recognition, development of vaccines, and elucidation of the mode of transmission.

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