Ultrastructural Observations on *Ehrlichia equi* Organisms in Equine Granulocytes

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Equine ehrlichiosis was reported in 1969 as a distinct disease of low mortality in horses residing in the foothills of the Sacramento Valley of Calif. (4, 14). The clinical signs included fever, anorexia, depression, edema of the legs, and ataxia. The hematological changes consisted of thrombocytopenia, leukopenia, and mild anemia. Inclusion bodies, representing the intracellular localization of the causal agent, occurred within the cytoplasm of neutrophils and eosinophils. The pathological alterations included edema, petechiae, and ecchymoses in the subcutaneous tissues and fascia of the legs and vasculitis (4).

The taxonomic position of the causal agent of equine ehrlichiosis has not been clearly defined. However, it has been suggested that the organism should be grouped with the agents of the genus *Ehrlichia* (14). The organism has been referred to as *Ehrlichia equi* (8), and the same designation will be used in this paper.

Based on the presence of inclusion bodies in peripheral blood smears, goats, sheep, dogs, cats, rhesus macaques (*Macaca mulatta*), and baboons (*Papio anubis*) have been found susceptible to experimental infection with *E. equi* (4, 8, 14). The disease was clinically mild or inapparent in most of these animals. Dogs infected with *E. equi* were not protected against challenge with *E. canis* (8).

Definition of ultrastructural features of *E. equi* was deemed necessary to further characterize it and compare it morphologically with other rickettsial agents.

MATERIALS AND METHODS

Two horses were each experimentally inoculated intravenously with 20 ml of *E. equi*-infected equine blood (provided by David H. Gribble, University of California, Davis) which had been maintained in liquid nitrogen. At day 7 postinoculation, during the acute phase of disease, heparinized blood samples were withdrawn from the horses and centrifuged to layer the cells. The plasma overlying theuffy coat was removed so as not to disturb the cells, and the buffy coat was overlayed with a 2.5% solution of glutaraldehyde in cacodylate buffer. After 30 min the disk of fixed leukocytes embedded in solidified plasma was removed, cut into 1-mm cubes, and fixed an additional hour in 2.5% glutaraldehyde. The tissues were postfixed in osmic acid for 1 h, dehydrated in graded alcohols, and embedded in araldite. Thin sections were cut from the blocks, stained with uranyl acetate and lead citrate, and examined with a Hitachi 11A electron microscope. Buffy coat smears were air dried, fixed in methanol for 10 min, and stained with Giemsa stain for light microscopic examination.

RESULTS

Both horses experimentally inoculated with *E. equi*-infected blood developed signs of acute equine ehrlichiosis characterized by pyrexia, anorexia, thrombocytopenia, and ataxia. The organisms occurring freely or as components of inclusion bodies were readily detected in the cytoplasm of granulocytes of Giemsa-stained buffy coat smears examined by light microscopy. They were pleomorphic, ranging from oval to rod-like forms, and stained dark blue to purple (Fig. 1–3).
Ultrastructurally, the *E. equi* organisms were detected within membrane-lined vacuoles in the cytoplasm of thin-section profiles of neutrophils (Fig. 4) and eosinophils (Fig. 5). Ovoid, round, rod-shaped, and irregular profiles of the organisms were observed. From 1 to 33 thin-section profiles of the organisms were evident within individual vacuoles, and single leukocytes contained multiple vacuoles with variable numbers of loosely arranged organisms (Fig. 6). The organisms lined the periphery of some vacuoles (Fig. 4). In some instances, one or more organisms were found tightly integrated within a cytoplasmic vacuole, lacking any clear space around or between the organisms and wall of the vacuole (Fig. 6). Vesicles were

![Fig. 1](image1.jpg)  
**Fig. 1.** Three neutrophils which contain *E. equi* inclusions (arrows). One neutrophil contains a single organism (S), and another contains an inclusion with a rod-shaped organism (I). ×1,600 Giemsa.

![Fig. 2](image2.jpg)  
**Fig. 2.** Neutrophils with a large inclusion (I). ×1,600 Giemsa.

![Fig. 3](image3.jpg)  
**Fig. 3.** Neutrophil with scattered *E. equi* organisms (arrow). ×1,600 Giemsa.

![Fig. 4](image4.jpg)  
**Fig. 4.** Neutrophil which contains two inclusions (I). The organisms line the periphery of the vacuole. ×33,000.
Fig. 5. Eosinophil with two vacuoles containing single organisms. ×35,000.

Fig. 6. Neutrophil containing several inclusions (I). Several single organisms are tightly bound by the membrane of the vacuole (S). Vesicles (V) are contained within and around the vacuoles. The cell wall (C) and plasma membrane (P) are evident around the organisms. ×41,000.
FIG. 7. Neutrophil with several inclusions (I). The inclusions bulge above the surface of the leukocyte. The membrane of the vacuole and plasma membrane of the leukocyte appear to be fused (F). The membrane of the vacuole (VM) is evident. ×32,000.
Fig. 8. Neutrophil with an inclusion that bulges above the surface of the leukocyte. The cell wall (C) and plasma membrane (P) of the organisms are evident as well as the membrane of the vacuole (VM). ×67,000.
found situated within and around some vacuoles (Fig. 6).

The plasma membrane of the cell and membrane of the vacuoles appeared to fuse and occasionally bulge above the surface of some cells (Fig. 7). Organisms of varying sizes were contained in such vacuoles.

Individual organisms were bound by two distinct membranes (Fig. 6, 8). The outer membrane, or cell wall, was rippled. The inner or plasma membrane was adherent to the internal constituents of the organism. Internally, the organisms consisted of electron-dense and lucid areas. The dense areas contained granules, apparently ribosomes, and in the lucid areas fine fibrils suggestive of deoxyribonucleic acid strands were observed. Occasionally small bodies with the consistency of organisms were observed beneath the cell wall adjacent to the organism (Fig. 9, 10). Multiplication by fission was indicated by the presence of dumbbell-shaped organisms and organisms in a state of almost complete division (Fig. 11).

Although the organisms varied considerably in size, the smallest about 0.18 μm in diameter and the largest about 1.40 μm along the greatest dimension, the internal structure remained constant.

**DISCUSSION**

The ultrastructure of *E. equi* is similar to that of agents in the genera *Rickettsia* (1) and *Ehrlichia* (6, 12, 13, 15), and the large particles of the genus *Chlamydia* (1, 2, 3, 10), as well as to the causal agent of bovine petechial fever (7).

*E. equi* differs morphologically from most of the organisms in the genus *Rickettsia* in that the latter are usually more rod shaped and do not occur within membrane-lined vacuoles, with the exception of *Rickettsia sennetsu*, an organism of uncertain classification (1, 11).

Examination of *E. equi* in blood leukocytes did not reveal a developmental cycle with elementary bodies and intermediate bodies typical of organisms in the genus *Chlamydia* (1, 3, 5).

The only mode of reproduction observed was that of fission of the organism. Condensation of small bodies within larger organisms, as described in bovine petechial fever (7), was not observed.

The significance of the small dense bodies beneath the cell wall was not determined.

Static evidence of the organism entering the cell was not observed. However, it seems likely that entrance may have been by a phagocytic process. Exit of the organisms from the cell probably occurred as a result of rupture of vacuoles after the membrane of the vacuole and plasma membrane of the cell came in close apposition, as has been indicated with other agents (7, 15). Although actual evidence of rupture of a vacuole was not observed in this study, the presence of organisms of all sizes in a single vacuole would suggest that there is no predilection for the release of a particular size of organism presumably to infect other cells.

Vesicles such as those observed around and in the vacuoles have been incriminated in the transfer of cellular material associated with the destruction of the constituents of the vacuole (7, 9, 15). However, degenerative changes were not observed in the organisms, nor were degenerative changes observed in neutrophils or eosinophils due to the presence of the organism.

The morphological characteristics of the
Fig. 10. Neutrophil with an inclusion (I) containing numerous organisms. Dense bodies (arrows) are present beneath the cell wall of some organisms. ×30,000.
causal agent of equine ehrlichiosis and the host cells parasitized are consistent with other organisms classified in the genus *Ehrlichia*.

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**LITERATURE CITED**


