BRIEF NOTE

Scanning and Transmission Electron Microscopic Observations of *Eperythrozoon ovis* (*E. ovis*).

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*Eperythrozoon ovis* (*E. ovis*), an erythrocytic parasite, was first recognized by Neitz et al. (1954) as a causal organism of ovine hemolytic anemia [7]. In Japan, the natural infection of *E. ovis* was recently reported by Sonoda et al. (1975) [10].

In July, 1980, the authors observed three cases of natural ovine eperythrozoonosis which showed pyrexia, anemia, and hemoglobinuria in main clinical signs and also succeeded experimental infection of *E. ovis* with the blood from a naturally infected sheep.

The present paper deals with the morphologic findings of *E. ovis* parasitism which has not been fully clarified up to date by scanning and transmission electron microscopy (SEM and TEM) with blood and aspirated bone marrow samples.

In experimental infection, a normal male sheep aged 4 months was intravenously injected with 20 ml of blood from the sheep naturally infected. As a result, *E. ovis* was initially detected not only in the erythrocytes of peripheral blood but also in the immature erythrocytes of the bone marrow on the 15th days after injection, and also parasitic rates of *E. ovis* were 4% and 35% in the peripheral blood and the bone marrow, respectively. The present results suggest that the bone marrow may be a main organ for multiplication of *E. ovis*. In addition, erythrocyte counts, hematocrit values, and hemoglobin concentrations decreased gradually in accordance with the increased parasitic rates of *E. ovis* and hemoglobinuria due to hemolytic anemia was observed in 33 to 35 days after injection as well.

In blood smear, *E. ovis* had pleomorphism such as spot, rod, coccoid, ring, and chain forms in morphology. The parasitic regions were marginal, central, and sometimes all over the surface of the erythrocytes (Fig. 1). *E. ovis* was often found in free form in plasma. These pleomorphism of *E. ovis* is considered to be related to the stage of multiplication [3–5, 7, 8, 10].

The morphology of *E. ovis* by SEM was rod (0.2–0.6 µm in length) (Fig. 2) or ring form (0.2–0.4 µm in diameter) (Fig. 3) and both the forms of *E. ovis* were located at the concave regions of erythrocytes. The rod-form organisms showed chain-like patterns, while the ring-form organisms were seen as single bodies.

The fine structure of *E. ovis* by TEM was similar to previous authors' findings [1, 3,
4, 6, 10]. They had a single limiting membrane (Figs. 4, 6, 8, and 10) and ribosome-like granular materials with high electron density were observed in the inner layer of E. ovis. The other organelles including nucleus, however, were not found. On the other hand, E. ovis bounded with a single limiting membrane which had electron lucent inner structure was observed as well (Fig. 5). These morphologic differences of E. ovis seem to be due to the stage of multiplication of E. ovis.

The mode of attachment of E. ovis to erythrocytes has not been fully understood up to the present. Mckee et al. [6] reported that only a part of the organism was attached to the erythrocyte membrane. A similar mode of attachment was also observed in Hemobartonella felis [2] and Hemobartonella bovis [9]. In the present investigations, the authors observed the erythrocytes attached by a thread-like structure on E. ovis membrane (Fig. 4).

As for the mode of parasitism of E. ovis, it has been considered that they parasitize only on the mature erythrocytes up to now. Mckee et al. [6] reported that Hemobartonella canis was attached to both the mature erythrocytes and reticulocytes, while E. ovis was attached only on the mature erythrocytes. In the present study, however, the attachment of E. ovis to reticulocytes (Fig. 8), immature form erythrocytes such as normochromic erythroblast (Figs. 9 and 10), and “ghost cells” (Figs. 7 and 9) were particularly confirmed in aspirated bone marrow samples in contrast to the results obtained by Mckee et al. [6].

From the above findings, it was considered that immature erythrocytes in bone marrow were already parasitized with E. ovis at an early stage of infection.

References


要約

Eperythrozoon ovis の走査電顕および透過電顕による観察 (速報)：一条 茂・細川 晃・金徳煕
・小西範雄（帯広畜産大学 家畜内科学教室）Eperythrozoon ovis 人工感染縛羊の血液と骨髄油的電顕的観察により、本虫体は大きさ 0.2–0.6 μm の楕円状およびリング状の寄生体で単細胞の寄生膜を有し、内部にリポソームに類似した顆粒を密に含持することを知った。また、虫体の周辺部には糸状構造物を持ち、それを介して付着する像を認めた。骨髄油の検索により、E. ovis は骨髄に増殖して赤芽球の時期からすでに寄生することが確認された。
Explanation of Figures

Figs. 1–6: peripheral blood.

Fig. 1. Heavy infection of _E. ovis_ (Giemsa stain). \( \times 2,500 \).

Fig. 2. The rod shaped _E. ovis_ (arrow) is located on the concave region of erythrocyte. \( \times 22,500 \).

Fig. 3. The ring shaped _E. ovis_ (arrow) is located on the concave region of erythrocyte. \( \times 15,000 \).

Fig. 4. The arrows show thread-like structure of _E. ovis_ attached to erythrocyte. \( \times 60,000 \).

Fig. 5. _E. ovis_ bounded with a single limiting membrane having electron lucent inner structure (arrow). \( \times 24,000 \).

Fig. 6. Budding figuration of _E. ovis_ (arrow). \( \times 60,000 \).

Fig. 7. _E. ovis_ (arrow) attached to the “ghost cell”. \( \times 20,000 \).

Fig. 8. _E. ovis_ attached to the reticulocyte are observed and arrow shows budding figure of _E. ovis_. \( \times 40,000 \).

Fig. 9. _E. ovis_ (arrow) attached to the normochromic erythroblast are observed and “ghost cell” is seen as well. \( \times 12,000 \).

Fig. 10. A magnification of Fig. 9.