EHRLICHIA-LIKE MICROORGANISMS IN THE SALIVARY GLANDS OF UNFED *IXODES RICINUS* (ACARI: IXODIDAE) NYMPHS

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RICKETTSIA-LIKE MICROORGANISMS
EHRLICHIA PHAGOCYTOPHILA
IXODES RICINUS
SALIVARY GLAND
ACINUS WALL AND LUMEN
TICK-BORNE FEVER

SUMMARY: A large number of Ehrlichia-like microorganisms was detected in acinus cells and lumen of salivary glands of host-searching unfed *Ixodes ricinus* nymphs. The results indicate that Ehrlichia-like microorganisms are able to multiply in the acinus cells and disseminate into acinus lumen before blood-feeding, and that the microorganisms can be transmitted into their vertebrate host once salivation begins during the very early period of blood-feeding. Although if the detected Ehrlichia-like microorganisms is one of strains of *Ehrlichia phagocytophila* remains uncertain, the results may give some clues to the further investigations into the tick relationship of the TBF agent.

MICROORGANISMES
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GLANDE SALIVAIRE
ACINI ET LUMIÈRE
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RÉSUMÉ : Un grand nombre de microorganismes présentant les caractères d'*Ehrlichia* ont été mis en évidence dans les cellules acineuses et la lumière des glandes salivaires de nymphes d'*Ixodes ricinus* non gorgées et en recherche d'hôte. Nos résultats indiquent que ces microorganismes sont capables de se multiplier dans les cellules acineuses et de se répandre dans la lumière des acini avant le repas de sang, et qu'ils peuvent être transmis à l'hôte vertébré aussitôt que la salivation commence, au tout début du repas. Bien qu'il ne soit pas certain que les microorganismes en question appartiennent à une souche d'*Ehrlicha phagocytophila*, ces résultats constituent des indices pour des recherches ultérieures sur les relations entre les tiques et l'agent de la « tick borne fever ».

Tick-borne fever (TBF) was first discovered in tick-infested pastures of Scotland (MACLEOD, 1932; GORDON *et al.*, 1932) but later reported from other parts of Great Britain (HUDSON, 1950; TUTT & LOVING, 1955). It was also recognized in Norway (OVERAS, 1959), Netherlands (BOOL & REINDERS, 1964), Finland (TUOMI, 1966), Ireland (COLLINS *et al.*, 1970), Austria (HINAIDY, 1973) and Switzerland (PFISTER *et al.*, 1987; LIZ *et al.*, 1991). In Switzerland this disease occurs in cattle in several isolated areas, mainly in the regions of veterinary importance (Vaud-Valais and Southern Berne) (LIZ *et al.*, 1991).

The causative agent of TBF is *Ehrlichia phagocytophila*, a rickettsia belonging to the genus *Ehrlichia* of the family Rickettsiaceae and having a great number of strains (WOLDEHIWET, 1983). In domestic animals, *E. phagocytophila* parasitizes the neutrophil and eosinophil granulocytes and, more rarely, monocytes (TUOMI & VON BONSDORFF, 1966). The spleen is heavily infected and possibly is the main site for the multiplication of the micoorganism (SNODGRASS, 1975). The developmental cycle of the pathogen is found to be simple (WOLDEHIWET & SCOTT, 1988). The microorganisms are phagocytosed by granulocytes and monocytes and then enclosed in the invaginating membrane which develops into a vacuole, giving rise to what appears as morulae or clusters. Some particles leave the vacuole to initiate new infections

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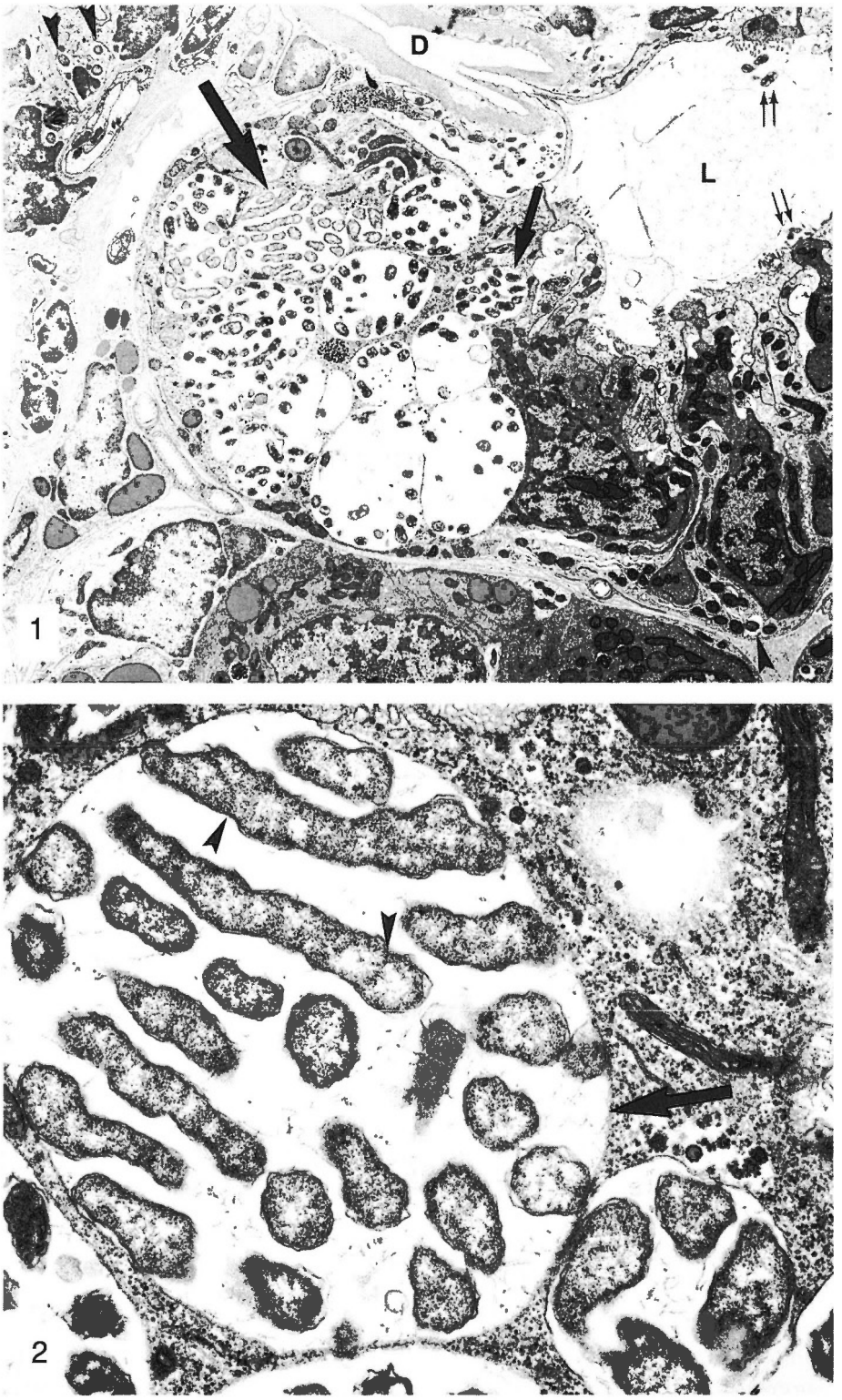
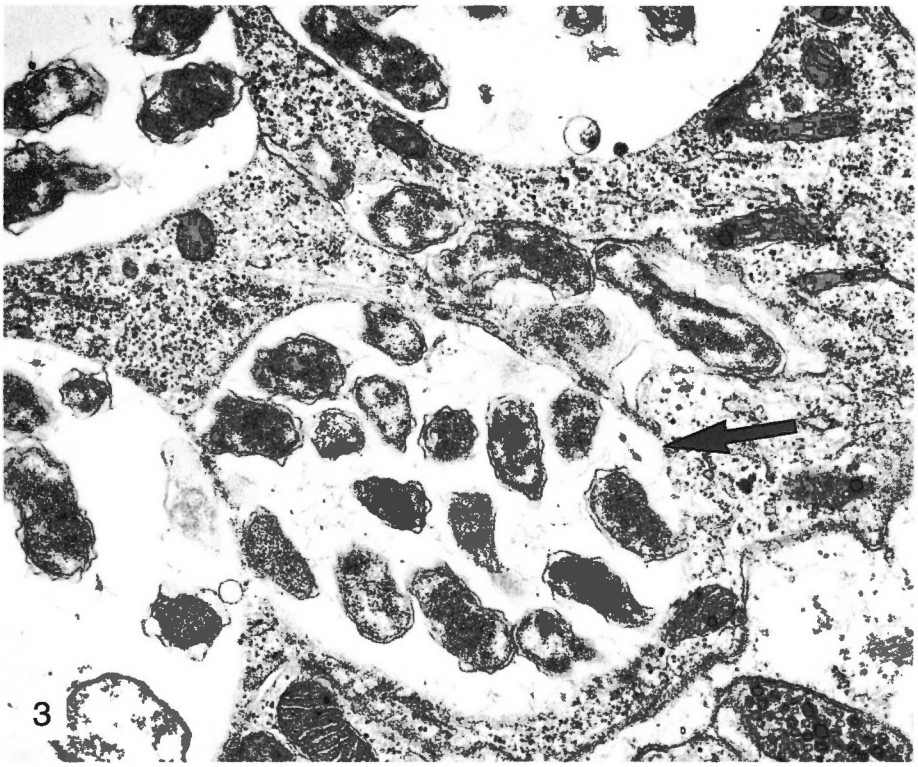


FIG. 1: Ultra-thin section of salivary gland tissue, demonstrating a large number of ehrlichia-like microorganisms within acinus cells (arrows) and lumen (double small arrows). Note several intracellular rickettsia-like microorganisms (arrowheads), assumed to be *Rickettsia helvetica* ("Swiss agent"). D: Salivary gland duct; L: Acinus lumen. 4300 ×.

FIGS. 2: Enlarged view of the two large groups of the microorganisms show in Fig. 1. Note that within the morula-like group demonstrated in Fig. 2 (arrow), some organisms are particularly long, which may result from the failure of separation of fissioning cells. The presence of cell wall-like septa (arrowheads) in these long forms supports this speculation. 25650 ×.



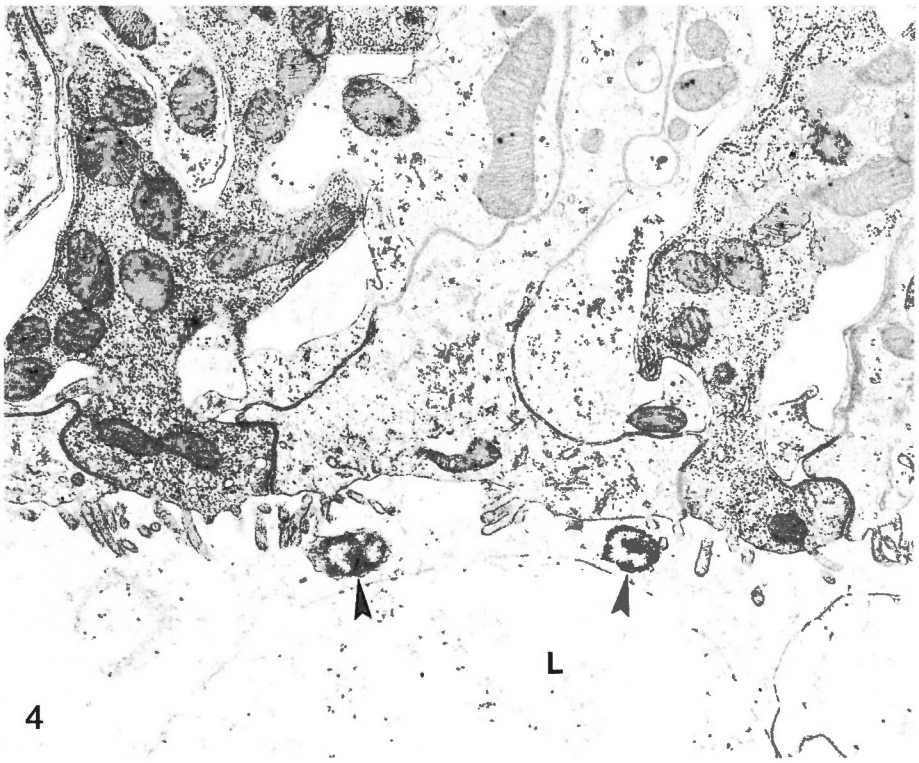
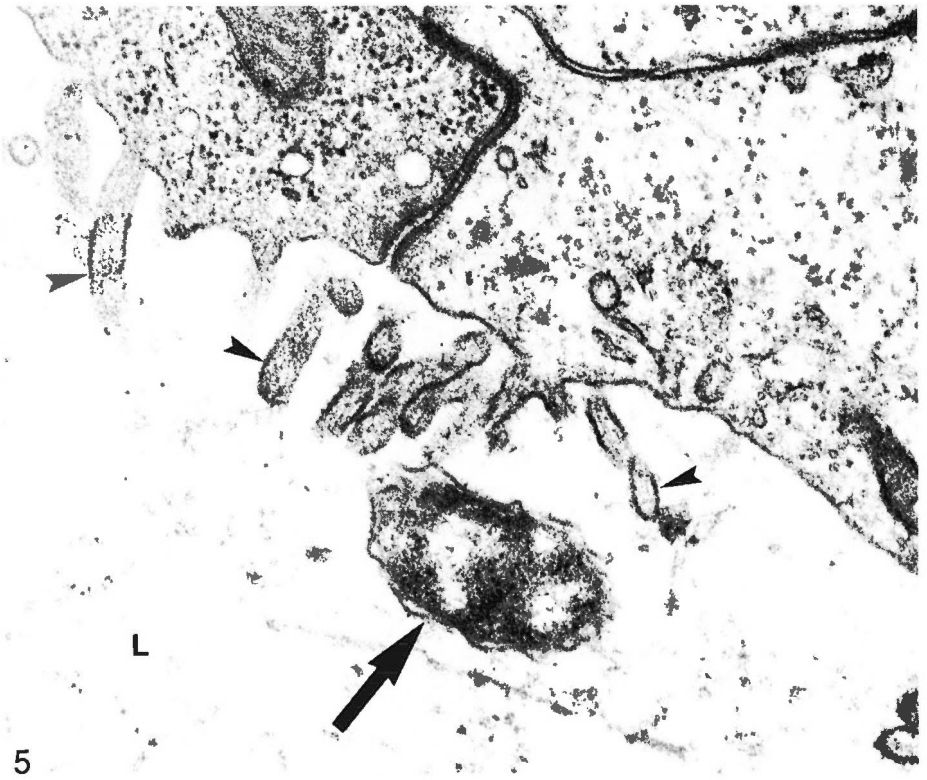


FIG. 3: Same legend as for figure 2.

FIG. 4: Ultra-thin section of acinus tissue, showing two microorganisms (arrowheads) in the acinus lumen (L). 17100 ×.



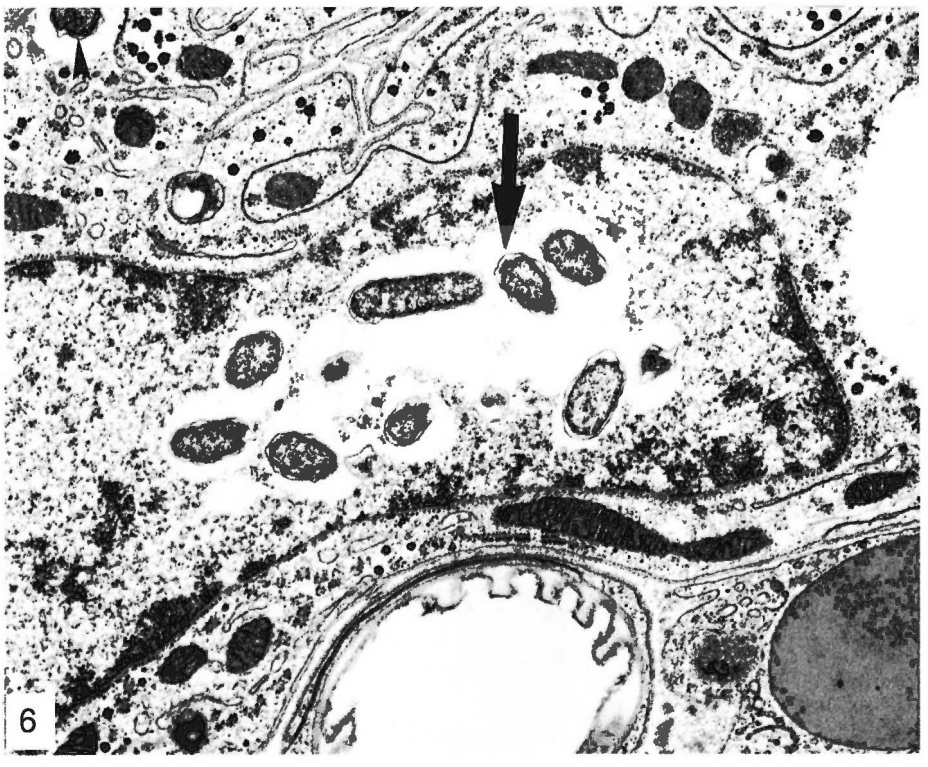


FIG. 5: Enlarged view of one of the microorganisms (arrow) in the acinus lumen (L) shown in Fig. 4. The microorganism is associated with microvilli (arrowheads) of acinus cells. 59850 ×.

FIG. 6: *Rickettsia helvetica* ("Swiss agent")-like rickettsiae within the cytoplasm (arrowhead) and nucleus (arrow) of a probable hemocyte. 17100 ×.

(WOLDEHIWET & SCOTT, 1988). Outside Europe, pathogens of TBF-like disease were often designated by other specific names or by the generic name *Cytoecetes*.

In Europe, the microorganisms are transmitted by the sheep tick, *Ixodes ricinus*, which is capable of maintaining infection transstadially but not transovarially (reviewed by FOGGIE, 1951). Although the morphology and the developmental cycle of the pathogens in vertebrate hosts have been elucidated (TUOMI & VON BONSDROFF, 1966; WOLDEHIWET & SCOTT, 1982), the tick relationship of the microorganisms still remains unclear. The rickettsia-like microorganisms detected in the ovaries of blood-feeding *I. ricinus* females, whose nymphal engorgement occurred on an infected sheep, were considered to be probably the causative agent of TBF (LEWIS, 1977; 1979). One of the remarkable characteristics of the reported microorganisms is their frequent association with mitochondria (LEWIS, 1979). In a light and microscopic investigation initially designed for the detection of *Borrelia burgdorferi* (Spirochaetales: Spirochaetaceae), the Lyme borreliosis spirochete, in *I. ricinus* tissues, rickettsia-like microorganisms displaying the same morphology as that described by LEWIS (1979) were demonstrated in the female gonads; and they could evidently be transmitted not only transstadially, but also transovarially (ZHU *et al.*, 1992). Because transovarial transmission of *E. phagocytophila* by its tick vector appears not to occur (MACLEOD *et al.*, 1933; MACLEOD, 1936), at present it cannot be assumed that the microorganisms detected in the female gonads of *I. ricinus* ticks collected from Neuchâtel, Switzerland, were the causative agent of TBF, despite the morphological resemblance.

It is interesting that, to date, among six species grouped in the genus *Ehrlichia* (*E. canis*, *E. phagocytophila*, *E. equi*, *E. sennetsu*, *E. ewingii* and *E. platys*), only *E. canis* could be demonstrated within hemocytes, midgut cells and salivary glands of its tick vector, *Rhipicephalus sanguineus* (SMITH *et al.*, 1976). However, subsequent investigations have failed to repeat the results reported by SMITH *et al.* (1976). Furthermore, even in labotatory-reared *R. sanguineus* that transmitted *E. canis* under experimental conditions, the microorganisms not been detected by microscopy have. Therefore, it is speculated that there may be a complex life cycle of *E. canis* in its tick vector. Whether a similar life cycle of *E. phagocytophila* also occurs in its *I. ricinus* tick vector remains unknown.

During the course of a light and electron microscopic detection of *B. burgdorferi* mainly using blood-feeding and replete ticks, Ehrlichia-like microorganisms were only found in female gonads but not in other organs (ZHU *et al.*, 1992). In a more recent ultrastructural investigation, a large number of Ehrlichia-like microorganisms were found in acinus cells and lumen of salivary glands in host-searching unfed nymphs collected from vegetation in a forest near Neuchâtel (Figs 1-6). It is particularly interesting that microorganisms within acinus cells were all grouped in large aggregate forms, while in the acinus lumen they are separated particles. The results reveal that Ehrlichia-like microorganisms are able to multiply in the acinus cells and disseminate into the acinus lumen before blood-feeding of nymphal *I. ricinus*, and suggest that the microorganisms can be transmitted to their vertebrate hosts once salivation begins during the very early period of blood-feeding. Although it remains uncertain whether the detected Ehrlichia-like microorganism was a of strain of *Ehrlichia phagocytophila*, the results may give some clues for further investigations into the tick relationship of the TBF agent.

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