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ATTEMPT TO CULTIVATE *RICKETTSIELLA PHYTOSEIULI* IN *DERMACENTOR RETICULATUS* TICKS : AN ELECTRON MICROSCOPIC STUDY

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Summary. — *Rickettsiella phytoseiuli* occurring in *Phytoseiulus persimilis* mites was cultivated in *Dermacentor reticulatus* ticks. Rickettsiella multiplied similarly as in mites exerting all six developmental stages: dense, intermediate, bacterial, giant, crystal-forming and small dark particles.

Key words: Dermacentor reticulatus; *Phytoseiulus persimilis*; *electron microscopy; Rickettsiella phytoseiuli*

Introduction

Rickettsiella phytoseiuli was discovered in *Phytoseiulus persimilis* ATHIAS-HENRIOT mites (Šuťáková, 1977 *a*, *b*), till now it was registered only in adult specimens (Šuťáková, 1988). Attempt to cultivate this microorganism in *Dermacentor reticulatus* (FABRICIUS) ticks is the subject of the presented study.

Materials and Methods

Adult mites P. persimilis were obtained from the breed of the Institute of Experimental Phytopathology and Entomology (Jedličková, 1983, 1985) and from the glasshouses of the Cooperative farm Kvetoslavov where they had been introduced previously. For feeding of *P. persimilis*, *Tetranychus urticae* KOCH mites were used in both cases. Under laboratory conditions they were fed on beans and in the glasshouse on cucumbers.

For the cultivation of rickettsiae in ticks, 30 adult mites *P. persimilis* were used from the laboratory breed and from the second locality.

Rickettsiae were cultivated in *D. reticulatus* ticks. They were collected by dragging a blanket in meadow forests near Bratislava. For these experiment females were included only, in the laboratory they were partially fed on rabbits.

After surface sterilization of P. persimilis mites in 70% ethanol and a thorough washing in distilled water, suspensions were prepared in a physiological solution of pH 7.2 (30 imagos in a 0.5 ml of solution). The suspension was administered to ticks intracoelomally in an amount of 0.01 to 0.03 ml according to the size of the recipient. On days 7, 14 and 21 after injection we verified by the haemocyte test (Řeháček *et al.*, 1971) whether rickettsial infection developed in the ticks. When the results of the haemocyte test were positive, the injected ticks were used for studying the developmental cycle, reproduction, morphology and ultrastructure of rickettsiae.

For electron microscopy the haemolymph was collected from *D. reticulatus* ticks and the alimentary tract plus the "fat body" were removed. The procedures of the material preparation for electron microscopy, i. e. the ultrathin sections and their contrasting were published elsewhere (Šutáková, 1988). The material was evaluated on the electron microscope TESLA BS 500 and photographes on glass plates ORWO EU 2.

Results

The six developmental stages of *R. phytoseiuli* found in *P. persimilis* mites were also seen in the alimentary tract, "fat body" and in the haemolymph of *D. reticulatus* ticks. They were most numerous in haemolymph, where they occurred single or mainly accumulated in round and oval shaped formations either not surrounded by an external membrane or membrane bound (Figs 1, 2). In these accumulations the rickettsiae were either of dense type only or of mixed types, i.e. dense, intermediate and bacterial forms (Figs 1, 2) as well as giant rickettsiae (Figs 3—5). Accumulations of dense forms were found with one (Fig. 4), two (Fig. 3) or more crystal-forming rickettsiae. In the lumen of the alimetary tract no rickettsiae were seen, however, they were present in the epithelial cells of the mid- and hind part of the central gut. The latter were mostly dense rickettsiae, occurring either single or in smaller accumulations lacking a membrane (complex), or enveloped by a membrane (Fig. 6). In the "fat body" the rickettsiae were more numerous. All morphologically different types of rickettsiae were observed, however, dense rickettsiae among which protein crystals were often recorded, prevailed (Fig. 7). Intermediate and bacterial forms of rickettsiae occurred frequently, the dividing bacterial rickettsiae similarly to the giant ones occurred only occasionally (Fig. 8).

Discussion

R. phytoseiuli injected into *D. reticulatus* multiplied intensively without causing any visible lesions in ticks. The physiological processes, such as a digestion, excretion, laying of eggs, etc., had a normal course. Similarly in *P. persimilis* mites tremendous infestation of almost all organs did not harm these arthropods, the mortality of infected mites did not differ from that of uninfected ones.

In the isolated alimentary tract, "fat body" and haemolymph the same developmental stages of *R. phytoseiuli* were observed as in mites *P. persimilis*, i.e. dense rickettsiae, intermediate, bacterial (binary dividing cells were found occasionally), giant, crystal-forming and those with small dark particles lacking the membrane complex. These were most frequent in the haemolymph, but massive infection was seen also in the "fat body" and alimentary tract. Among rickettsiae, the dense stage prevailed but a great amount of crystal-forming rickettsiae with dark particles and crystals within them was observed as well, confirming that *D. reticulatus* ticks represented a suitable substrate for the development of *R. phytoseiuli*. This seems the major contribution of the presented work, indicating that rickett-

siae infeting entomophagous mites are able to multiply in haematophagous arthropods—ticks known as vectors of pathogenic rickettsiae for man and animals similarly as in their natural hosts.

Concerning isolation of rickettsiae belonging to family *Rickettsiella* the report of *R. grylli* isolated from naturally infected *Zonocerus variagetus* is of interest (Henry *et al.*, 1986); the latter rickettsiae were successfully used to infect *Melanoplus sanquinipes* and *M. differentialis* in vivo and in vitro. In both cases only three developmental stages were detected (initial, intermediate and elementary). Various species of *Rickettsiella* appear in 3—5 developmental stages. The crystal, which always occurs during propagation of these rickettsiae, has been seen only in the original host, i.e. in *Z. variegatus*. However, in our experiments the crystal was found as a part of crystal-forming rickettsiae in *D. reticulatus*.

The presented results have also a practical significance, since upon the cultivation of *R. phytoseiuli* in ticks a sufficient number of these organisms can be obtained for antigen preparation as well as for immunization of laboratory animals. These ticks themselves can be used for maintaing the isolated strain of these rickettsiae and also as a "laboratory animal" in experimental studies, i.e. the study of morphology, developmental cycle, virulence and pathogenicity.

The results may have also a theoretical significance for the study of the phylogenesis of mites on the basis of the same reproductive cycle of *R. phytoseiuli* in *P. persimilis* mites as well as in *D. reticulatus* ticks — because a common phylogenetic origin of these arthropods may be presumed.

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Explanation of Electron Micrographs (Plates XIII—XVI):

Fig. 1. The occurrence of dense rickettsiae in the haemolymph of the tick *Dermacentor reticulatus*. DR — dense rickettsiae. × 13,5400.

Figs 2—5. Morphologically different rickettsiae in haemolymph of *D. reticulatus*. BR — bacterial rickettsiae, CR — crystal-forming rickettsiae, DR — dense rickettsiae, GR — giant rickettsiae, SP — small dark particles. 2 = × 11,300; 3 = × 6,420; 4 = × 10,560; 5 = × 19,200.

Fig. 6. Rickettsiae in the epithelia cell of the midgut of the tick *D. reticulatus*. DR — dense rickettsiae. × 15,500.

Fig. 7. Dense rickettsiae and protein crystals in the "fat body" of the tick *D. reticulatus*. Cr — — protein crystals, DR — dense rickettsiae. × 17,450.

Fig. 8. Different types of rickettsiae in "fat body" of *D. reticulatus*. BR — bacterial rickettsiae, GR — giant rickettsiae, IR — intermediate rickettsiae. × 41,900.