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INTERACTION BETWEEN *DERMACENTOR RETICULATUS* CELLS AND *COXIELLA BURNETII IN VIVO*

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Summary. — By electron microscopy the distribution of *Coxiella burnetii* was followed in females of *Dermacentor reticulatus* injected intracoelomally in a dose of about 10^3 EID_{50} per tick. The heaviest infestation with coxiellae was noticed in the cells of haemolymph, fat body, Malpighian tubulus and tracheal complex. No rickettsiae were found in Gene's organ. Unexpected was the propagation of rickettsiae in muscle fibres. *C.burnetii* multiplied in all organs affected. Heavy infection resulted at the marked damage of cell components. Coxiellae were evident in the haemocytes; in organs they formed small and large cell variants; endospore formations were observed free in the haemolymph.

Key words: Dermacentor reticulatus; Coxiella burnetii; haemolymph; tick organs; electron microscopy

Introduction

It is generally accepted that ticks are the principal vectors of *Coxiella burnetii*, the agent of Q fever. This statement comes from relatively frequent findings of coxiellae in ticks in the nature and was many times confirmed by experimental studies demonstrating the transmission of the agent to the host by various developmental stages of different ticks species and its transmission during developmental stages of tick metamorphosis. *C. burnetii* causes a generalized infection of ticks, it undergoes its developmental cycle and is released via secretions (saliva, faeces, coxal fluid) into the external environment.

Less is known of the fate of coxiellae in the tick body on their distribution in its organs and cells. Generalized infection *D. reticulatus* of ticks with *C. burnetii* was already studied in the course of dual infection of coxiellae and tick-borne encephalitis virus (Řeháček *et al.*, 1987). This study provided further data on the localization and biology of coxiellae in various organs of this tick species on cellular level and the occurrence of different forms of

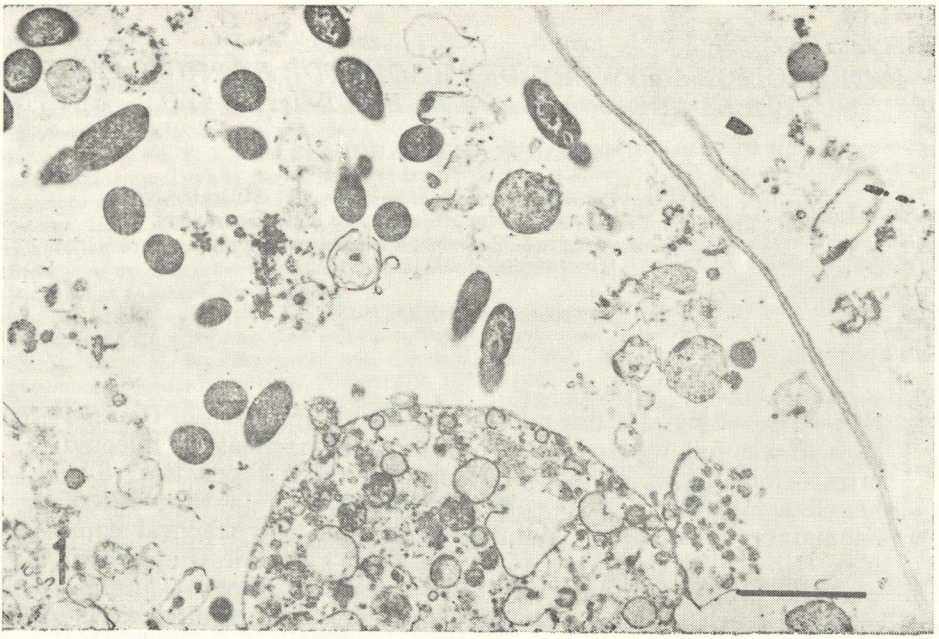


Fig. 1. *C. burnetii* free in the haemolymph. Bar = 2 μm.

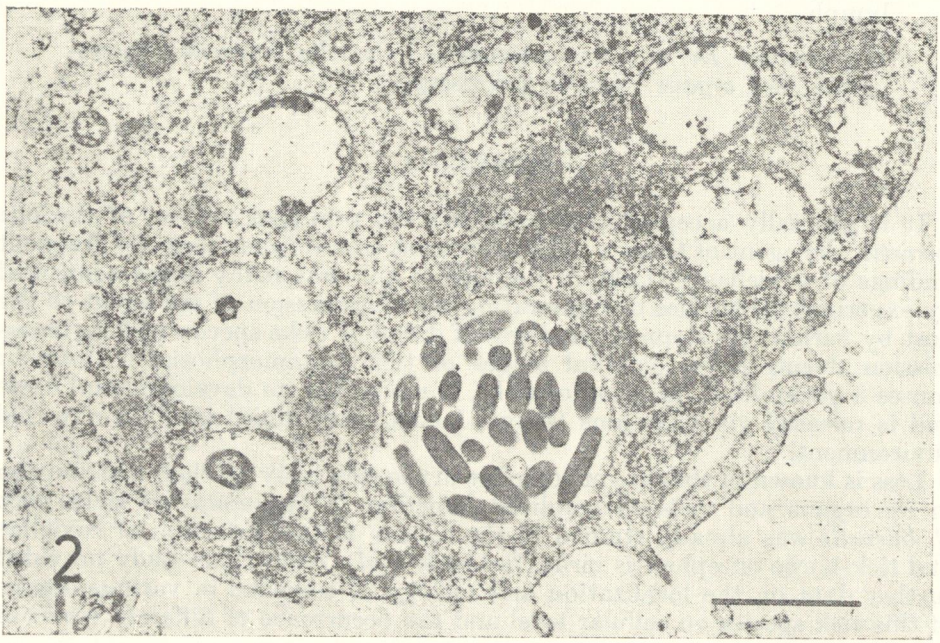


Fig. 2. C. burnetii in the vacuole of the haemocyte. Bar = 1 μm

rickettsiae, i.e. small cell variants (SCVs), large cell variants (LCVs), endospore formation (McCaul and Williams, 1981) in tick organs as detected by electron microscopy.

Materials and Methods

Eighteen half-engorged tick females on laboratory rabbits were employed. The ticks were inoculated into the body cavity with an approximate dose per tick of 10^3 EID_{50} of *C. burnetii* strain Nine Mile, I phase suspension, in the 3rd passage on embryonated hen egg yolk sacs. On the twenty first day following infection the ticks were bled for haemolymph, and their organs were dissected for electron microscopic investigation. Following dissection, the material was placed into fixative for about 20 hr at 4 °C (Ito and Karnovsky, 1968). Further procedures on the processing of tick material for electron microscopy were described in previous papers (Šuťáková, 1988; Šuťáková and Řeháček, 1988). Tick sections were examined in electron microscope TESLA BS 500 and photographed on glass plates ORWO EU 2.

Results

The results obtained repeatedly confirm the generalized spread of *C. burnetii* in *D. reticulatus* ticks. Nevertheless, we found marked differences in the capacity or frequency of various organs to become infected. In some organs the infestation was comprehensive, in some is less abundant and in some organs it did not occur. The heaviest infection with *C. burnetii* was found in the haemolymph namely in haemocytes, then in the cells of "fat body", Malpighian tubules and tracheal complex. The least infestation with the pathogen was noted in cells of the intestinum, ovaries, synganglion, and salivary glands, while no infection was observed in Gene's organ of arthropods.

Haemolymph

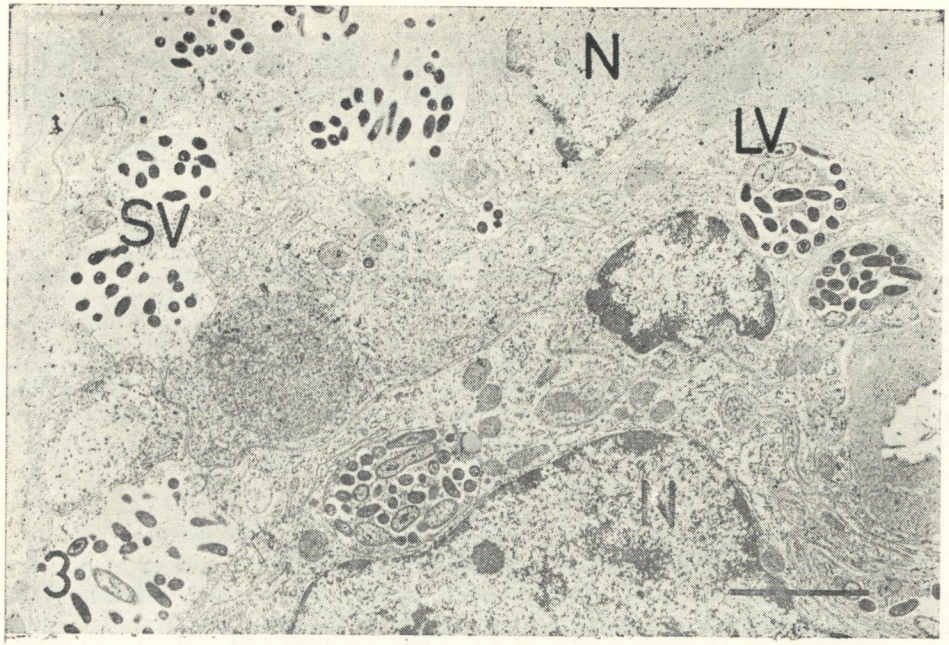
Both types of coxiella bodies, i.e. SCVs and LCVs, were seen frequently in the haemolymph either free without vacuolar enclosure (Fig. 1) or enclosed in the vacuoles. Most frequently *C. burnetii* attacked haemocytes, where propagated always in the vacuoles (Fig. 2). The infestation of haemocytes was always massive and permanent which confirms the competence of the application of the haemocyte test (Řeháček *et al.*, 1971*a*) as a proof of rickettsial infection of ticks.

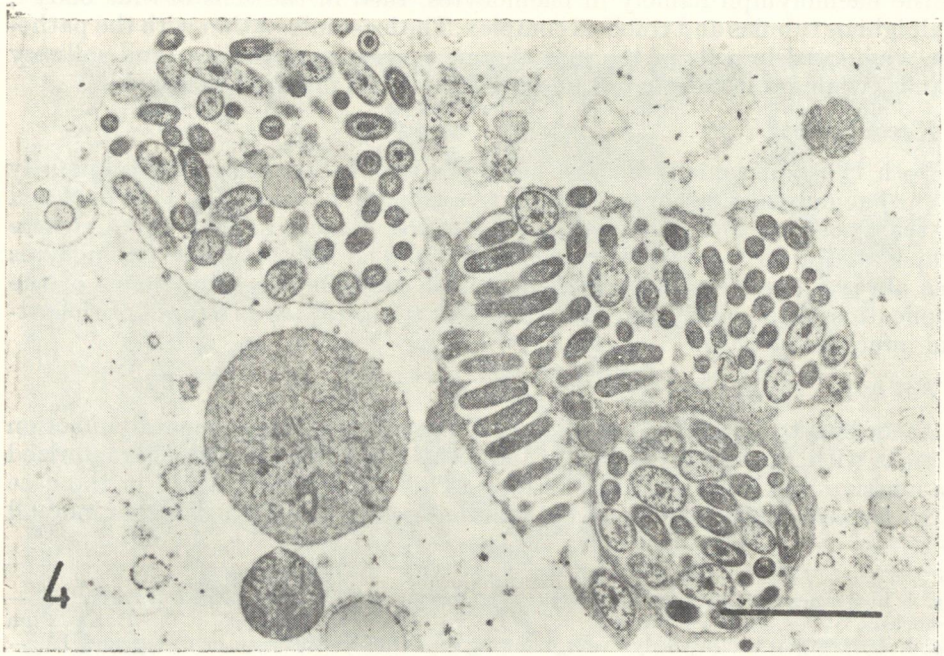
Fat body

According to our prior experience in natural and experimental infection of ticks with *R. slovaca* (Řeháček, 1984) this organ was always characterized by massive infestation with rickettsiae. This was attested again in the case of *C. burnetii* infection, however, it is necessary to emphasize that not all

Fig. 3. Less extensive infection of *C. burnetii* in the "fat body". LV — large cell variant, N — nucleus, SV — small cell variant Bar = 2 μm

Fig. 4. Destruction of "fat body" cells following an extensive infection of *C. burnetii*. LV — large cell variant; SV — small cell variant Bar = 1 μm





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cells of "fat body" complex were infected regularly in high degree but that there existed also places with limited number of rickettsiae or even free of infection. It was evident in some areas (Fig. 3) that the cells had become infected not long time ago, because only SCV coxiellae were seen in the both small and large vacuoles. Also the host cells were not markedly damaged and the majority of cellular components was still preserved. During extensive infection (Fig. 4) the second type of coxiellae, i.e. LCVs were visible. Such infection was characterized by the destruction of host cells. It is interesting to note that in the case of massive infection of cells of "fat body" with coxiellae, the pathogens appeared to form lines.

Other organs

What the extent of infection concerns, the Malpighian tubules could be compared with that of "fat body" as some areas of the organs were either massively or less frequently infested or were uninfected. However, in the majority of cells there occurred an extensive infection with a total destruction of host cells (Fig. 5). *C. burnetii* was not seen in lumen of Malpighian tubules.

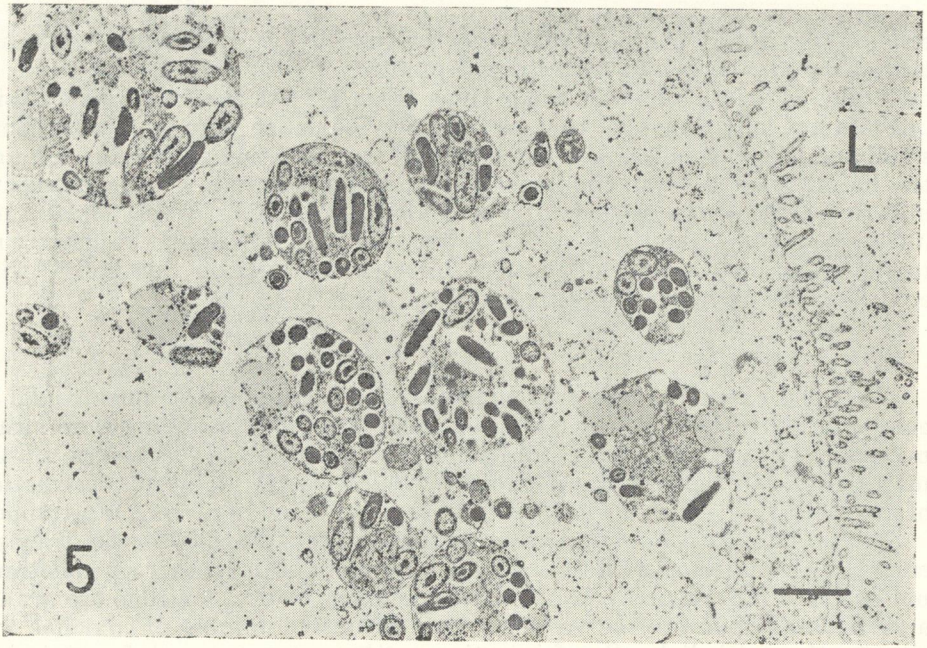
Similarly to both above mentioned tissues also the cells of the tracheal complex were infested with coxiellae; *C. burnetii* of both types were sometimes not enclosed into vacuoles. At massive infection, of course, coxiellae are always and regularly found in the vacuoles. Less extensive number of coxiellae was seen in the epithelial cells of intestinum; the agent was not found in the lumen. Areas with a limited number of rickettsial bodies without any vis ble damage to host cells were seen. Similarly to the intestinum, in the ovarial cells only small vacuoles were observed with low number of coxiellae. The infestation was situated in epithelial cells of the lateral and central oviduct.

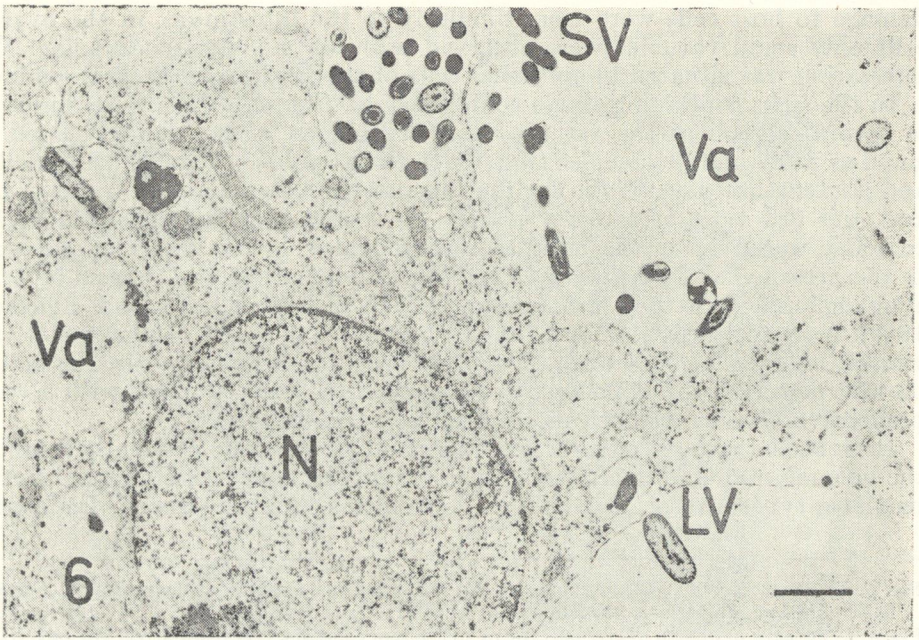
In the ticks containing single coxiellae only, the pathogens were observed in several alveoli of the 1st type salivary glands where they occurred in small as well as large vacuoles (Fig. 6). In ticks double infected with coxiellae and *Rickettsiella phytoseiuli*, the first agents were seen also in alveoli of the 2nd and 3rd type of salivary glands (Šuťáková and Řeháček, 1989). No coxiellae were seen in the brain of ticks inoculated with coxiellae only, but in the presence of *Rickettsiella phytoseiuli* they attacked the cortical layer of synganglion where they propagated in vacuoles (Šuťáková and Řeháček, 1989). Noteworthy is the fact, that *C. burnetii* was found in fibres of dorsoventral muscles. Groups of muscle fibers prepared together with "fat body" on 21st day after infection revealed longitudinal vacuoles filled with a comprehensive number of both morphological variants (Fig. 8).

Both basic types of coxiellae, i.e. SCVs and LCVs, were frequently found in the haemolymph and in almost all internal organs tested. Transitional (intermediate) types also occurred. Both basic forms showed division. Endospore

Fig. 5. Destructed cells of Malpighian tubules following the extensive infection of *C. burnetii*. L — lumen of Malpighian tubules Bar = 1 μm.

Fig. 6. C. burnetii in alveoli of the 1st type of the salivary glands. LV — large cell variant; SV — small cell variant; Va — vacuole Bar = 1 μm.





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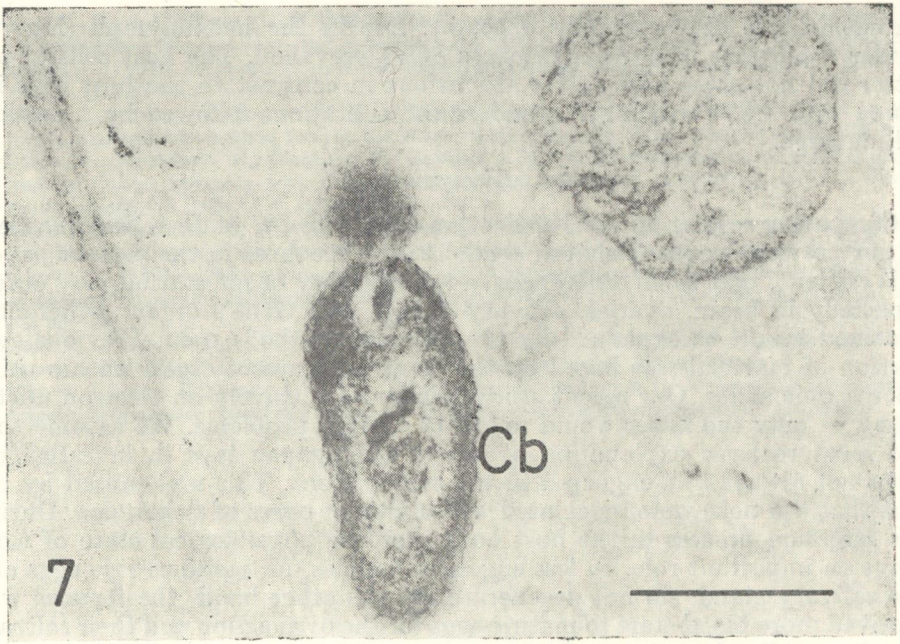

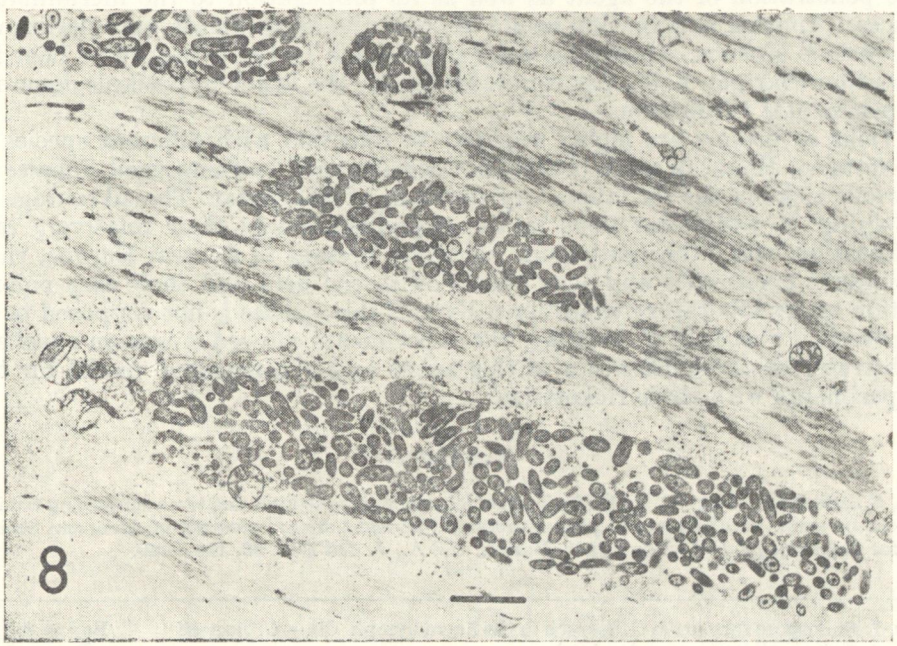

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formation was observed only once, i.e. free in the haemolymph (Fig. 7). Under conditions of our experiments SCVs prevailed. The host cells of the latter did not show any visible alterations in contrast to the host cells infested with LCVs which often underwent alterations followed by a marked cell damage.

Discussion

Comparing results on the localization of *C. burnetii* in *D. reticulatus* ticks in our previous paper (Řeháček *et al.*, 1987) with those in the present paper, it is evident, that some differences in the intensity of infestation may occur, especially in brain, ovaries, salivary glands, and Gene's organ. Differences obtained would be explained by the different methods used. Previous evaluation of material was based on the Gimenez stain of organ smears using microscopic slides. Our recent study is based exclusively on electron microscopy — only the latter would follow the target problems. We assume that the most realistic explanation of above discrepancy lays in investigating ticks not always half-engorged in the same extent. This was caused by the fact that the ticks were inoculated with different doses of rickettsiae. During the infection process in the host body also the physiological state of ticks plays an important role. In less engorged females, for instance ripe eggs and the salivary glands do not degenerate, on the other hand, the females who received more blood start to mature eggs for the oviposition and their salivary glands rapidly degenerate. Such condition, of course, markedly influences the penetration of the agent as well as its propagation to the incriminated organs. Another explanation would be, that the experiments were performed in various seasons, which also may play a role in the intensity of host infestation (Skripal, I. G., per com.). However, the latter hypothesis demands experimental verification.

Ticks as vectors are highly sensitive to infection with different species of rickettsiae. Řeháček *et al.* (1971*b*) successfully used half-engorged females of various tick species for laboratory titration of rickettsiae. The advantage of applying *D. reticulatus* ticks in the experiments was their relatively rare infestation with other pathogens naturally present in Central Europe, their abundant occurrence and easy collection in the field and their easy colonization under laboratory conditions. Even if this species is not supposed to be a potential vector of *C. burnetii*, our experiments showed it was an excellent substrate for the cultivation of this agent and a convenient model for the experiments with these rickettsiae.

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Fig. 7. Endospore formation *C. burnetii* in the haemolymph. Cb — *C. burnetii* Bar = 0.5 μm
Fig. 8. C. burnetii in the muscle of ticks. Bar = 2 μm

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