Acta virol. **33** : 577—581, 1989

VIRUS-LIKE PARTICLES IN *DERMACENTOR RETICULATUS* TICKS

J. ŘEHÁČEK, G. ŠUŤÁKOVÁ

Institute of Virology, Slovak Academy of Sciences, 817 13 Bratislava, and Institute of Experimental Phytopathology and Entomology, Slovak Academy of Sciences, 900 28 Ivanka pri Dunaji, Czechoslovakia

Received September 21, 1988

Summary. — Virus-like particles were found in the brain and salivary glands of *Dermacentor reticulatus* ticks double infected with *Coxiella burnetii* and *Rickettsiella phytoseiuli*. The particles were spherical (diameter of about 70 nm) and consisted of an electron-dense inner core and a dense external coat.

Key words: Dermacentor reticulatus; Coxiella burnetii; Rickettsiella phytoseiuli; virus-like particles; double infection; electron microscopy

Introduction

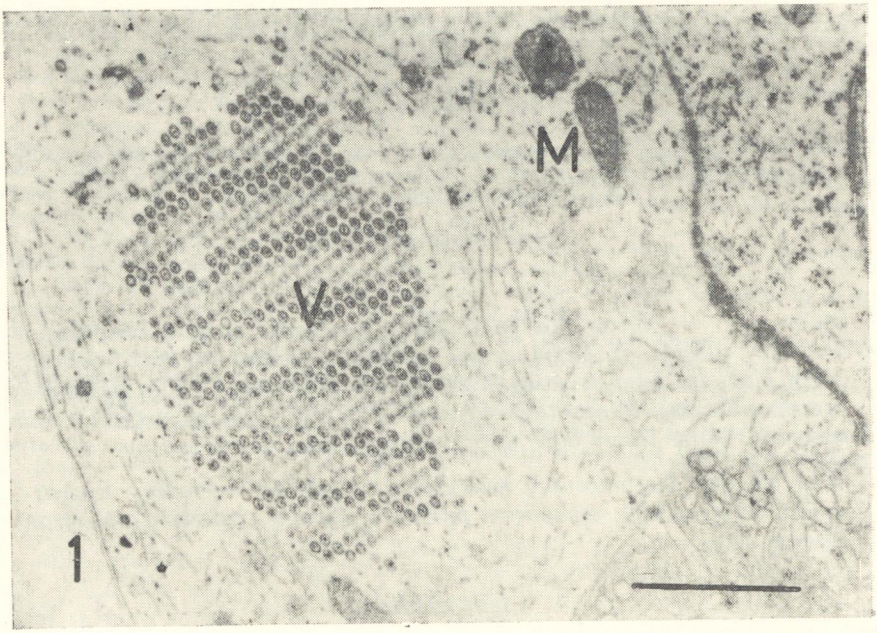
The detection of virus-like particles (VLPs) by electron microscopy is rare in ticks collected directly in the field. This happens occasionally in the course of other studies, i.e. when following the ultrastructure of various organs or investigating the dynamics of experimental infection with rickettsiae or other pathogens. Čiampor and Nosek (1976) found VLPs in cytoplasm of gut cells of *Dermacentor marginatus* ticks. Megaw (1978) demonstrated VLPs in salivary glands of the tick *Boophilus microplus* and Diehl *et al.* (1980) reported a virus in salivary glands of *Dermacentor marginatus* ticks when studying their natural infection with *Rickettsia slovaca*. The present paper deals with finding of the VLPs in *Dermacentor reticulatus* ticks in experimentally infected ticks with *Coxiella burnetii* and *Rickettsiella phytoseiuli*.

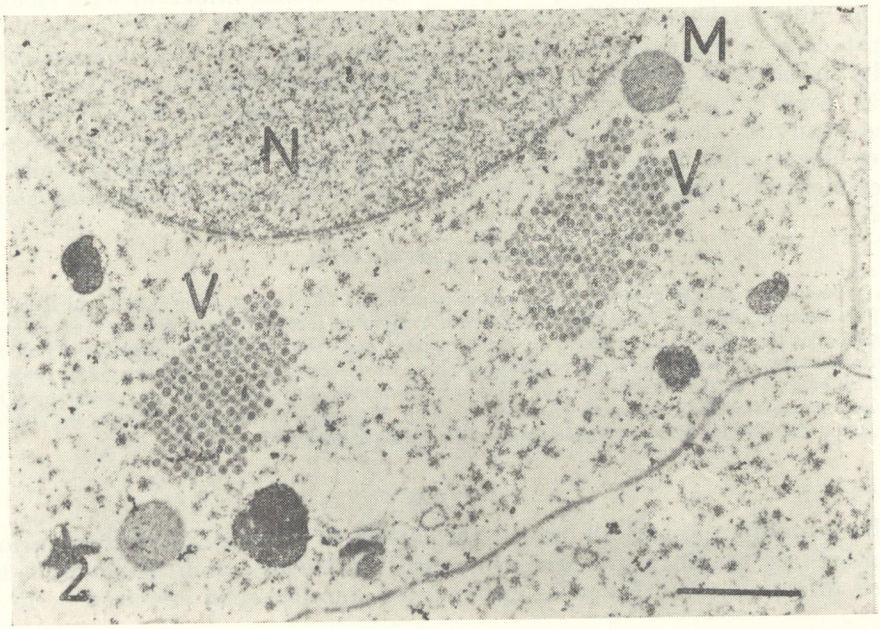
Materials and Methods

In experiments on mixed infections with *R. phytoseiuli* and *C. burnetii* (Šuťáková and Řeháček, 1989) we used half-engorged females of *D. reticulatus* ticks on day 21 following their intracoelomal injection. For the electron microscopy the haemolymph and all internal tick organs were removed and immediately after the dissection immersed into the fixative (Ito and Karnovsky, 1968) for about 20 hr at 4 °C. Further processing of this material for electron microscopy, sectioning and contrasting was described elsewhere (Šuťáková, 1988). The sections were examined in the electron microscope TESLA BS 500 and photographed on glass plates ORWO EU 2.

Results

The haemolymph and all internal organs of 15 females infected in various variations, i.e. the single infection either with *C. burnetii* or *R. phytoseiuli*, the mixed infection of both agents either after simultaneous infection or in 14


M
V
1


M
N
V
V
2

days interval apart, were investigated. From this material VLPs were demonstrated only in salivary glands and the synganglion in 12 female ticks belonging to all experimental variants.

The VLPs were distributed mainly in alveoli of salivary glands of the 2nd and 3rd type where they formed paracrystaline accumulations (Fig. 1). The infected cells did not show any marked signs of damage. The synganglion contained the VLPs in the cortical layer but they were not found in the neuropile. In contrast to the salivary gland, the size of VLPs crystalline accumulations in the brain was less extensive; however, one cell contained several arrangements of this kind. In the latter case the cellular components were damaged undergoing complete lysis of the cytoplasm (Fig. 2). VLPs in salivary glands and synganglion were morphologically identical, they were of spherical shape, of the same size, having a diameter of about 70 nm and consisted of an electron-dense inner core with a dense outer coat.

In one vacuole of the 2nd type salivary gland alveolus of one female tick which had been infected simultaneously with *C. burnetii* and *R. phytoseiuli* VLPs were found together with *C. burnetii*. It appeared that the morphology of the rickettsial cells was altered and damaged in the presence of VLPs (Fig. 3). Besides of this vacuole, in the same cell of this alveola, single forms of *R. phytoseiuli* were not accompanied with any morphological changes. This may be the reason for what it consisted in the absence of VLPs as well as *C. burnetii* cells.

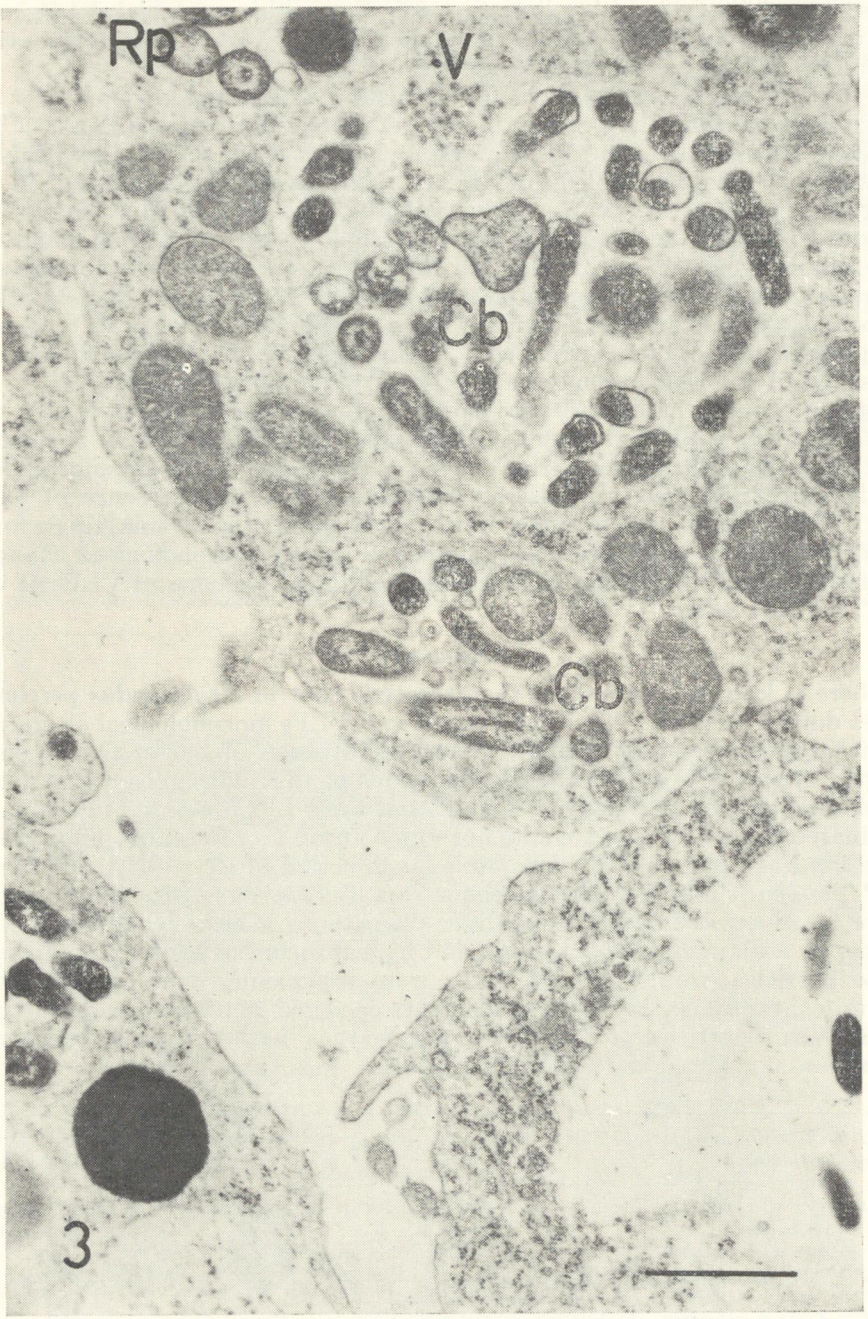
Discussion

There is known a case from the literature that in *Phytoseiulus persimilis* mites double infected with *R. phytoseiuli* and VLPs morphological alterations of rickettsiae were noted such as irregular division of bacterial and dense forms of rickettsiae and different alterations of this morphology, etc. (Šuťáková and Rüttgen, 1978). It is evident that the VLPs presented in the paper originated from ticks and could not come from *P. persimilis* mites which were used as donors of *R. phytoseiuli* for infection of ticks. VLPs occurring in *P. persimilis* mites might also come from *Tetranychus urticae* mites, which serve for them as nourishment, differ from those in ticks by an icosahedric shape, by a diameter of 30—40 nm and by a translucent centre. In addition, these particles even very numerous, were prevalently found scattered in the mites, rarely crystalline arrangements occurred (Šuťáková and Rüttgen, 1978). Virus particles of such size and feature as found in *D. reticulatus* ticks were not found in *P. persimilis* mites.

Fig. 1. Crystalline accumulation of virus-like particles in the salivary gland of the tick *Dermacentor reticulatus* Bar = 1 μm
M — mitochodrion; V — virus-like particles

Fig. 2. Crystalline accumulation of virus-like particles in the cortical layer of the synganglion of *D. reticulatus*. Bar = 1 μm
M — mitochondrion; V — virus-like particles; N — nucleus

Fig. 3. Virus-like particles and altered morphological forms of *Coxiella burnetii* in common vacuole of the salivary gland of *D. reticulatus* during triple infection. Bar = 1 μm
Cb — *C. burnetii*; Rp — *Rickettsiella phytoseiuli*; V — virus-like particles.


Rp
V
Cb
Cb
3

The taxonomy of VLPs in *D. reticulatus* ticks is still open. In diameter they differ from the tick-borne encephalitis virus occurring in different species of ticks living in Central Europe but they resemble those of *Bunyaviridae* or reoviruses. Nevertheless, their exact identification may be hampered by probably low percentual infestation of ticks regardless of the relatively common occurrence, by the lack of suitable sera for immunofluorescence tests, and by problems in application of a convenient medium for their isolation and cultivation.

References

Čiampor, F., and Nosek, J. (1976): Virus and rickettsia-like structures in gut cells of *Dermacentor marginatus* nymphs. *Acta virol.* **20**, 439—441.

Diehl, P., Řeháček, J., and Bázliková, M. (1980): Occurrence of virus in the salivary glands of *Dermacentor marginatus* ticks collected in nature. *Proceedings Intern. Symposium "New Aspects in Ecology of Arboviruses"*, Smolenice near Bratislava, June 11—15, 1979, p. 265—269.

Ito, S., and Karnovsky, M. I. (1968): Formaldehyde-glutaraldehyde fixatives containing trinitro components. *In Abstracts of Papers Presented at Eight Annual Meeting American Society Cell Biology*, 11. Nov. 1968, Boston, U.S.A. *J. Cell Biol.* 39, 168—169.

Megaw, M. J. W. (1978): Virus-like particles pathogenic to salivary glands of the tick *Boophilus microplus*. *Nature* (*Lond.*) **271**, 483—484.

Šuťáková, G. (1988): Electron microscopic study of the developmental stages of *Rickettsiella phytoseiuli* in *Phytoseiulus persimilis* ATHIAS-HENRIOT (*Gamasoidea*/Phytoseiidae) mites. *Acta virol.* **32**, 50—54.

Šuťáková, G., and Řeháček, J. (1988): Mixed infection of *Rickettsiella phytoseiuli* and *Coxiella burnetii* in *Dermacentor reticulatus* female ticks: electron microscope studies. *J. Invertebr. Pathol.* (in press).

Šuťáková, G., and Rüttgen, F. (1978): *Rickettsiella phytoseiuli* and virus-like particles in *Phytoseiulus persimilis* (*Gamasoidea*: Phytoseidae) mites. *Acta virol.* **22**, 333—336.