

## A RED MOSQUITO IRIDESCENT VIRUS IN Aedes PUNCTOR IN CZECHOSLOVAKIA

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**Abstract.** A first case of a red mosquito iridescent virus was found in *Aedes punctor* larvae, with icosahedral particles 200 nm in diameter. The pathology of this MIV is compared with the pathology of the bluegreen MIV in *Aedes cantans*.

The iridescent virus of mosquitos (MIV) was first detected in *Culex tarsalis* by Kellen et al. (1963) but first misinterpreted as "polyhedrosis". At the same time an iridescent virus was identified in spring *Aedes* mosquitos in Czechoslovakia and brought to the attention of Dr Kellen (Weiser 1964). Initiated by this an emended description was published (Clark et al. 1965) and our own observation was published in a mimeographed edition of the WHO (Weiser 1965). The virus in *Aedes cantans* and *A. annulipes* in Europe was considered as identical with the virus described from California also from another host, *A. taeniorhynchus*. But late instars of larvae infected with the virus in California were iridescent orange, sometime they were only milky white in the late stage of infection. This iridescence was different from the classical type represented by the *Tipula* iridescent virus or the MIV recorded from Czechoslovakia. Details of the pathology of this MIV in the mosquito host were published in the monograph by Weiser (1966, 1969). The different type of color was first connected with the size of virus particles, but further isolates did not show any strict coincidence. Further collections in the USA (Chapman et al. 1968) brought evidence of MIV in several new hosts and in *A. taeniorhynchus* another yellow green MIV was recorded beside the orange one. Matta and Lowe (1970) identified the fat body and the imaginal discs as the only tissue infected, without any sign of replication elsewhere in the host. Weiser (1966, 1969) demonstrated virogenic stromata in cells of most tissues except the midgut and the salivary glands. MIV infections were recorded later from different regions of Europe and Central Asia, with bluegreen iridescence as the leading symptom. Recently we discovered a first case of an orange type MIV from Czechoslovakia.

### MATERIAL AND METHODS

In spring of 1984 a road-side locality with *Aedes punctor* Kirby was studied near Bílek (E. Bohemia, ČSSR). The locality was an elongated trench with stagnant water, 20 cm deep and 25–30 cm broad, with remains of grass on the bottom. Water was dark brown from dissolved humic substances. A population of *A. punctor* as the only mosquito developed there from end of April till May 10 when most larvae were pupated and emerged. Of this population 0.5 % of late larvae were infected with *Amblyospora* (Microsporidia). A short dry period in May reduced the water volume to a minimum and new rains filled the rim again to the spring level and a new population of *A. punctor* developed there. On June 23, besides another series of larvae infected with *Amblyospora* (0.7 %), a single red opaque larva was found in samples which were collected from the habitat and inspected in a pan with black bottom.

The larva, *A. punctor* 4th instar, was cut transversally into three parts; the central part was used for water mounts, the thorax was dissected in 2 % glutaraldehyde in phosphate buffer and was processed for electron microscopy. The posterior part was fixed in Bouin's for

histology, embedded in paraffin and cut into sections 6  $\mu$ m thick. Dry smears fixed in metanol and section preparations were stained with Giemsa. Material in glutaraldehyde was postfixed in 4 % osmic acid in PB. Thick sections were stained with methylene blue and ultrathin sections after staining in uranyl acetate and lead citrate were inspected in the Tesla 613 TEM.

## RESULTS

The larva was filled with a fat body which was opaque with a red iridescence, the lobes of the fat body were distinct and coherent. The larva was motile without difficulties, of the same size as other larvae of the same population. After pressure on the cover slip, a turbid liquid appeared with minute particles shining in the dark field. Parts of the dark field image were masses of particles in clusters. The lobes of the fat body appeared as grapelike clusters of spherical cells connected only loosely together, with prominent oval nuclei. The cell wall was very fragile, in water they bursted open and they released masses of granules. On smears these cells were without fat droplets. Lymphocytes were on the smear without any sign of virogenic stromata in their interior. Isolated cells of the fat body stained in their cytoplasm dark red virogenic stromata.

In thick sections of the Vestopal-embedded material, the infected fat body cells had no remains of any fat droplets and their cytoplasm was filled with a virogenic stroma divided into irregular clusters and deposits under the cell wall. The same picture was in cells of the hypodermal tissue. In cells from the posterior segments, the virogenic stromata were concentrated in the centre of the host cell in a dense mass. With methylene blue the whole mass of the virus was stained blue (Plate I, Figs. 1, B, C).

In Giemsa-stained paraffin sections of the infected mosquito the virogenic stromata were stained only on their surface, whereas the interior with virus particles was not stained. The picture in infected cells was representing a system of surface stained "empty" spheres or foamy structures. Infected tissues were mainly the fat body and the hypodermal layer. To a limited extent the stromata were in some cells of the tracheal matrix and in some oenocytes deposited on the midgut wall. There was no sign of development of the stromata in any part of the digestive tract or in silk glands. The neural ganglia were not infected. Muscle cells were not infected. (Plate II, Fig. 1, A.)

On ultrathin sections the interior of infected cells was filled with icosihedral virus particles, pentagonal or hexagonal in cross section. Their diameter was 190 to 210 nm. Cytoplasmic structures of the cells were dissolved, with a few remains of mitochondria. The nuclei were not affected. (Plate I., Fig. 1, A).

## DISCUSSION

There are big differences between the observations of Matta and Lowe (1970) and of Hall and Anthony (1971) in the characterization of the infected tissues which were explained by the ultrastructural evaluation by the second authors. Compared with the data of the American authors, evidence of the tissue affinity given by European authors is much broader. The MIV in *A. cantans* (Weiser 1966, 1969) infected most tissues except the intestinal tract and the salivary glands, with evident stromata in cells of the muscle sheet and cells on the surface of neural ganglia. The virogenic stromata in the bluegreen MIV were spherical, without minute foamy structures, formed in the centre of the infected cells. The icosihedral particles

measured 175—185 nm in diameter. The individual virus particles were deposited in the infected tissue in regular distances from each other, not in dense masses. (Plate II., Figs. 2, B, C).

In the red MIV in *A. punctor* there is no spacing visible between individual particles. The virogenic stromata have a multiple foamy character, filling the whole interior of the host cytoplasm. There is lack of evidence of development of stromata in cells on the surface of muscles and neural ganglia and the infection of tracheae is only limited in extent. Lymphocytes in smears of the hemolymph of the infected larva are not infected and they are less numerous than in healthy individuals. (Plate II., Figs. 2, A.)

The difference in color of both types of MIV can be explained by differences in size of both virus particles, the 180 nm with blue-green and that of 200 nm with red iridescence. Compared with the wide spread of the MIV in *A. cantans* larvae, the red MIV is rather rare and its distribution is limited. The population investigated in June was of the same egg deposit on the grass of the habitat. There is no evidence of any coincidence of the infection and its late appearance in second set larvae.

## КРАСНОВАТО РАДУЖНЫЙ ВИРУС У КОМАРОВ ВИДА *Aedes* *PUNCTOR* В ЧЕХОСЛОВАКИИ

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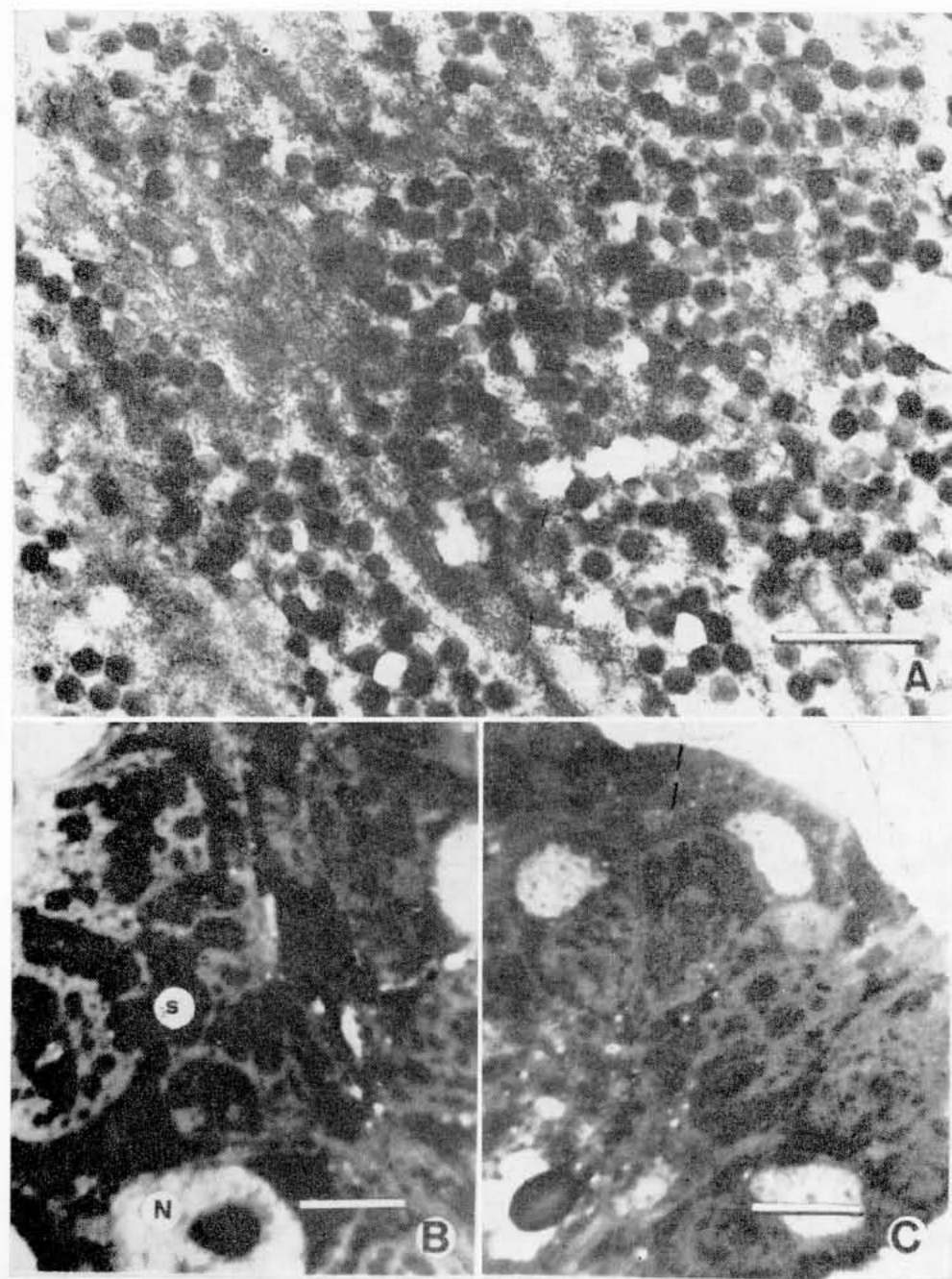
**Резюме.** Впервые обнаружено наличие красновато радужного вируса в личинках комаров вида *Aedes punctor*, с икосаэдрическими частицами диаметром 200 нм. Дано сравнение патологии этого радужного вируса с патологией синевато-зелёного радужного вируса у комаров вида *Aedes cantans*.

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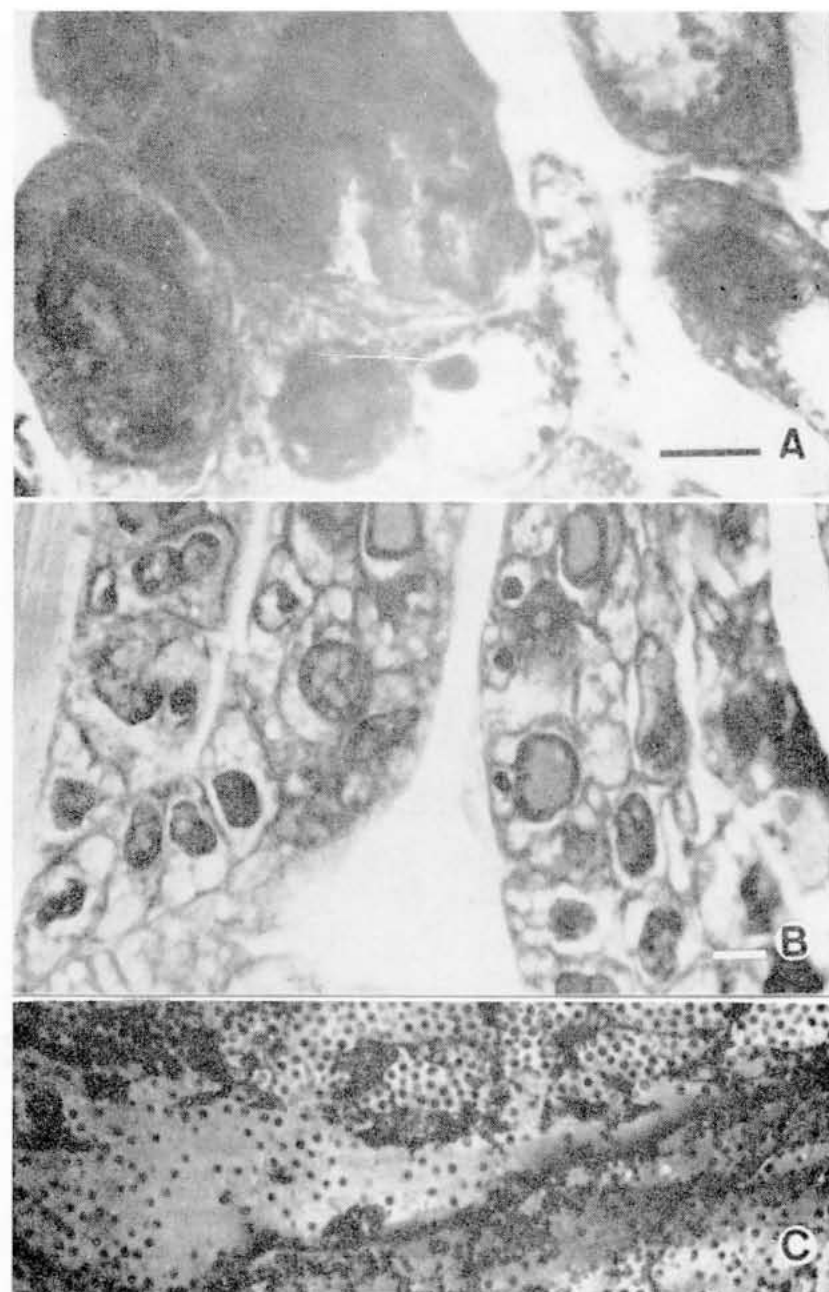
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Red Mosquito iridescent virus in *Aedes punctor*. **Fig. A.** Virus particles from a fat body cell. Rod = 500 nm. **Fig. B, C.** Virogenic stromata (s) of the R-MIV in the fat body and hypodermal tissue of the mosquito. N = nucleus of the host cell. Rod = 10 μm.



**Fig. A.** Red Mosquito iridescent virus in *Aedes punctor*, fat body cells with viral stromata. Rod = 5 μm. **Fig. B.** Bluegreen MIV in *A. cantans* fat body cells with viral stromata. Rod = 10 μm. **Fig. C.** Virus particles of bluegreen MIV in the stroma of *A. cantans* fat body cell. See the regular spacing of particles. Rod = 1 μm.