THE OCCURRENCE OF A VIRUS OF THE POX GROUP IN A FIELD POPULATION OF *CHIRONOMUS SALINARIUS* KIEFFER (DIPTERA: CHIRONOMIDAE) IN ITALY

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A larval population study on *Chironomus salinarius* Kieffer in two saltwater lakes surrounding the city of Orbetello, central Italy, was recently reported by Ali and Majori (1984). In this study, a part (18%) of the *C. salinarius* larval population in a sample taken from one station in June 1982 was patently infected with an entomopoxvirus. Further sampling of that station in June-July 1982 showed the overall disease incidence to be 6% (A. Ali, unpublished). A detailed description of the two lakes (East Lake and West Lake) is available in Ali and Majori (1984). The diseased larvae occurred at station 8 of sampling row G in the West Lake where the lake received domestic and industrial discharges. Patently infected larvae were recognized in the laboratory by the appearance of whitish areas beneath the integument of the entire body caused by massive accumulations of virus polyhedral bodies (PB). Infected 4th instars were randomly separated and placed in a rearing chamber (Biever 1965) to study their survival. Wet mount preparations (in saline) of larval smears of diseased larvae were observed under a phase-contrast microscope and the PB from cells of different host specimens were randomly measured.

For electron microscopy, infected 4th instar *C. salinarius* were fixed in a 2.5% glutaraldehyde in Millonig buffer (pH 7.4) for 1 hour. After rinsing in the buffer, the tissues were postfixed for an hour in 1% OsO_4, dehydrated in acetone series and embedded in Spurr's resin. Thin sections were examined with a Siemen's Elmiskop 102 and Zeiss EM 10 C electron microscope. Suspensions of purified virions (Huger et al. 1970) were negatively contrasted with 2% phosphotungstic acid at pH 7.2 and placed on carbon grids.

Microscopic examination of smears of infected larvae revealed oval shaped PB with a mean size of 7.30 × 5.52 μm but ranging in length from 3.4—13.23 μm and width of 2.47—9.26 μm. Phase-contrast microscopy showed that a variable number of PB may

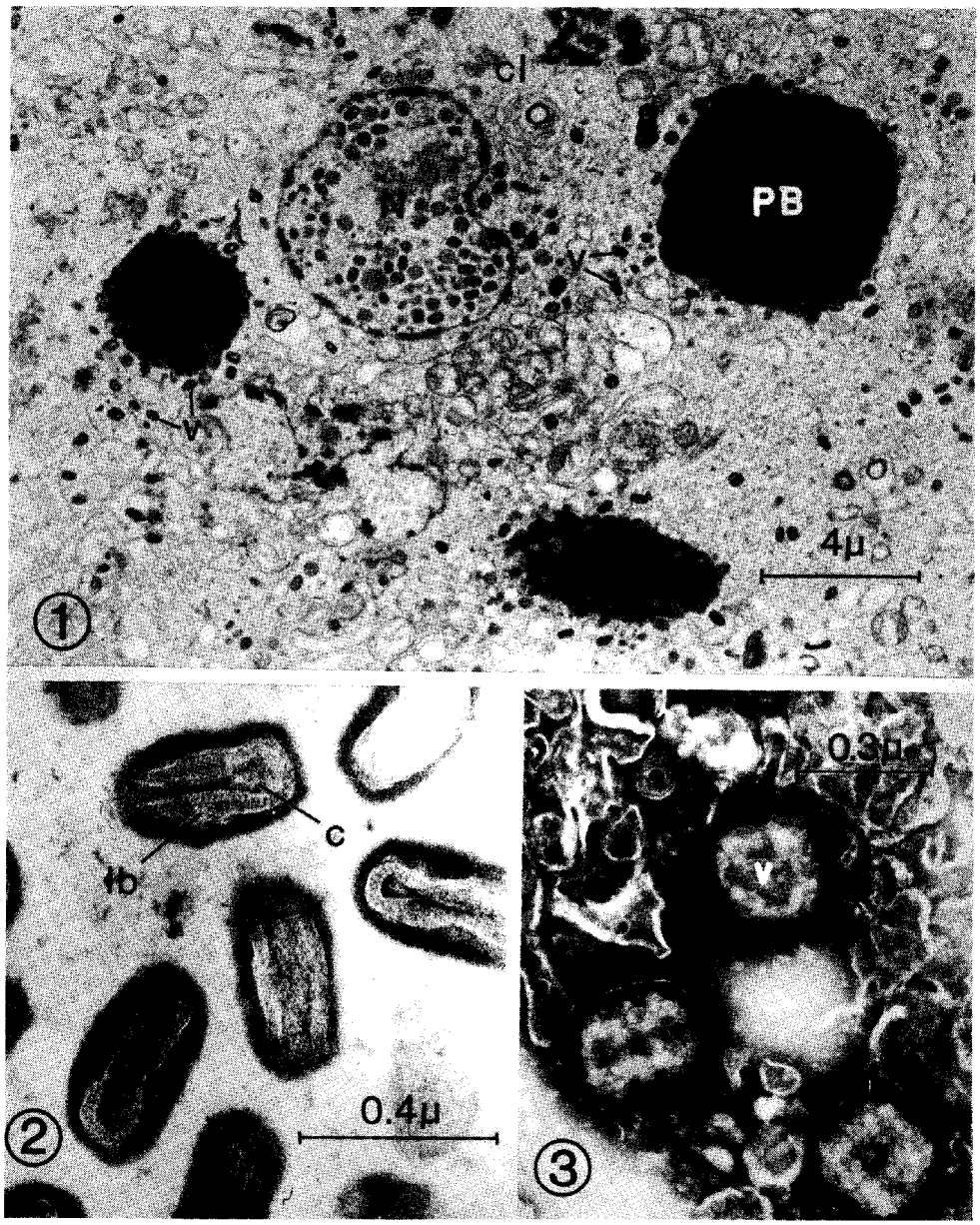

Fig. 1. Poxvirus infected hemocyte from 4th instar *Chironomus salinarius:* at least three polyhedral bodies (PB) are developing in this cell. Inclusion bodies are seen in the nucleus (N) with the chromatin marginated. Immature virions (v) are seen developing in the viral stroma. Concentric cytoplasmic lamella (cl) appears in the cytoplasm.

Fig. 2. Poxvirus virions in polyhedral body of *C. salinarius* showing a dumbbell shaped core (c) and lateral body (lb).

Fig. 3. Negatively stained free virions (v) illustrating the beaded surface characteristic of the poxvirus group.

develop per cell. As many as eight developing PB were observed in a single cell. In accordance with previously available descriptions of chironomid poxviruses, PB development appeared to be in the cytoplasm of hemocyte and fat body cells. The host cell pathology appears to be similar to that already described by Stoltz and Summers (1971). There are inclusion bodies of two types in the nuclei of infected cells and a margination of the chromatin (Fig. 1). Structures similar to the "concentric cytoplasmic lamellae" can be seen in the cytoplasm of infected cells. As with other entomopoxvirus infections, the most distinctive aspect of the cell is the dark staining polyhedral body. The virion appears to mature in the viral stroma surrounding the PB before being encased in the matrix of the PB, apparently at random. Accumulations of infected cells appear as white spots scattered irregularly throughout the body of the diseased larva. With the progression of infection, the normally deep red color of the larva gradually changes to a pale white color. Recognition of poxvirus infected *Chironomus* sp. midge larvae in early stages of disease is aided by the tendency of infected cells to collect in the dorsal region of the 9th abdominal segment just posterior to the heart (Harkrider & Hall 1975).

The cytological examination of infected larval cells revealed the structure of the virion corresponding to that of the subgroup 3 in the genus *Entomopoxvirus* previously described from chironomids and reviewed by Granados (1973). Each virus particle appeared to be composed of three distinct parts: the envelope, the two lateral bodies sandwiched between the envelope, and the centrally located core (Fig. 2). The core, as seen in longitudinal section, appeared typically dumbbell shaped. The overall dimensions of the virions were ca. 320 × 140 nm. The negatively stained whole mount of virions showed the presence of sphaeroid structures on the envelope surface (Fig. 3).

Among aquatic insects, only chironomid midges are reported to be hosts of entomopoxviruses (Anthony 1975). Specifically, Weiser (1969), Huger et al. (1970), Stoltz and Summers (1971), Federici et al. (1974), and Harkrider and Hall (1975) have reported entomopoxviruses in *Camptochironomus tentans*, *Chironomus luridus*, *C. attenuatus* (a junior synonym of *C. decorus*) and *C. plumosus*, *Goeldichironomus holoprasinus*, and *C. decorus*, respectively. The present study documents *C. salinarius* as an additional host species of the virus.

Entomopoxviruses have been shown to play a significant role in the natural regulation of nuisance midge populations (Harkrider and Hall 1978). In laboratory studies, Harkrider and Hall (1979) had demonstrated the important role of high midge population density in the precipitation of an epizootic of entomopoxvirus. However, the reported larval mortalities of field populations of chironomid species infected by poxviruses in different habitats range from <1% to 100% (Weiser 1948, Huger et al. 1970, Anthony 1975). In the present study, all infected 4th instar *C. salinarius* reared in the laboratory died before pupation. In the 2 lakes, 113 sites were systematically sampled, yet only one station (8G) supported significant numbers of diseased larvae. This site (8G) is located in an area receiving large quantities of domestic and industrial discharges. Perhaps pollutants in water and sediments in this area act as stress factors conducive to the viral disease. Alternatively, high nutrient availability in such a location may support dense larval populations resulting in overcrowding which initiates an epizootic; site 8G, by far, supported the highest density (33,330 larvae/m^2) compared to a mean larval density of 4213/m^2 for all sites (Ali and Majori 1984). While the entomopoxvirus appears to have some potential as a biological control agent, the understanding of the epizootiology of the virus is needed before this potential can be assessed.

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REFERENCES CITED

ALI, A., AND G. MAJORI. 1984. A short-term investigation of chironomid midge (Diptera: Chironomidae) problem in saltwater lakes of Orbetello, Grosseto, Italy. Mosq. News 44: 17-21.

ANTHONY, D. W. 1975. Use of viruses for control of aquatic insect pests. Environ. Protect. Agency EPA-600/3-75-100. pp. 23-47.

BIEVER, K. D. 1965. A rearing technique for the colonization of chironomid midges. Ann. Entomol. Soc. America 58: 135-136.

FEDERICI, B. A., R. R. GRANADOS, D. W. ANTHONY, AND E. I. HAZARD. 1974. An entomopoxvirus and nonoccluded virus-like particles in larvae of the chironomid *Goeldichironomus holoprasinus*. J. Invert. Pathol. 19: 27-31.

GRANADOS, R. R. 1973. Insect poxviruses: pathology, morphology, and development. Misc. Publ. Entomol. Soc. America 73-94.

HARKRIDER, J. R., AND I. M. HALL. 1975. The occurrence of an entomopoxvirus in a field population of *Chironomus* sp. Proc. and Pap. California Mosq. Contr. Assoc. 43: 103-106.

HARKRIDER, J. R., AND I. M. HALL. 1978. The dynamics of an entomopoxvirus in a field population of larval midges of the *Chironomus decous* complex. Environ. Entomol. 7:858-862.

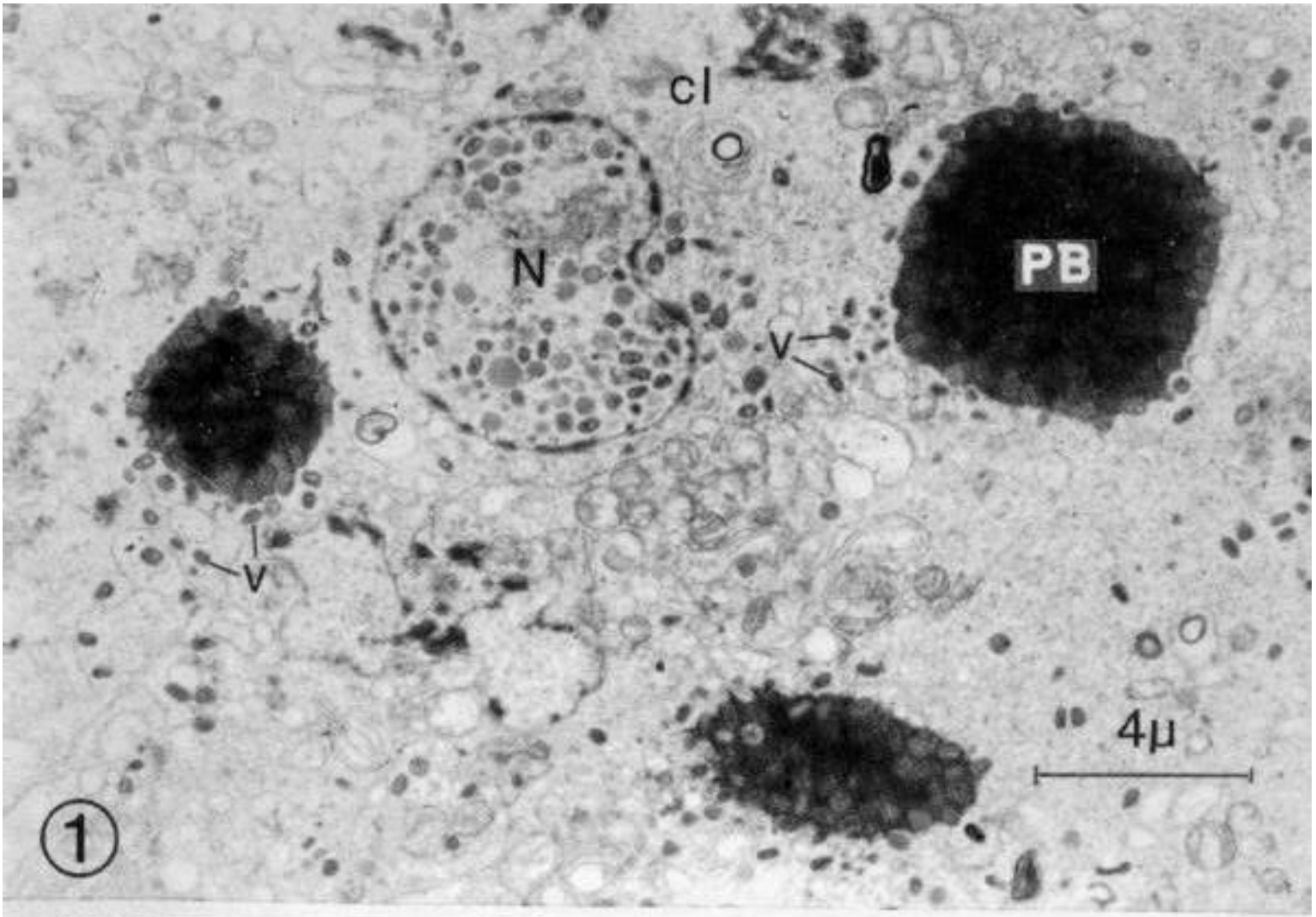
HARKRIDER, J. R., AND I. M. HALL. 1979. The effect of an entomopoxvirus on larval populations of an undescribed midge species in the *Chironomus decorus* complex under laboratory conditions. Environ. Entomol. 8:631-635.

HUGER, A. M., A. KREIG, P. EMSCHERMANN, AND P. GOTZ. 1970. Further studies on *Polypoxvirus chironomi*, an insect virus of the pox group isolated from the midge *Chironomus luridus*. J. Invert. Pathol. 15: 253-261.

STOLTZ, D. B., AND M. D. SUMMERS. 1971. Observations on the morphogenesis and structure of a hemocytic poxvirus in the midge *Chironomus attenuatus*. J. Ultrastr. Res. 40: 581-598.

WEISER, J. 1948. Zwei neuartige Erkrankungen bei Insekten. Experientia (Basel) 4: 317-318.

WEISER, J. 1969. A poxlike virus in the midge *Camptochironomus tentans*. Acta Virol. 13: 549-553.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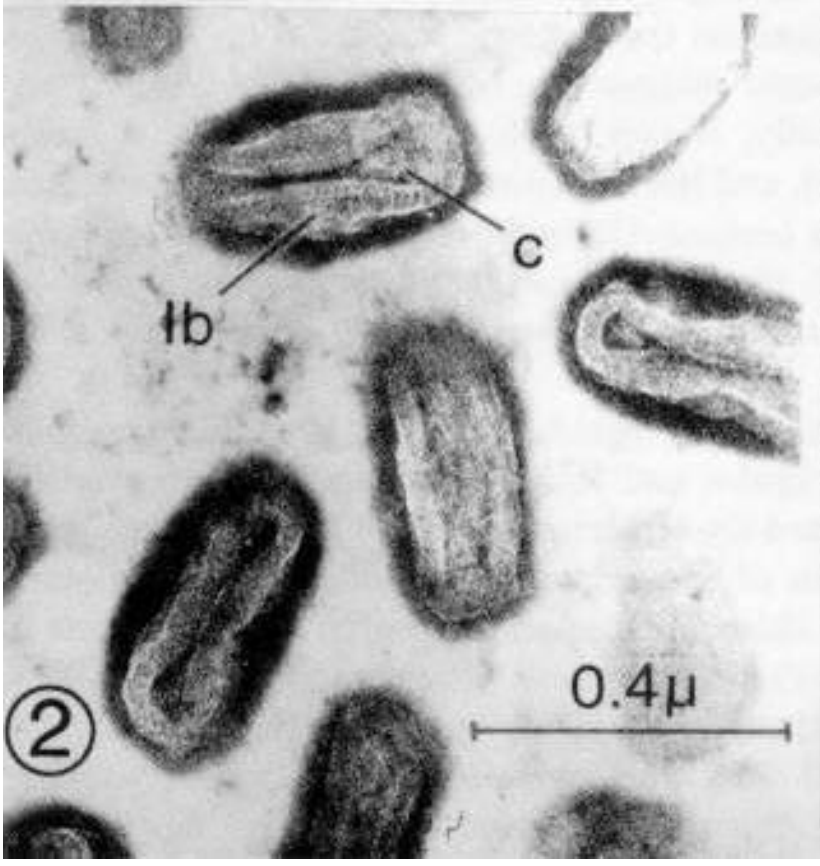
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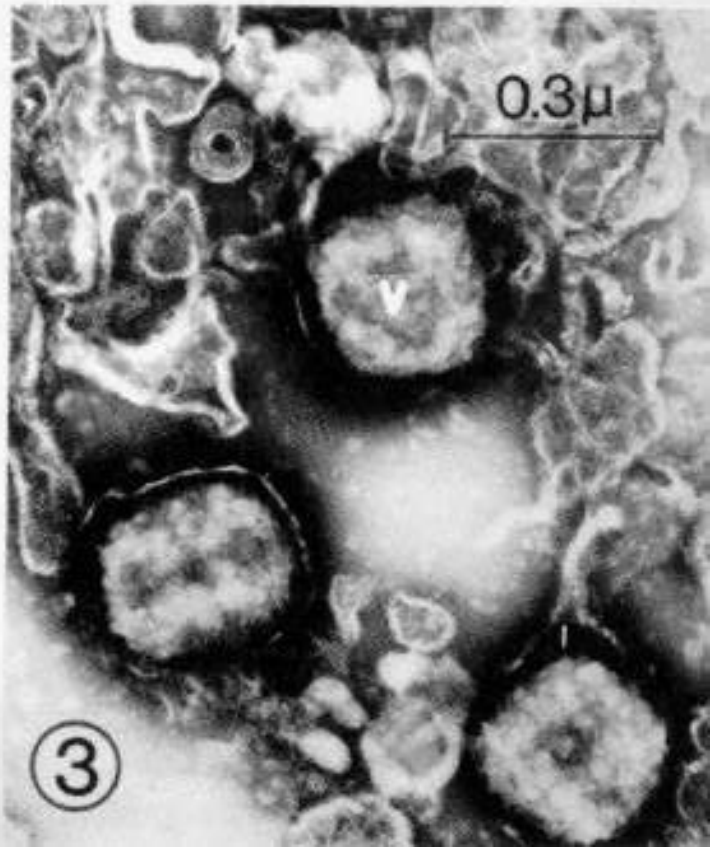
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