APPLIED MICROBIOLOGY, Nov. 1974, p. 885-886
Vol. 28, No. 5

Printed in U.S.A.

NOTES

Rickettsia-Like Organism Causing Disease in a Crangonid Amphipod from Florida

BRIAN A. FEDERICI, EDWIN I. HAZARD, AND DARRELL W. ANTHONY

Division of Biological Control, University of California, Riverside, California, 92502, and Insects Affecting Man Research Laboratory, Gainesville, Florida 32604

Received for publication 9 September 1974

A rickettsia-like organism causing high mortality and gross pathology has been found infecting over 40% of a population of the freshwater crustacean, *Crangonyx* (near) *floridanus* Bousfield, in Florida. This is the first report of a rickettsia-like organism from a strictly aquatic animal. Morphological and developmental stages of the organisms range in size and shape from small, dense bacilliform cells measuring 150 nm in diameter by 395 nm in length, to large sausage-shaped cells 0.5 by 3 μm.

Since shortly after the turn of the century rickettsiae causing chronic and acute diseases have been described from insects, birds, man, and other animals (1-3). However, only recently has a rickettsial disease been reported from crustacea. This was a report of a *Rickettsiella* in a terrestrial isopod from France (4). In this preliminary report we describe a pathogenic rickettsia-like organism infecting the freshwater amphipod, *Crangoynx* (near) *floridanus* Bousfield.

Diseased and apparently healthy amphipods were collected from a large woodland pond near Gainesville, Fla. Patently infected individuals exhibited an opaque, pale green iridescence; healthy amphipods were grey and slightly translucent. Initially, 270 amphipods were collected. Of these 43% (116/270) were patently infected and an additional 10% showed slight iridescence in the legs when examined with a dissecting microscope. Preliminary mortality rates were obtained from 20 iridescent field-collected adults maintained in the laboratory at 27 C on a wet leaf-litter diet in pond water held in cardboard cups. Twenty healthy adults were maintained in a similar manner. After 6 weeks 90% (18) of the patently infected adults had died whereas only 15% (3) of the healthy adults died during the same period. Accurate levels of infection for the field population were not determined. However, 96 of the 127 amphipods which originally showed no gross signs of infection were still alive after being maintained in the laboratory for 12 weeks. At this time 42 of these showed signs of infection, usually iridescence in the legs. This indicates that the level of infection in in the field may have seen significantly higher than 43%.

In patently infected individuals the rickettsia-like organisms were usually confined to large membrane-bound vesicles averaging 5 μm in diameter (Fig. 1). These vesicles, packed closely together in the cytoplasm of infected cells, could be observed easily by examining pieces of infected tissue in a drop of saline under phase contrast. In the most iridescent specimens these vesicles were observed throughout the entire epidermis, and in many cases could be found in the gastric caeca and floating freely in the hemolymph.

The rickettsia-like nature of the causal agent of the disease was confirmed by electron microscopy. Healthy and infected amphipods were dissected and fixed in 3% glutaraldehyde in 0.1 M phosphate buffer, postfixed in 1% OsO_4, dehydrated in an ethanol-propylene oxide series, and embedded in epoxy resin. Examination of ultrathin sections through the vesicles in the cytoplasm of infected cells showed them to consist of a single, limiting, unit membrane. The vesicles contained different developmental stages of the rickettsia-like organisms. The organisms within any one vesicle were at approximately the same stage of development. Early stages (Fig. 2), which averaged 0.5 μm in diameter and ranged from 1 to 3 μm in length, were characterized by a diffuse cytoplasm with a denser centrally located nucleoid. These cells

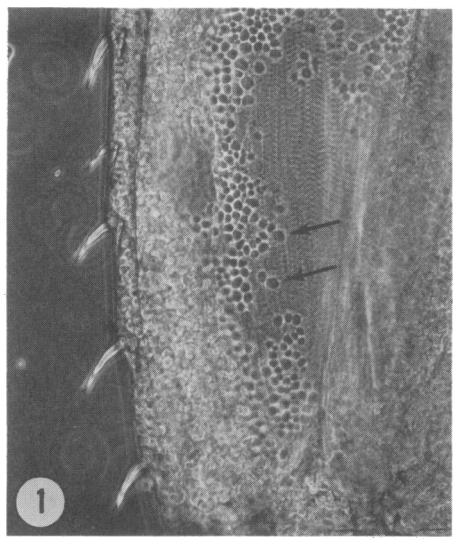

FIG. 1. *Portion of a basipodite from a patently infected amphipod. Note the vesicles (arrows) which are in the epidermis. These vesicles contain different developmental stages of the rickettsia-like organisms.* ×200.

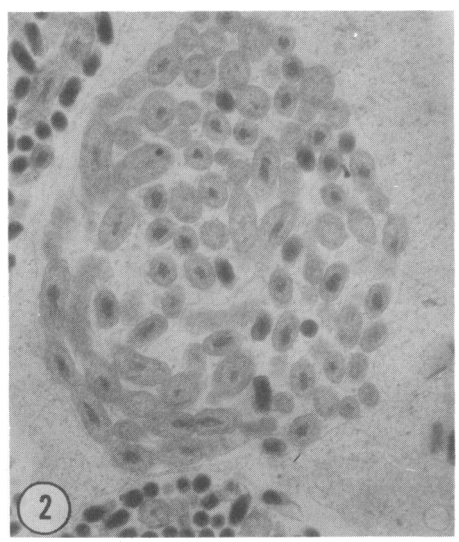

FIG. 2. *Ultrathin section through a vesicle filled with rickettsia-like organisms. These early stages of the organism produce smaller cells by binary and multiple fission.* ×12,500.

were bound by two distinct membranes; an inner one, unit-membrane like in structure, and a thicker outer membrane which resembled a bacterial cell wall. Both membranes had a trilaminar structure. The larger forms divided by binary and multiple fission eventually producing smaller, dense bacilliform cells which averaged 150 nm in diameter by 395 nm in length (Fig. 3). These small forms had a very dense nucleoid core surrounded by the above mentioned membranes. The orientation of these small cells into paracrystalline arrays within the vesicles was apparently responsible for the iridescent color of the patently infected amphipods.

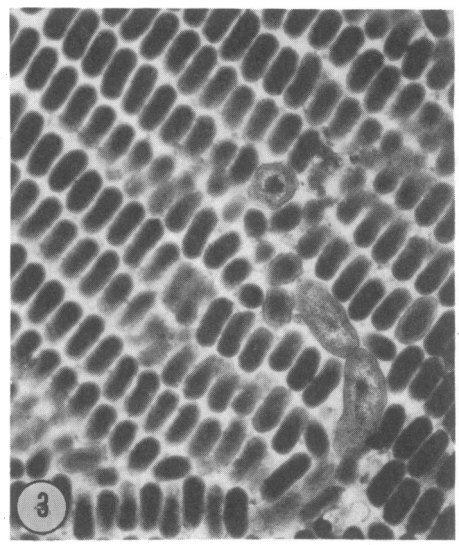

FIG. 3. *Ultrathin section through a portion of a vesicle demonstrating small dense cells packed in a paracrystalline array.* ×17,500.

Although the taxonomic placement of this organism is not certain at present, it appears to be related to members of the genus *Rickettsiella* (tribe *Wolbachieae*). The morphology and development of the rickettsia-like organism described above is similar to the rickettsiae in this genus reported from insects (1) and an isopod (4). However, the proteinaceous crystals usually associated with members of this genus have not been observed in infected amphipods.

We thank Edward Bousfield of the National Museum of Canada for identification of the amphipod.

LITERATURE CITED

1. Krieg, A. 1963. Rickettsiae and rickettsioses, p. 577–617. *In* E. A. Steinhaus (ed.), Insect pathology, an advanced treatise. Academic Press Inc., New York.
2. Ormsbee, R. A. 1969. Rickettsiae (as organisms). Annu. Rev. Microbiol. **23**:275–292.
3. Philip, C. B. 1957. Rickettsiae, p. 933–984. *In* R. S. Breed, E. G. D. Murray, and N. R. Smith (ed.), Bergey's manual of determinative bacteriology. Williams and Wilkins Co., Baltimore.
4. Vago, C., G. Meynadier, P. Juchaulf, J. Legrand, A. Amargier, J. Hoffman, and J. Duthoit. 1970. Une maladie rickettsienne chez les Crustacés Isopodes. C. R. H. Acad. Sci. Ser. D N. **271(22)**:2061–2063.