Failure of Certain Clams and Oysters to Serve as Intermediate Hosts for *Angiostrongylus cantonensis*¹

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Several species of invertebrate animals have been reported as intermediate or as paratenic hosts for the rat lungworm, *Angiostrongylus cantonensis*. Intermediate hosts which have been found naturally infected include: the land snails, *Bradybaena similaris*, *Opeas javanicum*, *Macrochlamys resplendens*, *Achatina fulica*, *Pupina complanata*, and *Subulina octona*; the slugs, *Deroceras laeve*, *Vaginella plebeius*, *Veronicella alte*, *Girasia peguensis*, and *Microparmarion malayanum*; and the amphipodous snail, *Pila ampullacea*. Naturally infected paratenic hosts include: the land planarian, *Geoplana septemlineata*; the freshwater prawn, *Macrobrachium* sp.; the land crab, *Cardioma hirtipes*; and the coconut crab, *Birgis latro*. Also, several land and aquatic snails and slugs have been reported as experimental host (Alicata, 1965).

Of particular interest have been the recent reports (Cheng and Burton, 1965 and Cheng, 1966) that the American oyster, *Crassostrea virginica*, and the soft-shell clam, *Mercenaria mercenaria*, could serve as intermediate hosts of *A. cantonensis* under experimental conditions. This finding could have special significance especially in some of the Pacific islands where the rat lungworm exists and clams and oysters may be eaten raw or imperfectly cooked.

In the present study, attempts were made to determine the following: (a) ability of local clams (*Venerupis philippinarum*) and oysters (*Crassostrea virginica*) to serve as intermediate hosts of *A. cantonensis*; (b) possible natural infection of certain species of clams and oysters in Hawaii and in the island of Ulong, Palau Islands, with third-stage larvae of *A. cantonensis*. These are areas in which murine angiostrongylosis is endemic.

**Materials and Methods**

Clams collected from Kaneohe Bay, Oahu, Hawaii, on 31 August 1965, were divided into 4 groups having 8 clams per group. Each group was placed in a glass aquarium containing 5,000 ml of continuously aerated sea water with a salinity of 15.1% and temperature of 20 ± 1 C. After 96 hr, the clams in the different aquaria were exposed to first-stage *A. cantonensis* larvae as follows: group 1, 38 larvae in 0.25 ml of water were injected into the mantle of each clam using a hypodermic syringe equipped with a 4-inch, 22-gauge needle which was inserted between the valves. The clams remained out of the water for 15 min after larval injection; group 2, 3,040 larvae were placed directly into the aquarium with the clams; group 3, similar to group 1 except that 76 larvae were injected into each clam; group 4, same as group 2 except that 6,080 larvae were placed in the water. No sand was used for a substrate so the clams rested on the glass bottom of their respective aquaria.

Oysters collected from Pearl Harbor, Oahu, Hawaii, on 1 February 1966, were divided into 6 groups having 5 oysters per group. Groups 1, 2, and 3 were placed in a glass aquarium containing 5 gallons of continuously...

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aerated sea water with a salinity of 15.3%/e and a temperature of 20 ± 1°C. Groups 4, 5, and 6 were placed in another similar aquarium with a salinity of 10.6%/e. After 96 hr, the body of each oyster of groups 1 and 3 was injected with 0.02 ml normal saline solution containing approximately 5,000 first-stage larvae of A. cantonensis. Each injection was carried out by using a hypodermic syringe equipped with a 4-inch, 22-gauge needle which was carefully inserted between the valves. The oysters remained out of the water for 15 min after larval injection. For oysters of groups 2 and 5, the valves of each were slightly opened with a knife blade to drain the containing sea water, and then with the aid of a fine Pasteur pipette, 0.1 ml of saline solution containing approximately 10,000 first-stage lungworm larvae was ejected into the cavity between the two valves. In this operation, the oysters remained out of the water for 1 hr before they were replaced in their respective glass aquaria. Oysters of groups 3 and 6 were removed into two small glass aquaria, each containing 2,000 ml of continuously aerated sea water (salinity 15.3%/e and 10.6%/e, respectively) to which approximately 150,000 freshly isolated first-stage rat lungworm larvae were added. After two days’ exposure to larval infection, the two groups of oysters were replaced in their former larger aquaria.

All of the oysters were examined for larvae of A. cantonensis 25 days after injection or exposure to infection. This was carried out both by press preparation of parts of the tissues of each oyster, and by digesting the remaining parts of the oysters with the use of the pepsin–HCl digestion technique.

Oysters and clams which were collected and examined for natural infection with larvae of A. cantonensis included the following: 150 clams, Venerupis philippinarum, from Kaneohe Bay, Oahu, Hawaii; 50 clams, Matra thaanumi, from Ulong, Palau Islands; 100 oysters, Crassostrea virginica, from Pearl Harbor, Oahu, Hawaii. All these mollusks were examined by use of the pepsin–HCl digestion method.

Results and Discussion

All the clams and oysters which were either injected or exposed to infection with first-stage larvae of A. cantonensis and examined by press preparation or artificial digestive methods 21 and 25 days later, respectively, failed to show either developing or infective third-stage larvae of the parasite. A few (1 to 2) dead and undeveloped first-stage larvae were recovered after artificial digestion of 3 clams from groups 3 and 4 which died 11, 12, and 17 days after exposure to larval infection. Similarly, 2 live and many dead and undeveloped first-stage larvae were found on press preparation of the tissue of an oyster (group 1) which died 4 days after injection with first-stage larvae.

It is concluded that under the conditions of these experiments, neither the clam, V. philippinarum, nor the oyster, C. virginica, serves as intermediate host of A. cantonensis. These data do not confirm the report of Cheng and Burton (1965) that C. virginica serves as an experimental host for this parasite. It is believed also that further tests are necessary to verify the reported claim that M. mercenaria can be infected with A. cantonensis. No natural infection with third-stage larvae of A. cantonensis was found among oysters or clams collected from the island of Oahu, Hawaii, nor from the island of Ulong, Palau Islands.

The failure either to infect or find natural infection of local clams and oysters with larvae of A. cantonensis suggests that these mollusks have little importance in the transmission of human angiostrongylosis in the Hawaiian Islands.

Summary

An attempt was made to infect the Hawaiian edible clam, Venerupis philippinarum, and the American oyster, Crassostrea virginica, with first-stage larvae of Angiostrongylus cantonensis. The clams were maintained in glass aquaria containing sea water with a salinity of 15.1%/e. The oysters were maintained in similar aquaria containing sea water with a salinity of 15.3%/e and 10.6%/e. These mollusks were exposed to infection by either injecting their tissues with first-stage larvae of A. cantonensis or placing the larvae in the water. When these mollusks were examined by press preparation or artificial digestive methods, none of them was infected with third-stage larvae of the parasite. A few undeveloped and dead first-stage larvae were found in the tissues of 3 clams and one oyster which died a few days after exposure to infection.
No evidence of natural infection with larvae of A. cantonensis was found among 150 clams (V. philippinarum) and 100 oysters (C. virginica) collected from the shores of the island of Oahu, Hawaii, nor among 50 clams collected from the island of Ulong, Palau Islands.

**LITERATURE CITED**


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**Myzotrema cyclepti gen. n., sp. n.** (Trematoda: Monogenea) from Gills of Cycleptus elongatus (LeSueur) from Alabama

**Wilmer A. Rogers**

The species described in this paper was collected as part of a survey of fish parasites being conducted by the Southeastern Cooperative Fish Parasite and Disease Project of the Agricultural Experiment Station, Auburn University. This species was collected using the 1:4,000 formalin field-collecting method described by Rogers (1966). Specimens were measured according to the procedure given by Mizelle and Klucka (1953). Measurements are expressed in microns and were made from specimens mounted in glycerin jelly or permount. Details of internal anatomy were determined from hematoxylin-stained specimens. Illustrations were prepared with the aid of a camera lucida. The keys to the genera of Ancyrocephalinae by Mizelle and Price (1964) and Yamaguti (1963) were useful in determining the status of the present species.

**Myzotrema gen. n.**

Generic diagnosis: Dactylogyridae, Ancyrocephalinae: Body large, elongate, with two pairs of eyespots, head organs poorly developed or lacking. Opisthohaptor well set off from body proper by stout peduncle, with two pairs of nearly similar anchors, each pair supported by a nonarticulate transverse bar; 14 marginal booklets present. Pharynx large, heavily muscularized, perfectly round in cross-section. Intestinal crura simple, united posteriorly. Testis and ovary equatorial, overlapping. Vas deferens looped around left intestinal crus, seminal vesicle formed by dilation of vas deferens. Two prostatic reservoirs present. Cirrus a U-shaped tube with complex accessory piece articulated to base. Ovary looping around right intestinal crus. Vagina present, opening dextroventrally; submedian or submarginal. Vitellaria coextensive with intestine. Parasitic on fresh water fish.

**TYPE SPECIES:** Myzotrema cyclepti sp. n.

**TYPE HOST:** Blue sucker, Cycleptus elongatus (LeSueur).

**LOCALITY:** Tombigbee River, Pickens County, Alabama.

**REMARK:** Myzotrema gen. n. is most closely related to Pseudomurraytrema Bychowsky, 1957 (nec Pseudomurraytrema Yamaguti, 1958) as shown by the structure of the copulatory complex and the reproductive system. It is readily separated from Pseudomurraytrema by