STUDIES ON EOSINOPHILIC MENINGITIS. 2. EXPERIMENTAL INFECTION OF SHRIMP AND CRABS WITH ANGIOSTRONGYLUS CANTONENSIS

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(Received for publication November 29, 1965)

Angiostrongylus cantonensis (Chen), a metastrongylid lungworm of rats, was recovered from the brain of a patient from Hawaii in 1961 (1) and since has become the principal suspect in the search for the etiologic agent of the eosinophilic meningitis seen on Pacific Islands (2-5). Since epidemiologic investigations (1, 4, 6) suggest that certain food animals may be involved in the transmission of this parasite to man, experiments were undertaken to determine if certain of these animals could serve as hosts for third-stage (infective) larvae of A. cantonensis. This report describes the findings for Macrobrachium lar (Fabricius), a freshwater shrimp, and Ocypode ceratophthalma (Pallas), an amphibious crab. These particular crustaceans were selected for study because of evidence linking them to cases of eosinophilic meningitis in Tahiti and Hawaii, respectively.

Materials and Methods

Source and maintenance of crustaceans. The M. lar were trapped in rivers on Tahiti, French Polynesia. They were identified and distinguished from the other species of Macrobrachium present in that locality by means of the descriptions and key published by Holt-huis (7). Some were used in experiments on Tahiti and others were shipped by air to Honolulu for experiments conducted there. They were maintained in the laboratory in aerated 20-gallon fresh-water aquaria and were fed a diet of “trout chow” (manufactured by the Ralston Purina Co.). Those used in the experiments ranged in length (anterior tip of rostrum to posterior edge of Telson) from 6.2 to 13.6 centimeters and averaged 9.0 centimeters in length.

The O. ceratophthalma were trapped on beaches on Oahu, Hawaii and identified by means of the description given by Edmondson (8). They were maintained in the laboratory in individual plastic cages containing 1 to 2 inches of moist sand. The crabs were moistened with sea water and given a few pellets of “trout chow” daily. Those used in the experiments ranged in length (anterior tip of rostrum to posterior edge of Telson) from 6.2 to 13.6 centimeters and averaged 9.0 centimeters in length.

Source of A. cantonensis larvae. First-
stage larvae used for infecting snails and for exposing shrimp were obtained from the feces of albino rats, Rattus norvegicus (Berkenhout), raised in the laboratory and infected with a strain of A. cantonensis originally isolated from wild rats on Oahu, Hawaii. Third-stage larvae were obtained from aquatic snails, Australorbis glabratus (Say), raised in the laboratory, which had usually been exposed in groups of approximately 50 to the feces of the infected rats.

Identification of third-stage larvae of A. cantonensis. Third-stage larvae of A. cantonensis were recognized by two different methods. In one, the presence and viability of the larvae were established by feeding shrimp or crab tissues or larvae themselves to albino rats. Food had been withheld from these rats for 24 to 48 hours and most of the test material was usually consumed within a few hours. All test material was eaten within 21 hours. The rats were killed 20 to 23 days later and the brain was examined for the presence of young adult A. cantonensis. All rats used had been raised only on commercially prepared food.

The other method consisted of identifying third-stage larvae of A. cantonensis by their morphologic characteristics. The features studied included overall length and width, shape of anterior and posterior extremities, sclerotized rods in the vestibule, and, in some cases, movement.

Exposure of shrimp to third-stage larvae. Shrimp were exposed to third-stage larvae of A. cantonensis by offering to them as food part of an infected A. glabratus snail in the following manner. After having been deprived of food for one to three days, shrimp were placed in separate plastic cages (12 inches long, 7 inches wide, and 5 inches deep) containing approximately four liters of water. Most of one infected snail, removed from its shell, was then placed near the mouth of each shrimp. The cages were covered with a cloth and examined at intervals to determine when each snail had been consumed. Most shrimp ate the snail within a few hours, except when preparing to molt. In the few instances in which the snail was not consumed within 48 hours it was replaced by a fresh snail. If the second snail was not eaten within 48 hours, the shrimp was excluded from the experiment.

After the infected snail had been eaten, the shrimp was thoroughly rinsed in fresh water. If it was to be examined within 78 hours it was returned to the plastic cage after the cage had been rinsed and filled with fresh water. If the shrimp was to be examined more than 78 hours later it was placed in an aquarium. The shrimp returned to the plastic cages were not fed thereafter, but those placed in the aquarium received the usual diet of "trout chow". In many experiments, an elevated piece of galvanized wire mesh ("hardware cloth") was placed on the bottom of the plastic cage after the shrimp had fed and the water had been changed. This was designed to raise the animal above the bottom of the container in order to reduce the opportunity for re-exposure to any active larvae which might be excreted during the experiment.

In most experiments, a portion of each snail was removed and the presence of third-stage larvae confirmed before the remainder was offered to a shrimp. This portion, consisting of approximately the posterior one-third of the snail, contained almost all of the viscera and a
small part of the musculature. It was known that in *A. glabratus* third-stage larvae are much more numerous in the musculature than in the viscera. At least five whole snails selected at random from each of the various groups used were examined and the total number of active third-stage larvae in each snail counted. The average count by group ranged from 320 to 671 per snail. The extremes found for individual snails ranged from 58 to 1559.

Shrimp were dissected and examined for the presence of third-stage larvae as follows. First, the legs, carapace, and abdominal exoskeleton were removed in that order. The stomach and intestine were then removed intact, when possible, and placed together or separately in a Petri dish with water. These organs were then teased into small pieces and the contents of the dish examined immediately and also, in most instances, after standing at room temperature for one and one-half to 16 hours.

After removal of the stomach and intestine, the cephalothorax and abdomen were washed thoroughly in running water and then fed separately to albino rats (usually 2 to 4 cephalothoraces or abdomens to each rat). It should be noted that in *M. lar* the stomach, a small portion of the intestine, and most of the other viscera are contained within the cephalothorax and the remainder of the intestine is contained within the abdomen (“tail”).

**Exposure of shrimp to first-stage larvae.** Individual *M. lar* were exposed to first-stage larvae of *A. cantonensis* in one of two ways. In one technique, each shrimp, after having been deprived of food for 48 hours, was placed in a plastic cage containing water and one pellet of rat feces, estimated by weight and dilution counts to contain between 13,000 and 18,000 active first-stage larvae. All of the shrimp consumed one-quarter or more of the pellet within six hours. At this time, the shrimp were removed from the plastic containers, rinsed, and placed in an aquarium located in a room with an almost constant temperature of 25 C.

In the other technique, shrimp were exposed by injecting approximately 300 active first-stage larvae contained in 0.1 ml of water into the mouth with a blunt needle. The same shrimp were also exposed by placing them in individual plastic cages containing one liter of water and dispensing approximately 6000 active first-stage larvae into the water directly over them. Twenty hours later the shrimp were removed, rinsed, and placed in the same aquarium with the 10 shrimp which had consumed the rat feces. Numerous active first-stage larvae were still present in the plastic containers at the time that the shrimp were removed.

**Exposure of crabs to third-stage larvae.** Crabs were exposed to third-stage larvae of *A. cantonensis* either by placing them in a clean dry plastic cage with an infected *A. glabratus* snail (removed from its shell) or, more often, by placing the snail under the maxillipeds. In the latter instance, the crab would usually move the snail into its mouth parts and consume it within a few minutes. If the crab was not to be examined within a few hours after feeding it was returned to its container and maintained on “trout chow”. As with the shrimp, in most experiments the posterior one-third of each snail was shown to contain third-stage larvae before the rest of the mollusc was fed to crabs. The average count of active third-stage larvae in the groups of snails fed to crabs varied from 313 to 856. The extremes for individual snails ranged from 101 to 1817.
Crabs were dissected and examined for the presence of third-stage larvae as follows. First, the legs and the dorsal portion of the carapace were removed, disturbing the viscera as little as possible. Before any of the viscera were removed, the body cavity was rinsed with water and the washings saved for examination. The heart, digestive gland, gonads, and gills were removed before the stomach and intestine to avoid, in so far as possible, contamination of the former organs with larvae which might be present in the alimentary tract. Since the stomach and intestine were very fragile, it was usually not possible to be certain that they were removed intact.

In some instances, the viscera, other than stomach and intestines, were pressed between glass plates and examined microscopically for the presence of third-stage larvae. In others, these viscera were fed to albino rats, using one rat for 2 or 3 crabs. The stomach and intestine were examined in a Petri dish with water as for shrimp. In almost all cases, the musculature from the merus of each walking leg, that from the merus and chela of the chelipeds, and that at the base of each leg inside the body were also fed to albino rats. Again, one rat was used for the musculature from 2 or 3 crabs. After the removal of all viscera, and before removal of the musculature inside the body, the body cavity was rinsed with water in an attempt to remove any larvae which might have been liberated from the viscera.

Results

Shrimp. A number of experiments were carried out to determine if *M. lar* could serve as a transport host of *A. cantonensis* by maintaining a viable state any third-stage larvae which might be ingested. Preliminary experiments indicated that third-stage larvae remained viable for a number of hours in the stomachs of shrimp after they had been fed infected molluscs. Therefore, nine further experiments were done to determine, *a*) how long larvae would remain alive in the alimentary tract and, *b*) whether or not such larvae would penetrate the walls of the alimentary tract and remain viable in other anatomical sites. A summary of the results obtained in the later experiments are presented in tables 1 and 2.

It will be noted from the data presented in table 1 that a fairly high proportion (23 per cent) of shrimp had living larvae resembling *A. cantonensis* in either their stomachs or intestines one to three days after consuming an infected mollusc. No other nematodes were seen in the stomachs and intestines. The number of larvae found in individual shrimp was very small, especially in view of the relatively large number which are presumed to have been ingested. The highest number of larvae found in the intestine of any one shrimp was seven (76 hours after feeding). In another instance, 13 larvae were found in the water containing both the stomach and intestine of a shrimp killed 26 hours after feeding. The identity and viability of the latter larvae were tested by feeding them by stomach tube to an albino rat. Ten young adult *A. cantonensis* were recovered from the brain of this rat 23 days later. The identity and viability of six larvae recovered from the stomach and intestine of one shrimp 49 hours after the infective meal was similarly tested and two *A. cantonensis* were recovered from the brain of the rat 23 days later. None of the shrimp had undigested material remaining in the stomach when examined 24 to 78 hours.
after feeding. It is also seen from table 1 that living larvae were recovered from the intestine of a relatively small proportion of shrimp as long as 29 days after the infective meal. The identity and viability of one larva recovered at this interval was proven by feeding it via stomach tube to an albino rat and recovering a young adult A. cantonensis from the brain of the rat 22 days later. Larvae were recovered more frequently and in higher numbers from the intestine of shrimp as compared with the stomach. The data also show that the percentage of shrimp with larvae and the number of larvae per positive specimen decreased with the passage of time. Only those experiments in which the stomach and intestine were examined separately are summarized in table 1. The experiments in which the stomach and intestine were examined together gave similar results.

It did not appear that all of the larvae found in the stomach and intestine were free in the lumen of these organs. In several instances no larvae were seen when the organs were first placed in water, but were found later after the tested portions had been allowed to stand for several hours. In some cases, larvae were found in a Petri dish containing the telson and an attached small piece of the distal end of the intestine. The frequency of this occurrence seemed out of proportion to the amount of intestine involved and suggested that the larvae might have a predilection for remaining in the distal end of the intestine or in the adjacent tissues in that anatomical region.

### Table 1

**Living larvae resembling third-stage A. cantonensis found in the stomach and intestine of experimentally infected M. lar by time after feeding**

<table>
<thead>
<tr>
<th>Time after feeding</th>
<th>Number positive</th>
<th>Percentage positive</th>
<th>Average number of larvae per positive specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-78 hours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>69 shrimp examined</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>4</td>
<td>6</td>
<td>1.5</td>
</tr>
<tr>
<td>Intestine</td>
<td>13</td>
<td>19</td>
<td>2.4</td>
</tr>
<tr>
<td>Stom. or Intest.</td>
<td>16</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>13-15 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31 shrimp examined</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestine</td>
<td>2</td>
<td>6</td>
<td>1.0</td>
</tr>
<tr>
<td>29 days</td>
<td>31 shrimp examined</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestine</td>
<td>3</td>
<td>10</td>
<td>1.0</td>
</tr>
</tbody>
</table>

### Table 2

**Infective larvae* of A. cantonensis in experimentally infected M. lar by time after feeding and anatomical location**

<table>
<thead>
<tr>
<th>Time after feeding</th>
<th>Number positive possible</th>
<th>Percentage positive possible</th>
<th>Average number of larvae per pos. specimen possible</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-78 hrs.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>128 shrimp examined</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cephalothorax</td>
<td>30</td>
<td>62</td>
<td>2.5</td>
</tr>
<tr>
<td>Abdomen†</td>
<td>13</td>
<td>18</td>
<td>1.0</td>
</tr>
<tr>
<td>13-15 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31 shrimp examined</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cephalothorax</td>
<td>2</td>
<td>2</td>
<td>1.0</td>
</tr>
<tr>
<td>Abdomen</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>29 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31 shrimp examined</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cephalothorax</td>
<td>2</td>
<td>3</td>
<td>1.0</td>
</tr>
<tr>
<td>Abdomen</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* Confirmed by development in rats.
† Abdomens of only 127 shrimp were fed to rats.
The results of feeding the cephalothoraces and abdomens of shrimp to rats are summarized in table 2. It will be noted that viable *A. cantonensis* were recovered from a relatively high proportion (23 to 48 per cent) of cephalothoraces 18 to 78 hours after the shrimp had consumed an infected mollusc and that some cephalothoraces were found positive as long as 29 days after the infective meal. Again, the number of larvae found in individual shrimp was relatively small. One rat which received the cephalothoraces of two shrimp killed 26 hours after exposure had nine young adult *A. cantonensis* in its brain and another rat which received the cephalothoraces of four shrimp killed 54 to 76 hours after exposure had 24 worms. In contrast to the findings from the direct examination of stomachs and intestines, larvae were recovered more frequently and in higher numbers from the cephalothoraces as compared with the abdomens. All percentages and numbers of larvae referred to in table 2 refer to the actual number of young adult *A. cantonensis* recovered from the brains of the experimental rats. Since parts of several shrimp were usually fed to a single rat, the results are given in terms of a range. For example, the minimum number positive was based on the assumption that all the worms found in a rat came from a single shrimp, whereas the maximum number positive was based on the assumption that if more than worm was found more than one shrimp served as the source.

A series of five experiments were also carried out to determine if viable third-stage larvae migrated out of or were excreted by the experimentally infected shrimp. This was done by examining at intervals all of the water from the plastic cages in which the shrimp had been placed after their initial feeding and subsequent rinsing. Each time the water was removed for examination the shrimp were rinsed before being placed in fresh water. In four of five experiments it was found that practically all shrimp (30 of 31) examined for at least 23 hours excreted larvae which resembled third-stage larvae of *A. cantonensis* and which appeared to be alive. In the other experiment, in which shrimp were examined for the same period of time, only 2 of 8 shrimp were found to have excreted larvae. There was no apparent reason for the discrepancy in these results. Many of the shrimp were also examined for longer periods of time (up to a total of 74 hours in some instances). The total number of larvae recovered in the water ranged from 1 to 69 with a mean of 11 per positive shrimp. Most of the larvae were recovered within the first 23 hours after feeding with the majority being found between 8 and 23 hours. This latter period was also the time interval during which the most fecal material was passed by the shrimp. Most of the shrimp were also examined for an additional 24 to 51 hours and a fairly high proportion (10 of 33) excreted larvae during this interval. However, the number of larvae recovered was relatively low (mean of 3 per positive shrimp). In two instances a single larva was found more than 60 hours after the shrimp had fed. Practically all the larvae observed appeared to be alive but only a few were tested for viability. In one instance a single young adult *A. cantonensis* was recovered from the brain of a rat fed 9 larvae found in water 5 to 15 hours after the shrimp had fed. In another, no worms were found in the brain of a rat fed 3 larvae recovered 23 to 47 hours after the shrimp had fed.
Living larvae resembling third-stage *A. cantonensis* found in the stomach, intestine, and body cavity washings of experimentally infected *O. ceratophthalma* by time after feeding

<table>
<thead>
<tr>
<th>Time after feeding (hours)</th>
<th>Number of crabs examined</th>
<th>Number of crabs positive</th>
<th>Percentage of crabs positive</th>
<th>Number of larvae in each positive crab</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-6</td>
<td>11</td>
<td>8</td>
<td>73</td>
<td>5, 11, 12, 17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>31, &gt;50, &gt;50, &gt;50</td>
</tr>
<tr>
<td>7-14</td>
<td>11</td>
<td>4</td>
<td>36</td>
<td>1, 1, 2, 2</td>
</tr>
<tr>
<td>23-79</td>
<td>57</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Twenty *M. lar* were exposed to first-stage larvae of *A. cantonensis*, 10 by each of the two methods described. A total of 13 shrimp survived for six weeks. At this time, all were fed to albino rats after the legs, carapace, and abdominal exoskeleton had been removed. No *A. cantonensis* were found in the brain of these rats upon subsequent examination.

Crabs. Five experiments utilizing a total of 79 *O. ceratophthalma* were carried out to determine if this species of crab could serve as a transport host of *A. cantonensis*. The results with respect to the recovery of larvae resembling *A. cantonensis* from the stomach, intestine, and body cavity washings are summarized in table 3. It will be noted that a high proportion of crabs had such larvae in the stomach a short time after feeding but that the proportion of crabs with larvae and the number of larvae per positive crab decreased rapidly with time. In contrast to the situation with the shrimp, many of the larvae seen in Petri dishes with the stomachs of crabs were moving relatively slowly. The movement of these larvae was noted to increase when they were placed in fresh water.

No larvae resembling *A. cantonensis* were seen in the viscera of 38 crabs whose organs were examined by pressing them between glass plates. Similarly, no *A. cantonensis* were found in the brain of the rats fed the viscera from the other 41 crabs used in the experiments. The musculature from 68 of the crabs was fed to rats and, with one exception, these rats were negative on later examination. Four young adult *A. cantonensis* were found in the brain of one rat which had received the musculature from 2 crabs. These crabs had been killed six hours after being fed an infected snail. No *A. cantonensis* larvae had been seen in the stomach, intestine, or body cavity washings of the two crabs. Numerous nematodes which did not resemble *A. cantonensis* were seen in many crabs.

**Discussion**

The present studies were undertaken in an attempt to obtain data on various ways in which man might become infected with *A. cantonensis*. Since this parasite had been recovered from the central nervous system of humans (1, 9) and has a geographic distribution which roughly coincides with the geographic distribution of the eosinophilic meningitis in the Pacific (10), it is the prime suspect as the etiologic agent of the condition in that area. However, epidemiologic investigations (11) have shown that very few persons on Pacific Islands who acquired eosinophilic meningitis deliberately consumed raw terrestrial or aquatic molluscs, the only known intermediate hosts of *A. cantonensis*. Furthermore, these studies indicate that it is unlikely that a significant proportion of the patients in most
areas could have consumed such molluscs unintentionally. The data do suggest, however, that most cases of eosinophilic meningitis in the Pacific are acquired by the ingestion of the etiologic agent. Consequently, attention has been focused on food animals, other than molluscs, which might serve as transport or intermediate hosts of *A. cantonensis*.

*Shrimp. M. lar* was selected for study because this shrimp was commonly consumed raw in Tahiti, an area where eosinophilic meningitis is common, and because third-stage larvae of *A. cantonensis* have been found among its stomach contents in nature (6, 11). The data obtained in the present study indicate that *M. lar* could serve as a transport host for *A. cantonensis*. However, it appears that the proportion of ingested third-stage larvae which remain in this crustacean is relatively low, and the number or viability of such larvae drops off relatively rapidly with time. It was surprising to find living third-stage larvae of *A. cantonensis* in the alimentary tract of shrimp for as long as 29 days. There was no apparent explanation for this phenomenon other than the possibility that the larvae found were actually embedded in the walls of the intestine.

Most of the infective larvae detected outside of the alimentary tract were found in the cephalothorax whereas the majority found within this tract were seen in the intestine. Hence, it is very probable that the larvae detected in the cephalothorax actually were located outside of the alimentary tract and were not found there as a result of the contamination of the tissues when the stomach and intestine were removed during dissection.

The situation with respect to larvae detected in the abdomen of shrimp is less clear. Relatively few larvae were detected in this location. With one exception, all shrimp abdomens found positive by rat feeding still had the telson attached when they were fed. In later shrimp experiments, after the possible concentration of larvae in the distal end of the intestine had been noted, the telson was routinely removed with the intestine. When the abdomens from these shrimp were fed to rats, only one rat was found positive (with one *A. cantonensis*). On the other hand, the cephalothoraces of the same shrimp were as highly positive or more so than those from the earlier experiments in which the abdomens with telsons had been fed. Thus, it is not certain that the relatively few larvae detected by the feeding of the abdomens to rats actually had penetrated the alimentary tract of the shrimp and were located in other tissues of the abdomen.

Detecting the presence and the number of third-stage larvae of *A. cantonensis* by feeding suspect tissue to rats is a relatively simple and accurate technique and, in addition, each larva is shown to be fully viable and capable of maturation. The dissection of rats as early as 23 days after feeding should preclude the possibility of young adult *A. cantonensis* having already left the brain and migrated to the pulmonary arteries. It is also unlikely that very many young adults were missed by not examining the spinal cord. Quantitative experiments carried out with small numbers of third-stage *A. cantonensis* larvae showed that each of ten rats fed a single larva had a young adult *A. cantonensis* on the surface of the brain when examined 22 days later. In the same experiments, two, three, or four larvae were fed to a total of 26 rats and 67 per cent of these larvae were recovered as
young adult forms on the surface of the brain after the same time interval. The procedure of examining the brain rather than the pulmonary arteries in using the rat to detect third-stage larvae was chosen for several reasons. First, it is relatively easier and less time-consuming to examine the brain thoroughly than it is the pulmonary arteries. At 20 to 23 days after feeding the young adult *A. cantonensis* are almost invariably found on the surface of the brain. Secondly, the results are obtained sooner since it is necessary to wait more than 30 days to be sure that all the *A. cantonensis* have reached the pulmonary arteries. Of course, one could examine the brain earlier than 20 days and still find the larvae by dissection or pressing the brain between glass plates. However, this would negate the advantage of having to look only for relatively large nematodes which are usually readily apparent upon removal of the brain from the cranial case. Finally, the detection of young adult forms in the brain serves as a possible additional control on the source of the infective third-stage larvae. Thus, if a rat had inadvertently been infected with *A. cantonensis* prior to its use in a given experiment and only the pulmonary arteries were examined, it would be relatively difficult to distinguish *A. cantonensis* from the inadvertent exposure from those acquired by experimental exposure. The relatively rapid growth of *A. cantonensis* in the brain furnishes an accurate index of the length of time elapsed since the ingestion of the third-stage larvae.

Although relatively large numbers of third-stage larvae were fed to the shrimp in the present experiments, it is not improbable that they could be exposed to similar dosages in nature. Certain species of slugs which are found along banks of rivers on many Pacific Islands are commonly infected in nature with several thousand third-stage *A. cantonensis* larvae each (12). Since *M. lar*, like most decapod crustaceans, is probably either predacious or a scavenger, or both, it is possible that it could consume such molluscs if they were washed into the rivers with heavy rains. *M. lar* has sometimes been observed in nature out of the water along the banks of rivers. Thus, it is possible this species could also consume infected terrestrial molluscs in their natural habitat. As yet, there is no evidence to suggest that fresh-water molluscs are naturally infected with *A. cantonensis* on Pacific Islands.

Although the possible importance of shrimp in general, and *M. lar* in particular, as a source of human infection with *A. cantonensis* on Tahiti will be discussed in detail elsewhere (11), it is pertinent to record here certain observations on the preparation and consumption of shrimp in that locality. First, it is the general practice to remove the stomach of the shrimp before any of the animal is eaten. The stomach is usually readily recognized by the layman since it is almost invariably full when a shrimp is captured in nature. Secondly, the intestine of the shrimp is usually also removed, but not the telson. After removing the stomach and intestine, it is common practice to rinse the crustacean in water to remove traces of debris which may have escaped from the stomach and intestine when they were removed. The part of the shrimp which is most commonly consumed raw is the abdominal musculature. However, the viscera, other than the stomach, in the cephalothorax are also consumed raw by some persons. Thus, if shrimp in nature were serving as transport hosts of
A. cantonensis in the manner of the shrimp in the present experiments, there would be a greater risk of infection from consuming the cephalothorax than from consuming the abdomen. There would be some risk from the abdomen, however, since the intestine is often broken in the process of removal, the telson is left attached, and the washing procedure is probably not always thorough. It should also be noted that shrimp are usually captured on Tahiti by spearing through the carapace, or, sometimes inadvertently, the abdomen. In some instances, the spear point traverses the stomach or intestine and thus could carry any larvae which might be present in these organs to other anatomical locations.

Crabs. O. ceratophthalma was selected for study for two reasons. First, this particular species of crab was chosen because it was the only type of animal food which had recently been consumed raw by a patient who acquired eosinophilic meningitis on Oahu, Hawaii in 1962 (11). It was thought desirable to investigate crabs in general as possible transport hosts of A. cantonensis because of epidemiologic data available (11) from Ponape, the island on which the first known outbreak of eosinophilic meningitis occurred. Aquatic crabs, Scylla serrata (Forskol), and amphibious crabs of the genus Sesarma (family Grapsidae), both of which live in mangrove swamps, had commonly been eaten raw by persons who had acquired the disease on that island.

Although it was demonstrated that third-stage larvae of A. cantonensis could survive for a short period of time in the alimentary tract of O. ceratophthalma, there was relatively little evidence that such larvae penetrate the walls of this tract and remain viable elsewhere in the animal. The one instance in which A. cantonensis was recovered from a rat fed the musculature removed from two crabs six hours after experimental feeding could have been due to the contamination of the musculature of one of the crabs with larvae which escaped from the alimentary tract on dissection.

There appeared to be a marked difference between the behavior of third-stage larvae of A. cantonensis in M. lar and O. ceratophthalma. One possible reason for this observed difference is the difference in the digestive organs of the two crustaceans. Both M. lar and O. ceratophthalma, like other decapod crustaceans, have gastric mills (chitinous teeth in the stomach used for triturating food). However, the gastric mill of O. ceratophthalma is much more developed than that of M. lar and it is possible that fewer larvae escape its grinding action. It is also possible that there are differences in the chemical digestive processes of the two species. Finally, it is possible that larvae might have migrated in the crab to an obscure organ or area which was not fed to rats. The entire cephalothorax of shrimp (including all of the exoskeleton except the carapace and legs) had been fed to rats, whereas the various organs and musculature had been removed from the crabs and fed.

Although it was not demonstrated unequivocally that third-stage larvae of A. cantonensis could penetrate the walls of the alimentary tract of O. ceratophthalma and remain viable elsewhere, it is still possible that this species of crab could serve as a source of human infection with that nematode. In view of the fragility of the stomach of O. ceratophthalma, persons consuming the viscera and musculature of this crab...
could ingest larvae which might escape from the stomach when the other viscera and musculature are removed. It is likely that *O. ceratophthalma* feeds to some extent on the giant African snail, *Achatina fulica* Bowditch, on Oahu since a species of *Ocypode* had been observed feeding on a similar species of *Achatina* elsewhere (14). *A. fulica* is commonly infected with very large numbers of *A. cantonensis* larvae on Oahu.

It has been reported (13) that several larvae morphologically identical to those of third-stage *A. cantonensis* were found in the stomach contents of two specimens of crabs from Saipan tentatively identified as *Ocypode* sp. The identity of these larvae was not confirmed by feeding them to rats and the identification of the crabs was later (15) changed to *Cardisoma hirtipes*. It was also reported (13) that two persons who acquired eosinophilic meningitis on Saipan had eaten raw land crabs prior to the onset of their illness. However, the significance of this latter observation is lessened by the fact that it is also known (11) that these particular patients were fond of eating many types of raw foods, and specifically, had eaten raw fresh-water shrimp and raw fresh-water fish shortly before the onset of their illnesses.

Third-stage larvae of *A. cantonensis* were also reportedly found (15) in the stomach and intestinal tract of coconut crabs (*Birgus latro*) on Saipan. This particular crab is not ordinarily eaten raw.

Since it has been demonstrated that third-stage larvae of *A. cantonensis* can penetrate the wall of the alimentary tract of *M. lar* and remain viable for a considerable length of time, it is obvious that this might also occur with other species of fresh-water shrimp, and, perhaps, in crabs other than *O. ceratophthalma*. The possible importance of such decapod crustaceans in the transmission of *A. cantonensis* to man would depend on a number of factors such as the opportunity which the crustaceans might have to feed on infected molluscs and the frequency and manner in which the crustaceans in turn are eaten by humans.

**Summary**

Molluscs infected with large numbers of third-stage larvae of the metstrongylid lungworm, *Angiostrongylus cantonensis*, were fed to a species of fresh-water shrimp, *Macrobrachium lar*, and a species of amphibious crab, *Ocypode ceratophthalma*, to determine if these crustaceans could serve as transport hosts of the parasite. Viable larvae were recovered in small numbers from the cephalothoraces of the shrimp after the stomach had been removed. Such larvae were found in a relatively high proportion of the shrimp up to three days after feeding and in a small proportion for as long as 29 days afterwards. Viable larvae were also recovered from both the stomach and intestine of some of the shrimp for as long as 29 days after feeding. Third-stage larvae were excreted or migrated out of the shrimp for several days after feeding.

Living third-stage larvae of *A. cantonensis* were detected in relatively large numbers in the stomach contents of most of the crabs for a few hours after feeding and in very small numbers in a few instances up to three days afterwards. However, with one possible exception, no viable larvae were detected.
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in the other viscera or musculature of the crabs.

Third-stage larvae were not recovered from *M. lar* which had been exposed to large numbers of first-stage larvae of *A. cantonensis* six weeks prior to examination.

Various ways in which shrimp and crabs might become infected in nature with third-stage larvae of *A. cantonensis* and in turn transmit these to man are discussed.

**REFERENCES**


