GLOBAL ADVANCES IN ECOLOGY AND MANAGEMENT OF
GOLDEN APPLE SNAILS

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Apple Snails as Disease Vectors

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Abstract

Apple snails (Ampullariidae) are intermediate hosts of parasites causing at least three diseases in humans: cercarial dermatitis ("swimmer's itch") caused by trematode cercariae, intestinal problems caused by flukes in the genus *Echinostoma*, and eosinophilic meningitis caused by the nematode *Angiostrongylus cantonensis*. Angiostrongyliasis is the most important of these diseases. Experiments show that apple snails do not acquire *A. cantonensis* as easily as certain other mollusks, and the natural parasite load of individual apple snails is generally much lower than in large species of land mollusks, such as the giant African snail (*Achatina fulica*) and veronicellid slugs. Nevertheless, apple snails are important carriers of *Angiostrongylus cantonensis* because of their widespread use as human food and high intraspecific variation in parasite load. Angiostrongyliasis caused by apple snails results primarily from consumption of raw or undercooked snail meat, but contact with the debris associated with preparation of the snails for eating may also cause infection. As pest species of apple snails continue to be spread, the potential exists for the concomitant spread of *A. cantonensis* into new regions and a resultant increase in the number of cases of angiostrongyliasis worldwide.

Key words: angiostrongyliasis, *Angiostrongylus cantonensis*, paratenic hosts, cercarial dermatitis, *Echinostoma*, *Achatina fulica,* Veronicellidae, susceptibility, *Pomacea canaliculata,* eosinophilic meningitis

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Introduction

Like many other mollusk species, apple snails (snails in the family Ampullariidae) are intermediate hosts of a number of disease organisms that reach maturity in vertebrate hosts. Apple snails are vectors of at least three such diseases affecting humans, two caused by trematodes (flukes, class Trematoda) and one caused by a nematode, *Angiostrongylus cantonensis*.

Cercarial Dermatitis

The least serious of these diseases is cercarial dermatitis ("swimmer's itch" or "rice paddy itch"). It is caused by various trematode species, whose cercariae (the infective stage) are released into water from the bodies of freshwater snails, and has been reported widely throughout the world, including Asia (e.g., Narain et al. 1994, Muller 2001, Zbikowska 2004). The parasite reaches maturity in the definitive host (usually waterfowl or small mammals), developing in the mesenteric arteries. In humans, the cercariae penetrate the skin but do not develop further (Muller 2001, Burke and Tester 2002). They cause irritation of the skin in the region of penetration, beginning as early as 30–90 minutes (Narain et al. 1994) or as long as about 48 hours after exposure, by which time the parasites have usually died (Burke and Tester 2002). Intense itching (pruritus), macules, and papules may develop (Muller 2001, Burke and Tester 2002). Symptoms may disappear after several days or may worsen and resolve only after several weeks (Burke and Tester 2002). However, symptoms are not usually severe, and medical intervention is generally not required. In the United States (US), the disease typically peaks in the summer in stagnant water with abundant vegetation on which the snail intermediate hosts feed. In Florida, *Pomacea paludosa* has been considered as the vector of dermatitis-producing cercariae in Lake Okeechobee (Hanning and Leedom 1978). *P. paludosa* is locally abundant and constitutes an important food source for several species of birds, including the limpkin (*Aramus guarauna*). Initial exposure to contaminated water caused burning and itching lasting about 2 hours. This was followed by a quiescent period of 8 hours, after which intense itching occurred for several days. Blisters formed 2 days after exposure and lasted for 2 weeks (Hanning and Leedom 1978, Leedom and Short 1981).

Intestinal Flukes

A second and more serious disease that can be carried by apple snails is caused by intestinal flukes in the genus *Echinostoma*. These flukes commonly cause infections in Southeast Asia, where infection rates average around 20% (Eveland and Haseeb 2003) but range from 15% to 90% (Huffman and Fried 1990, Eveland and Haseeb 2003, Park et al. 2004). Infections result from the consumption of raw or undercooked freshwater snails, clams, and fish containing the metacercariae. Adult parasites are found in the small intestine of the definitive host (birds or mammals). There are many species of *Echinostoma* (Huffman and Fried 1990, Eveland and Haseeb 2003), but *E. ilocanum*, *E. revolutum*, and *E. malayanum* are among the most widespread species infecting humans in Asia (Muller 2001, Eveland and Haseeb 2003). Infections are usually asymptomatic, but moderate infections can cause headache, dizziness, anemia, gastric pain, and diarrhoea; and heavy infections may be associated in addition with anorexia, nausea, postprandial burning, epigastric pain with hematemesis, vomiting, fever, flatulence, constipation, abdominal pain, eosiniphilia, edema, and hepatomegaly (Eveland and Haseeb

2003). The disease is diagnosed by examining stool samples for the presence of eggs (Keawjam 1986, Huffman and Fried 1990, Eveland and Haseeb 2003). *E. ilocanum* is widespread in the Philippines, and average infection rates of 11% have been reported in Ilocano populations of northern Luzon, although rates as high as 44% were recorded (Cross and Basaca-Sevilla 1986). Infection in this area was probably related to the fact that the ampullariid *Pila luzonica* (probably a synonym of *Pila conica*) is eaten, sometimes uncooked, by the people; metacercariae from these snails developed into adults in laboratory animals (Cross and Basaca-Sevilla 1986, Eveland and Haseeb 2003).

Angiostrongyliasis

By far the most serious disease known to be vectored by apple snails is angiostrongyliasis, manifested in humans as eosinophilic meningitis and caused by the rat lungworm, *Angiostrongylus cantonensis* (Nematoda: Metastrongylidae). Although there are other causes of eosinophilic meningitis, infection by *A. cantonensis* is the most common cause worldwide (Lindo et al. 2002). This disease, which can be fatal, is an "emerging disease" because its geographical range and the list of animals affected has been expanding rapidly in recent years (Prociv et al. 2000). Human infections with *A. cantonensis* are traditionally associated with Southeast Asia and the Pacific (e.g., Cheng and Alicata 1965, Alicata 1991, Kliks and Palumbo 1992, Prociv et al. 2000), but the disease, or at least rodent infection, has been reported from other parts of the world, including India, Africa, Mauritius, Madagascar, the Caribbean, and the southeastern US (Campbell and Little 1988, Alicata 1991, Kliks and Palumbo 1992, Prociv et al. 2000, Lindo et al. 2002). The giant African snail, *Achatina fulica*, may have played an important role in the pan-Pacific spread of *Angiostrongylus cantonensis* (Alicata 1991, Kliks and Palumbo 1992), although this role has probably been exaggerated (Wallace and Rosen 1969b, Civeyrel and Simberloff 1996, Prociv et al. 2000), and rats, the definitive hosts of the pathogen, will continue to be the principal agents of expansion of the parasite beyond the Indo-Pacific region (Prociv et al. 2000). The natural geographical range of apple snail species includes primarily humid tropical and subtropical habitats in Africa, South and Central America, and Southeast Asia. However, apple snails have been intentionally introduced for use as food in other parts of Asia (Mochida 1991, Lai et al. 2005) and a number of Pacific islands, including Guam and Hawaii (Smith 1992, Cowie 2002). Ampullariids also occur in some of the same Caribbean countries infested with *A. cantonensis*, including Cuba, the Dominican Republic, Jamaica, and Puerto Rico (Cowie 2002). Thus, the potential of apple snails to vector *A. cantonensis* is an urgent concern because their ranges overlap and both are rapidly spreading into new areas of the world (Prociv et al. 2000; Cowie, 2002).

Angiostrongyliasis in humans can result in transient meningitis (inflammation of the meninges of the brain and the spinal cord) or a more serious disease involving the brain, spinal cord, and nerve roots, with characteristic eosinophilia of the peripheral blood and cerebrospinal fluid (Kliks and Palumbo 1992). The term "eosinophilia" indicates that the level of eosinophils (a type of white blood cell) in the blood is above the normal range, a condition that can occur in response to allergic reactions or parasitic infections (Lindo et al. 2002). When humans become infected by *A. cantonensis*, the nematodes can sometimes be found in the cerebrospinal fluid; they eventually reach the meninges, the tissues surrounding the brain. There they develop to the fifth developmental stage and cause inflammation and swelling, but they die instead of reproducing. Occasionally the fifth-stage nematodes will migrate to the lungs of infected humans, but they apparently do not reproduce there either (Alicata and Jindrak 1970, p. 25). In mild cases, disease symptoms include headache, neck stiffness, vomiting, and arm and shoulder

pain; more severe infections may also involve bowel and bladder dysfunctions, and weakness and hyporeflexia of the legs, indicating involvement of the brain, spinal cord, and nerve roots; characteristic lesions may be seen in brain scans (Kliks et al. 1982, Kliks and Palumbo 1992). Symptoms usually begin within 18 days after infection but sometimes within 1–6 days (e.g., Kliks et al. 1982). Most cases are not fatal, and usually symptoms abate within 10 weeks (e.g., Kliks et al. 1982). Fatal cases are associated with ingestion of high numbers of nematodes (hundreds or thousands), for instance when veronicellid slugs or *Achatina fulica* are consumed raw (e.g., Kliks et al. 1982).

Definitive diagnosis of angiostrongyliasis in humans is generally difficult. A spinal tap (lumbar puncture) is sometimes carried out to relieve pressure on the brain, but the nematodes are often not detectable in the cerebrospinal fluid (Prociv et al. 2000, Chye et al. 2004). Elevated eosinophil count in the blood is another, but less definitive, indication of disease. An enzyme-linked immunosorbent assay test is available (Cross and Chi 1982), but this test sometimes produces inaccurate results because of delays in the appearance of antibodies after infection and the persistence of antibodies after recovery of the patient. An immuno-polymerase chain reaction test has been developed that has 100% specificity and sensitivity (Chye et al. 2004) and may soon become the assay of choice for patients suspected of having the disease.

Discovery of Angiostrongyliasis

The history of the events leading to the discovery of *Angiostrongylus cantonensis* as the causal agent of eosinophilic meningitis in humans has been outlined by Alicata and Jindrak (1970) and Alicata (1991). *A. cantonensis* was first discovered in rats, in Canton, China, in 1935. It was recognized as a disease agent in humans in 1944, but the scientific report, published in Japanese in 1945, attracted relatively little attention (Kliks and Palumbo 1992). A decade later Mackerras and Sandars (1955) recognized that slugs and snails were the intermediate hosts of a related nematode, *A. mackerrasae*, although at that time it was not recognized as a different species (Prociv et al. 2000). In the early 1960s, outbreaks of eosinophilic meningitis occurred in the human population of a number of Pacific islands, including Tahiti. University of Hawaii parasitologist Joseph Alicata correctly surmised that *A. cantonensis* infection was the cause of those outbreaks, and he and other workers subsequently collected data to show that *A. cantonensis* was the usual cause of eosinophilic meningitis in Southeast Asia and the Pacific islands (Alicata 1991).

Life Cycle of the Pathogen

The life cycle of *A. cantonensis* has been summarized by Alicata and Jindrak (1970), Alicata (1991), and Kliks and Palumbo (1992) among others. Rodents, principally rats, are the definitive hosts for the parasites. Rats become infected by eating slugs or snails infested with third-stage larvae of the parasite. Most of the larvae enter the venules and eventually (≤ 4 days later) end up concentrated in the tissues of the central nervous system (brain and spinal cord). In the brain, the larvae grow and undergo two molts. The young adult (fifth-stage) worms then migrate through the venous system to the pulmonary artery (lungs) and heart (Chao et al. 1987), where they develop to sexual maturity. The time from infection of the rat host to sexual maturity is about 1 month. The adult nematodes mate, and the females lay eggs that hatch into first-stage larvae, which make their way from the terminal branches of the pulmonary arteries to the pharynx. There they are swallowed and pass in the feces. Mollusks become infected either through ingestion of first-stage nematodes present in rat feces or through burrowing of these nematodes through the body wall or respiratory pores (Cheng and Alicata 1965, Chao et al. 1987).

Visceral organs that can be infected in slugs and snails include the lungs, liver, and kidneys, although in most species, mature nematode larvae are most common in the head-foot region by the time the nematodes reach the infective (third) stage. For example, in snails of the genus *Oncomelania*, the majority of third-stage larvae were found in the kidney and intestine region; in *Achatina fulica*, the majority were found in the mantle; and in *Ampullarium* sp., which is an invalid name (Cowie 1997b, Cowie et al. 2006) but presumably refers to *Pomacea canaliculata*, the majority were found in the head and foot (Uchikawa et al. 1986, Iwanaga 1995). The minimum developmental time required for first-stage larvae to reach the third (infective) stage in the intermediate host is about 12 days; by the third to fourth week following infection of a suitable host, third-stage larvae are the most common stage present. These quiescent third-stage larvae can live for many months in mollusk tissue. Quiescence is broken when an infected mollusk is consumed by a rodent, and the cycle begins again.

The prevalence of *Angiostrongylus cantonensis* in intermediate hosts can be assessed by digesting slug or snail tissue in artificial gastric juice (1% pepsin with 1% HCl). The third-stage larvae can be identified microscopically by their size and shape using the key of Ash (1970). However, most researchers confirm the presence of *A. cantonensis* in intermediate hosts by feeding the infective stage to albino rats, followed by examination of the adult worms infesting the lung tissue approximately 2 months later (Asato et al. 2004).

Paratenic Hosts

Although slugs or snails are the obligatory intermediate hosts for *A. cantonensis*, supporting parasite development from the first to the third larval stage, first-stage to third-stage *A. cantonensis* can also be found in the tissues of other kinds of hosts that are passive carriers. In these animals, called paratenic hosts, development of the nematodes does not progress (Kliks and Palumbo 1992). However, third-stage nematodes in paratenic hosts can cause angiostrongyliasis if they are consumed by humans. The most important paratenic hosts are crustaceans (such as prawns and land crabs) and predacious land planarians (such as flatworms in the genus *Platydemus*). *Platydemus* spp. are predators of slugs and snails, excreting digestive juices that externally digest their prey. These flatworms are strongly implicated as vectors of the disease in humans, as they are small; commonly found on lettuce, cabbage, and fruits; yet easily overlooked while preparing food. It has been hypothesized that these flatworms vector the disease when the whole animal, or pieces of it, are accidentally consumed in salads (Alicata and Jindrak 1970, p. 45; Ash 1976; Asato et al. 2004). *Platydemus manokwari*, an invasive species thought to be native to New Guinea, has been introduced widely across the Pacific in ill-conceived attempts to control *Achatina fulica*, the giant African snail (e.g., Hopper and Smith 1992, Cowie and Robinson 2003). Although this flatworm is a general predator of snails and has been implicated in the extirpation of native species, there is reason to doubt that it has played a significant role in controlling the target pest (Lydeard et al. 2004). Freshwater prawns are a favorite food in certain Pacific islands, and infected prawns in Tahiti are thought to have been responsible for the outbreak of eosinophilic meningitis there that spurred the study of this disease, beginning in 1961 (Alicata and Jindrak 1970, p. 43; Alicata 1991).

Disease Transmission to Humans
and Other Warm-Blooded Nonhost Animals

People get infected with *Angiostrongylus cantonensis* by intentionally consuming raw or undercooked snail meat. From case histories of angiostrongyliasis involving apple snails, we know that the disease potential for humans is high in regions of the world where apple snails are consumed as food items. For example, in Thailand, native apple snails in the genus *Pila* are abundant in rice fields and shallow bodies of water supporting succulent vegetation. The large snails are favored as food items and are commonly eaten raw. Numerous cases of angiostrongyliasis have resulted (Alicata 1964, Wallace and Rosen 1969b, Keawjam 1986). In Hawaii, a case of angiostrongyliasis was traced to the consumption of apple snails of the genus *Pila* (Wallace and Rosen 1969b). In Taiwan, where *A. cantonensis* is one of the most important zoonotic parasites, and where natural infections in introduced populations of *Pomacea canaliculata* have been documented, apple snails are collected for food but are rarely eaten raw. Most patients did not acquire their infections from ingesting the snails but were thought to have become infected through exposure to the debris associated with the preparation of the snails for eating (Yen et al. 1990).

It is possible that people may also become infected by accidentally consuming a small slug, snail, or other (paratenic) host, or a portion of one, in uncooked vegetables (e.g., Alicata 1964, Ash 1976). If the host present in the produce is damaged or decomposed, there is a greater chance that it will not be noticed during food preparation and consumption, particularly in the case of green leafy vegetables. This underscores the importance of preventing infestation of the commodity by potential hosts and thoroughly inspecting and washing such produce before consumption.

It has been suggested that the mucous trail of slugs and snails left behind on fresh produce (such as lettuce) may contain sufficient numbers of *A. cantonensis* larvae to cause angiostrongyliasis in people (see Ash 1976). However, there is no definitive evidence of human infection via this route, and the available evidence indicates that the number of *A. cantonensis* larvae shed in slime is probably too low to cause disease. In preliminary studies (J. Sullivan, Centers for Disease Control, Atlanta, Georgia, US, personal communication, 2005), third-stage larvae of *A. cantonensis* were recovered from slime of *Parmarion martensi* after the slugs were induced to produce slime by gentle prodding, but the numbers of larvae were very low with respect to the numbers of larvae recovered from the same animal by digestion.

Ash (1976) found that veronicellid slugs rarely shed larvae in their mucus, and when they did, the larvae survived for only a few hours. Campbell and Little (1988) detected no larvae shed from heavily infected *Limax flavus* (slugs, family Limacidae). Chen et al. (2005) reported a study that failed to detect *A. cantonensis* in slime rinsed off infected *Achatina fulica*. The identification of *Angiostrongylus cantonensis* in the slime of the Malaysian slug *Microparmarion malayanus* (Heyneman and Lim 1967) was probably a misidentification of *A. malaysiensis* (Prociv et al. 2000), and the numbers of larvae found were low. *A. costaricensis*, the cause of human abdominal angiostrongyliasis, has been found in mucus secreted by slugs but only in very small numbers (Bonetti and Graeff-Teixeira 1998).

Contaminated drinking water is another potential source of infection for humans (e.g., Wallace and Rosen 1969b). Cheng and Alicata (1964) found that infective-stage *A. cantonensis* were released into water from damaged and even undamaged *Achatina fulica* and *Subulina octona* (Gastropoda: Subulinidae) and survived in water for up to 72 hours. Similar results have been found for *Angiostrongylus costaricensis* released from the freshwater snail *Biomphalaria glabrata* (Ubelaker et al. 1980). Theoretically, nematodes could also infect a human through an

open wound (Angus 2005). Again, however, there is no evidence for human infection via these additional routes.

A. cantonensis infections have been reported in many animals, including dogs, marsupials, horses, primates, flying foxes, and alpacas (Gardiner et al. 1990, Reddacliff et al. 1999, Prociv et al. 2000, Angus 2005). The organism has been considered as causing the deaths of livestock in New Caledonia, Niue, Tonga, and Vanuatu (Angus 2005). A relatively large number of reported infections have been in horses, perhaps a reflection of their high value and better supervision rather than greater susceptibility (Angus, 2005).

The number of infective third-stage A. cantonensis necessary to cause disease in humans is not known (Alicata and Jindrak 1970, Prociv et al. 2000). When pigs and calves were infected with 20,000–70,000 larvae, respectively, cerebral pathology resulted in one of five pigs and in all of the calves tested, although only the calves manifested clinical signs of disease; a dog developed temporary partial paralysis of the hind legs 12-16 days after infection from a dose of 2,000 larvae, but a dose of several hundred larvae produced eosinophilic meningitis in monkeys (Alicata and Jindrak 1970). It is not unusual for individual Achatina fulica and veronicellid slugs to harbor several thousand infective larvae (Alicata and Jindrak 1970, Prociv et al. 2000).

Inherent Susceptibility to Infection of Apple Snails and Other Mollusks

Where mollusks, such as apple snails, are used as food items, transmission of Angiostrongylus cantonensis is mostly passive and presumably occurs with high efficiency whenever the mollusks are consumed. In such cases, the competency of the vector (i.e., the ability to acquire and transmit the parasite) becomes a question of inherent susceptibility, that is, the ability of the intermediate host to acquire the pathogen.

Numerous species of terrestrial and freshwater mollusks are known to act as intermediate hosts of A. cantonensis (Cheng and Alicata 1965, Higa et al. 1986, Campbell and Little 1988). Wallace and Rosen (1969a) tested a range of terrestrial and aquatic mollusk species from various Pacific islands for their susceptibility to A. cantonensis. Mollusks were exposed in petri dishes to suspensions of first-stage larvae. Of the terrestrial species studied, the veronicellid slug Laevicaulus alte was the most susceptible, while the agriolimacid slug Deroceras laeve was moderately susceptible. Land snails from atolls, including the subulinid snails Subulina octona and Prosopeas javanicum (the latter now known as Paropeas achatinaceum; see Cowie 1997a), were relatively resistant. With the exception of Biomphalaria glabrata, the aquatic snails were either completely or partly resistant. In a separate experiment, the relative susceptibilities of Biomphalaria glabrata and Pomacea paludosa were compared, exposing each group in an aquarium. After 3-4 weeks, the number of third-stage larvae found in B. glabrata averaged 562 per snail. Seven of ten P. paludosa became infected with third-stage larvae, and all but two of these snails had fewer than ten larvae each. However, one of these two snails had 272 larvae and the other had 691. Most P. paludosa were therefore concluded to have a natural resistance to A. cantonensis, with a minority of individuals having moderate-to-high susceptibility. In general, larger snails and slugs harbor more A. cantonensis larvae (e.g., Campbell and Little 1988) than smaller ones.

Chao et al. (1987) studied the life cycle and distribution of A. cantonensis in artificially infested P. canaliculata, an apple snail species that is an extremely important pest of rice in Southeast Asia. Snails that were 15–25 mm in size were infested with first-stage larvae in petri dishes. Each day, a group of three snails was sacrificed and their bodies were separated into four parts: head/foot, mantle, digestive tract, and visceral organs. Excised tissues were digested in pepsin-HCl and examined under a dissecting microscope for juvenile A. cantonensis. During

the first 4 days following infection, nematodes were found only in the digestive tract. This indicated that the mouth was the primary means of infection of *P. canaliculata*, in contrast to the general finding that mollusks can become infected either by ingestion of contaminated food or by active penetration of the body surface (Cheng and Alicata 1965). The first molt was observed on the fifth day following infection, and the second molt (to the infective third stage) was first observed on day 12. By day 27, the majority of worms recovered were third-stage larvae, and at about this time approximately 46%, 31%, 21%, and 2% were recovered from the head/foot, mantle, digestive tract, and viscera, respectively.

Natural Levels of Infection by *A. cantonensis* in Apple Snails and Other Mollusks

Natural infection rates in a mollusk species will be determined largely by its inherent susceptibility and the level of exposure to first-stage larvae in the environment. Wallace and Rosen (1969b) studied the prevalence of *A. cantonensis* in a variety of mollusk species from the islands of Oahu (Hawaiian Islands), Tahiti (Society Islands), Rarotonga (Cook Islands), and Majuro (Marshall Islands). As might be expected, they found that very young and juvenile mollusks contained fewer *A. cantonensis* larvae than more mature mollusks. A large proportion of the veronicellid slugs and *Achatina fulica* were infected, often containing more than 1,000 infective larvae per animal (see also Cheng and Alicata 1965). These large species were considered important in the natural disease cycle and geographical spread of the parasite. Wallace and Rosen (1969b) also examined natural infection levels in the apple snail "*Pomacea paludosa*" (probably misidentified and more likely a different species of *Pomacea* or a species of *Pila*) collected from an irrigation ditch on Oahu (Hawaii). The snails were being reared for human consumption and were tested in the course of an investigation into a case of human eosinophilic meningitis. Of 128 apple snails examined, 27% were infected with *Angiostrongylus cantonensis*. The number of larvae per individual snail ranged from 3 to 34, with an average of 5. Wallace and Rosen (1969b) examined 78 *Pila* sp. from Maui (Hawaii), including adults and juveniles. None were infected with *A. cantonensis*. In all, five species of aquatic snails were examined on Oahu, but only "*Pomacea paludosa*" was infected. In Tahiti, the slug *Deroceras laeve* was found to contain intermediate levels of the nematode, with an infection rate of 65% and a mean number of 30 larvae per slug.

Yen et al. (1990) collected *Pomacea canaliculata* monthly from five irrigation canals in Taiwan and examined the snails for *A. cantonensis* in order to study among-site variation, seasonality of infection, and influence of snail size on parasite load. The number of third-stage larvae per snail increased from 11 to 40 as snail size increased from 1.0 to ≥4.0 cm shell height. Infection rates in the different irrigation canals varied from 13.6% to 30.8%, and the average number of larvae per infected snail was 23 during the rainy season and 33 in the dry season, a statistically significant difference (Yen et al. 1990).

In Thailand, Keawjam et al. (1993) collected *P. canaliculata* once a month for a year from two localities (a canal and a pond) to search for natural parasites. Of 1,181 snails collected, 200 were infected with three types of metacercariae, but none was infected with *A. cantonensis*.

Lim and Heyneman (1965) studied natural infection of rodents and mollusks in Malaysia. They found that *A. cantonensis* was a common parasite in two species of field rats. From rice fields, they collected *Pila scutata* (probably a synonym of *Pila conica*), of which 12.8% were positive for *A. cantonensis*, with an average of 16 larvae per host (range 4-124). Rats in the areas around the rice fields had the remains of aquatic species of snails in their nests and were infected with *A. cantonensis*. However, there is some doubt regarding the correct identification of the nematodes, which were probably *A. malaysiensis* (Prociv et al. 2000).

In Okinawa, Japan, Asato et al. (2004) studied the prevalence of *A. cantonensis* in a wide variety of mollusk species and other organisms in an effort to find the probable cause of an outbreak of angiostrongyliasis in 2000. A total of 3,764 apple snails (referred to as "*Ampullarium*" sp.) were examined, but none were found to be infected. The identity of these snails is not known, since "*Ampullarium*" is not a valid name (Cowie 1997b, Cowie et al. 2006); they may have been species of *Pomacea*, *Pila*, or both, but most likely were *Pomacea canaliculata*. Based on prevalence levels of the disease agent, seasonal correlations, and other considerations, the species that were suspected to be primarily responsible for the outbreak included the flatworm *Platydemus manokwari* and the slug *Parmarion martensi*. Both are recent immigrants to Okinawa. The latter species is also a recent immigrant to the Hawaiian Islands (Cowie 1997a) and is thought to be responsible for a recent outbreak of angiostrongyliasis cases on the island of Hawaii (R.G. Hollingsworth, unpublished). In the area where the disease outbreak occurred, *P. martensi* were very numerous, and some of them were heavily infected with third-stage *Angiostrongylus cantonensis* larvae (J. Sullivan, Centers for Disease Control, Atlanta, Georgia, US, personal communication, 2005).

Effect of *A. cantonensis* on Apple Snails

Little is known about the potential negative effects of *A. cantonensis* on infected slugs and snails. The aquatic snail *Physa elliptica*, exposed to first-stage larvae of *A. cantonensis*, exhibited a high mortality rate relative to snails in a control group (Wallace and Rosen 1969a). For other species of snails and slugs tested by Wallace and Rosen (1969a), including *Pomacea paludosa*, increases in mortality following infection by *A. cantonensis* were not apparent. Similarly, *A. cantonensis* appears not to cause mortality in *Pomacea canaliculata* (Chao et al. 1987). As yet there is therefore no evidence that infection by *A. cantonensis* causes mortality in apple snails.

Acknowledgment

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