Pathways for Transmission of Angiostrongyliasis and the Risk of Disease Associated with Them

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Abstract
Angiostrongylus cantonensis, the rat lungworm, is a major cause of eosinophilic meningitis in humans. This short paper reviews what is known about the pathways of infection and assesses the probable importance of each in causing disease. Rats are the definitive hosts. People can become infected by eating, both deliberately and inadvertently, raw or under-cooked intermediate hosts (snails or slugs) or paratenic hosts such as freshwater shrimp, crabs and frogs. Food preparation prior to cooking can leave debris from which infection can also occur. It may be possible to become infected by consuming snail/slug slime (mucus) on produce or by transferring mucus from hands to mouth after handling snails/slugs. Infection from consuming drinking water contaminated by snails/slugs and infection via open wounds may be theoretically possible but no cases have been reported. The severity of the disease is probably related to the number of infective larvae ingested as well as to the precise location of the worms in the host and the host's inflammatory response. Strategies for reducing human infection should include snail and slug control to reduce chances of accidental ingestion, cooking of intermediate and paratenic hosts, and public education on food preparation.

Keywords
Angiostrongyliasis, Angiostrongylus cantonensis, Emerging infectious disease, Eosinophilic meningitis, Parasitology, Rat lungworm disease, Snails, Slugs

Introduction
Eosinophilic meningitis caused by the parasitic nematode Angiostrongylus cantonensis is an emerging infectious disease. The natural life cycle involves rats as the definitive host and snails and slugs (Figure 1) as the intermediate hosts. Adult worms reproduce in the pulmonary arteries of rats, where the females lay eggs. The eggs travel passively via the circulatory system to the lung, where they hatch into first stage larvae (L1). The larvae break through the walls of the bronchioles and alveoli, move up the trachea, and are swallowed and passed out in the feces. Feces containing L1 larvae are then ingested by the intermediate hosts and develop to the third, infective larval stage (L3) in the snail or slug. The natural cycle then involves rats ingesting the infected intermediate hosts. The L3 larvae migrate from the rat’s gut via the circulatory system to the central nervous system, eventually reaching the brain. In the brain they develop to the fifth stage (young adult) before returning via the circulatory system to the pulmonary arteries, where they mature fully.

Human infection (angiostrongyliasis) results when L3 larvae are ingested by people, as accidental hosts. The cycle then follows the same trajectory as in the definitive rat host, with worms migrating to the brain and developing to the young adult stage. However, at this stage instead of returning to the circulatory system and continuing the cycle, they die. Tissue damage is caused by the movements of the worms in the brain, and an inflammatory immune response is triggered, predominantly by dead worms. The damage and inflammation result in eosinophilic meningitis, the symptoms of which range from severe headaches, through various neurological malfunctions, to coma, and occasionally death.

Eosinophilic meningitis caused by rat lungworm infection is a potentially serious disease. It is spreading geographically and the number of cases reported is increasing. To reduce the risk of people becoming infected, it is important to understand the pathways via which infection may occur. This short paper reviews what is known about these pathways and assesses the probable relative importance of each in causing disease.
Raw or Under-cooked Snails or Slugs

Deliberate Ingestion

People become infected with *A. cantonensis* through intentionally consuming raw or under-cooked snails and slugs. The majority of reported cases of disease are from southern China and other parts of South-east Asia, notably north-east Thailand, where raw or slightly cooked snails are a delicacy.\(^{14}\) An early report from Thailand implicated the apple snail *Pila ampullacea* as the cause of eosinophilic menigitis, after the snails had been consumed raw.\(^{15}\) *Pila* species continue to be important in causing angiostrongyliasis in Thailand.\(^{15-17}\) However, with the introduction to Asia around 1980 of non-native apple snails from South America (*Pomacea canaliculata* and *P. maculata*) followed by their spread and dramatic increase in abundance,\(^{3,18,19}\) these snails, especially *P. canaliculata*, became the popular dietary choice, especially in southern China where there is a strong association between the presence of *P. canaliculata* and *A. cantonensis*.\(^{3}\) Disease outbreaks have occurred in Taiwan among immigrant Thai workers as a result of eating raw *P. canaliculata*,\(^{20}\) although this is not a common practice among Taiwanese people. An outbreak occurred in 1980 among a group of Korean fishermen aboard a boat docked in American Samoa, following ingestion of raw giant African snails (*Achatina fulica*); eating raw snails is not normal among Samoans but is traditional in Korea.\(^{11}\)

In other countries, snails are not widely eaten raw, but are cooked, rendering them non-infective. Occasionally, however, there have been instances of infection and resultant disease following deliberate ingestion of raw snails or slugs. The first clinically diagnosed case in Hawai’i was in 1961 when a man became ill after eating two raw veronicellid slugs for health reasons.\(^{20}\) Also in Hawai’i, a case of eosinophilic menigitis was traced to the consumption of apple snails\(^{21}\) (identified as *Pomacea paludosa*, which may be incorrect as this species is not known for certain to have ever been present in the wild in Hawai’i\(^{22}\)). In Brazil, the first recorded cases of eosinophilic menigitis caused by *A. cantonensis* occurred following ingestion of a live veronicellid slug,\(^{23,24}\) on a dare when drunk. Shortly thereafter two cases were associated with eating raw snails, probably giant African snails.\(^{25}\) Similarly, a man in Australia became ill with eosinophilic menigitis following ingestion of a slug on a dare,\(^{26}\) and the first case in North America was of a boy who consumed a snail on a dare.\(^{26}\) More recently, a man in Hawai’i contracted the disease after eating a live giant African snail for a bet.\(^{27}\) Children may deliberately swallow snails\(^{28}\) and this may have been the cause of a fatal case of a 14-month old boy in Jamaica, although it may also have resulted from inadvertent ingestion of contaminated fruit or vegetables.\(^{29}\)

Inadvertent Ingestion

Snails are safe to eat once thoroughly cooked. However, it may be possible to become infected via contact with the debris associated with preparing the snails for cooking. This was thought to have been the case in Taiwan, where *A. cantonensis* is one of the most important zoonotic parasites. Natural infections have been detected in introduced populations of the apple snail *Pomacea canaliculata* in Taiwan, but, although these snails are collected for food, they are rarely eaten raw.\(^{30,31}\) Crushing giant African snails (*Achatina fulica*) by hand, and presumably ingesting debris or mucus, has been implicated in Okinawa.\(^{28}\) However, where snails are not deliberately eaten raw, the most likely pathway of infection is by accidentally consuming a small slug or snail, or portion of one, in uncooked vegetables, especially salads.\(^{2,32,28,33-35}\) This was the possible cause of an outbreak of eosinophilic menigitis in Jamaica, where a small slug was found in a head of lettuce purchased in a market.\(^{3,35}\) This pathway also appears to be the most likely mode of transmission in most cases in Hawai’i.\(^{36,37}\) In Taiwan, an outbreak occurred associated with drinking raw vegetable juice.\(^{33}\) Small species and especially juveniles may be most easily overlooked in produce.\(^{36,37}\) If the host present in the produce is damaged or decomposed, with infective larvae still viable, there may be a greater chance that it will not be noticed during food preparation and consumption, particularly in the case of green leafy vegetables.\(^{33}\)

Mucus

It has been suggested that shedding of *A. cantonensis* larvae in the mucus of infected slugs and snails may result in pathways for human infection.\(^{32,38}\) The main pathway has been thought to be through consumption of vegetables or other produce contaminated by infected mucus.\(^{12,33,35,39}\) It has also been suggested that infection may result from handling slugs and transferring larvae in the mucus from hand to mouth, especially among children playing with snails.\(^{5}\) Most cases of eosinophilic menigitis in Taiwan were among children, mostly associated with contact with the giant African snail, *Achatina fulica*, including raising them as pets.\(^{40-42}\) Handling *A. fulica* has also been implicated in transmission of the parasite in Okinawa.\(^{28}\)

Although larvae have been found in the mucus of two veronicellid slug species\(^{33}\) and the semi-slug *Parmarion martensi*,\(^{39}\) the numbers of larvae were low and this may not represent a significant mode of transmission. In other species (the slug *Limax maximus*)\(^{43}\) and the giant African snail\(^{44}\), no larvae were detected in mucus slime trails. Identification of *A. cantonensis* larvae in the slime of the Malaysian slug *Microparmarion malayanus*\(^{38}\) was probably a misidentification of *Angiostrongylus malaysiensis*,\(^{12}\) and the numbers of larvae found were low (less than 1 per infected slug). *Angiostrongylus costaricensis*, the cause of human abdominal angiostrongyliasis, has been found in mucus secreted by slugs but also only in very low numbers.\(^{45}\) While slime trails may represent a source of sporadic infections the evidence suggests that this is not one of the main routes of transmission of the parasite nor a major cause of disease.

Contaminated Water

Contaminated drinking water has been suggested as another potential source of infection for humans.\(^{9,21}\) When damaged and even undamaged *Achatina fulica* and another snail species, *Subulina octona*, were immersed in water, infective stage *A.
cantonensis were released and survived in the water for up to 72 h.\textsuperscript{46} Similar results were found for A. costaricensis released from the freshwater snail Biomphalaria glabrata.\textsuperscript{47} However, only few larvae were released from the veronicellid slug Lacciviaulis alte when drowned.\textsuperscript{46} In a market in Jamaica vendors rinse vegetables in buckets of water prior to displaying them, and it has been suggested that if larvae are present in slime or feces of snails they could be washed off into the water.\textsuperscript{55} Similarly, if dead infected snails were rinsed into the buckets, larvae could be released into the water. In both cases, produce could be cross-contaminated.\textsuperscript{35}

Open Wounds
Theoretically, A. cantonensis could also infect a person through an open wound.\textsuperscript{33} However, there is no evidence for human infection via this route.

Parateneic Hosts
Parateneic (or transport or carrier) hosts are animals that can be infected with larval parasites but in which the parasite does not develop through the stages it would in its normal intermediate host. People can become infected by ingestion of a parateneic host infected with third stage A. cantonensis larvae. This was considered the most likely pathway of infection in Tahiti in the early days (1960s) of developing an understanding of the causes of eosinophilic meningitis, with freshwater prawns being the major source of infection.\textsuperscript{2,9} Freshwater shrimp have also been postulated as a source of infection in Jamaica.\textsuperscript{33} In addition to prawns and shrimp, various other animals have been identified as parateneic hosts, including land crabs, frogs and toads (including tadpoles), monitor lizards, and planarians.\textsuperscript{5,17,25,41,48} Some of these parateneic hosts are eaten intentionally by people in certain parts of the world, and have been identified as pathways of infection by A. cantonensis, for instance frogs in Taiwan, China, and the USA,\textsuperscript{2,49} and monitor lizards in Thailand, Sri Lanka, and India.\textsuperscript{2} Some species of planarians are snail predators and become infected by feeding on infected snails.\textsuperscript{33} Such planarians have been thought to be important in transmission to people,\textsuperscript{2} as they are readily consumed inadvertently in vegetables, fruit, and other produce, especially green salad vegetables, for example, in New Caledonia\textsuperscript{32} and Okinawa.\textsuperscript{28}

Other animals that are important human food resources have been experimentally infected with A. cantonensis, including fish, pigs, and calves.\textsuperscript{38,50} If these were to become infected naturally, then they might pose a threat of infection to humans, although only if eaten uncooked or inadequately cooked.

Risk Associated with Pathways
The number of infective third-stage Angiostrongylus cantonensis larvae necessary to cause disease in humans is not known.\textsuperscript{2,25,51} When pigs and calves were infected with 20,000 to 70,000 larvae, respectively, cerebral pathology resulted in one of five pigs and in all of the calves tested, although only 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.\textsuperscript{51} It is not unusual for individual Achatina fulica and veronicellid slugs to harbor several thousand infective larvae.\textsuperscript{12,51}

It has been implied that the number of infective larvae ingested is important in the severity of disease,\textsuperscript{2,9} which seems likely. The greater the number of worms ingested, the more that are likely to reach the brain, and if large numbers reach the brain, greater damage will ensue and the more serious the symptoms that will be manifested. But it is not known what parasitic burden is necessary to cause a particular degree of severity of symptoms; nor is it known how many must be ingested for a certain number to reach the brain. Nonetheless, the risk associated with the various possible pathways of infection is probably correlated with the number of infective larvae likely to be ingested. Mild cases of eosinophilic meningitis, the mildest involving just a short-lived headache, may be so mild as to not be diagnosed as eosinophilic meningitis and probably result from very few larvae reaching the brain. Severe cases probably result from a large number reaching the brain.

Therefore, the most important pathways of infection likely to result in disease are those involving ingestion of the hosts (intermediate or parateneic), and especially those host species that carry large parasite loads. So, deliberate ingestion of a large host such as a giant African snail, apple snail (Pomacea spp., Pila spp.), or veronicellid slug, which can carry thousands of infective larvae,\textsuperscript{21} probably has the highest likelihood of causing serious disease. Infection via inadvertently eating a much smaller snail or slug, containing a smaller total number of infective larvae, may pose less of a disease risk, but in many parts of the world where raw snails are not eaten, is probably the most likely path for infection. Nonetheless, even small snails or slugs may carry thousands of infective larvae. The potential parasite load of the various possible parateneic hosts is not known, but if it is as much as that of the snail and slug intermediate hosts, then eating these parateneic hosts raw poses a great risk.

The only pathway that has received much attention other than eating intermediate and parateneic hosts, is ingestion of snail and slug mucus, either by direct transfer from hand to mouth after handling these hosts or through ingestion of produce contaminated with mucus. Although there are implications that the latter pathway has been important, particularly among children,\textsuperscript{2,28,40-42} no case of disease caused by infection via mucus on produce has been definitively demonstrated. And given the low numbers of larvae in the mucus of those species so far investigated, these mucus mediated pathways are probably less likely to cause serious disease. However, in cases in which disease is ascribed to ingestion of contaminated produce it is rarely possible to determine definitively whether the contamination was snails/slugs or only mucus.\textsuperscript{3} Given the limited knowledge of these mucus pathways, it would be irresponsible to consider them negligible.

Contaminated drinking water probably poses little risk, depending on the number of hosts and volume of water involved. Because of dilution, the number of larvae likely to be ingested
may be low and insufficient to cause serious illness. No cases have been linked to this pathway of infection.

No cases of disease caused by infection through open wounds have been reported. Though it may be theoretically possible to become infected via this route, the chance of a large number of larvae entering the blood stream seems minimal. The risk of contracting serious disease therefore seems very low.

**Recommendations**

Strategies for reducing human infection should include public education so that people do not deliberately eat raw intermediate and paratenic hosts and that they take care to clean vegetables/produce prior to consuming them. People, especially children, should be cautioned not to handle snails and slugs, and if they do to wash their hands thoroughly afterwards. Control of definitive and intermediate hosts, and management of intermediate and paratenic hosts to reduce chances of accidental ingestion, may also be undertaken.

**Conflict of Interest**

The author identifies no conflict of interest.

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