Effects of Washing Produce Contaminated with the Snail and Slug Hosts of *Angiostrongylus cantonensis* with Three Common Household Solutions

Norine W. Yeung PhD; Kenneth A. Hayes PhD; and Robert H. Cowie PhD

Abstract

The emerging infectious disease angiostrongyliasis (rat lungworm disease) is caused by ingesting snails and slugs infected by the nematode Angiostrongylus cantonensis. The definitive hosts of A. cantonensis are rats and the obligatory intermediate hosts are slugs and snails. Many cases result from accidentally ingesting infected snails or slugs on produce (eg, lettuce). This study assessed three readily available household products as washing solutions for removing snails and slugs from produce (romaine lettuce) to lower the probability of accidentally ingesting them. The solutions were acetic acid (vinegar), sodium hypochlorite (bleach), and sodium chloride (domestic salt). Snail and slug species known to be intermediate hosts and that are common in the Hawaiian Islands were used in the experiments: the alien snail Succinea tenella, the alien semi-slug Parmarion martensi, and the alien slugs Veronicella cubensis and Deroceras laeve. None of the products was any more effective than washing and rinsing with tap water alone. Most snails and slugs were removed after treatment but some remained on the lettuce even after washing and rinsing the produce. Only washing, rinsing, and then rinsing each leaf individually resulted in complete removal of all snails and slugs. The study did not address removal of any remaining slime left by the snails and slugs, nor did it address killing of worms.

Keywords

Angiostrongyliasis, Eosinophilic meningitis, Food safety, Hawaii, Infectious disease, Lettuce, Postharvest produce, Rat lungworm

Introduction

Emerging infectious diseases are having an increasing impact on the global economy and on public health, brought about by a diversity of social, economic, and environmental factors.[1] Proper public health management, including management of hosts of parasites that cause disease, is a key component of the prevention of future proliferation of such diseases.

Infection by the nematode *Angiostrongylus cantonensis* (angiostrongyliasis) is one of the main causes of eosinophilic meningitis, the disease commonly called rat lungworm disease.[2,3] As a disease that is not widely known or understood by medical practitioners, and because mild cases may not be diagnosed and the aetiological agent therefore remains undetected, it may spread rapidly without being noticed.[4] Severe cases may result in long term neurological damage, coma, or death. The parasite and cases of the disease have been reported from many Pacific Islands as well as widely in southern and eastern Asia and elsewhere,[5] with recent outbreaks leading to considerable attention in Hawai'i.[6,7]

Rats are the definitive hosts and the intermediate hosts of the parasite are snails and slugs (henceforth "snails", as slugs are simply snails that have lost or internalized their shell through evolution). Humans become infected as accidental hosts by ingesting third stage *A. cantonensis* larvae carried by snails. As in the natural definitive rat host, these third stage larvae penetrate the intestine wall and enter the circulatory system, from where they cross into the central nervous system, eventually reaching the brain where they mature to the fifth (subadult) stage.[8] However, instead of returning to the circulatory system to mature and reproduce in the pulmonary artery, as in the rat, with newly hatched first stage larvae passing up the rat's trachea to be swallowed and expelled in the feces, these subadult worms move around within the human brain, eventually dying there. The resulting neurological damage within the brain, combined with the immunological response to the worms, especially the dead worms, causes eosinophilic meningitis.[8,9]

People can become infected by intentionally eating raw or under-cooked snails, which in some countries are considered a delicacy, notably Thailand[10] and China.[11] Other cases have involved acquiring the infection by ingesting a snail on a dare or for a bet.[12,13] More widely, however, infection results from accidental ingestion of raw snails, or paratenic hosts such as flatworms, on some types of produce.[14-17] New thrusts to eat more fruit and vegetables, grow your own, and buy produce from local growers, for instance at farmers markets, mean that it is important to increase public awareness of the possibility of being infected by this route and of how to reduce that possibility.

To prevent infection it is therefore important to ensure that produce is free of infected hosts. Careful washing of produce is recommended, especially of green leafy vegetables that grow in close proximity to places where snails live.[18,19] The aim of the present study was to test various commonly available household products to assess their efficacy, compared to tap water, as washing solutions for removing snails from produce, in the present case from romaine lettuce.

Methods

Snails

When snails are active, they produce mucus that allows them to adhere better to produce. Therefore, it was necessary that the experimental snails were active during the washing experiments. However, some of the snails were to be placed within the lettuce head such that it would not be possible to observe their activity. Preliminary experiments were therefore performed to determine the mean time from being placed on a lettuce head for the snails to become active. A fresh romaine lettuce head was used for each trial. Having been stored in a refrigerator to keep them fresh, lettuce heads were first allowed to reach room temperature (around 25 °C). The snails were then placed on top of the lettuce head and the time it took for all snails to become active was recorded. Snails were considered active

when their tentacles were out and they were crawling. Adults and juveniles of four gastropod species were used (Table 1), all of which are introduced agricultural, horticultural, or garden pests in Hawai'i and commonly encountered.[20,21] Each trial consisted of three snails from one of the eight categories (4 species, 2 age classes) and each trial was replicated three times (72 snails in total). The mean time to activity of the nine snails in each category was used in the washing experiments as the time from placing the snails in/on the lettuce head to the start of the washing treatment.

Washing Experiments

The three washing solutions used were sodium hypochlorite (0.09%), acetic acid (1%), and sodium chloride (3%), with tap water as the control. These concentrations were chosen because experimentally 0.09% sodium hypochlorite (equivalent to 1.5% household bleach) and 1% acetic acid both reduce infectivity of the closely related parasite *A. costaricensis* by 100%, and a 3% cooking salt solution inactivates other parasites.[22] Dilutions of all solutions were prepared with tap water.

Four 15 l tubs were used for the washing experiments, one per wash solution, with 7.5 l of each solution in each designated tub, maintained at room temperature (ca. 25 °C). Each of the eight snail categories was tested separately. As above, a fresh lettuce head was used for each trial. Three individuals of the same age class were placed on/in the lettuce head: one in the center of the lettuce head, one outside the center but among the inner leaves, and one on the outside of the lettuce head. Each trial was replicated three times. Thus, for each treatment 72 snails were used, for a total of 288 snails across the four treatments. For each trial, the lettuce head with the snails was submerged completely under the wash solution, allowing all areas of the produce to be exposed to the solution, and left to soak for 10 min. Since the lettuce heads tended to float, they were turned over after 5 min so that the entire head was thoroughly soaked. The lettuce head was then removed and rinsed under cold flowing tap water for 10 s (from top to base of lettuce). The lettuce was then turned upside down and shaken. The numbers of snails that fell off during the washing and rinsing

Table 1. Gastropod species used in the experiments, with age class and size.		
Species	**Juvenile**	**Adult**
Veronicella cubensis (slug)	10-20 mm	21-40 mm
Succinea tenella (snail)	4-5 mm	6-7 mm
Deroceras leave (slug)	10-15 mm	16-25 mm
Parmarion martensi (semi-slug)[a]	10-20 mm	21-35 mm

[a]Semi-slugs have a rudimentary shell into which they are not able to retract

and their locations were recorded. Each leaf was then removed individually to record snails that had not been removed. Lastly, each individual leaf was rinsed under cold running water and the snails still remaining recorded. Each snail and lettuce head was used for one trial only. Logistical regression (MiniTab 16) was used to determine how removal rates varied according to species, age class, location on produce, and wash solution.

Results

Complete removal of all snails was not achieved for any species using any of the wash solutions (Table 2). However, several solutions were successful in completely removing certain age classes of particular species after washing and rinsing (Table 2). Washing and rinsing with tap water was effective in completely removing juveniles of *Veronicella cubensis* and *Deroceras laeve* and both juveniles and adults of *Parmarion martensi*. Bleach was effective in completely removing adult *Succinea tenella* and juvenile *Veronicella cubensis*. Vinegar was only effective in completely removing juvenile *Veronicella cubensis*. Salt was effective in removing adult *Succinea tenella* and *Veronicella cubensis*. All (100%) snails were removed only after washing, rinsing the whole lettuce head under cold running tap water, and then washing each individual leaf under cold running tap water. Almost all the snails placed on the outside of the lettuce heads were removed by the washing procedure (Table 3); only seven of the 96 snails were not removed by the washing procedure: two in the tap water control, four in the sodium hypochlorite, and one in the sodium chloride treatments (Table 3). However,

Table 2. Percentage of snails removed by species and age class after washing only and after washing and rinsing.									
Snail species	**Age class**	**Percentage of snails removed**							
		Tap Water		**Bleach**		**Vinegar**		**Salt**	
		After wash	After wash + rinse	After wash	After wash + rinse	After wash	After wash + rinse	After wash	After wash + rinse
Succinea tenella	Juvenile	44	78	22	56	33	56	44	67
Succinea tenella	Adult	22	33	22	100	44	89	33	100
Veronicella cubensis	Juvenile	11	100	22	100	33	100	0	100
Veronicella cubensis	Adult	33	78	33	89	22	89	33	67
Deroceras laeve	Juvenile	44	100	33	89	33	78	33	78
Deroceras laeve	Adult	22	89	33	67	33	78	33	89
Parmarion martensi	Juvenile	89	100	44	67	33	67	78	89
Parmarion martensi	Adult	67	100	56	67	67	67	44	78

Table 3. Numbers of snails removed by washing alone and numbers removed by washing and rinsing.								
Location of Lettuce	Numbers of snails removed							
	Tap Water		Bleach		Vinegar		Salt	
	After wash	After wash + rinse	After wash	After wash + rinse	After wash	After wash + rinse	After wash	After wash + rinse
Center	2	17	0	15	2	10	1	13
Inner	8	22	2	20	11	23	6	22
Outter	22	24	20	24	24	24	23	24

these seven remaining snails were subsequently removed during the tap water rinsing process. In contrast, of the 96 snails in the center of the lettuce head, only five were removed by the washing procedure: two in the control, two in the acetic acid, and one in the sodium chloride treatments. Another 50 were removed during the subsequent tap water rinsing (Table 3) but this meant that 41 out of the 96 snails remained in the center of the lettuce head following the entire washing and rinsing procedure.

The results of the logistic regression indicated very few significant differences among species, adults and juveniles, or position on/in the lettuce head with regard to the likelihood of being removed. However, there were a small number of significant differences among categories regarding the probablity of being removed after only the washing: juvenile *Veronicella cubensis* ($P = .026$) and *Parmarion martensi* ($P = .043$), *Parmarion martensi* in the inner ($P = .001$) and outer ($P = .015$), but not center locations, and *Succinea tenella* ($P = .011$) on the outer location all had a higher probability of being removed than other snail/age class/position combination.

Discussion

The three experimental washing solutions were chosen as being readily available in most domestic residences, sodium hypochlorite (bleach), acetic acid (vinegar), and sodium chloride (cooking salt). Simply washing and rinsing with water was just as effective overall as washing and rinsing with any of the experimental solutions. The procedure was not perfect, as some snails did remain in the lettuce head following washing and rinsing.

Although a few snail species and age class combinations were identified by the logistic regression analysis as being more readily removed than most others, overall, there were no general trends. However, snails in the center of the lettuce heads tended to be less readily removed, presumably because the solutions did not fully penetrate to the center and/or they were less readily dislodged by washing and rinsing, being wedged more tightly between the leaves.

Numerous snail species can act as hosts of *A. cantonensis*[23] and they probably differ considerably in the ease with which they can be removed from produce by washing and/or rinsing. The fact that some individuals of all species were not removed remains a cause for consideration. Only just over half the snails

placed in the center of the lettuce were removed by the washing and rinsing. Thus if each leaf were not washed and rinsed separately, snails would remain in the lettuce head. If the lettuce, or similar produce, were subsequently chopped up and prepared for consumption, these snails would go unnoticed. Even if the snails themselves were chopped up the worms would probably remain viable and infective for some time. This may be particularly important when blending raw produce for drinks.[24] During the experimental washing treatments, in particular the acetic acid and to a lesser extent the sodium chloride treatments, the snails produced a lot of slime. Ingestion of slime containing infective *A. cantonensis* on produce has been thought of as a possible way people could become infected, but is generally considered of minor significance.[25-27] Nonetheless, production of large amounts of slime, possibly containing infective larvae, during washing with these solutions further indicates that washing with water alone, although some slime may still remain, is the most appropriate treatment. This study did not address killing the worms.

Conflict of Interest

None of the authors identifies any conflict of interest.

Authors' Affiliation:
- Pacific Biosciences Research Center, University of Hawai'i, Honolulu, HI

Correspondence to:
Robert H. Cowie PhD, Pacific Biosciences Research Center, University of Hawai'i, 3050 Maile Way, Gilmore 408, Honolulu, HI, 96822; Ph: (808) 956-4909; Email: cowie@hawaii.edu

References
1. Morse SS. Factors in the emergence of infectious diseases. *Emerg Infect Dis*. 1995;1(1):7-15.
2. Wallace GD, Rosen L. Studies on eosinophilic meningitis: experimental infection of rats and other homeothermic vertebrates with *Angiostrongylus cantonensis*. *Am J Epidemiol*. 1969;89:331-344.
3. Weller PF, Liu LX. Eosinophilic meningitis. *Semin Neurol*. 1993;13:161-16
4. Longbottom H. Emerging infectious diseases. *Communicable Diseases Intelligence*. 1997;21(7): 89-93.

5. AltoW. Human infections with *Angiostrongylus cantonensis*. *Pacific Health Dialog*. 2001;8:176-182.
6. Hochberg NS, Park SY, Blackburn BG, et al. Distribution of eosinophilic meningitis cases attributable to *Angiostrongylus cantonensis*, Hawaii. *Emerg Infect Dis*. 2007;13(11):1675-1680.
7. Hochberg NS, Blackburn BG, Park SY, et al. Eosinophilic meningitis attributable to *Angiostrongylus cantonensis* infection in Hawaii: clinical characteristics and potential exposures. *Am J Trop Med Hyg*. 2011;85(4):685-690.
8. Graeff-Teixeira C, da Silva ACA, Yoshimura K. Update on eosinophilic meningoencephalitis and its clinical relevance. *Clin Microbiol Rev*. 2009;22(2):322-348.
9. Tsai H-C, Liu Y-C, Kunin CM, et al. Eosinophilic meningitis caused by *Angiostrongylus cantonensis* associated with eating raw snails: correlation of brain magnetic resonance imaging scans with clinical findings. *Am J Trop Med Hyg*. 2003;68(3):281-285.
10. Eamsobhana P, Yoolek A, Yong H-S. Effect of Thai 'koi-hoi' food flavoring on the viability and infectivity of the third-stage larvae of *Angiostrongylus cantonensis* (Nematoda: Angiostrongylidae). *Acta Trop*. 2010;113:245-247.
11. Lv S, Zhang Y, Steinmann P, et al. The emergence of angiostrongyliasis in the People's Republic of China: the interplay between invasive snails, climate change and transmission dynamics. *Freshw Biol*. 2011;56:717-734.
12. New D, Little MD, Cross JH. *Angiostrongylus cantonensis* infection from eating raw snails. *N Engl J Med*. 1995;332:1105-6.
13. Kwon E, Ferguson TM, Park SY, et al. A severe case of angiostrongylus eosinophilic meningitis with encephalitis and neurologic sequelae in Hawaii. *Hawaii J Med Public Health.* 2013;72(6 Suppl 2):41-45.
14. Ash LR. Observations on the role of mollusks and planarians in the transmission of *Angiostrongylus cantonensis* infection to man in New Caledonia. *Rev Biol Trop*. 1976;24:163-174.
15. Asato R, Taira K, Nakamura M, Kudaka J, Itokazu K, Kawanaka M. Changing epidemiology of angiostrongyliasis cantonensis in Okinawa Prefecture, Japan. *Jpn J Infect Dis*. 2004;57:184-186.
16. Waugh CA, Shaffir S, Wise M, Robinson RD, Eberhard ML, Lindo JF. Human *Angiostrongylus cantonensis*, Jamaica. *Emerg Infect Dis*. 2005;11(12):1976-1977.
17. Cowie RH. 2013. Pathways for transmission of angiostrongyliasis and the relative risk of disease associated with each of them. *Hawaii J Med Public Health.* 2013;72(6 Suppl 2):70-74.
18. Saulo A. Avoid contracting angiostrongyliasis (rat lungworm infection): wash fresh fruits and vegetables before eating! *Food Safety and Technology, College of Tropical Agriculture and Human Resources, University of Hawai'i at Manoa*. 2009;35:1-3.
19. Hollyer JR, Troegner VA, Cowie RH, et al. Best on-farm food safety practices: reducing risks associated with rat lungworm infection and human eosinophilic meningitis. *Food Safety and Technology, College of Agriculture and Human Resources, University of Hawai'i at Manoa*. 2010;39:1-8.
20. Hollingsworth RG, Kaneta RK, Sullivan JJ, et al. Distribution of *Parmarion* cf. *martensi* (Pulmonata: Helicarionidae), a new semi-slug pest on Hawai'i Island, and its potential as a vector for human angiostrongyliasis. *Pac Sci*. 2007;61:457-467.
21. Cowie RH, Hayes KA, Tran CT, Meyer, WM III. The horticultural industry as a vector of alien snails and slugs: widespread invasions in Hawaii. *Int J Pest Manage*, 2008;54:267-276.
22. Zanini GM, Graeff-Teixeira C. Inactivation of infective larvae of *Angiostrongylus costaricensis* with short time incubations in 1.5% bleach solution, vinegar or saturated cooking salt solution. *Acta Trop*. 2001;78:17-21.
23. Kim JR, Hayes KA, Yeung NW, Cowie RH. 2013. Definitive, intermediate, paratenic, and accidental hosts of *Angiostrongylus cantonensis* and its molluscan intermediate hosts in Hawai'i. *Hawaii J Med Public Health*. 2013;72(6 Suppl 2):10.
24. Ash LR. Observations on the role of mollusks and planarians in the transmission of *Angiostrongylus cantonensis* infection to man in New Caledonia. *Rev Biol Trop*. 1976;24:163-174.
25. Cowie RH. Pathways for transmission of angiostrongyliasis and the risk of disease associated with them. *Hawaii J Med Public Health*. 2013;72(6 Suppl 2):70-74.
26. Qvarnstrom Y, Sullivan JJ, Bishop HS, Hollingsworth R, da Silva AJ. PCR-based detection of *Angiostrongylus cantonensis* in tissue and mucus secretions from molluscan hosts. *Appl Environ Microbiol*. 2007;73:1415-1419.
27. Jarvi SI, Farias MEM, Howe K, Jacquier S, Hollingsworth R, Pitt W. Quantitative PCR estimates *Angiostrongylus cantonensis* (rat lungworm) infection levels in semi-slugs (*Parmarion martensi*). *Molec Biochem Parasitol*. 2012;185:174-176.