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◆研究報告

Prevalence of *Angiostrongylus cantonensis* in terrestrial mollusks of Port Island, Hyogo Prefecture, Japan

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Abstract

Angiostrongylus cantonensis, also known as the rat lungworm, is a parasitic helminth that infects both rodents and snails as its definitive and intermediate hosts, respectively. In 2012, Sakamoto and Uga found that 46% of examined rodents (19 of 41 infected) on Port Island were infected with *A. cantonensis* adult worms. Given the extent of infection, we investigated the prevalence of *A. cantonensis* in terrestrial mollusks of Port Island. Snails and slugs were collected in seven different sites of Port Island between August and September of 2015. These mollusks were then subjected to artificial digestion for nematode larval collection. Nematode larvae were found in 9% of examined snails (24 of 257 infected) and 23% of examined slugs (11 of 48 infected). *Allopeas clavulinum* and *Lehmannia valentinana* were positive for nematode larvae. In addition, the site harboring infected mollusks was near universities, residential buildings, and parks, which could lead to the transmission of parasites. Morphological examination revealed that the nematode larvae recovered were different from *A. cantonensis*. Further identification of the parasite is needed to assess the risks it poses on the environment, humans, and animals.

Keywords: *Angiostrongylus cantonensis*, Port Island, second-intermediate host, third-stage larva

Introduction

Angiostrongylus cantonensis is a zoonotic helminth whose life cycle involves infection of both rodents and mollusks. This parasite causes the disease angiostrongyliasis in humans, and it is one of the major causes for tropical eosinophilic meningoencephalitis (Prociv *et al*. 2000; Wang *et al*. 2008). Parasitic infection occurs by ingesting the third-stage larvae of *A. cantonensis* found in snails and slugs. Humans can be infected by eating raw or undercooked infected mollusks, materials contaminated by infected mollusks such as soil and vegetables (Kilks and Palumbo, 1992; Marquardt *et al*. 2000) and paratenic hosts such as crabs and frogs (Wang *et al*. 2008).

The parasite is believed to have spread from East Asia to other countries (Kliks and Palumbo, 1992). Its wide distribution is facilitated by the spread of its hosts, particularly rodents, on ships and boats. Thus, its distribution increases over time, causing infections and outbreaks even in regions where it was not previously reported. Cases of angiostrongyliasis have been reported in Japan, particularly in Okinawa Prefecture (Toma *et al*. 2000; Hattori *et al*. 2001; Asato *et al*. 2004). Rodents and slugs infected with *A. cantonensis* have been found and these infections have extended from harbor areas to mainland Japan (Yoneda *et al*. 2001; Ohta, 2008).

Port Island is one of the main international trade ports of Japan (Sakamoto and Uga, 2010) harboring both local and international vessels. A study in 1981 (Uga *et al*. 1981) revealed that no rodents positive for *A. cantonensis* adult worm were found in Port Island. However, in 2010, Sakamoto and Uga found that 19 out of 41 (46%) rodents captured in Port Island were positive for the parasite. This confirms that the parasite has invaded Port Island. With definitive hosts present in the area, it is important to know which intermediate hosts contribute to the transmission of *A. cantonensis*, as these species carry its infective stage. Thus, this study was conducted to determine the prevalence of *A. cantonensis* in terrestrial snails and slugs of Port Island.

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Materials and Methods

1. Study site

The study was conducted in Port Island, Hyogo Prefecture, Japan from August to September 2015. Port Island is an artificial island located at the south of Port of Kobe and is linked to mainland Japan by a bridge and a tunnel. It is one of the major ports for Japanese global trade with almost 37,000 foreign and local vessels entering in 2010 (Sakamoto and Uga, 2010). Port Island was divided into seven different sites for this study (Figure 1).

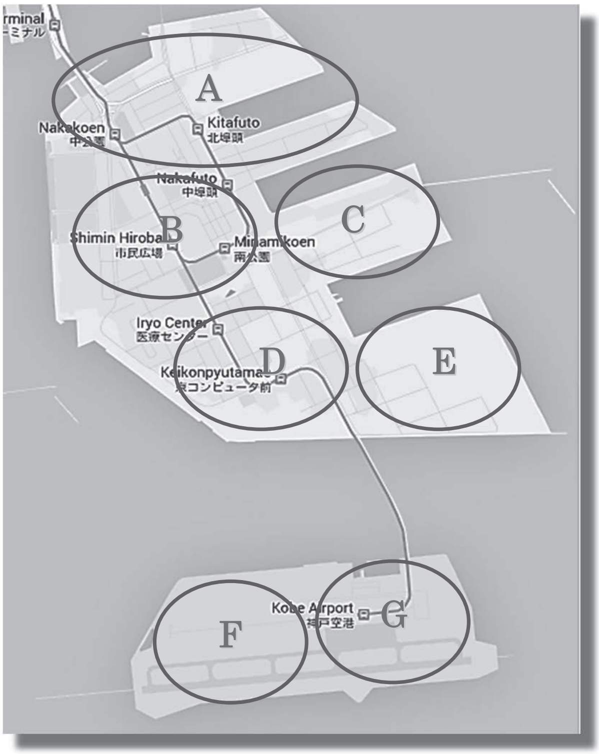

Figure 1. Map of Port Island (Google Maps, 2015).

2. Collection of terrestrial snails and slugs

Snails were collected by sweeping the topmost layer of soil, including fallen leaves and twigs, using a broom. Collected soil was put into a small plastic bag and brought back to the laboratory for examination of snails. The snails collected were identified according to their shape and size and then placed in petri dishes with wet filter paper for further examination.

Slugs were collected using the beer trap method. A paper cup was filled with beer and covered with gauze before leaving it for one to two hours. The slugs attached to the cup were collected and placed in petri dishes with wet filter paper before larval examination.

3. Detection of larvae in terrestrial snails and slugs

Artificial digestion was conducted to examine the snails and slugs for nematode larvae. Each snail or slug was chopped into small pieces and digested with 0.5% pepsin in 0.7% HCl solution (Ash, 1970) for 12 to 18 hours. After digestion, each specimen was observed under the stereoscopic microscope for nematode larvae. Live nematode larvae seen were collected and preserved in AFA (acetic-formalin-alcohol) for morphological examination.

Results

A total of 641 snails were collected from seven different sites of Port Island and 257 (40%) were revealed to be alive. These snails belong to three different species: *Allopeas clavulinum*, *Strobilops aenea*, and *Zonitoides arboreus*. The most abundant species of live snail collected was *S. aenea* (209 individuals; 81%), followed by *A. clavulinum* (40 individuals; 16%), and *Z. arboreus* (8 individuals; 3%).

Out of the seven sites surveyed on Port Island, sites A, B, and C had the highest percentage of collected live snails with over 40% each (Table 1). Moreover, only site B had snails infected with nematode larvae. A total of 24 nematode larvae were recovered from *A. clavulinum* collected from site B.

Table 1. Terrestrial snails collected and examined for nematode laevae in seven different sites

	No. of			No. of larvae
Site	soil sample	collected snail	live snail(%)	recovered
A	20	129	59 (46)	
B	20	280	116 (41)	24
C	20	150	67 (45)	
D	10	21	3 (14)	
E	10	51	8 (16)	
F	10	8	4 (50)	
G	10	2	0 (0)	
Total	100	641	257 (40)	24

Forty-six slugs were collected using the beer trap method and all were identified as *Lehmannia valentiana*. In addition, 17 *A. clavulinum* were also attracted to the trap and collected (Table 2). Both terrestrial snails and slugs exhibited parasitic infection. However, *A. clavulinum* has the highest prevalence of infection, with nine individuals (53%) found to be positive.

Table 2. Terrestrial mollusks collected and examined for nematode larvae using the beer trap method

Site	Species	No. of snails collected	No. of snails infected	No. of larvae recovered
At the back of Kobe Women's University	*Lehmannia valentiana*	31	2 (6)	3
	Allopeas clavulinum	17	9 (53)	21
Naka Park	*Lehmannia valentiana*	15	1 (7)	2

Nematode larvae recovered from terrestrial snails and slugs were approximately 670 µm long and 20 µm wide (Figure 2). This was different from the reported measurements of *A. cantonensis* third-stage larvae, which were 450 by 30 µm (Cowie, 2013). Moreover, compared to *A. cantonensis*, the larvae recovered do not exhibit rod-like structures in their heads and also have straight tails (Figure 3).

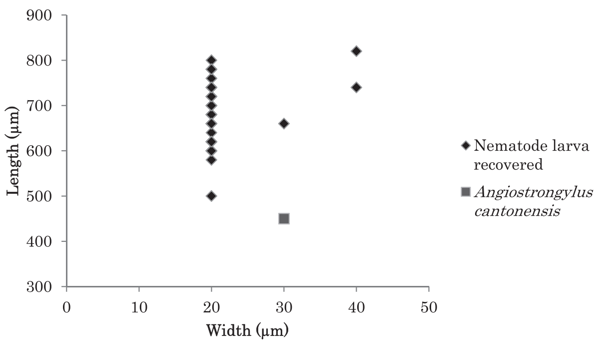

Figure 2. Measurement of nematode larvae recovered from terrestrial mollusks in Port Island and the third stage larva of *Angiostrongylus cantonensis*.

Figure 3. Nematode larva (A) recovered from a terrestrial snail showing a close-up view of the head (B) and tail (C). Bar indicates 160 µm .

Discussion

Mollusks have a significant role in the life cycle of *A. cantonensis*. These animals harbor the third-stage larvae of the parasite, which is infective to rodents and other animals. Thus, they play a critical role in the spread of the parasite and its disease. In this study, the prevalence of nematode larvae was 9% in snails (24 out of 257) and 23% in slugs (11 out of 48). However, the recovered larvae were different from the third stage larva of *A. cantonensis*. Even though rodents from Port Island were infected with *A. cantonensis*, the intermediate hosts in the area remain unknown. The reason for this is still not clear. However, other possibilities exist, as the prevalence of mollusks infected with *A. cantonensis* is relatively low (Asato *et al*. 2004; Ibrahim, 2007; Lv *et al*. 2009; Tesana *et al*. 2009; Vitta *et al*. 2010). Furthermore, mollusks are easily affected by changes in their environment (Parent, 2008), which is why they are used as models for monitoring environmental changes (Bayne *et al*. 1979; Dyke *et al*. 1996; Taylor *et al*. 1998; Moullac and Haffner, 2000; Rittschof and McClellan-Green, 2005). Thus, changes in the environment would result in changes in the population of these hosts and also in the epidemiology of *A. cantonensis*. This demonstrates how difficult it is to detect infected intermediate hosts in their natural habitat.

Artificial infection of possible intermediate hosts in Port Island revealed that *A. clavulinum*, *Z. arboreus*, and *L. valentiana* are candidate hosts for *A. cantonensis* (Sakamoto and Uga, 2010). However, in this study, both *A. clavulinum* and *L. valentiana* were infected with another nematode larva. The identity of the parasite is unknown as confirmation by artificial infection is required, but this clearly proves that there is transmission of parasites on Port Island. Site B, where infected mollusks were found, is near university campuses, residential buildings, and parks. These places have tended gardens and backyards where mollusks are likely to be found as pests (Hollingsworth *et al*. 2002; Flint and Wilen, 2009). This could have led to the high percentage of live mollusks collected at the site. Moreover, possible parasite hosts such as humans and other animals are abundant in Site B, which could have contributed to the transmission of parasite in mollusks. It is therefore important to identify the species of the nematode larvae recovered in order to specifically know its hosts and also assess the risks it poses in the environment as well as in humans and other animals.

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兵庫県の人工島に生息する軟体動物における広東住血線虫第３期幼虫の有病率調査

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2015 年 8-9 月の間にポートアイランドで陸産貝とナメクジの採取を行った．これらはその後に人工消化液を用いて消化し，感染している第 3 期幼虫の検索を実施した．幼線虫は検査した陸産貝の 9%（24/257）とナメクジの 23%（11/48）から検出された．陽性ナメクジは大学や公園の周辺から検出された．得られた幼線虫を形態学的に検査したところ，それらは広東住血線虫の第 3 期幼虫とは異なっていることが明らかとなった．現時点では検査した幼虫数も限られており，今後さらに調査を持続する予定である．

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