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Research Note

First report of *Angiostrongylus cantonensis* in the Giant African Land Snail *Achatina fulica* in French Polynesia detected using the SSU rRNA gene

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Abstract. The 5' end of the small subunit ribosomal RNA gene was used to determine whether 3rd larval stage *Angiostrongylus cantonensis* are present in populations of the giant African land snail *Achatina fulica* from French Polynesia. Two populations, one from Moaroa Valley, Tahiti (n=5) and the other from Haapiti Valley, Moorea (n=10), were examined. All snails from Tahiti were infected with nematodes, with parasite load ranging from 12 to 28. A total of 92 nematodes were found, of which 91 were positively identified as *A. cantonensis*. No nematodes were found in the snails from Moorea. We report for the first time the presence of *A. cantonensis* in *A. fulica* snails from French Polynesia, indicating a viable route of human infection of *A. cantonensis* in the region through the handling of *A. fulica* or consumption of the snail or contaminated food crops associated with the snail.

Angiostrongylus cantonensis has a pantropical distribution (Marquardt *et al.*, 2000) and is a major concern from a human health perspective as it causes food-borne eosinophilic meningoencephalitis (EME) or cerebral angiostrongyliasis (Kliks & Palumbo, 1992, Prociv *et al.*, 2000). The life cycle of *A. cantonensis* involves a gastropod intermediate host and a murid rodent definitive host; the parasite is not highly specific to its intermediate host, and it has been known to infect a wide range of gastropods (Anderson, 2000). The consumption of contaminated salad crops or raw/undercooked snails (Kliks & Palumbo, 1992, Marquardt *et al.*, 2000) has led to sudden outbreaks or isolated cases of eosinophilic meningoencephalitis due to 3rd larval stage *A. cantonensis* infection (Kliks & Palumbo, 1992). Some of these outbreaks were attributed to the giant African land snail, *Achatina fulica* (Kliks & Palumbo, 1992), which Alicata (1966) suggested as primarily responsible for the spread of the parasite to Southeast Asia and the Pacific due to the ease with which the snail established itself into these areas via human-aided means. The ability of the parasite to readily infect *A. fulica*, as shown in Brazil (Maldonado Jr. *et al.*, 2010) and in China (Lv *et al.*, 2009), highlights the risk of human infection of *A. cantonensis* through this snail. Larval stages of *A. cantonensis* are difficult to identify with certainty morphologically (Wallace & Rosen, 1969), rendering surveys of *Angiostrongylus* incidence in intermediate hosts difficult. Molecular techniques provide a reliable alternative method for nematode identification that can be applied to all life stages. The small subunit (SSU) or 18S ribosomal (r) RNA gene has become the molecule of choice for

distinguishing nematode species (Floyd *et al.*, 2002, Bhadury *et al.*, 2006, Santos *et al.*, 2006), including *A. cantonensis* (Qvarnstrom *et al.*, 2007; Hollingsworth *et al.*, 2007; Fontanilla & Wade, 2008; Foronda *et al.*, 2010; Chen *et al.*, 2011). Here we use the method developed by Fontanilla & Wade (2008) to detect the presence of *A. cantonensis* from the giant African land snail, *A. fulica*, in two French Polynesian territories, namely Haapiti Valley in Moorea and Moaroa Valley in Tahiti.

Extraction of nematodes from snail tissues, the extraction of DNA from each individual nematode and the PCR and direct sequencing of an approximately 480 bp fragment of the 5' end of the SSU rRNA follow the protocols described by Fontanilla & Wade (2008) and are briefly summarized here. Snails were cut into small pieces and digested overnight in a Petri dish containing Ash's digestive fluid (Ash, 1970) containing 0.7% pepsin in 0.5% HCl. Individual nematodes were then collected and stored in TE buffer at -20°C until use. Each nematode was subjected to the NaOH lysis method of DNA extraction (Floyd *et al.*, 2002), followed by PCR amplification using the F07 and R09 primers (Blaxter *et al.*, 1998) under the following conditions: two minutes at 94°C and 38 cycles of 94°C at 30 seconds, 45°C at 30 seconds, and 65°C at 60 seconds. Purified PCR products were then sent to the Department of Biochemistry of Oxford University for sequencing. Individual nematode sequences were processed using the STADEN package (Staden *et al.*, 2000), and the presence of *A. cantonensis* was determined by BLAST search in GenBank based on the 5' end of the SSU rRNA gene.

A total of five and ten snails, respectively, were sampled from Tahiti and Moorea. The small number of snails sampled was due to the patchy distribution of the snail and the relative difficulty of finding them (T. Coote, pers. comm.). This could be a reflection of the state of the snail population in French Polynesia. Mead (1979) noted that the snail frequently undergoes three phases after establishment in a new area: (1) an exponential increase characterized by large individuals; (2) a stable phase of variable duration; and (3) decline exemplified by small individuals. It is highly likely that the snail populations in Tahiti and Moorea are already in the third phase, though this needs further confirmation.

Only the Tahitian samples yielded nematodes, with all five snails being infected. A total of 92 nematodes from the Tahitian snails were DNA-extracted and subjected to direct sequencing, of which 91 gave 100% identity with *A. cantonensis* (GenBank AY295804) based on BLAST results using the 5' end of the SSU rRNA gene. The sequence of the remaining nematode did not match any named taxon in GenBank and its exact species identification could therefore not be definitively known. Table 1 summarises the parasite load of each of the five snails as well as the identity of the nematodes present based on BLAST.

Historically, cases of rat, gastropod and human *Angiostrongylus* infections in Tahiti have been recorded *(*Alicata, 1966; Wallace & Rosen, 1969; Bronstein *et al.*, 1977). Although Wallace & Rosen (1969) surveyed Tahiti for the presence of *A. cantonensis* in gastropod intermediate hosts between 1965 and 1966 and found 3rd stage larvae in areas where the rats were known to be infected with the parasite, they did not confirm the identity of the larvae by feeding them to laboratory rats. Their survey also did not include *A. fulica* among the gastropods they examined as they did not encounter the snail, noting that it was only reported in Tahiti in 1967 (Raut & Barker, 2002). This study therefore

Table 1. Parasite load of the Tahitian *A. fulica* and identity of nematodes based on BLAST (GenBank Release No. 191)

Snail Number	Nematode found	Nematode frequency
1	*A. cantonensis*	11
	Unknown*	1
2	*A. cantonensis*	13
3	*A. cantonensis*	21
4	*A. cantonensis*	28
5	*A. cantonensis*	18
	TOTAL	**92**

* closest match: *A. cantonensis* GB AY295804 with 98% identity (GB Release No. 191)

provides the first ever reported case of *A. cantonensis* infection in *A. fulica* in Tahiti. The first recorded case of human infection in Tahiti by the nematode parasite occurred in 1957 and rat infection in 1961 (Alicata, 1966), several years before *A. fulica* was supposedly introduced. In the case of Tahiti, Alicata (1966) hypothesized that *A. cantonensis* was not brought there by *A. fulica*. Nevertheless, the results show that the Tahitian *A. fulica* is also used as an intermediate host of *A. cantonensis*. This study presents a further possible route of human infection of *A. cantonensis* in Tahiti through handling of *A. fulica* or consumption of the snail or contaminated food crops associated with the snail. A more comprehensive survey of *A. fulica* populations in French Polynesia for the nematode parasite in *A. fulica* is therefore warranted.

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