Molecular Detection of Taeniid Eggs in Beetles Collected in an Area Endemic for *Taenia solium*

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Abstract. The aim of this study was to demonstrate the presence of *Taenia solium* eggs in beetles collected from sources within the natural environment through molecular techniques. Fifty-four pools of beetles were collected in three villages in Piura, Peru. DNA was extracted using the FastDNA spin kit for soil. Molecular identification of *Taenia* species was then performed through partial amplification of the mitochondrial cytochrome C oxidase subunit I gene. Finally, positive samples were sequenced to determine the tapeworm species. Seven positive samples were obtained through polymerase chain reaction amplification. Sequencing confirmed that two samples were from *T. solium* and three samples were from *Taenia hydatigena*. The other two samples could not be specifically identified. Our findings demonstrate that dung beetles ingest *T. solium* and *T. hydatigena* eggs under natural conditions and suggest that beetles may play a role in the dynamics of transmission of these cestodes.

INTRODUCTION

Dynamics of transmission of *Taenia* eggs have been evaluated in scientific works, and these have demonstrated that eggs can use different routes of dissemination such as its use of vertebrates and invertebrates. Regarding vertebrates, some studies have showed that seagulls, chickens, crows, and sparrows can ingest *Taenia saginata* eggs and expel them intact.^{1,2} Likewise, another study suggested that pigs become infected by eating contaminated feces from other pigs that had previously become infected with *Taenia solium*.³

Different groups of arthropods can be found in human excrement.⁴ For this reason, several studies have been conducted to demonstrate the role of these insects as mechanical vectors for parasite eggs. Blowflies and earthworms were found to be able to support dissemination of *Taenia* eggs.^{5,6} Other experimental findings have suggested that beetles are a good vector for transporting *Taenia* eggs.⁷

Beetles of the families Carabidae (*Carabus granulatus* and *Pterostichus vulgaris*), Scarabaeidae (*Aphodius fimetarius* and *Aphodius luridus*), and Dermestidae (*Dermestes maculatus*) can ingest *T. saginata* eggs.^{8,9} Likewise, beetles of the genus *Heliocopris* sp. and *Onitis* sp. can keep 30.8 eggs of *T. saginata* on average in their guts on the first day post-infection.¹⁰ Also, *Ammophorus rubripes* beetles can have viable *T. solium* eggs until 27 days postinfection.¹¹ As is known, several species of beetles are obligate intermediate hosts of some *Spiruridae* nematodes such as *Ascarops strongylina* and *Physocephalus sexalatus*, which are common parasites of pigs.¹² An association between these parasites and porcine cysticercosis has been demonstrated, thus also supporting a possible role for beetles in the transmission of *Taenia* eggs.¹³

So far, the role of beetles in the transmission of *Taenia* eggs has only been demonstrated experimentally. To ascertain whether this can also occur under natural conditions, molecular techniques were used in the present study to evaluate the

presence of *T. solium* eggs in beetles that were collected from environmental sources within the natural environment in an area that is endemic for porcine cysticercosis.

MATERIALS AND METHODS

This study was carried out in three small villages chosen at random in the district of Paimas, in Piura, Peru (4°37'S; 79°56'W), named Culqui, Minas de Jambur, and Algodonal. Domestic animals, such as pigs, turkeys and dogs, were raised freely in the village. This region has a dry season (April to October) and a rainy season (November to March).

Beetles were collected directly from the feces of domestic animals, using tweezers, every 2 months for 1 year, from April 2014 to March 2015. All beetles of the same genus that were found within a radius of 5 m and were collected at the same time were put into the same pool. The beetles were killed with chloroform and were then kept in 96° ethanol.

All beetles from each pool were dissected and their digestive tract and hemocoele were removed and put in 2 mL vials. The samples were placed in an oven at 37°C for 30 minutes to evaporate the ethanol completely. We then added 200 µL of phosphate buffered saline (PBS) buffer and 10 µL of proteinase K (40 mg/mL), and the vials were incubated at 55°C for 2 hours. Finally, DNA was extracted using the FastDNA spin kit for soil (Qbiogene Inc.[®], Carlsbad, CA) in accordance with the manufacturer's protocol.

The partial cytochrome C oxidase subunit I (COI) gene (~400 base pairs, bp) was amplified by means of PCR using the primers JB3 (5'TTTTGGGCATCCTGAGGTTTAT3') and JB4.5 (5'TAAAGAAAGAACATAATGAAAATG3').¹⁴ The PCR template consisted of 0.2 µM of each primer, 6 µL of DNA, ultrapure water, and master mix (GoTaq[®] Green Master Mix, Promega, Madison, WI) in a total volume of 50 µL. PCR amplification was carried out in a thermocycler (GeneAmp 9700, Applied Biosystems, Carlsbad, CA) using an initial denaturation step at 94°C for 5 minutes, followed by 36 amplification cycles, each consisting of a denaturation step at 94°C for 30 seconds, annealing at 50°C for 30 seconds, elongation at 72°C for 30 seconds, and extension at 72°C for 10 minutes.

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TABLE 1

Number of beetle pools collected in the locality of Paimas, department of Piura, Peru

Characteristic	N (%)	Median (IQR)
Beetle		
<i>Aphodius</i> spp.	48 (88.9)	4 (4)
<i>Ammophorus rubripes</i>	6 (11.1)	4 (3)
Village		
Culqui	33 (61.1)	4 (3)
Algodonal	11 (20.4)	4 (2)
Minas de Jambur	10 (18.5)	5 (5)
Season		
Dry	24 (44.4)	6 (6)
Rainy	30 (55.5)	4 (3)

IQR = interquartile range.

The 400-bp PCR product was purified and sequenced (Macrogen, Seoul, Korea). Then the sequences were aligned using the Bioedit program (Biosoft, Ferguson, MO) and the ChromasPro program (Technelysium, South Brisbane, Australia). The molecular identification of *Taenia* tapeworm specimens was based on the similarity of their nucleotide sequences to those of tapeworms that had previously been published in GenBank using the Blast software (National Library of Medicine, Bethesda, MD).

RESULTS

The Global Health Center reported the presence of 14 cases of porcine cysticercosis in Paimas, Peru, during the collection period for our study. A total of 309 beetles were collected and allocated into 54 pools according to geographical location, time, and beetle genus. The median number of beetles per pool was 4 (interquartile range: 3). The largest number of beetle pools (30; 55.5% of the total) was collected during the rainy season. The largest numbers of samples were collected from Culqui (33; 61.1%), followed by Algodonal (11; 20.4%) and Minas de Jambur (10; 18.5%). All the beetles were identified as belonging to two genera, *Ammophorus* and *Aphodius*, as detailed in Table 1.

Seven samples amplified the partial fragment of the COI gene. Of these, two were compatible with *T. solium*, and were found to be 99.5% identical through sequencing (Figure 1). Both positive samples were found in *Aphodius* spp., one in

each season. Another three sequences corresponded to *Taenia hydatigena*, and were more than 99.7% identical, whereas the remaining two samples were not compatible with any sequence in GenBank.

DISCUSSION

The role of beetles as mechanical vectors for some species of *Taenia* has been studied since several years ago, probably because these beetles feel a natural attraction to the feces and are part of the cycle of other parasites.^{12,15} Some species of beetles can ingest the eggs of the genus *Taenia* and keep them viable in their guts^{8–11}; however, the present study is the first to show the presence of *T. solium* and *T. hydatigena* in beetles under natural conditions, molecularly.

PCR products were successfully amplified in seven of the 54 pooled beetle samples. The DNA was identified in five of these as *T. solium* in two and as *T. hydatigena* in three. This provided evidence that beetles ingest eggs of these species and supported the theory that beetles might be involved in the mechanics of transmission of taeniid species. Our findings could also indicate the existence of endemic stability for both tapeworms in pigs, given that experimental evidence demonstrates that *Taenia* eggs may survive in a good condition in the gut of these beetles¹¹ and that pigs can become infected with cysticercosis due to *T. solium* when they ingest infected beetles.¹⁶ As is known, pigs are coprophagic and can become infected through direct ingestion of eggs or proglottids in contaminated stools. However, the presence of an invertebrate host could help to explain the wide dispersion of pigs that are seropositive for cysticercosis in endemic villages, and the fact that the vast majority of pigs infected with cysticercosis harbor fewer than five cysts in the entire animal.

Many areas in north Peru are endemic for the taeniasis-cysticercosis complex and for echinococcosis. Studies have reported prevalences of porcine cysticercosis of 45% due to *T. solium*¹⁷ and 28% due to *T. hydatigena*.¹⁸ Our results also demonstrated sympatric presence of *T. solium* and *T. hydatigena*; this can commonly occur in places where dogs and pigs are living together.¹⁹ This is a known problem in relation to making the immunodiagnosis of porcine cysticercosis because these species have antigenic proteins in common,

FIGURE 1. Molecular sequencing of detected *Taenia solium* DNA. Positive samples (9 and 13) are compared with *T. solium* sequences from Tanzania (AB066493.1) and Ecuador (AB066491.1) and showed 99.5% identity. The points (.) denote nucleotides identical to the first sequence. This figure appears in color at www.ajtmh.org.

which affects detection, both of circulating parasite antigens and of some of the most frequent diagnostic antibodies.²⁰

Seven samples were positive for the COI gene. However, it was not possible to obtain the sequences of two samples. The sequencing obtained in those samples was not clean because it showed multiple peaks overlapping one another. This suggests to us that beetles in the same group could have ingested more than one helminth at the same time.

Moreover, this study had other limitations. We were unable to determine how many beetles were positive because detection was performed for groups of beetles, all at the same time. Second, we did not determine the numbers of tapeworm eggs in the digestive systems of the beetles. Finally, we do not know whether the eggs were viable or not because the samples had to be fixed in alcohol.

Our study showed the presence of *T. solium* eggs in beetles from the natural environment, thus providing proof to support the theory that beetles are mechanical vectors for some cestodes. Although only two samples were positive for *T. solium* in this series, techniques of greater sensitivity and further assessment of the presence of *T. solium* DNA in different beetle species and at different seasons, as well as its correlation with exposure to or infection with porcine cysticercosis, could help assess whether *T. solium* egg ingestion by dung beetles is a casual event or whether beetles are in some way involved in the dissemination of this parasite into the porcine population.

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