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Ants as first intermediate hosts of *Mesocestoides* on San Miguel Island, USA

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Abstract

This study tested the hypotheses that ants (Formicidae) function as a first intermediate host of *Mesocestoides* (Cestoda: Mesocestoididae) and that deer mice (*Peromyscus maniculatus*) develop metacestode infections after ingesting cysticercoid or procercoid-infected ants. Field studies were conducted at an island fox (*Urocyon littoralis littoralis*) breeding facility located on San Miguel Island, California Channel Islands National Park, USA, where >40% of captive foxes were infected with adult *Mesocestoides*. Eight percent (8%) of deer mice at the fox pen site were infected with *Mesocestoides* metacestodes while none were infected at a distant site where foxes were absent (campground), thereby indicating the potential localized presence of a first intermediate host. To test whether ants from San Miguel Island contained *Mesocestoides* DNA, a polymerase chain reaction (PCR)-based diagnostic assay was developed using nested primers that could detect a single hexacanth larva within pooled samples of ten ants. Ants (*Lasius niger* and *Tapinoma sessile*) collected near the fox breeding facility were tested using the nested-PCR assay. Seven of 223 pooled samples of *L. niger* (3.1%) and 2 of 84 pooled samples of *T. sessile* (2.4%) tested positive for *Mesocestoides* DNA, while none of the ants were positive at the campground site. Positive samples were sequenced and found to match DNA sequences from *Mesocestoides* obtained from island fox and deer mice. Finally, to determine whether ants function as a first intermediate host for *Mesocestoides*, colony-raised deer mice ($n = 47$) were fed *L. niger* ($n = 3860$) or *T. sessile* ($n = 339$) collected from the San Miguel Island fox breeding facility. No mouse became infected with *Mesocestoides* metacestodes after ingesting ants. While both *L. niger* and *T. sessile* from SMI were positive for *Mesocestoides* DNA, they were not infective to deer mice in the laboratory.

Introduction

Despite over 60 years of research on the life cycles of *Mesocestoides* tapeworms, it remains unclear how intermediate hosts such as mice, lizards, and domestic dogs acquire metacestode infections. While most cyclophyllidean tapeworms (e.g. *Taenia*, *Hymenolepis* and *Monezia*), require two hosts, a vertebrate or arthropod intermediate host and a vertebrate definitive host, *Mesocestoides* may require three hosts (Rausch, 1994). Experimental results have shown that *Mesocestoides* proglottids are not directly infectious to vertebrates (Webster, 1949; Loos-Frank, 1991; Padgett & Boyce, 2004), thereby indicating the presence of an additional intermediate host in the life cycles of these tapeworms.

To identify the first intermediate host of *Mesocestoides*, over 40 arthropod species have been fed gravid proglottids of *Mesocestoides* and checked for development of immature tapeworms, i.e. cysticercoids or procercoids (Webster, 1949). In particular, oribatid mites have been

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suggested as possible intermediate hosts of *Mesocestoides* due in large part to the experimental results of Soldatowa (1944), who reported cysticercoid-infected oribatid mites at a fox fur farm in Russia; nonetheless, she was unable to complete the life cycle using these mites. Despite numerous inoculation trials with oribatid mites in Europe and North America, no other researcher has successfully infected any species of oribatid mite with *Mesocestoides* cysticercoids or procercoids (Webster, 1949; Loos-Frank, 1991).

Empirical evidence, including prey analyses, suggests that ants may function as the first intermediate host of *Mesocestoides*. Horned lizards (*Phyrnosoma* spp.) have the highest prevalence of *Mesocestoides* metacestode infection among reptiles surveyed in North America. For example, in southern California, horned lizards (*P. m'calli* and *P. platyrhinos*) were found to have prevalences of 26% and 27%, respectively (Mankau & Widmer, 1977). These two lizard species are almost exclusively myrmechophagous (i.e. ant eaters) (Pianka & Parker, 1975). Ants are also a first intermediate host for several *Raillietina* tapeworm species, primarily those that are parasites of chickens and rabbits (Jones & Horsfall, 1935; Bartel, 1965).

A recent study identified San Miguel Island, California Channel Islands National Park, USA as a unique site to conduct *Mesocestoides* life cycle analyses (Padgett & Boyce, 2004). At least 40% of captive foxes on San Miguel Island were naturally infected with adult *Mesocestoides* (Padgett & Boyce, 2004). Foxes were the only known definitive host on this small island and because all foxes were in captivity, we hypothesized that transmission of *Mesocestoides* to the first intermediate host likely occurs at the captive fox facility.

The primary aim of this study was to test if ants function as an arthropod first intermediate host of *Mesocestoides*. The specific objectives were: (i) to compare the prevalence of *Mesocestoides* infections in mice between sites where foxes were present and absent; (ii) to develop a sensitive and specific polymerase chain reaction (PCR)-based diagnostic assay for detection of *Mesocestoides* DNA within ants; (iii) to test ants from the *Mesocestoides*-endemic site for the presence of *Mesocestoides* DNA; and (iv) to feed mice potentially infected ants and examine for metacestode development.

Materials and methods

Study site

Field studies were conducted on San Miguel Island, California Channel Islands National Park (34°4′N, 120°3′W), USA. This small island (37 km^2) is located 100 km off the coast of southern California and is the most remote of the northern Channel Islands. The flora of San Miguel Island is dominated by native bush lupin (*Lupinus albifrons*), coyote brush (*Baccharis douglasii*) and giant coreopsis (*Coreopsis gigantea*), with native and introduced grasses; trees are absent. The only terrestrial mammals on the island are two endemic species: the San Miguel deer mouse (*Peromyscus maniculatus streatori*) and the San Miguel island fox (*Urocyon littoralis littoralis*). Between 1995 and 1999, the fox population declined from 450 to 17 animals, prompting park service personnel to relocate all remaining animals into ten pens for protection and captive breeding purposes in 1999 (Tim Coonan, National Park Service, personal communication).

One of the reasons for selecting the San Miguel Island site for this research is that adult *Mesocestoides* tapeworms were detected in foxes from all ten pens (Padgett & Boyce, 2004). Although foxes were infected with other helminths (*Spirocerca, Angiocaulus* and *Uncinaria*), *Mesocestoides* was the only tapeworm species found in this fox population (Faulkner *et al.*, 2001; L. Munson, personal communication). In addition, foxes on San Miguel Island were infected with a single genetic strain of *Mesocestoides*, Clade A, the most common strain involved in canine peritoneal larval cestodiasis in dogs on mainland California (Crosbie *et al.*, 2000).

Another factor in favour of testing ants on San Miguel Island is that the fauna is depauparate, a common characteristic of small oceanic islands. San Miguel Island supports only five ant species (*Lasius niger, Tapinoma sessile, Monomorium ergatogyna, Solenopsis molesta* and *Leptothorax andrei*) (K. Padgett & P. Ward, unpublished results), compared to 34 ant species on nearby Santa Cruz Island (Wetterer *et al.*, 2000). *Lasius niger* and *T. sessile* were the most common ant species found throughout the island.

The daily diet of captive foxes consisted of dried dog food and cooked poultry or other meat as well as assorted nuts, fruits, and vegetables. Faeces were removed from each fox pen every 2 days and deposited at one of four faecal dump sites that were located adjacent to the fox pens. Because the pens were not impermeable to the surrounding environment, it is possible that foxes may have occasionally ingested wild birds and mice within their cages.

To augment their diet, every 2–3 days, captive foxes were fed live deer mice trapped at a site where foxes were absent (campground site). The campground was located approximately 3 km north of the fox pen site and served as a control for the presence of *Mesocestoides*-infected foxes. With the exception of the lack of foxes, the campground site did not differ markedly from the fox pen site in terms of fauna and flora.

In spring 2002, half of the foxes were moved to a second pen site on the island to protect this fox subspecies against potential extinction from catastrophic events (e.g. disease outbreak or fire). This site was located approximately 5 km from the original fox pens. No studies were conducted at this site.

Rodent trapping

Deer mice (*P. maniculatus streatori*) ($n = 99$) were trapped in Sherman traps (H. B. Sherman Traps, Tallahassee, Florida) at the San Miguel Island fox pen site (Padgett & Boyce, 2004) and at the campground site ($n = 98$). Mice were anaesthetized with isoflurane (IsoFlo, Abbott Laboratories, North Chicago, Illinois) and euthanized by cervical dislocation. The body cavities (peritoneal and pleural), lungs, liver, heart and spleen were checked for metacestodes. Tapeworms were preserved in 95% ethanol and tested by a polymerase chain reaction–restriction fragment length polymorphism assay (PCR–RFLP) that can identify *Mesocestoides* tapeworms (Crosbie

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et al., 1998). The stomach contents of all euthanized deer mice were checked for the presence of ants and other easily identifiable arthropods.

PCR diagnostic assay

A nested-PCR diagnostic assay was developed to amplify *Mesocestoides* DNA within pooled samples of ten ants. Three primers were designed that are fully embedded in the highly variable ITS-2 region of the nuclear rDNA of Clade A *Mesocestoides*. The primers MS1 (5′-GCGTTAGACAGCGATGGCTT-3′) and MS4 (5′-AACACTAAGCCACGGCTTGT-3′) were used to amplify a 270 bp portion of DNA. A 1 μl aliquot of product from the first PCR reaction served as the DNA template for a second PCR reaction with an internal primer MS3 (5′-GCGTTAGACAGCGATGGCTT-3′) and MS4, one of the first-round primers. Total PCR volume for reactions with each pair of primers was 50 μl, comprised of 36.3 μl sterile water, 1 μl of extract or PCR product, 2.5 μl of each primer (10 μM), 1 μl of 10 mM dNTP mix, 5 μl of 10 × PCR buffer, 1.5 μl of MgCl (1.5 mM), and 0.2 μl (5 units μl^{-1}) *Taq* polymerase (Invitrogen, Carlsbad, California). For both sets of primers, PCR conditions were as follows: denaturation at 95°C for 3 min; two cycles of 94°C for 30 s, 65°C for 30 s, and 72°C for 30 s; followed by 28 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s; and a final extension at 72°C for 4 min before storage at 4°C. The presence and size of amplified DNA products were visualized on 1.8% agarose gels containing 0.1 μg ml^{-1} ethidium bromide.

To determine the lowest possible amount of *Mesocestoides* DNA that could be amplified by the nested-PCR assay, PCR was performed using serial dilutions of *Mesocestoides* DNA with and without ant tissue. To prepare *Mesocestoides* DNA, alcohol-preserved *Mesocestoides* proglottids ($n = 17$) from San Miguel Island foxes were dissected on glass microscope slides and 10,000 hexacanth larvae (HL) were removed and pipetted into a clean Eppendorf tube (mean HL per proglottid = 577.4; S.D. = 140.4; range = 304–771). Alcohol and water were removed from the HL with a speed-vacuum. DNA from HL was extracted with Qiagen DNeasy Tissue Kit (Qiagen, Hilden, Germany) using the manufacturer's protocol. The 10,000 HL extract (26 ng μl^{-1} DNA) was diluted serially 10 times adding 10 μl of each sample to 90 μl of AE buffer (elution buffer, Qiagen). The resulting 100 μl samples contained DNA representative of 10,000, 1000, 100, 10, 1, 0.1, 0.01, 0.001, 0.0001 and 0.00001 *Mesocestoides* hexacanth larvae.

Ants (*L. niger*) collected from the campground site (fox-absent site) were used in development of the diagnostic assay because they had a lower probability of *Mesocestoides* infection than ants from the fox pen site. Ants were placed in disposable plastic Petri dishes, washed three times with 70% ethanol, and air dried. Pooled samples of ten ants from the campground site were ground in a small amount of liquid nitrogen with disposable plastic pestles. Genomic DNA was extracted from ground ants using the Qiagen DNeasy Tissue Kit.

The following samples were tested: (i) sterile water; (ii) ant extract diluted 1:1 with sterile water; (iii) serially-diluted *Mesocestoides* HL extract diluted 1:1 with water; and (iv) serially-diluted *Mesocestoides* HL extract diluted 1:1 with ant extract. All samples were tested using the nested-PCR assay and 5 μl of the resulting PCR products were electrophoresed on an agarose gel to visualize the results. The sensitivity of this assay was measured by the lowest dilution level of *Mesocestoides* hexacanth larvae with ant extract that was identified by a visible band.

In order to assess the specificity of the nested-PCR assay for *Mesocestoides*, the following cestodes were tested: *Taenia pisiformis*, *T. serialis*, *T. saginata* and *Diphyllobothrium (Spirometra) mansonoides*. *Taenia pisiformis* (20 ng μl^{-1} DNA) and *T. serialis* (4 ng μl^{-1} DNA) were obtained from wild-caught coyotes in northern California; *T. saginata* (243 ng μl^{-1} DNA) was obtained from a person infected in Columbia; *D. mansonoides* (93 ng μl^{-1} DNA) was isolated from a dog in Florida, USA. Genomic DNA was extracted from the four tapeworm isolates as described above and tested by the nested-PCR assay. Water and ant extract served as negative controls. Diluted samples of *Mesocestoides* from foxes on San Miguel Island (Clade A) and nearby San Nicolas Island (Clade C) were used as positive controls; Clade C *Mesocestoides* are genetically distinct from Clade A and can be differentiated by PCR–RFLP (Crosbie *et al.*, 1998).

Testing ants for Mesocestoides *DNA*

Ants were collected by hand at the four faecal dump sites adjacent to the fox pens between the months of August and October in 2000 ($n = 500$ *L. niger*; $n = 500$ *T. sessile*), 2001 ($n = 1200$ *L. niger*; $n = 1000$ *T. sessile*), and 2002 ($n = 1500$ *L. niger*). *Lasius niger* were also collected at the campground site (2000, $n = 1000$; 2001, $n = 500$; 2002, $n = 1500$). Due to low numbers at the study site, no other ant species were used in this study. Ants were preserved in 70% ethanol at room temperature and tested within a year of collection.

To test for *Mesocestoides* DNA, ants were processed and DNA extracted as described in the previous section. Ten to 13 pooled samples of ants ($n = 100$–130 ants) were tested in a single nested-PCR assay. As a positive control for each assay, a sample of ten ants from the campground site was spiked with DNA equivalent to one hexacanth larva of Clade C *Mesocestoides* from San Nicolas island fox (0.0027 ng μl^{-1}). Water was used as a negative control in each assay. At least one pooled sample of ten ants from the campground site (not spiked with Clade C *Mesocestoides* DNA) was included in each assay.

Ants (*L. niger*, $n = 1060$; *T. sessile*, $n = 840$) collected from the fox pen site in 2001 were tested using the nested-PCR assay; each assay included one or two pooled unspiked samples of ants from the campground site (total $n = 210$ *L. niger*). Subsequently, ants (*L. niger*, $n = 1170$) collected at the fox pen site in 2002 were tested using in parallel with ants from the campground site (*L. niger*, $n = 850$). To mitigate against potential laboratory contamination with amplified DNA, ants were tested with the nested-PCR assay in three different laboratories.

Any ants that tested positive by the nested-PCR assay were sequenced. To prepare samples for sequencing, amplified DNA was purified with a QIAquick PCR purification kit (Qiagen) and the concentration of DNA

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was estimated by running 5 μl of sample on an agarose gel adjacent to a DNA Molecular Mass Ladder (EZ Load Precision Molecular Mass Standard, Bio-Rad Laboratories, Hercules, California). Purified DNA was sent to a local facility for sequencing. All isolates were sequenced in two directions. Complementary strands were compared using the program Sequencer 3.0 (GeneGodes Corporation, Ann Arbor, Michigan) and sequence ambiguities evaluated. Sequences were aligned with Clustal X (Thompson *et al.*, 1997) for comparison.

Rodent inoculation

Animal inoculations were conducted to determine if deer mice that ingested potentially infected ants acquired *Mesocestoides* metacestode infections. Because *T. sessile* were difficult to collect in large numbers and a larger percentage of *L. niger* were found positive for *Mesocestoides* DNA, inoculation studies were focused on *L. niger*. Ants collected in July 2002 from the fox pen site (*L. niger*, $n = 2500$; *T. sessile*, $n = 500$) and October 2002 (*L. niger*, $n = 2000$) were placed into 10 ml test tubes containing phosphate buffered saline (PBS), and transported to Davis, California. Ants in PBS were kept refrigerated for up to 1 week prior to inoculation. Because *Mesocestoides* metacestodes were found in San Miguel Island deer mice (Padgett & Boyce, 2004), deer mice (*P. maniculatus sonorensis*) obtained from the *Peromyscus* Genetic Stock Center (University of South Carolina, Columbia, South Carolina) were used in inoculation trials. Mice were housed individually and given mouse chow and water *ad libium*. To feed mice with ants, 10–25 ants were removed from PBS and mixed with 1.5 g of peanut butter on a glass microscope slide. Slides were placed into each mouse cage and removed after peanut butter and ants were ingested. Mice were fed ants over a period of 3–5 days.

In July 2002, 25 mice were fed 2055 *L. niger* collected from the San Miguel Island fox pen site ($\bar{\chi}$ ants per mouse = 82.2, S.D. 12.1, range 60–100) and five mice were fed 339 *T. sessile* from the fox pen site ($\bar{\chi}$ ants per mouse = 67.8, S.D. 10.1, range 54–65). As negative controls, six mice were fed peanut butter without ants. In October 2002, 17 mice were fed 1805 *L. niger* from the fox pen site ($\bar{\chi}$ ants per mouse = 106.2, S.D. 18.7, range 45–125); three non-inoculated mice served as negative controls. Sixty to 75 days after feeding ants, mice were anesthetized with isoflurane and euthanized by cervical dislocation. The pleural and peritoneal cavities were opened and flushed with PBS and all major organs (e.g. liver, spleen and heart) were carefully examined for the presence of metacestodes.

Results

Rodent trapping

As reported previously (Padgett & Boyce, 2004), 8% of deer mice trapped at the fox pen site were infected with *Mesocestoides* metacestodes (i.e. 'tetrathyridia'). In contrast, none of 98 deer mice trapped at the campground site during the same time period were infected with *Mesocestoides* (this study). Metacestodes were found embedded in the livers of four mice at the campground site and these were analysed using a PCR–RFLP assay; the resulting RFLP patterns differed from three molecular strains of *Mesocestoides* found in California (Clades A, B, and C) (Crosbie *et al.*, 1998, 2000). Based upon morphological characteristics, these metacestodes were identified as *Paruterina* spp. (USNPC No. 093314, identified by E. Hoberg, curator, US National Parasite Collection, Beltsville, Maryland).

There was no detectable difference in the percentage of deer mice that ingested ants at the fox pen site and campground site. Ants and ant parts were found in the stomachs of deer mice at the fox pen site (12.1%) and the campground site (11.2%). We were able to identify *L. niger* workers and queens in several of these samples. Although other arthropods such as flies and beetles were detected, none were identifiable to genus.

Diagnostic assay

The nested-PCR assay was able to distinguish between two distinct molecular strains of *Mesocestoides* found on different islands. The nested primers amplified a 210 bp region of DNA for the San Miguel Island strain of *Mesocestoides* (Clade A) and 225 bp for the San Nicolas Island strain (Clade C). Because a dilution representing 1HL of the San Nicolas strain was used as a positive control in all nested PCR trials, potential contamination with amplified DNA from the positive control could be detected. The primers were specific for *Mesocestoides*; no positive bands were observed with genomic DNA from ants or from other tapeworm species tested (i.e. *T. pisiformis*, *T. serialis*, *T. saginata* and *D. mansonoides*).

The nested PCR assay was highly sensitive in detecting *Mesocestoides* spp. DNA within ants. As little as 0.01 HL was detected within pooled samples of ten ants during serial dilution trials (0.000026 ng μl^{-1} HL DNA) (fig. 1). Thus, this assay is sensitive enough to detect a single ant infected with a cysticercoid or procercoid.

Ant collection and testing

Both *L. niger* and *T. sessile* collected at the San Miguel Island fox pen site in 2001 were found positive for

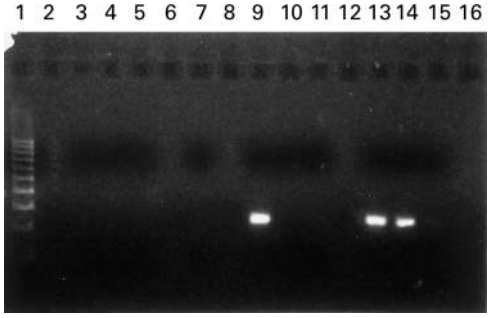

Fig. 1. Nested PCR amplification using primers developed for the ITS-2 region of *Mesocestoides* DNA. Lane 1 is a 100 to 1000 bp ladder; lanes 3, 4, 5, 7 and 10 are negative controls (water); lane 9 is a positive control (Clade C); lane 11 is a negative control (10 ants); lane 13 is 1 HL + 10 ants; lane 14 is 0.1 HL + 10 ants; lane 15 is 0.01 HL + 10 ants; lanes 2, 6, 8, 12 and 16 are blank.

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Table 1. Ants (*Lasius niger* and *Tapinoma sessile*) from San Miguel Island, Channel Islands National Park tested by PCR for *Mesocestoides* DNA.

Collection date	Site	Species	Number (pooled samples of 10 ants)	Number of pooled samples positive (%)
October 2001	Campground	*L. niger*	21	0
	Fox pen	*L. niger*	106	6 (5.7%)
	Fox pen	*T. sessile*	84	2 (2.4%)
October 2002	Campground	*L. niger*	85	0
	Fox pen	*L. niger*	117	1 (0.9%)

Mesocestoides DNA (table 1). Six pooled samples of *L. niger* tested positive by the nested-PCR assay (total tested, $n = 1060$ ants). Similarly, two pooled samples of *T. sessile* tested positive (total tested, $n = 840$ ants). These PCR results were confirmed by comparing the DNA sequences from the ants with *Mesocestoides* isolates taken from mice and foxes. None of the 21 pooled samples of ants collected in 2001 (total tested, $n = 210$) from the campground site tested positive for *Mesocestoides* DNA.

The prevalence of *Mesocestoides*-positive *L. niger* was lower in 2002. Only a single pooled sample of *L. niger* was positive for *Mesocestoides* DNA (total tested, $n = 1170$). This sample was confirmed by sequencing. All ants tested from the campground site (collected in 2002) were negative for *Mesocestoides* DNA (total tested, $n = 850$).

Rodent inoculation

None of the 42 colony-raised deer mice that ingested 3860 *L. niger* from the San Miguel Island fox pen site developed a detectable *Mesocestoides* metacestode infection. Similarly, the six mice fed 339 *T. sessile* remained uninfected.

Discussion

Molecular techniques have become increasingly valuable as a first step in identification of cryptic parasites within arthropod hosts in disease surveillance. Here, a PCR-based diagnostic assay is presented that can detect *Mesocestoides* DNA within pooled samples of ants. These results provide evidence that ants can harbour *Mesocestoides* DNA, and are potential candidates for an arthropod first intermediate host for *Mesocestoides*. Although the life cycle of this tapeworm was not completed using potentially-infected ants from a *Mesocestoides*-endemic site, these negative results cannot be regarded as definitive, and suggest that additional experimental studies of ants are justified.

Interest in identifying the first intermediate host of *Mesocestoides* is fuelled by the fact that domestic dogs can develop serious and potentially fatal *Mesocestoides* metacestode infections, characterized by masses of metacestodes in the peritoneal cavity (Crosbie *et al.*, 1998, 2000). Experimental evidence suggests that dogs do not acquire these infections after ingesting proglottids or metacestodes (Padgett & Boyce, 2004) but acquire metacestodes after ingesting an infected arthropod first intermediate host. Although dogs may ingest a wide range of arthropods, there is a high probability that they may occasionally ingest ants, which are common household insects that often infest pet food.

Focal transmission of *Mesocestoides* tapeworms was demonstrated at the island fox pen site on San Miguel Island during the study period. At least 40% of captive foxes were infected with adult tapeworms and there is evidence that the prevalence had increased since foxes have been placed in captivity (Padgett & Boyce, 2004). Foxes born in captivity have subsequently become infected with adult tapeworms (L. Munson, personal communication), providing strong evidence that foxes can acquire adult tapeworm infections within their cages. *Mesocestoides*-infected mice were found in the immediate environment surrounding the fox pens (Padgett & Boyce, 2004) but not at the campground site where foxes were absent. Collectively, these results indicate that the life cycle of *Mesocestoides* occurs at the fox pen site but not at the campground site. The small number of ants from the campground site that were analysed precludes any definitive conclusion regarding their exposure to *Mesocestoides.*

The prevalence of *Mesocestoides* infection in deer mice and foxes strongly supports the premise that the first intermediate host is present at the fox pen site. Foxes shed gravid proglottids into the immediate environment surrounding the fox pen site. These proglottids have the ability to move but, due to the hard cuticle surrounding the paruterine organ, the hexacanth larvae are not deposited in the environment in the manner of other tapeworms (e.g. *Taenia* spp.) (Sharpilo & Kornyushin, 1995; K.A. Padgett, personal observation). It is likely that the first intermediate host ingests the proglottid with an intact paruterine organ that contains approximately 500 infective hexacanth larvae. Previous studies have shown that these proglottids are not infective to mice (Webster, 1949; Loos-Frank, 1991; Padgett & Boyce, 2004). Deer mice and potentially other small vertebrates apparently serve as second intermediate hosts after ingesting infected first intermediate hosts. Within an unknown time period, deer mice develop metacestode infections and eventually are preyed upon by foxes, the definitive host. Within carnivores such as dogs, tetrathyridia develop into adult tapeworms within 3 to 4 weeks (Eckert *et al.*, 1969; Padgett & Boyce, 2004).

There is empirical evidence in support of the hypothesis that ants are first intermediate hosts of *Mesocestoides*. The strongest support thus far is the detection of DNA within ants that matches DNA

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sequences of *Mesocestoides* isolated from deer mice and foxes at the San Miguel Island fox pen site. This hypothesis is also supported by observations of foraging behaviour of ants and deer mice at the fox pen site. In our study, at least 10% of deer mice on San Miguel Island were found to ingest ants. As further empirical support, in every case when *L. niger* and *T. sessile* worker ants were offered fresh proglottids, the proglottids were grasped in their mandibles and taken down into their nests (unpublished data). It is unknown if worker ants ingest proglottids or whether the proglottids are offered to larval ants such as the case of *Raillietina loweni* tapeworms (Bartel, 1965).

Although *Mesocestoides* DNA was detected within ants at the fox pen site, these results do not unequivocally demonstrate that ants are a first intermediate host of *Mesocestoides* spp. Several potential factors may have compromised completion of the life cycle in this study. For example, this study focused on worker ants, the most abundant life stage. It is possible that a different ant stage functions as the first intermediate host (e.g. larvae, male or queen). Another explanation is that ants may not have been collected during the appropriate time of year. Here, ant collection was focused in late summer/early autumn to coincide with a peak in reported cases of *Mesocestoides* metacestode infections in dogs (unpublished data). It is possible that the first intermediate host is only infective during a specific season other than late summer/early autumn. Another important factor that may have affected these results was the death of one of the most heavily infected foxes in winter 2001 and the movement of foxes in spring 2002. Thus, the decrease in available proglottids may have reduced the prevalence of infection in the first intermediate host at the fox pen site prior to collection and deer mouse inoculation. Lastly, we acknowledge that ants may not be the first intermediate host of *Mesocestoides* tapeworms. *Lasius niger* and *T. sessile* may simply ingest proglottids, thereby having *Mesocestoides* DNA in their digestive systems without becoming infected with cysticercoids or procercoids.

To describe life cycles of enigmatic parasites like *Mesocestoides*, there is no alternative to conducting methodical studies that focus on a small number of potential host species. The identification of the elusive first intermediate host of *Mesocestoides* has been problematic due to the large number of potential arthropod hosts. In this study, a site was identified where the first intermediate host of *Mesocestoides* has a high probability of infection (San Miguel Island, fox pen site), ants were targeted as the most likely intermediate host, and a PCR-based assay was developed to test whether ants at this site contained *Mesocestoides* DNA. The PCR-based assay proved to be sensitive enough to detect a single hexacanth embryo within ants and could distinguish between two different genetic strains of *Mesocestoides*. While the detection of *Mesocestoides* DNA within ants from the San Miguel Island fox pen site provides evidence consistent with ants as a first intermediate host of *Mesocestoides*, we acknowledge that we have not rejected our null hypothesis. Although we were unsuccessful in completing the life cycle of *Mesocestoides*, additional studies involving ants are clearly warranted.

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