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BRIEF REPORTS

First report of *Toxocara cati* in the domestic land snail *Rumina decollata*

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Abstract The prospective role of the land snail *Rumina decollata* as a potential paratenic host of *Toxocara cati* for domestic cats was studied. *R. decollata* specimens and cats' feces were collected from the open spaces of a Buenos Aires city hospital. Cats' feces were analyzed and snails were digested to identify *T. cati* stages, by morphological and molecular analyses. *T. cati* larval eggs were recovered from 23.5% (4/17) of the sampled feces. Twenty percent of snail pools (5/25) were confirmed to be positive for *Toxocara* spp. third larval stage (L3) by PCR. The mean value of total larvae recovered per gram of snail in all positive pools was 5.1, with a maximum 33 L3/pool. This is the first report of *T. cati* infective larvae in *R. decollata* domestic snail as a paratenic host, since the relationship between infection in snails and in cats' feces could be demonstrated in a common environment.
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PALABRAS CLAVE
Toxocara cati;
Rumina decollata;
Hospedador paraténico

Primer reporte de *Toxocara cati* en el caracol doméstico *Rumina decollata*

Resumen Se estudió el rol prospectivo de *Rumina decollata* como potencial hospedador paraténico de *Toxocara cati* para los gatos domésticos. Se recolectaron caracoles *R. decollata* y heces de gatos de un hospital de la Ciudad Autónoma de Buenos Aires. Se procesaron las heces y los caracoles fueron digeridos para identificar estadios de *T. cati* por análisis morfológico y molecular. El 23,5% (4/17) de las muestras de heces resultaron positivas a huevos larvados de *T. cati*. El 20% (5/25) de los pooles de caracoles fue positivo a larvas de tercer estadío (L3) de *Toxocara* spp. por PCR. El promedio de larvas totales recuperadas por gramo

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de caracol en todos los pooles positivos fue de 5.1, con un máximo de 33 L3/pool. Se trata del primer reporte de *R. decollata* como hospedador paraténico de *T. cati*, puesto que ha sido demostrada la infección en caracoles y gatos en un ambiente común.
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Toxocara spp., the common roundworms of dogs and cats, produce eggs that are eliminated with their feces to the environment, which become a source of infection for paratenic hosts like mammals (including humans), birds and invertebrates[4]. *Toxocara* spp. larvae are able to migrate through the tissues of their hosts, and in man cause human Toxocariasis[12]. In some countries there are cultural dietary preferences for raw or undercooked meat of paratenic hosts and it became a risk factor for acquiring the human disease. Most human infections are asymptomatic but the infection may lead in clinical syndromes known as visceral larva migrans (VLM), ocular larva migrans (OLM) and common or covert toxocariasis (CT)[12].

Rumina decollata (Linnaeus, 1758) is a pulmonate land snail native to southern Europe, northern Africa and western Asia, and it borders the Mediterranean Sea. In America, it was accidentally and intentionally introduced as biological control of the garden snail *Helix aspersa*[2]. In Argentina, it has been recorded in urban areas of Buenos Aires, Mendoza and La Pampa provinces[2]. The snail is omnivorous; they also prey upon other land snails, eggs, worms and insects and feed from decaying fresh vegetable and organic matter like animal faeces[5].

This study was aimed at reporting the presence of *T. cati* in a stray cat population living in a public institution of Buenos Aires city and the evidence of infection in *R. decollata* snail, as potential paratenic host of the parasite in the same environment.

The study site was the open spaces of a public Hospital from the city of Buenos Aires, inhabited by a feline stray population. The general characteristics of the surrounding fences do not allow the entrance of dogs but permit cats' access. Seventeen samples of cats' feces were collected individually from the environment of the open spaces surrounding the buildings of this public Institution. They were stored at 4 °C before being processed. *R. decollata* snails were identified according to the descriptions of Dundee[5], and 75 adult specimens were collected from the same environment and were kept in plastic bags[2].

Fifteen grams of each cat sample feces was weighted and processed by Benbrook's technique, with modification according to Dolcetti[3], *T. cati* eggs were identified under optic microscope (10×), according to Sprent[14] descriptions. *R. decollata* snails were processed in 25 pools of three adult snails each one, since its low weight made it difficult for individual processing. They were cleaned individually by brushing the foot and shell in order to remove free living nematodes attached or their eggs. Snails were killed by immersion in tepid water for 24 h; shells were removed and bodies were weighed, minced with scissors and processed by artificial digestion technique[7]. Each snail macerated pool was placed in an Erlenmeyer with the digestive fluid (per 1 g of snail tissue it was used 0.15 g, pepsin, 0.15 ml concentrated HCl and 15 ml tap water), incubated in a magnetic shaker (1000 *g*), for one hour, at 37 °C. Afterwards, the fluid of the digestion was filtered in a 500 μ mesh and centrifuged at 1500 rpm for three minutes. The supernatant was discharged and total larvae were counted under the light microscope (10×). Larvae were identified according to morphological characteristics described by Sprent[14], like the anterior end with a mouth dorsally inclined and a spine-like cuticular thickening forms the ventral margin of a shallow buccal capsule[14].

Polymerase chain reaction (PCR) amplification of larval DNA was carried out to confirm *Toxocara* spp. infection. DNA isolation and extraction was performed using lysis buffer and proteinase K for two hours at 65 °C. The PCR mixture (total volume of 25 μl) consisted of 2.5 μl of 5 μl of a single larvae preparation, 2.5 μl of 10× PCR Buffer, 0.2 μl of 2.5 mM each deoxynucleotide triphosphate, 2 U Taq polymerase and 1 μl (6.25 μM) of two pair of primers designed in silico by González Prous et al.[8] (5′-ACGTATGCGTGAGCCG-3′ and 5′-GTGTTTTTGGTTTTTGGCG-3′). The bioinformatic analysis was performed using ITS-1 from ribosomal DNA from *T. canis* and *T. cati* obtained from the public database, GenBank. It has been designed in-silico primers in order to amplify a region that could, after sequencing, reveals species of *Toxocara*. Direct sequencing was performed on an automated Sequencer, using forward and reverse primers and the Big Dye Terminator kit from Applied Biosystems, according to the manufacturer's instructions. Sequences were analyzed by alignment with reference sequences from Basic Local Alignment Search Tool (BLAST) algorithm optimized for highly similar sequences (megablast) at the National Center for Biotechnology Information (NCBI, Bethesda, MD, USA) (www.ncbi.nlm.nih.gov/blast/).

DNA was amplified for 35 cycles, each cycle consisted of 1 min at 94 °C, 1 min at 55 °C, and 1 min at 72 °C. Previously to the first cycle and after the final cycle longer denaturation (3 min at 95 °C) and extension (10 min at 72 °C) steps were applied, respectively. Amplification products were visualized after electrophoresis on 2.5% agarose gel and visualized under an UV transiluminator after staining with ethidium bromide.

Prevalence of *T. cati* positive feces was calculated. Proportion of *R. decollata* positive pools and mean value of *T. cati* total larvae (L3) per gram and per pool of snail was reported. The estimation of the confidence intervals (CI) was created at the 95% confidence level. Statistical analysis was performed using InfoStat software.

T. cati larval eggs were recovered in 23.5% (4/17) of the cat sample feces collected. Most of *R. decollata* snails were found on buried feces (Fig. 1). Third *T. cati* larval stages (L3) were recovered from the 20% (5/25) of snails'

Figure 1 *R. decollata* snails feeding on buried feces from the open spaces of a public hospital, Autonomous City of Buenos Aires, Argentina.

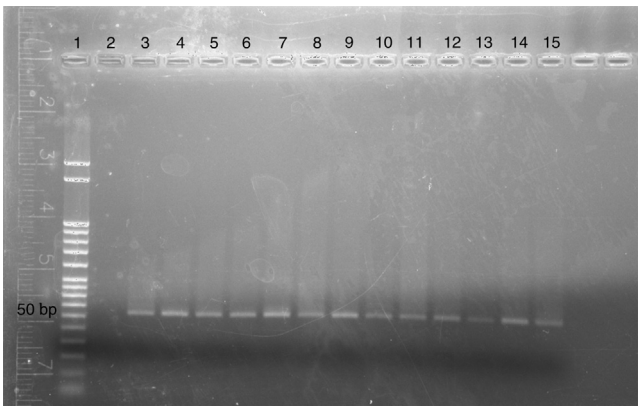

Figure 3 DNA amplification of *Toxocara* spp. isolated from *R. decollata* snails by PCR on 2.5% agarose gel, using the pair of primers 5′-ACGTATGCGTGAGCCG-3′ and 5′-GTGTTTTTGGTTTTTGGCG-3′: lane 1: marker 50 bp; lane 2: negative control; lane 3–14: amplification of DNA from different isolated larvae, each lane corresponds to a single larva; lane 15: DNA from *T. cati* larvae isolated from cats feces, positive control.

pools (Fig. 2). The mean value of *T. cati* total larvae per gram of snail recovered in total positive pools was 5.1 larvas (CI 95%: −2.5 to 12.7), Mean value of total L3/pool was 8.4 (CI 95%: −8.8 to 25.6), and the maximum was 33 L3/pool. There have been sequenced two amplicons of 297 bp in length (Fig. 3) from individual larvae that were deposited into the GenBank database under Accession Nos. KR337264 and KR337265. BLASTN searches against GenBank reference sequences for *T. cati* with a query cover of 100% displayed high degrees of similarities (100–98%). The DNA sequence GenBank Accession No KR337264 in study revealed the highest nucleotide identity to *T. cati* (100%, GenBank Accesión No. KJ777179; GenBank Accesión No. AB571303; GenBank Accesión No. AB110025; 99% , GenBank Accesión No. KJ777163; GenBank Accesión No. JF837172; GenBank Accesión No. AJ002436; 98%, GenBank Accesión No. AJ002437), whereas the nucleotide sequence GenBank Accession No KR337265 showed a slightly lower similarity to this *Toxocara* species (99%, GenBank Accesión No. KJ777179; GenBank Accesión No. AB571303; GenBank Accesión No. AB110025, GenBank Accesión No. KJ777163, GenBank Accesión No. JF837172, GenBank Accesión No. AJ002436; 98%, GenBank Accesión No. AJ002437).

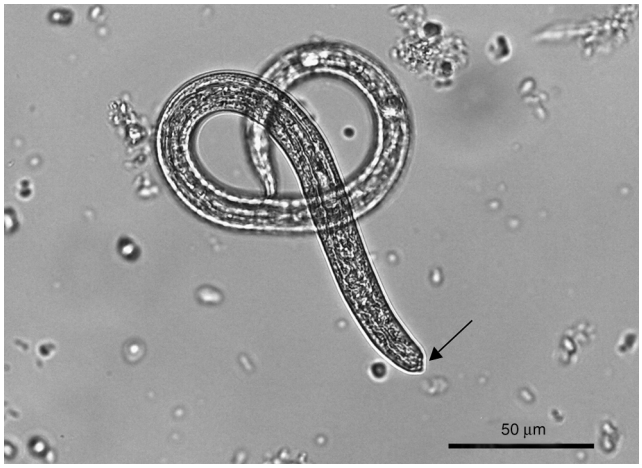

Figure 2 *T. cati* larva 3 obtained from digested snails collected from the open spaces of a public hospital, Autonomous city of Buenos Aires, Argentina. The arrow shows the anterior end with a mouth dorsally inclined (45×).

In the city of Buenos Aires, there are free cats populations living in semi-wild conditions in public institutions which offer them shelter and protection[13]. The presence of *T. cati* (23.5%) in cats feces, confirmed that the life cycle of the parasite takes place in the area. Most of snails were found feeding on buried feces so coprophagus habits of *R. decollata* were confirm. It seems that feces provide food for snails and protective substrate for *T. cati* eggs by perpetuating the snail and parasite cycle in contaminated environments. These conditions would represent a risk for rodents and birds as paratenic hosts and also for cats. Dubinsky et al.[4] reported the importance of earthworms and mice as natural reservoirs of *Toxocara* spp. larvae in nature, even more, as sentinels of environmental contamination, especially in urban areas, representing an important source of infection for hunter cats.

Sprent[14] recovered *T. cati* larvae by experimental infection of earthworms and cockroaches. Mizgajska et al.[10], obtained an 87% of earthworms, collected from urban gardens and courtyards, positive to *Toxocara* spp. eggs. Umeche et al.[15] found *Toxocara* spp. eggs in domestic flies captured in Nigeria.

Human cases of toxocariasis acquired by eating raw snails were reported in Spain[11] and in Italy[1]. In all cases, the snail species involved probably was *H. aspers*; since there is a popular belief in eating raw snails as a good treatment and prevention of gastric ulcer so it was traditionally medicinally used[1]. Romeu et al.[11] suggested that the likely mechanism of transmission might have been the ingestion of *Toxocara* spp. eggs mechanically attached to snail bodies, eaten raw without a thorough cleaning.

Molecular identification revealed the highest nucleotide identity to *T. cati* (100–98%), and was coincident with that previously reported[6,9]. Specific PCR assays were both sensitive and specific, and provide molecular tools for diagnosis and epidemiological surveys with species-specific primers designed, using the ITS-1 and ITS-2 sequences for the identification and differentiation of dog and cat *Toxocara* species[6,9]. The increase of cats' populations which live in semi-wild conditions in urban areas without health care needs special attention from sanitary authorities. They may consider the role of these cats in the circulation of emerging pathogens and as reservoirs of other diseases, which include some zoonotic infections like toxocariasis. Moreover, it is necessary to improve epidemiological surveillance of ecological species such as *R. decollata*, which could become intermediate hosts of animals' parasites and contribute to maintain them in the environment.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflict of interest

The authors declare that they have no conflicts of interest.

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