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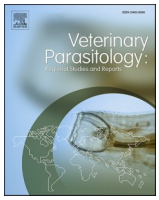

Original Article

Emerging *Lagochilascaris minor* infections in domestic cats from Costa Rica: A zoonotic threat for the region

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ABSTRACT

Two cases of lagochilascariasis minor in domestic cats from Costa Rica within a period of two months are recorded for the first time in Central America. Clinical findings included purulent discharge and a tumor in the left ear in one of the cats, whereas the other cat had an ulcerated cervical lesion. Both patients underwent surgical procedures during which nematode worms were collected and analyzed. The collected nematodes were identified using a combination of morphological and molecular assays, which revealed a 99.1% similarity in the cytochrome oxidase subunit 1 with *L. minor* from Mexico. The lack of information on this parasitosis, as well as the enormous harm it does to animal and human hosts, highlights the need for more research and awareness in Costa Rica and Central America. Furthermore, the unexpected occurrence of these instances in the same location emphasizes the imminent zoonotic risk to humans and the active circulation of the parasite.

1. Introduction

Lagochilascaris spp. are ascarid parasitic nematodes of vertebrates. There are six accepted species in the genus, whose adult stages are known to infect wild and domestic felid hosts as well as other carnivores like bush dogs (*Speothos venaticus*) and raccoons (*Procyon lotor*) (Bowman et al., 1983; Craig et al., 1980; Falcon-Ordaz et al., 2016; Trindade et al., 2019). *Lagochilascaris minor* was described for the first time producing subcutaneous abscesses in two human patients in Trinidad (Leiper, 1909). Since then, the parasite has been reported in domestic cats, domestic dogs, cougars (*Puma concolor*) (Falcon-Ordaz et al., 2016), raccoons (*Procyon lotor*) (Craig et al., 1980), and Geoffroy's cat (*Leopardus geoffroyi*) (Trindade et al., 2019). Notably, *L. minor* is the only species of the genus infecting humans (Campos et al., 2017).

This parasite is distributed from Mexico to Argentina, including the Caribbean. In addition, most human lagochilascariasis minor cases have been reported in Brazil and Mexico (Barrera-Perez et al., 2012; Barreto et al., 2018; Cardoso et al., 2020; Guimarães et al., 2010; Paco et al., 1999; Roig et al., 2010). Furthermore, reports in domestic cats are rare, and come predominantly from Brazil, with another few other cases in Uruguay (Sakamoto and Cabrera, 2002; Volcan and Medrano, 1990).

The life cycle of *L. minor* has not been fully elucidated and is mostly based on experimental observations (Fig. 1). Transmission of the nematode to the definitive host occurs via ingestion of an intermediate host, since cats are not likely infected when they directly consume the parasite's eggs (Campos et al., 1992; Paco et al., 1999). Humans and cats act as definitive hosts, while rodents have been suggested as intermediate hosts in experimental infections since cats are infected only when fed from L3-encysted rodent tissues and not by administration of *L. minor* embryonated eggs (Campos et al., 1992; Paco et al., 1999). However, no natural intermediate hosts of this parasite have been discovered to date, and it is unknown how the infection occurs in nature. Eggs are infective to rodents when they contain early third-stage larvae (L3) protected with one or two residual egg sheaths, as the result of intraovular moult remnants. Advanced L3 larvae are found during the first hours to days after infection in the liver and lungs of the definitive host. Several weeks post-infection, L4 and adults develop within nodules in different anatomical locations in cats, predominantly in the neck, oropharynx,

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cervical musculature, ears, or mastoid region (Campos et al., 1992; Cardoso et al., 2020; Paco et al., 1999). Autoinfection, which has been experimentally demonstrated in cats infected with *L. minor*, may lead to chronic infections and provide a stronger stimulus for inflammation since adult copulation and egg laying commonly occur inside nodules (Falcon-Ordaz et al., 2016; Lucio and Flores, 2021; Vargas-Ocampo and Alvarado-Aleman, 1997).

The distribution and epidemiology of *L. minor* in wild fauna, domestic animals, and humans from Costa Rica and Central America is unknown. Only two cases of *Lagochilascaris* have been reported in the Central American region: an unidentified *Lagochilascaris* sp. in the larynx of an ocelot (*Leopardus pardalis*) from Costa Rica and a human lagochilascariasis report in Costa Rica (Brenes-Madrigal et al., 1972; Brenes and Brenes, 1961). Herein, we describe two clinical cases with the zoonotic nematode *L. minor*, a rarely diagnosed parasite affecting two domestic cats from the same region of Costa Rica, both within a short period of time, and discuss the importance of this parasite in domestic cats and its zoonotic risk to humans.

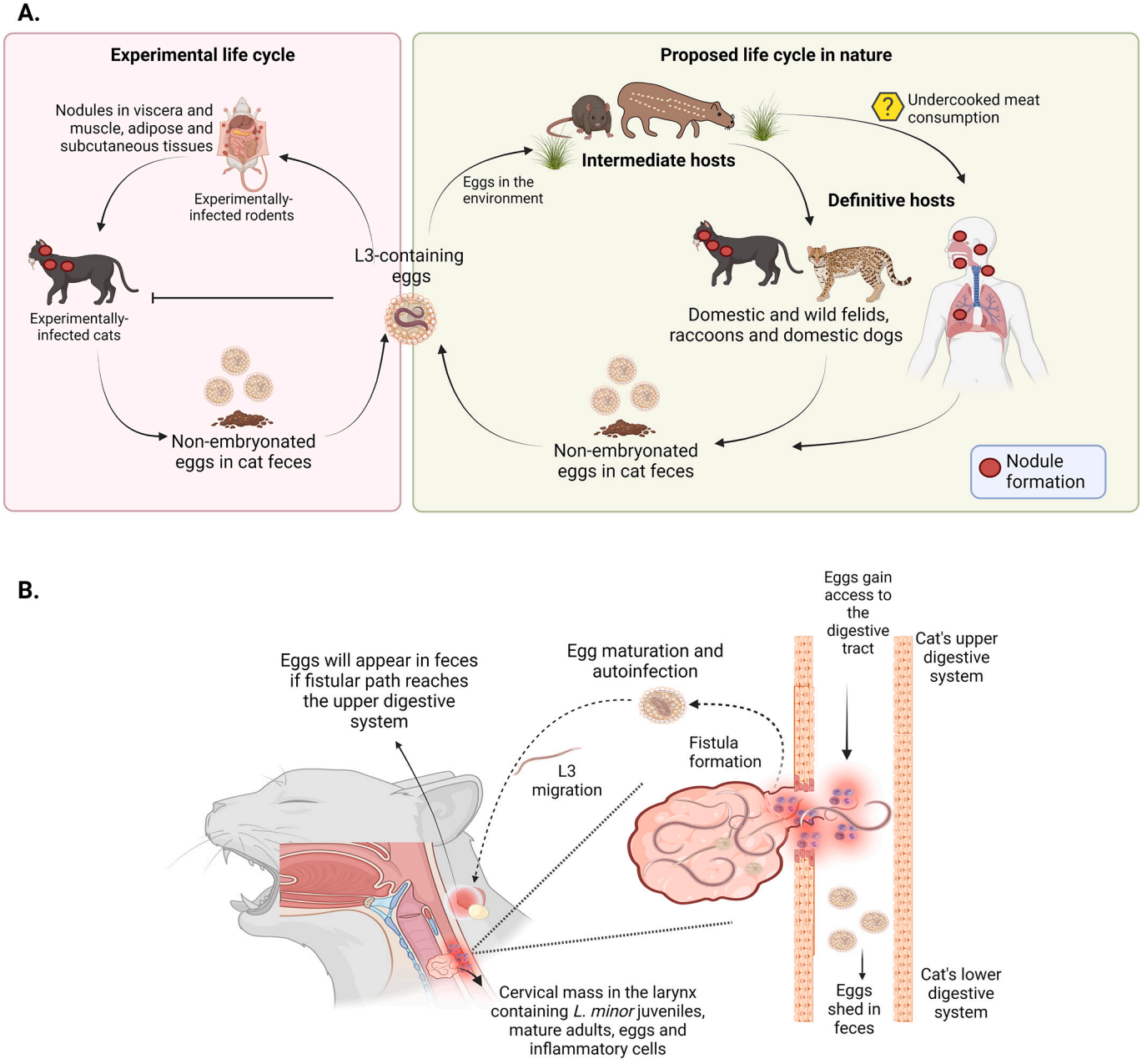

Fig. 1. Life cycle of *Lagochilascaris minor*. A. Eggs remain in a favorable environment for developing L3 in their interior. Identity of natural intermediate hosts and their infection pathways are not well understood, but experimental data support different mouse strains, agoutis (*Dasyprocta leporina* and *Dasyprocta agouti*) and vesper mice (*Calomys callosus*) as potential hosts (Campos et al., 1992; Paco et al., 1999; Volcán and Medrano, 1990). L3s are released from the eggshell when administered orally to rodents, and migrate mainly to muscular tissues, but also to viscera and adipose and subcutaneous tissues where they encyst in nodules (Campos et al., 1992; Paco et al., 1999; Semerene et al., 2004). Definitive hosts (felids, canids, raccoons, and humans) most likely become infected by ingesting raw meat of intermediate hosts. Experimental data has revealed that L3-containing eggs are not infective to definitive cat hosts (Paco et al., 1999). B. Inside the definitive host, L3 larvae excyst from nodules and migrate through blood and lymphatic circulation to several tissues, preferentially to cervical and mastoid regions (Barbosa et al., 2005). The presence and maturation of the worms promotes chronic inflammation, nodule formation and granulomatous reactions (Vargas-Ocampo et al., 1997). Worms inside nodules mate and shed eggs which stimulate further inflammation and fistulae formation. Eggs and worms gain access to the upper digestive tract through fistular pathways. Later during infection, eggs can appear in the feces of definitive hosts (Campos et al., 1992; Volcan et al., 1992).

2. Materials and methods

Two domestic and owned cats, both of which had access to the outdoor environment, were attended in a veterinary clinic in the province of Heredia, Costa Rica. The first case was presented in April 2020, and the other in September 2019. Both cats lived in the same region and belonged to different owners.

2.1. Clinical presentation

2.1.1. Patient #1

In April 2020, a common European female adult cat was taken to the veterinary clinic. The cat's head was tilted to the right without any other neurological signs noted. On examination of the ears, a deep nodular lesion in the right external auditory canal was observed. It was suggested to the owner that surgery be performed in order to remove the mass. The patient returned to the clinic in January 2021 presenting with a purulent discharge in the right ear that drained outwards. A mass completely obliterating the external auditory canal was observed and subsequently removed by performing right external auditory canal ablation. The excised tissue was fibrous and extended deep into the horizontal canal of the auditory meatus. The mass was dissected, and approximately nine living nematodes were extracted (Supplementary video 1). The oropharynx, oral cavity, and left ear of the cat were examined but no lesions or parasites were detected. The cat was treated with oral ivermectin 1 mg/kg (Endovet CES®, Riverfarma, Mexico) every 15 days and topical treatment of the external auditory canal once a day for 10 days with ciprofloxacin (0.80 g/100 ml of product), ketoconazole (2.00 g/100 ml of product) and prednisone (0.25 g/100 ml of product) (Otiflex C®, Labyes, Argentina). Immediately after extraction, parasites were placed in 70% ethanol and sent to the Laboratory of Helminthology in the Universidad de Costa Rica for taxonomical identification. In July 2021, the cat was reported healthy without any evidence of intra-aural lesions.

2.1.2. Patient #2

In September 2019, a common European female adult cat from the same location as Patient #1 was taken to the same veterinary clinic. A lesion in the cervical area, compatible with a cat bite, was found upon clinical examination. The lesion was ulcerated, purulent and located in the ventral zone of the neck. Wound sepsis was initially suspected and oral treatment with amoxicillin-clavulanate 12 mg/kg (Clavaseptin ®, Chemie S.A., Chile) for 10 days, chlorhexidine lavages as well as topical ciprofloxacin, ketoconazole and prednisone (Otiflex-C®) were administered. However, in March 2021, the cat returned to the clinic with a cervical purulent nodule. A foreign body in the lesion was suspected. Therefore, radiological analyses were performed without revealing any observed alterations. During surgery, excision of the cervical nodule resulted in the release of purulent material. In addition, several live worms were observed exiting the wound from the ventral side of the neck near the trachea and between the brachiocephalic and sternocephalic muscles (Supplementary video 2). Twelve live worms were collected in 70% ethanol and sent to the Laboratory of Helminthology in the Universidad de Costa Rica for taxonomical identification. The surgical area was washed with chlorhexidine. Both ears, the oropharynx, and the mouth cavity were thoroughly examined, and no other lesions or parasites were found. A single dose of oral ivermectin 1 mg/kg (Endovet CES) was administered every 15 days for 1 month. By the end of June 2021, the cat had improved clinically although the persistence of a mild exudative discharge from the cervical nodule was evident.

2.2. Microscopic analyses

The helminths were visualized and measured by means of light microscopy and the total body length of both sexes, length of the male spicule, and number of pits in the equator of the egg were registered. Worms were kept in 70% alcohol for molecular analyses and scanning electron microscopy (SEM).

SEM was performed at the Institutional Laboratory of Microscopy in the Instituto Tecnológico de Costa Rica. Worms were initially fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in phosphate buffer 0.1 M, pH 7.4 for 48 h. Then, the nematodes were washed with phosphate buffer (0.1 M, pH 7.2) and post-fixed with 1% osmium tetroxide (OsO_4). Thereafter, the worms were washed with distilled water and dehydrated using ethanol. Fixed worms were dried using a critical point dryer model EM CPD300 (Leica, Wetzlar, Germany) and mounted on aluminum holders with a carbon double-sided adhesive tape. Samples were sputter-coated with gold using an EMS 150R ES sputter coater (Electron Microscopy Sciences, Philadelphia, United States) and observed in a Scanning Electron Microscope model TM-3000 (Hitachi, Tokyo, Japan) at 7.5 kV accelerating voltage.

2.3. Molecular identification

DNA extraction was performed using the Qiagen DNeasy Blood and Tissue DNA kit following the manufacturer's instructions. Then, an endpoint PCR was performed to amplify a 650 bp fragment of the cytochrome oxidase subunit 1 (*cox*1) (positions 317 to 967 bp of the *cox*1 gene, hereinafter referred as fragment A) with primers NTF (5′-TGATTGGTGGTTTTGGTAA-3′) and NTR (5′-ATAAGTACGAGTATCAATATC-3′) (Casiraghi et al., 2001) and a 389 bp fragment of the *cox*1 (807 to 1201 bp of the *cox*1 gene, hereinafter referred to as *cox*1 fragment B) using primers JB3 (5′-TTTTTTGGGCATCCTGAGGTTTAT-3′) and JB4.5 (Bowles et al., 1995)(5′-TAAAGAAAGAACATAATGAAAATG-3′) as previously described (Rojas et al., 2018). Obtained amplicons were purified and Sanger sequenced. Then, sequences were cleaned and compared to reference databases using BLAST.

3. Results

3.1. Morphological analysis

Nematodes collected from both cats were identified as *Lagochilascaris* sp. based on the morphology of the buccal capsule and labia (Fig. 2A and B), presence of lateral alae, cuticular striations, male's spicule measurements and papillae number and distribution in the posterior region of the body (Fig. 3A). Only one male could be measured, with a total body length of 11.4 mm while females were in the range of 11.1 to 18.5 mm. The buccal region showed three labia separated from each other by a deep groove interrupted by interlabial projections.

The dorsal lip had two papillae while the two sub ventral lips had one small papilla and one amphid (Fig. 2B). Lateral alae and small cuticular transverse striations were observed along the body's length (Fig. 2B). The position of the excretory pore was mid anterior, just before the beginning of the transverse striations, and in females, the vulvar opening was located midventral in the middle of the body. The male ventral posterior end was characterized by a long row of 29 papillae on each side (right and left) of the body, and those immediately posterior to the cloaca were double (Fig. 3A), while the female posterior region lacked ventral papillae (Fig. 3B). A single spicule was observed (fallen, cut from its base during preparation for SEM), measuring 0.43 mm (Fig. 3A). In both sexes, the anus was subterminal. Eggs (35–62 × 34–59 μm) were rounded oval and had a typical pitted pattern on the equator with 26 pits evident both in SEM and light microscopy (Fig. 4A and B).

3.2. DNA analysis

Fragments A and B of the *cox*1 gene were successfully obtained from worms of patient #1, whereas only fragment B sequences were obtained from nematodes of patient #2. Fragment A sequence was 523 bp long and 99.13% identical to *L. minor* found in a human case of Mexico (accession number MH571139.1) with 90% of coverage (Gonzalez-Solis

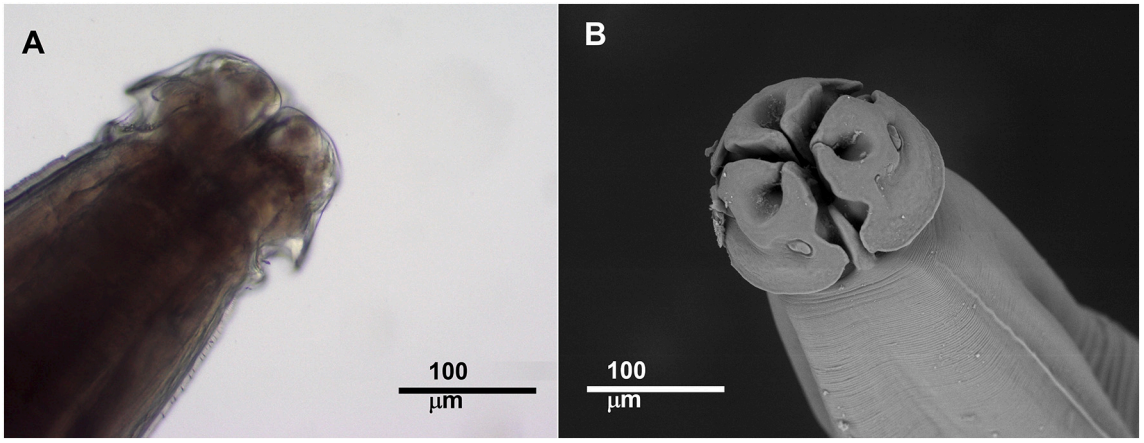

Fig. 2. Anterior region of *Lagochilascaris minor* adults collected from naturally-infected cats. A. Light microscopy observations of three well-developed, wider than long lips. Deep postlabial and interlabial groves are present (400×). B. Micrographs of the parasite in SEM. Lateral alae are seen on both sides of the body.

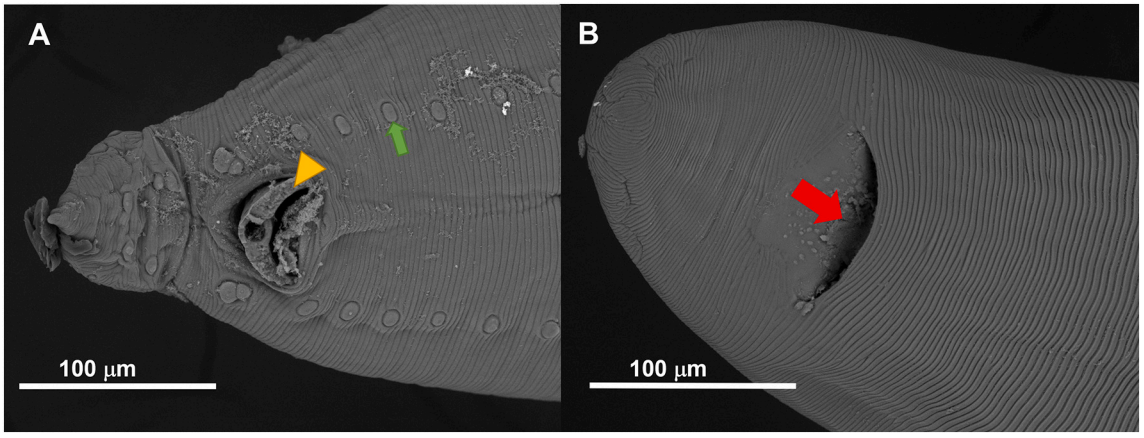

Fig. 3. Posterior ends of male and female *Lagochilascaris minor*. A. Scanning electron micrography at 600× magnifications showing the ventral posterior region of the male, 29 paired anal papillae (light green arrow) were counted in total (not shown). The base of the spicule is seen (yellow arrowhead) and fell during preparation. B. Ventral posterior region of *L. minor* female, lacking anal papillae with the subterminal anal opening (red arrow). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

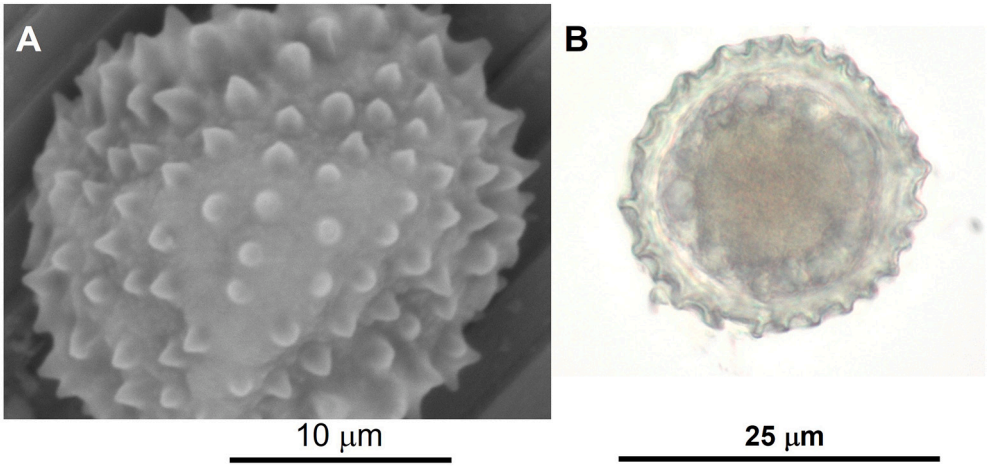

Fig. 4. *Lagochilascaris minor* eggs. A. SEM image of *L. minor* egg surface showing the spike-pitting pattern in detail. B. Light microscopy demonstrates the spike-pitting pattern found in *L. minor* eggs, with 26 pits around its equator.

et al., 2019). Moreover, Fragment B sequences rendered fragments of 364 bp and were 92.31% similar to *Toxascaris leonina* (MZ505444) collected from a wolf in Kazakhstan with 100% of coverage, suggesting the absence of *L. minor* fragment B sequences in GenBank. Fragment A sequence obtained from the worm of patient #1 was deposited in GenBank under accession number OL331249, and fragment B sequences

from the worm of patients #1 and #2 were deposited with accession numbers OL331248 and OL331250, respectively.

4. Discussion

These cases represent the first report of the zoonotic agent *L. minor* in domestic cats from Costa Rica, and, to our knowledge the only report of this species in Central America. Definitive diagnosis of *L. minor* in cats relies mainly on the identification of eggs in feces, which may be confused with eggs of other ascarid species like *Toxocara cati*. In addition, clinical manifestations in the animals, such as the presence of cervical nodules, ear masses, or skin fistulae are not frequently observed or are overlooked by the owners or clinicians in the early stages of the infection. Therefore, diagnosis based solely on clinical signs is not recommended. As this disease is considered rare, and eggs are not always evident in feces or misdiagnosed with *T. cati*, the correct diagnosis in the cat is usually delayed with the possibility of advancing to a severe illness (Barbosa and Campos, 2001; Cardoso et al., 2020; Fehlberg et al., 2014).

Clinical manifestations of both patients were compatible with *L. minor* infection. Adults that shed eggs inside nodules produce an intense infiltration of leukocytes, that may result in fistulous tracts and purulent discharge in the neck, ear, trachea and skull bones (Cardoso et al., 2020). Granulomatous abscesses in lymph nodes and skin lesions usually have a necrotic center surrounded by histiocytes. In addition, fistulous tracts and a hemorrhagic discharge can also be observed in the mucous wall of the esophagus and trachea during larval migration on their way to anterior regions of the digestive system (Campos et al., 1992; Cardoso et al., 2020; Paco et al., 1999). These fistulous paths facilitate the release of *L. minor* eggs in the cat feces (Paco et al., 1999). In the present report, both cats presented with fistulous tract associated with a purulent discharge from which eggs could have been shed. However, eggs were not found in feces.

Both animals from this study were free-range domestic cats. This lifestyle may have facilitated the ingestion of rodent intermediate hosts, which may be numerous in the region due to the presence of secondary growth forest (Campos et al., 1992; Paco et al., 1999; Volcan et al., 1992), although rodent infections were not tested in the present study. Rodents have been previously suggested as paratenic or intermediate hosts of *L. minor*. In experimental studies, cats fed with eggs containing advanced L3 larvae do not develop the infection, and only cats fed with rodent carcasses containing encysted L3 larvae develop the adults. This implies that rodents can be considered intermediate hosts of *L. minor*, as some unknown changes must occur for the infection to be successful in the cat (Fig. 1) (Campos et al., 1992; Paco et al., 1999).

Dozens of *L. minor* cases in humans have been reported in Brasil, Mexico, Colombia, Bolivia and Paraguay (Aquino et al., 2008; Assy et al., 2020; Barrera-Perez et al., 2012; Barreto et al., 2018; Campos et al., 2017; Costa et al., 1986; de Aguilar-Nascimento et al., 1993; Draper, 1963; Guimarães et al., 2010; Moncada et al., 1998; Moraes et al., 1985; Ollé-Goig et al., 1996; Roig et al., 2010; Vargas-Ocampo and Alvarado-Aleman, 1997). Anatomical localization of nodules, pathological findings and clinical presentation in most cases reported in humans are similar to those in cats. Neck and head involvement is frequent, and complicated chronic or even fatal infections have been reported (Aquino et al., 2008; Do Carmo Cupertino et al., 2020; Guimarães et al., 2010; Moncada et al., 1998; Rosemberg et al., 1986). The short time between both cases and the fact that both cats came from the same region of Costa Rica highlight the risk for human infection. These findings should encourage further investigation of lagochilascariasis epidemiological knowledge in definitive hosts, including humans in Costa Rica.

Reports of *L. minor* as natural parasites of domestic cats are scarce, and mainly come from Brazil and a few cases reported in Uruguay (Amato et al., 1990; Cardoso et al., 2020; Fehlberg et al., 2014; Lucio and Flores, 2021; Sakamoto and Cabrera, 2002; Sprent, 1971). Moreover, there have been several cases of lagochilascariasis minor in humans in countries outside South America, primarily in Mexico, but there have been no reports of this nematode in cats in these areas. This could suggest that the infection in cats is underestimated. More epidemiological research is needed to determine the causes of these differences and to better understand the epidemiology of this zoonotic disease.

The collected specimens were morphologically identified as *L. minor* by comparing them to previous descriptions of the species (Gonzalez-Solis et al., 2019; Lanfredi et al., 1998; Sprent, 1971). The presence of lateral alae and the morphology of buccal lips (three strongly indented lips, a deep postlabial groove separating the labia from the rest of the body, and lanceolate projections or interlabia) are typical for the genus. The size and appearance of the eggs, as well as measurements of male and female adults (Lanfredi et al., 1998), supported the specific diagnosis of *L. minor*. In comparison to *L. multipapillatum* (males 50–65 mm, females 50–78 mm), *L. sprenti* (males 13.1–20.8 mm, females 14–26.5 mm), and *L. turgida* (males 22.5 mm, females 22–26 mm), *L. minor* adults are smaller (males 5–17 mm, females 6–20 mm). Definitive morphological diagnosis of *Lagochilascaris* spp. may be a challenging task to perform due to the wide intraspecific morphological variation and incomplete morphometric separation between *L. major, L. turgida,* and *L. minor*. Hence, molecular analyses are crucial for an accurate identification (Amato et al., 1990; Barreto et al., 2018; Gonzalez-Solis et al., 2019; Guimarães et al., 2010; Paco et al., 1999; Scioscia et al., 2018).

Integration of careful morphometrical and molecular analyses is necessary for the identification of *L. minor*. Molecular information for the 18S rDNA gene and *cox*1 mitochondrial genes is available only for *L. minor* and not for other *Lagochilascaris* spp. In this report, the identity of the worms was confirmed by using sequences from two independent PCRs amplifying two different *cox*1 fragments. The utility of *cox*1 sequences to correctly identify this ascarid has been previously demonstrated with the identification of *L. minor* from Mexico (Gonzalez-Solis et al., 2019).

5. Concluding remarks

In the present study, we report the zoonotic nematode *L. minor* in domestic cats for the first time in Costa Rica and Central America. Interestingly, both cases originated from the same geographical region of the country in a time frame of two months. This nematode is likely underdiagnosed in cats in the region due to the lack of awareness, and the nonspecific clinical manifestations of *L. minor* infection. Therefore, the actual prevalence of this parasite in the country is currently unknown and further epidemiological research is needed. This report should draw the attention of human and veterinary health authorities of Costa Rica and Central America since *L. minor* greatly affects cat health and threatens human populations due to severe or fatal consequences.

Ethical statement

This study did not require any ethical statement. Data from patient were completely anonymized.

Declaration of Competing Interest

The authors declare that they have no financial or personal competing interests that may have affected the writing and conclusions of this study.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vprsr.2022.100797.

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