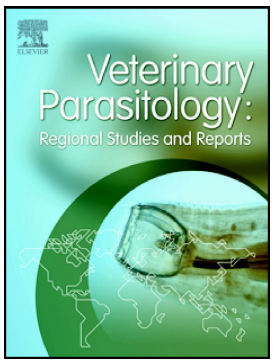

Dioctophyme renale in a domestic cat (Felis catus): Renal location and nephrectomy

M.J. Butti, M.I. Gamboa, J.D. Terminiello, G.R. Franchini, N. Giorello, L.L. Maldonado, L. Kamenetzky, M.F. Luna, M. Lopez Merlo, N.E. Radman

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Dioctophyme renale in a domestic cat (*Felis catus*): Renal location and nephrectomy

Butti MJ[a]; Gamboa MI[a,b]; Terminiello JD[c];GR. Franchini GR[d];Giorello N[d], Maldonado LL[g] ; Kamenetzky L[g] ; Luna MF[e]; Lopez Merlo M[f], and Radman NE[a]

[a] Cátedra de Parasitología Comparada, Laboratorio de Parasitosis Humanas y Zoonosis Parasitarias, Universidad Nacional de La Plata, Argentina.

[b] Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC), Argentina.

[c] Cátedra de Cirugía I, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, Argentina.

[d] Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP), Facultad de Ciencias Médicas, Universidad Nacional de La Plata, Argentina

[e] Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, Argentina.

[f] Métodos complementarios de Diagnóstico, Área Ultrasonografía, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, Argentina.

[g]IMPAM-CONICET, Facultad de Medicina, Universidad de Buenos Aires, Argentina.

Corresponding author: Marcos J. Butti

Contact email: mbutti@fcv.unlp.edu.ar

Telephone : +54 9 221-4236663/4 int 413

ABSTRACT

Dioctophymosis is caused by *Dioctophyme renale*, nematode with indirect life cycle. Its intermediate host is a freshwater oligochaete and its definitive host is a wild or household carnivore. The adult nematode develops in the definite host, generally locating itself in the kidney. This article was meant to describe the first nephrectomy performed in a domestic cat due to renal dioctophymosis in Argentina. The subject showed a non-specific appearance of generally feeling ill, hematuria and mild diarrhea. It was diagnosed through abdominal ultrasound, followed by exploratory celiotomy and nephrectomy. After verifying absence of free specimens, the right kidney was removed. This organ was found to be enlarged in a spheroidal manner in contrast to the left kidney, with significant thickening of the renal capsule, excessive congestion of vessels and adhesions involving the caudal vena cava. An adult nematode was removed from the right kidney and identified as *Dioctophyme renale*. Reports of feline dioctophymosis are scarce being most of them necropsy findings. In this we are presenting a confirmed case of *D. renale* removed by surgery from a live cat. The results presented here reinforces the fact that cats are also appropriate definitive hosts for this parasite.

KEYWORDS: dioctophymosis, renal parasitosis, felines, parasitic nematode, molecular diagnosis.

1. Introduction

Dioctophymosis is a cosmopolitan food borne zoonotic helminthiasis caused by the nematode *Dioctophyme renale* (Goeze, 1782) (Enoplida: Dioctophymatidae), commonly known as the "giant kidney worm". It develops in, and completely destroys

mammalian kidneys, and is thereby a debilitating and potentially lethal parasite of humans, domestic animals and endangered wildlife. Natural hosts are mainly mustelids (Dyer, 1998; Mech and Tracy, 2001) and other carnivores (Acosta et al., 2008; Ribeiro et al., 2009). Among domestic animals it is particularly pathogenic and common in dogs (Acha & Szyfres, 1986; Nakagawa et al., 2007; Mesquita et al., 2014; Pereira et al., 2016). On the other hand, reports of feline dioctophymosis are scarce being most of them necropsy findings (Paras et al., 2018; Pedrassanni et al., 2014; Verocai et al., 2009; Goldman and Pérez Tort, 2008.) Regarding human infections, *D. renale* is a zoonotic since unequivocal cases in human beings have been documented, including fatal cases and in relation with renal carcinoma (Li, 2010; Katafigiotis et al., 2013; Venkatrajaiah et al., 2014; Kuehn et al., 2016; Yang et al.; 2019).

Eggs are dispersed via urine in the environment, where an annelid (oligochaete) ingests the embrionated eggs allowing them to develop to L3 stage (Measures & Anderson., 1985). Circumstantially a wide range of vertebrates (e.g. fishes, toads and turtles) were reported as hosts of L3, suggesting that they may act as a paratenic hosts (Mascarhenas et al., 2019; Pedrassani et al., 2009). Inside the definite hosts, L3 larval stages are released, they pass through the duodenum and migrate to the liver, where they molt to L4. Then they proceed to the peritoneal cavity, molt to L5, reach the right kidney, where they become sexually mature, copulate, and start oviposition (Hallberg, 1953; Mace and Anderson, 1975). Although extrarenal localizations are rare in mustelids (Dyer, 1998) they are frequent in canines. This might be explained by the route followed by the L3s larvae depending on the side of the gastric wall they are going through: i) at the minor curvature of stomach, adults would locate themselves in the abdominal cavity, ii) at the major curvature, they would be found in the left kidney (Fiorentini and Negro, 2009). Worms can cause severe damage to the renal parenchyma destroying the cortex and the

medulla and leaving just the capsule. The course of dioctophymosis can be asymptomatic, if the healthy kidney is able to compensate the damage, or can present with renal colics, hematuria, progressive weight loss, vomiting, diarrhea, dehydration, etc. The number of specimens per host and ectopic localizations modify the clinical profile (Fyvie, 1971; Luna et al., 2003; Pereira et al., 2006; Burgos and Radman, 2008; Pedrassani et al., 2010). The pre-patent period in canines ranges from 135 to 180 days; however, the pre-patent period in felines is unknown (Karmanova, 1968; Mace and Anderson, 1975; PAHO, 2003).

This parasitosis is endemic in the Northeastern region of Argentina and in the coastline of the Rio de la Plata. Recent studies performed in the area show a 35.3% prevalence of *D. renale* in canines (Radman et al., 2017).

In this work we are describing the first case of diagnosed renal dioctophymosis in a domestic cat from Argentina. It was also the first registered nephrectomy performed so far allowing to monitor the cat after surgery. We were able to confirm this case by morphology and molecular studies.

2. Material and Methods

2.1 Patient and specimen

As part of an educational and sanitary workshops organized to diagnose parasitoses in canines and felines, the neighborhood "El Molino", in the City of Ensenada, Province of Buenos Aires, Argentina (34º 49' S, 57º 58' W) was visited monthly in 2017.

A 3-year-old, mixed-breed, intact male feline, was brought to consultation with a nonspecific profile of generally ill condition, hematuria and mild diarrhea. His owners informed the clinical profile had a two-month evolution. A blood sample was drawn by

cephalic venipuncture (with and without anticoagulant), and a urine sample was taken by urethral catheterization. The tests done included full blood count, blood biochemistry screen -urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, globulins, albumin/globulin ratio-, as well as serology of Feline Leukemia Virus (FeLV) antigen, and Feline Immunodeficiency Virus (FIV). Seriate stool samples were taken for parasitological analysis. A complete abdominal sonogram was performed with a Sonoscape A6 ultrasound machine (SonoScape Medical Corp., China) with a 4-8 MHz microconvex transducer. Additionally, urine was microscopically inspected for eggs.

After diagnosis, an exploratory celiotomy through xiphopubic approach and nephrectomy were performed (Williams and Niles, 2009; Fossum, 2004). Skin, subcutaneous tissue and muscle were incised at the midline (*linea alba*), and the abdominal cavity was approached for exploration. The left parieto-mesocolon region was explored first, the colon came next, followed by the anterior and posterior regions, to finish at the right parieto-mesocolon, in search of parasites and lesions in organs and peritoneum. The right kidney (located in the right parieto-mesocolon region) was approached next. After exploration of the organ, a blunt divulsion of the renal capsule was started to approach the organ's hilum. The capsule was separated from the kidney entirely, freeing up the renal artery and vein, as well as the right ureter. The renal vessels were ligated with non-absorbable material 2-0 (nylon), and a double ligation was performed over artery and vein. The ureter was ligated close to the bladder with non-absorbable suture 2-0 (nylon), with prior skeletonization and later resection.

The final step was the resection of the renal vessels with subsequent excision of the organ (nephrectomy). The patient had a favorable recovery after the surgical intervention.

2.2 Molecular techniques

For genetical identification total parasite genomic DNA was prepared from 70% ethanol preserved isolate using the DNeasy Blood & Tissue Kit (Qiagen GmbH, Hilden, Germany). Two molecular markers were used to confirm *Dioctophyme renale* species. PCR reaction and sequence primers were previously design by Tokiwa et al., 2014 for amplification of small subunit ribosomal RNA (SSU rRNA; Genbank accession nº AB842276) and mitochondrial cytochrome subunit c oxidase (COX1; GenBank accession nº AB854727) genes. The PCR products were sequenced at Macrogen Inc. (Korea).

3. Results

The ultrasound findings corresponded especially to the circular cross-sectional images with hyperechoic margin and hypoechoic center in the projection area of the right kidney. These findings were pathognomonic for *Dioctophyme renale* infection (Figure 1). The left kidney, as well as the rest of the abdominal viscera, showed no alterations at the moment of the study.

Full blood count, blood chemistry screen and renal parameters showed no alterations. Serology for detection of FeLV-P27 antigen and anti-FIV antibodies (GP40) through immunochromatography resulted negative.

A moderate number of *Dioctophyme renale* eggs and plenty of red and white blood cells were observed in the urinary sediment (Fig 2).

After verifying absence of free specimens in the exploratory celiotomy, the right kidney was approached. Compared to the left kidney, the right one was found enlarged and spheroidal (Fig. 3A), with significant thickening of the capsule, abundant congestive vessels and adherences involving the caudal vena cava.

During the nephrectomy, an 18 cm long and 0.4 cm diameter viable adult nematode, identified by its morphological features (Pedrassani & do Nascimento, 2015; Urano et al., 2001) as a female *D. renale* specimen, was removed (Fig 3B and Fig 4A). The anterior end showed a small lipless mouth, surrounded by six papillas (Fig 4 B). A transversely grooved cuticle was observed, as well as the obtuse caudal end with terminal anus. The specimen was stored in 70% ethanol and scised for molecular identification. Part of the worm was deposited in the invertebrate collection at the Natural History Museum of La Plata (FCNyM, UNLP) with catalogue nº MLP-He 7526.

The SSU and COX1 gene of the specimen showed 100 % and 90.4 % identity with AB842276 and AB854727 sequences respectively, previously described as *D. renale* (Tokiwa et al., 2014). The obtained sequences for SSU and COX1 have been deposited at Genbank with accessions codes: FcatLp1_SSU MN304734 and FcatLp1_COX1 MN304733, respectively.

4. Discussion

Dioctophyme renale affects a great number of carnivorous species, including both wild and domestic, occurring worldwide. It causes considerable damage to their hosts kidneys being sometimes lethal (Mascarenhas et al., 2019; Pedrassani et al., 2017). Due to changes in climatic conditions, environmental degradation, deforestation, and compromised sanitation many natural places considered biodiversity hotspots have undergone severe habitat fragmentation forcing wild species to move and face different threats and hence becoming more vulnerable to infections caused by parasites. During these migrations wild species interact with domestic animals spreading and increasing

the risk of infections of both and also to humans in the case of zoonosis (Myers et al., 2010). In this sense dioctophymosis represents several concerns: it is increasingly affecting household animals though this might be considered as a veterinary problem and it is also affecting endangered wild species though it may have ecological impact as well. Moreover, given that this is a confirmed zoonosis, it is a human sanitary problem with particular danger for people living in poverty and poor sanitary conditions. Unfortunately this is a frequent observed situation in many parts of the world including South America.

With this scenario, study and report of new cases on both, animals and humans, is of great importance since it contributes towards the better knowledge of this zoonosis and its cycle in different ecosystems.

The study presented here is of veterinary concern and confirms that domestic cats are possible definitive hosts for *D. renale*. Additionally, it also gives evidence for nephrectomy as a radical therapy for pets. Household and stray cats present independent life habits and explore different areas in the surroundings of their place of settlement presenting an important role in dissemination of eggs in the environment. Moreover, the intermediate host has not been identified yet for South America (Mascarenhas et al., 2019; Mascarenhas et al., 2018; Pedrassani et al., 2017; Radman et al., 2017) though this might represent a danger for humans´ health since it is extremely often to observe a close interaction with humans.

In South America *Dioctophyme renale* severely affects wild fauna like bush dog (*Speothos venaticus*), southern two-toed sloth (*Choloepus didactylus*) (Rocha et al., 1965), crab-eating fox (*Cerdocyon thous*) (Ribeiro et al., 2009) and maned wolf (*Chrysocyon brachyurus*) (Giovannoni et al., 1960; Kumar, 1972; Duarte, 2013) among others. In the case of domestic animals *D. renale* has been diagnosed mostly in dogs

that live close to rivers and the infection is diagnosed only by urine analysis, ultrasonography, surgery, or at necropsy (Pedrassani et al., 2015). However, diagnosed dioctophymosis cases in domestic cats are rarely found. It is important to note that the difficulty posed by performing diagnostic maneuvers in cats probably leads to a sub-diagnosis of this parasitosis in felines.

Though the domestic cat is not frequently reported as the definitive host of *D. renale*, cases are mentioned by several researchers (Janicki, 1934; Stefanski et al., 1936a, 1936b; Langenegger and Lanzieri, 1965; Mace, 1976a; Karmanova, 1968; Golman et al., 2007; Verocai, 2009; Pedrassani et al., 2014; Trindade et al., 2017; da Silva, 2017). It has long been hypothesized that cats are not good definitive host for *D. renale*. In 1953, Hallberg exposed four cats to larvae of *D. renale* coming from a naturally infected *Ictalurus nebulosus* fish, but none of them became infected. However, he was able to infect a ferret (*Mustela putorius*) using the same method. Hallberg suggested that the larvae were not yet infective, or the cats were not appropriate hosts. On the other hand the work by Verocai (2008) also suggests that due to the abnormal location of the worm, felines would not be the appropriate definitive hosts. In the present work the naturally occuring renal localization of the adult parasite and the presence of eggs in urine give strong evidence that felines could be appropriate hosts.

It is likely that household cats wandering freely might include fish and frogs in their diet, and thus they can be at risk of getting this parasitosis. However, the habit of not drinking stagnant water, though, reduces the chances of ingesting annelids infected with the parasite's larvae.

5. Conclusions

This is the first report of a diagnosed and genetically confirmed case of dioctophymosis in a domestic cat in Argentina. The fact of observing eggs in urine gives evidence not only for considering cats as appropriate definitive hosts but also as another source of egg dissemination that might be unconsidered. Given that it is extremely common to observe families living with cats as pets, it is mandatory to perform more epidemiologic studies in the area to determine the prevalence of this parasitosis in felines.

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The authors declare no conflicts of interest.

Authors contributions

Butti MJ: Methodology, Writing - original draft
Gamboa MI: Writing - review & editing, Funding acquisition.
Terminiello JD: Methodology
Franchini GR: Methodology, Writing – review & editing
Giorello AN: Methodology
Maldonado LL: Data curation; Formal analysis
Kamenetzky L: Methodology, Formal analysis
Luna MF: Methodology
Lopez Merlo M: Methodology
Radman NE: Conceptualization, Funding acquisition

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Figure Captions

Figure 1. Ultrasonographic image of the right kidney containing *Dioctophyme renale*

Figure 2. Eggs from *Dioctophyme renale* found in urine sediment. 40X

Figure 3. A) Right kidney removed after nephrectomy showing enlarged and spheroidal shape B) Dissection of right kidney capsule and collection of the *D. renale* specimen.

Figure 4. A) Adult *D. renale* female specimen, 18 cm long and 0.4 cm diameter B) Anterior end of the *D. renale* specimen showing six papillae around the lipless mouth.

Highlights

- This is the first report of a diagnosed and genetically confirmed case of dioctophymosis in a domestic cat in Argentina
- The renal localization of the adult parasite and eggs in urine makes the domestic cat an appropriate host

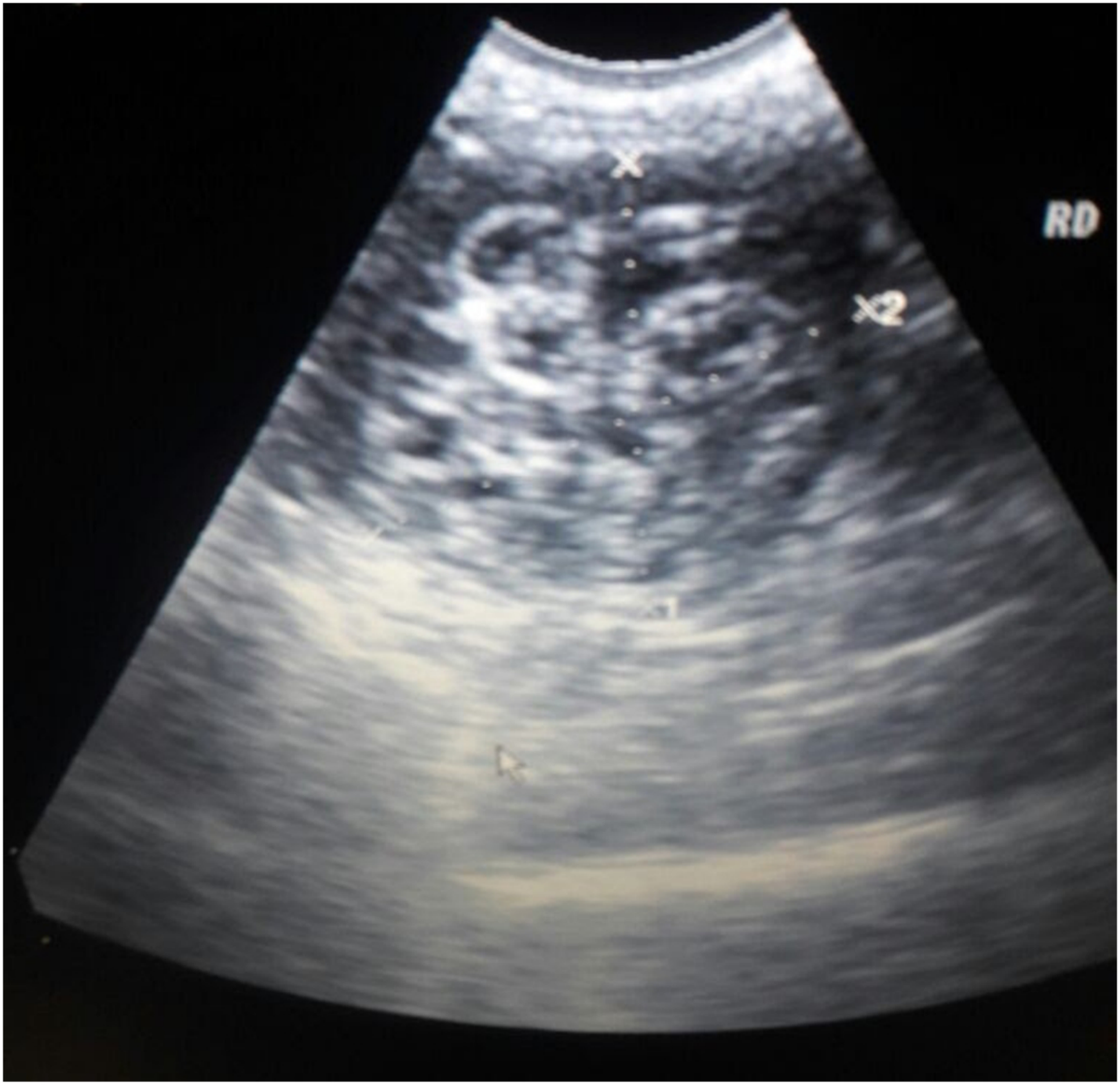

Figure 1

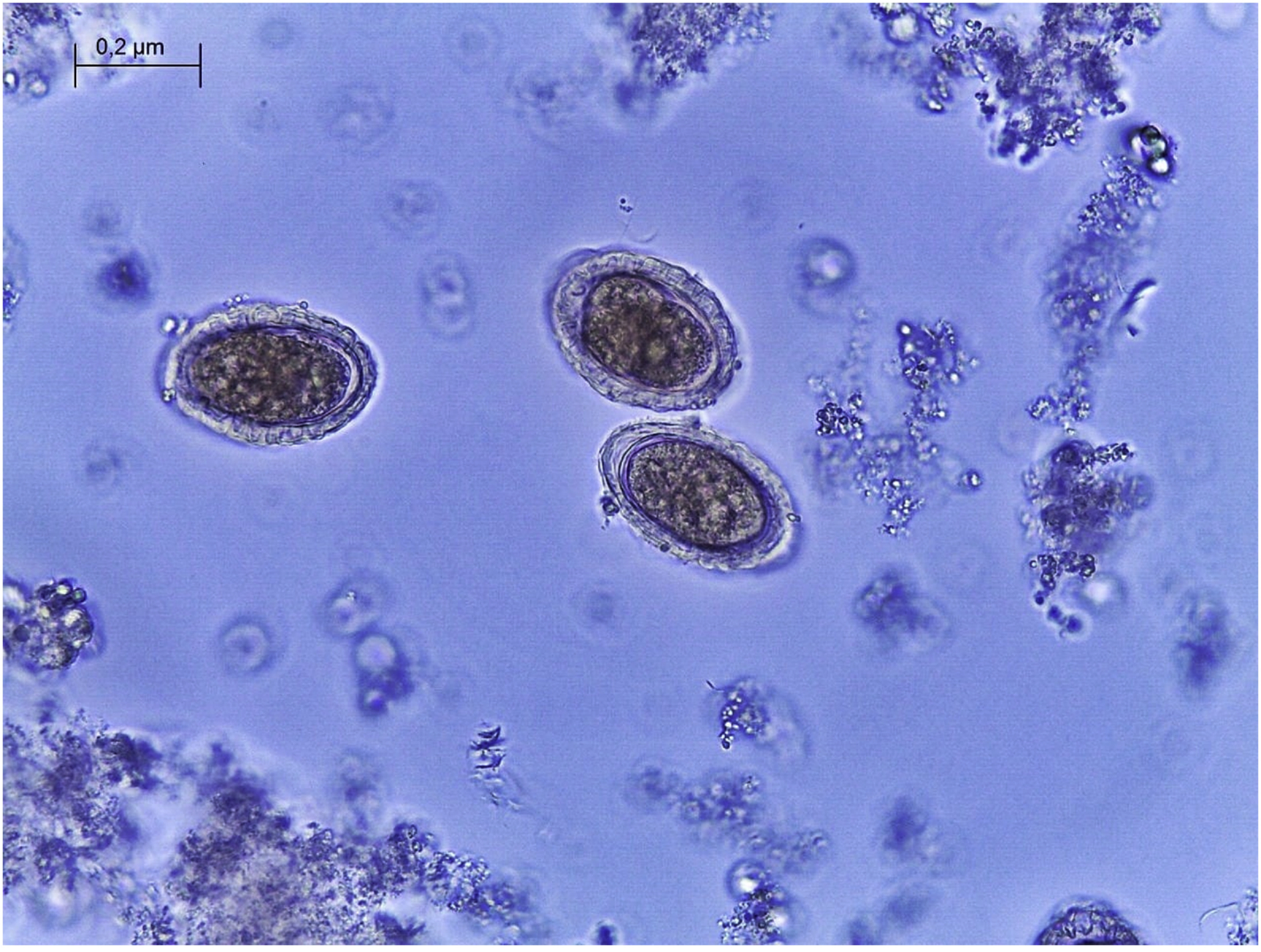

Figure 2

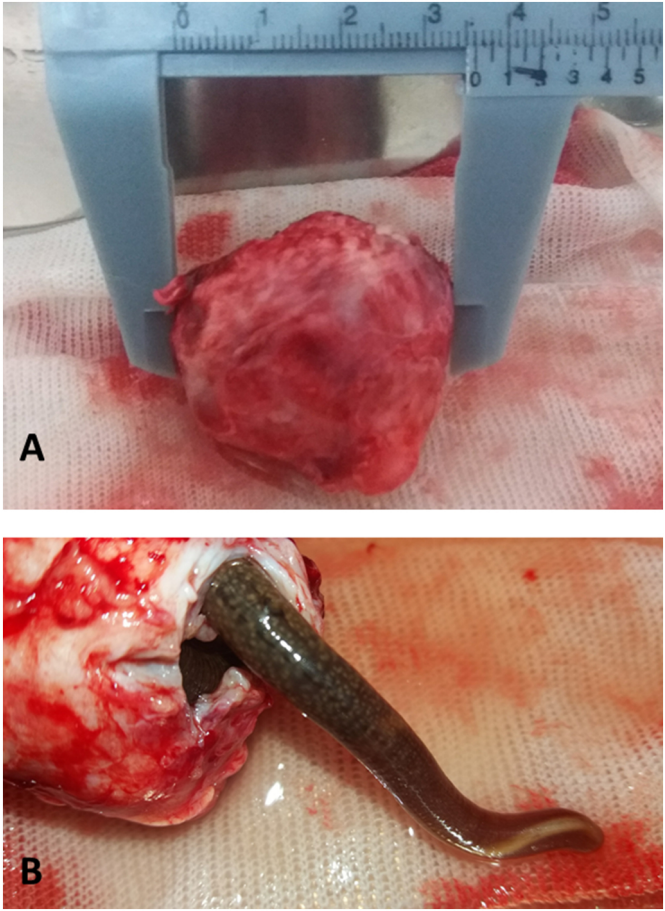

Figure 3

Figure 4