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PII: S1383-5769(23)00107-1

DOI: https://doi.org/10.1016/j.parint.2023.102829

Reference: PARINT 102829

To appear in: *Parasitology International*

Received date: 24 August 2023

Revised date: 21 November 2023

Accepted date: 22 November 2023

Please cite this article as: S.L. Meister, F.C. Origgi, F.S. Wyss, et al., Angiostrongylus dujardini infection in a coconut lorikeet (Trichoglossus haematodus) from a zoological garden in Switzerland, *Parasitology International* (2023), https://doi.org/10.1016/j.parint.2023.102829

Angiostrongylus dujardini infection in a Coconut lorikeet (*Trichoglossus haematodus*) from a zoological garden in Switzerland

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Abstract

Angiostrongylus spp. (Metastrongyloidea) can cause severe disease in several animal species and humans. This report describes an infection with *Angiostrongylus dujardini* in a captive Coconut lorikeet (*Trichoglossus haematodus*) from a zoo in Switzerland. The bird was reported being attacked by conspecifics, removed from the flock, and hospitalized. It showed lethargy, moderately reduced body condition, and lack of reaction to visual stimuli. Analgesic and antibiotic treatment were initiated but because of worsening of its general condition the bird was euthanized the following day. Necropsy revealed multifocal, subcutaneous hemorrhages, diffusely reddened

lungs and a moderately dilated right heart with several intraluminal nematodes embedded in a coagulum. Four worms were collected and microscopically examined. They were identified as adult females, measuring 19-21 mm long x 0.4-0.5 mm wide, with general morphological and morphometric characteristics consistent with angiostrongylid nematodes. In lung sections, multifocal collection of thin-walled embryonated eggs in variable stages of development was observed along with fully developed nematode larvae within the lumina of alveoli and lung vessels. Associated granulomatous infiltrates indicated a severe, multifocal, chronic, granulomatous pneumonia. The diagnosis of *A. dujardini* infection was formulated by morphological examination of adult and larval stages, supported by molecular analysis (PCR-amplification and sequencing of the ITS2, 5.8S and 28S rDNA flanking regions). This is the first report of *A. dujardini* infection in an avian species, providing evidence that birds can serve as accidental hosts of this parasite in addition to mammals, and that the parasite can reach maturity and multiply in the avian cardiorespiratory system.

Keywords

Angiostrongylus, Bird, Lorikeet, PCR, ITS2

1. Introduction

Cardiopulmonary nematodes of the genus *Angiostrongylus* are heteroxenous parasites within the superfamily Metastrongyloidea, which use mammals as definitive hosts (DH), and mainly gastropod mollusks (snails and slugs) as intermediate hosts (IH) [1-3]. To date 22 *Angiostrongylus* species have been described, infecting a broad range of mammal families including cricetid, heteromyid, soricid, glirid, sciurid

and murid rodents, as well as tupaiids, mephitids, mustelids, procyonids, felids and canids [4, 5]. *Angiostrongylus* spp. may also cause aberrant infections in avian, marsupial, and eutherian hosts, including humans [2, 6].

Angiostrongylus species inhabit primarily the pulmonary arteries and the right heart (e.g., *Angiostrongylus vasorum* and *A. dujardini*), or the mesenteric arteries (e.g., *Angiostrongylus costaricensis*) of mammals, and most commonly affect carnivores and rodents. In these locations, female worms produce eggs that (i) embolize and embryonate in the pulmonary capillaries, in which first-stage larvae (L1) hatch, cross into the alveoli and are subsequently carried up the mucociliary escalator along bronchi and trachea to be finally ingested and shed with the feces (e.g., *A. vasorum*, *A. dujardini*), or (ii) embryonate in the mesenteric arteries, and L1 are shed directly into the intestine (e.g., *A. costaricensis*) [6]. Eggs and L1 are responsible for important inflammatory reactions either in cardiorespiratory or intestinal tissues, respectively. Regardless of the adult location in the DH, L1 reach the gastrointestinal tract, are excreted with the feces, and are subsequently ingested by gastropod IH (a broad variety of slugs, terrestrial and aquatic snails). There is also some evidence that L1 of some *Angiostrongylus* species such as *A. cantonensis* and *A. costaricencis* might penetrate the mollusks' tegument while they feed or crawl on feces [2, 6]. Within the IH, L1 develop through a second larval stage into infective third-stage larvae (L3). There is also a wide spectrum of animals that may serve as paratenic hosts, in which infective larvae may persist in the tissues without undergoing further development (e.g., planarians, prawns, crabs, amphibians and lizards for *A. cantonensis* [1, 2, 7], or amphibians and chickens for *A. vasorum* [8]), which may play an important role in the epidemiology of these parasitic infections. The parasite's life cycle is completed when infected intermediate or paratenic hosts are ingested by the DH. A secondary pathway of infection is the ingestion of food items contaminated

with spontaneously released L3 by the IH within the mucous, as it has been described for *A. cantonensis* [9] and *A. vasorum* [10, 11].

Angiostrongylus dujardini was first described in the rodents *Apodemus sylvaticus* (wood mouse) and *Clethrionomys glareolus* (bank vole) in France [12, 13], followed by further descriptions in murid and cricetid rodents from Portugal [13], Hungary [14], Finland [15] and the Iberian Peninsula [16]. The adults are located in the pulmonary arteries and the heart, where the females lay eggs, which embolize in the pulmonary capillaries. First-stage larvae develop within the eggs, hatch in the lung, move up the airways and are swallowed and shed with the feces after a prepatent period of 24-26 days [2]. Gastropods, mainly aquatic snails, then serve as IH [1, 2, 13]. Although this nematode had been mainly considered a parasite of rodents (DH), it was later determined as the cause of death of 14 captive monkeys belonging to the family Callitrichidae, namely four cotton-top tamarins (*Saguinus oedipus*), five Goeldi's tamarins (*Callimico goeldii*), one white-lipped tamarin (*Saguinus labiatus*), three white-headed marmosets (*Callithrix geoffroyi*), and one pygmy marmoset (*Callithrix pygmaea*) in four zoos in France [17]. This was the first report of *A. dujardini* in vertebrates other than rodents and in individuals kept in human care. Since it seems that the parasite's life cycle cannot be completed in these animal species, they were considered accidental hosts in comparison to the rodents, which function as DH. The authors assumed a significant health risk for New World primates in zoological settings, which was reinforced by further fatal cases in a captive *S. oedipus*, a *C. goeldii* and two suricates (*Suricata suricatta*) in a zoo in Central Italy [18]. This showed that other animal species kept in zoos, besides non-human primates, are at risk of life-threatening angiostrongylosis due to *A. dujardini*.

The infection with *A. dujardini* in a captive Coconut lorikeet (*Trichoglossus haematodus*) in a zoo in Switzerland is the first report of this parasite in an avian

species and thus provides evidence that this nematode may not only exploit mammals but also birds as hosts.

2. Case description

At the Zoo Basel, a captive, 15-year-old, female Coconut lorikeet (*Trichoglossus haematodus*) was found sitting on the floor of an indoor aviary being attacked by several individuals of the flock. The clinical examination revealed a lethargic animal with moderately reduced body condition and lack of reaction to visual stimuli. The bird was separated from its conspecifics in a quiet room under a heat lamp and treated with meloxicam (1 mg/kg s.c.) and enrofloxacin (10 mg/kg s.c.) but was found in lateral recumbency and stupor the next day. Euthanasia was performed by intravenous injection of pentobarbital under general anesthesia. The carcass was submitted to the Institute of Animal Pathology (Vetsuisse Faculty, University of Bern) for postmortem examination.

Necropsy revealed multifocal, 2-5 mm in diameter sized, subcutaneous hemorrhages in the area of the left scapula and on the right side of the occipital and parietal bones, diffusely and moderately reddened lungs as well as a moderately dilated right heart in which several intraluminal nematodes were embedded in a coagulum (Fig. 1A). Four worms could be recovered and examined microscopically. They presented general morphological and morphometric characteristics of angiostrongylid nematodes (Fig. 1B). They measured 19-21 x 0.4-0.5 mm and corresponded to adult females, presenting a papilla in the tail extremity (Fig. 1C, arrow), subterminal anus (Fig. 1C, arrowhead), vulva near anus (Fig. 1C, asterisk), a prominent ovojector (Fig. 1C), and a uterus filled with eggs in various stages of development (Fig. 1D). No male worms were recovered.

A parasitological examination of intestinal content using fecal flotation technique was performed but no parasitic structures were identified. Due to the scarce amount of material, a Baermann test to detect the presence of larvae could not be additionally performed. However, after the post-mortem diagnosis was stated, pooled fecal samples of the remaining Coconut lorikeets were analyzed by the Baermann method on three consecutive days, without detection of *Angiostrongylus* larvae.

The main histopathological finding was a multifocal collection of ovoid, thin-walled embryonated eggs at different developmental stages, as well as elongated, full-developed nematode larvae with a thin cuticle and a primitive intestinal tract within the alveolar lumina and the pulmonary arterioles (Fig. 2A and 2B). The tunica intima was multifocally thickened by moderate amounts of fibrous connective tissue and some lymphocytes and plasma cells consistent with a moderate, multifocal, chronic, proliferative endarteritis. The parasitic stages were associated with multifocal, occasionally perivascular infiltration composed of numerous concentrically arranged epitheloid macrophages, multinucleated giant cells, lymphocytes, and plasma cells consistent with a severe, multifocal, chronic, granulomatous perivasculitis with many intravascular and intra-alveolar nematode eggs and larvae (Fig. 2C), followed by regional consolidation with the features of an overall granulomatous pneumonia. Additionally, moderate pulmonary congestion and interstitial to perivascular edema were observed (Fig. 2D). The liver displayed a multifocal, randomly distributed infiltration of few lymphocytes and macrophages (mild, multifocal, lymphohistiocytic hepatitis). The heart, spleen, kidney, proventriculus, ventriculus (gizzard), small and large intestine as well as both eyes were histologically unremarkable. A mild to moderate amount of mixed bacterial flora was isolated in liver and kidney tissue, respectively. The bacterial culture of spleen tissue yielded no bacterial growth.

For molecular characterization of the observed nematodes, total DNA extraction was performed from formalin-fixed, paraffin-embedded lung tissue as previously described [19, 20], as well as from one of the adult worms collected from the heart using the DNeasy® Blood & Tissue Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's protocol. Subsequently, a PCR targeting the ITS2 plus 5.8S and 28S rDNA flanking regions of lungworms [21] was performed. The reaction mix included 0.5 µl each of the NC1 and NC2 primers in a 100 µM working solution, 12.5 µl of a commercial master mix (QIAGEN Multiplex PCR Kit, Hombrechtikon, Switzerland), 9.0 µl of ddH_2O and 2.5 µl of DNA template to a total volume of 25 µl per tube. The PCR amplification was performed under the following thermocycling conditions: 94 °C for 15 min followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min and extension at 72 °C for 1 min, followed by a final extension at 72 °C for 10 min. Total DNA from *A. vasorum* recovered from a dog was used as a positive control. The amplification products were resolved by gel electrophoresis in a 1.5% agarose gel containing ethidium bromide and purified using the Zymo Research purification kit (DNA Clean & Concentrator™-5, Zymo Research Europe GmbH, Lucerne, Switzerland). Bidirectional Sanger sequencing with the same primers used in the PCR reaction was performed (Microsynth AG, Balgach, Switzerland). The chromatograms were analyzed, and consensus sequences obtained, by using Geneious software (Geneious Prime® 2023.0.4). Sequences were compared with those from other nematodes available on GenBank using the Nucleotide BLAST algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Both analyzed samples provided an amplification product of the expected size and identical sequences; the sequence obtained from the adult worm was deposited in GenBank (**OR268984**). This sequence showed 99.6 % (472/474 bp) identity with a sequence of *A. dujardini* (GQ181113) isolated from *A. sylvaticus* in France. The percentage of

identity with deposited sequences from *A. vasorum* and other *Angiostrongylus* species was lower than 82.2%.

3. Discussion

To the authors' knowledge, this is the first report of *A. dujardini* in an avian host. *Angiostrongylus dujardini* has heretofore been considered a parasite of rodents [2, 3]; however, little is known about the epidemiology of this parasite. In recent years, infection in other host species was observed, and several cases of angiostrongylosis were reported in zoological institutions in France and Italy involving callitrichid monkeys and suricates as accidental hosts [17, 18]. Clinical signs ranged from anorexia, dyspnea, malaise, lethargy to sudden death. The bird described in this case report was euthanized; however, the poor general condition and lethargy along with the severity and extent of the pathomorphological findings, were strongly consistent with a pre-terminal clinical stage. The observed clinical signs were non-pathognomonic but consistent with the presence of adult nematodes in the right heart ventricle as well as the myriads of intralesional eggs and larvae markedly compromising the cardiac and pulmonary function. The mild hepatitis and the mixed flora isolated from the liver and kidney are non-specific findings, and of questionable clinical relevance. Since numerous *A. dujardini* larvae were observed in lung tissue, it is possible to assume that the infection occurred more than one month earlier.

The source of infection could not be conclusively clarified, but the infection by ingestion of an infected intermediate host is likely. Given that the flock had access to a naturalistic outdoor aviary, contact with gastropod mollusks was possible. In one of the French zoos, in which fatal *A. dujardini* infection in callitrichid monkeys was reported, infection and L1 shedding was also detected in rodents (*A. sylvaticus* and

Microtus agrestis) captured or found dead on zoo premises [17]. Therefore, the presence of free-ranging rodents in zoos represents a possible source of environmental contamination with this parasite.

Morphological identification of angiostrongylid nematodes to the species level is mainly based on the morphology of the bursal rays [2, 3]. Unfortunately, no male worms were recovered. Nonetheless, morphological and morphometrical examination of the recovered females is consistent with their classification as *A. dujardini*. In addition, molecular analysis revealed 99.6% identity with reported sequences of *A. dujardini* (GQ181113) from *A. sylvaticus* and also from callitrichids and suricates from an Italian zoo [18], and less than 92.2% sequence identity with other *Angiostrongylus* species.

Because of the small amount of gastrointestinal content, a Baermann test to detect the presence of first-stage larvae could not be performed; therefore, we could not state whether the lorikeet was shedding these stages with the feces or not. Analyses of pooled fecal samples of the remaining Coconut lorikeets in the group, did not reveal *Angiostrongylus* larvae. Interestingly, L1 were also not identified in captive callitrichids by the Baermann technique although adult worms as well as a verminous pneumonia and pulmonary endarteritis were described in these individuals [17]. Based on the lack of infectious L1 in the fecal samples, it was hypothesized that these animals might have served as accidental hosts. In our opinion, *A. dujardini* displayed obvious parasitic potential in these species, which allowed the development of the parasite to the adult stage, as well as its reproduction [17, 18]. This is in accordance with the lorikeet case, as we observed development of eggs in adult females as well as the presence of eggs and larvae in lung tissues, confirming the reproduction of the parasite in this avian host. Although the presence of adult parasites and developed larvae in cardiorespiratory tissues suggest that this animal

species may be able to act as DH, until shedding of L1 with the feces is proven it is more advisable to consider them as accidental hosts. Further coprological studies should be conducted to confirm the excretion of larvae in these host species and to better understand their epidemiological role.

In conclusion, *A. dujardini* can infect different classes of warm-blooded vertebrates, including birds, causing severe disease and even mortality in captive animal collections. Therefore, the parasite's host range and health risk should not be underestimated, especially when captive animals have access to possible IH in naturalistic outdoor enclosures.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

Figure Captions

Fig. 1: Morphological examination of adults of *Angiostrongylus dujardini*. Gross appearance of an adult female (arrow) within the opened right ventricle of a formalin-fixed heart (asterisk) of a Coconut lorikeet (A), with the anterior extremity showing the esophagus, scale bar = 100 µm (B), the caudal extremity presenting a papilla (arrow), subterminal anus (arrowhead) and vulva (asterisk), scale bar = 100 µm (C), and a uterus filled with eggs at different developmental stages, scale bar = 100 µm (D).

Fig. 2: Histopathological lesions observed in the pulmonary tissue of a female Coconut lorikeet infected with *Angiostrongylus dujardini*. Diffusely moderately

congested lung with multifocal collection of ovoid, thin-walled embryonated eggs at different developmental stages and elongated, full-developed nematode larvae within the alveolar lumina and the pulmonary arterioles (dashed lines), scale bar = 250 µm (A), scale bar = 50 µm (B). Parasitic aggregates are surrounded by concentrically arranged epitheloid macrophages, multinucleated giant cells, lymphocytes, and plasma cells consistent with a severe, multifocal, chronic, granulomatous perivasculitis, scale bar = 25 µm (C). Multifocally, perivascular inflammatory infiltration and edema (asterisk), scale bar = 50 µm (D). H&E staining.

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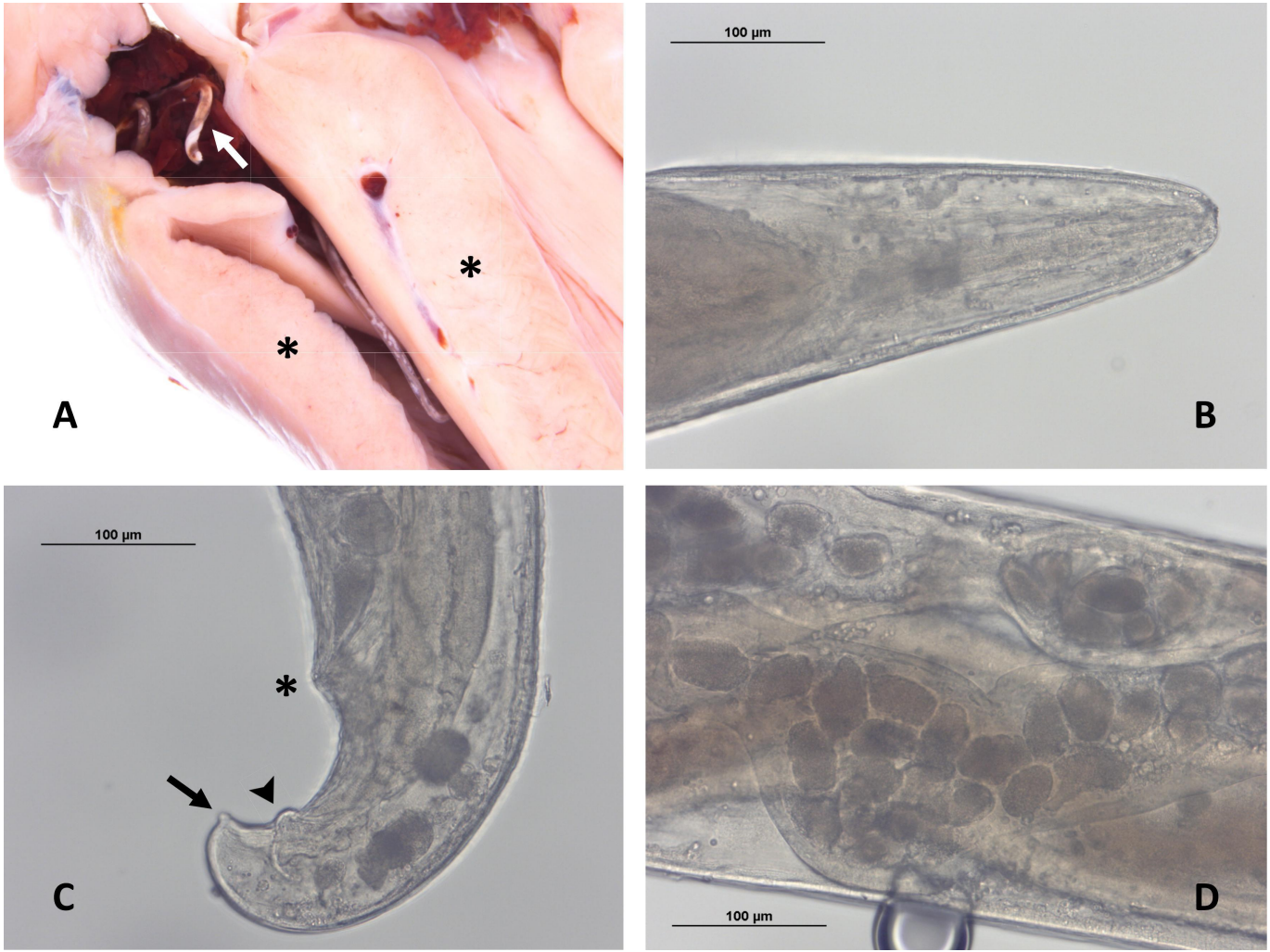

Figure 1

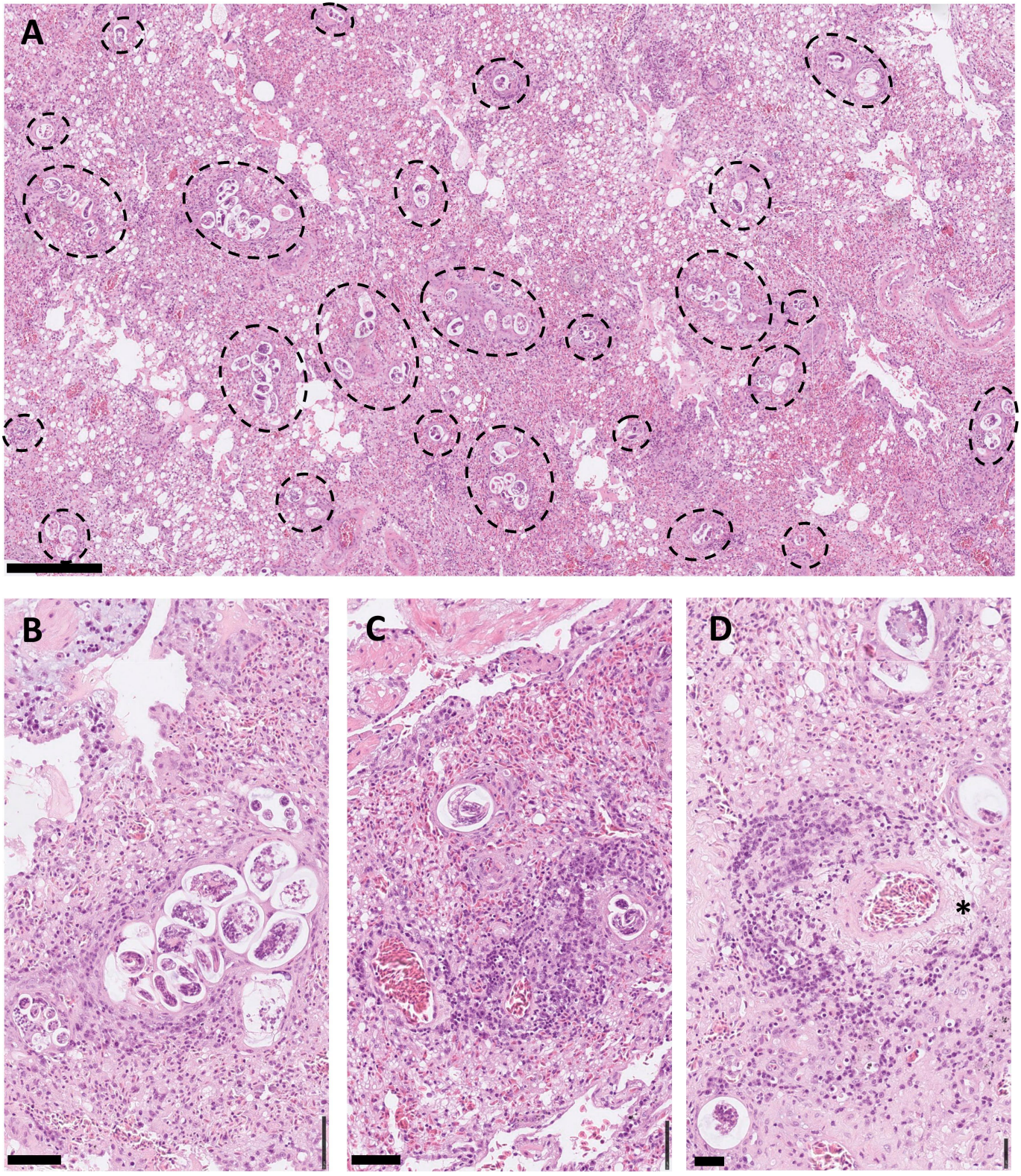

Figure 2