

Case Report

A zoonotic parasite, *Linguatula serrata*, infection in a dog imported from Ethiopia to the United StatesYoko Nagamori^{a,*}, Akhilesh Ramachandran^b, Carrie Kuzma^c, Laura Nafe^c, Eileen M. Johnson^a^a Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK 74078, United States^b Oklahoma Animal Disease Diagnostic Laboratory, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK 74078, United States^c Department of Veterinary Clinical Sciences, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK 74078, United States

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ABSTRACT

A moderate number of oval-shaped, $114.7 \times 61.3 \mu\text{m}$ in size, amber-colored, arthropod-like eggs that had chitinous, smooth, semi-thickened outer wall and 2–4 short appendages armed with 2 terminal hook-like structures were detected in multiple fecal samples from an approximately 9-month-old, intact female, collie-mixed dog that had been recently imported from Ethiopia to Oklahoma, United States. Initially the unusual arthropod-like eggs were considered to be a pseudoparasite, most likely mite eggs. However, based on the history of the dog, morphology of the eggs, and presence of the eggs in repetitive fecal flotations, a pentastomid, *Linguatula serrata*, was suspected. DNA extraction and PCR analysis of the partial 18S rRNA gene were performed on the eggs, and nucleic acid sequence showed 100% homology to *L. serrata*, a parasite of dogs, and *L. arctica*, a parasite of Norwegian reindeers. Rhinoscopy and head CT scan on the dog failed to demonstrate adult parasites or detect any pathologic changes. At this time, pentastomid eggs were no longer observed on fecal flotation. Due to the possibility of juvenile stages of the parasite still migrating in the dog, fluralaner (Bravecto®, Merck) was administered and continuing treatment recommended for at least 6 months. A follow-up fecal examination conducted a month after the treatment did not reveal any parasites or eggs. This is a case report of canine linguatuliiasis diagnosed in Oklahoma, United States.

1. Introduction

Pentastomids are often referred to as tongue worms because adult forms resemble the shape of a tongue (Blagburn et al., 1983; Drabick, 1987; Haugerud, 1989; Acha and Szyfres, 2003; Tappe and Buttner, 2009; Shamsi et al., 2017; Villedieu et al., 2017). Due to their unique morphology, pentastomids have been historically classified under various diverse taxa, including cestodes, nematodes, trematodes, and arthropods as well as in a separate phylum, Pentastomida (Haugerud, 1989); classification is still disputed. Phylogenetic studies indicated that Pentastomida is an intermediate group between the Arthropoda and some Nematelminth and shares common morphological characteristic of annelids and arthropods (Almeida et al., 2008; Gjerde, 2013; Mohanta and Itagaki, 2017). Several pentastomid parasites such as *Linguatula* spp., *Armillifer* spp., *Leiperia* spp., *Raillietiella* spp., and *Porocephalus* spp., are obligate zoonotic parasites (Acha and Szyfres, 2003). Most (> 90%) cases of human pentastomiasis are caused by

either *A. armillatus* or *L. serrata* (Tappe and Buttner, 2009). Many pentastomids use carnivorous reptiles as definitive hosts. Others use birds and mammals (Acha and Szyfres, 2003).

Linguatula serrata uses carnivores, canids and rarely felids, as definitive hosts. The adult parasite can be found in the nasal airway, frontal sinus and tympanic cavity. Herbivores, such as ruminants and lagomorphs, become infected by ingesting eggs shed in the feces or nasal discharge of infected carnivores and serve as intermediate hosts by harboring encysted larvae (nymphs) in the internal organs and lymph nodes. Definitive hosts become infected by ingestion of the nymphal stages found in infected intermediate hosts (Acha and Szyfres, 2003). The prepatent period of this parasite is approximately 6–7 months, and adults live in definitive hosts for about 15 months (Acha and Szyfres, 2003; Rezaei et al., 2016). Eggs passed in the feces or nasal discharges are immediately infectious to intermediate hosts (Haugerud, 1989). Humans become infected by ingesting either eggs excreted by definitive hosts or nymphs in raw, undercooked viscera of intermediate hosts

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(Abadi et al., 1996; Acha and Szyfres, 2003; Gjerde, 2013; Attia et al., 2017). Although the prevalence of this parasite is thought to be worldwide, most cases have been reported in Africa, the Middle East, and southern Asia (Abadi et al., 1996; Acha and Szyfres, 2003). Oluwasina et al. (2014) reported 37.45% of client-owned dogs were infected with *L. serrata* in Jalingo, Nigeria. In Egypt, several coprological surveys demonstrated the prevalence of *L. serrata* in dogs ranged from 0 to 25% (Khalil, 1970, 1973; Attia et al., 2017). Feline linguatuliiasis has also been reported sporadically throughout Africa (Young, 1975; Khalafalla, 2011; Mukarati et al., 2013). Recently, unexpectedly high prevalence of *L. serrata* in wild dogs has been reported in south-eastern Australia (Shamsi et al., 2017). To our knowledge, canine linguatuliiasis has been reported only twice in the United States (Ehrenford and Newberne, 1981; Blagburn et al., 1983). This is a case report of *L. serrata* infection in a dog brought from Africa into the United States that provides photographs of the eggs and first larval stage.

2. Case presentation

A fecal sample from an approximately 9-month-old, intact female, collie-mixed dog was submitted to the parasitology diagnostic laboratory at Oklahoma Animal Disease Diagnostic Laboratory (OADDL), Center for Veterinary Health Sciences (CVHS), Oklahoma State University (OSU) in September 2017 for recheck evaluation post-treatment against persistent *Dipylidium caninum* and *Sarcocystis* spp. infections. The dog had been rescued and imported from Ethiopia to the United States 3 months prior. The dog had been vaccinated against canine distemper and rabies, and a heartworm antigen test (SNAP® 4Dx® Plus, IDEXX) was negative. She was on monthly heartworm preventative (Heartgard® Plus, Merial) since August 2017 and received pyrantel, fenbendazole, praziquantel, sulfadimethoxine (Albon®, Zoetis), and amprolium (Corid®, Merial) for the persistent *Sarcocystis* and tapeworm infections.

The submitted fecal sample was normal in color and consistency. Centrifugal fecal flotation with Sheather's sugar solution (specific gravity, 1.25) was performed and revealed a low number of *Sarcocystis* spp. sporocysts and a moderate number of fairly large, oval-shaped, amber-colored, arthropod-like eggs (Fig. 1A and B). Four arthropod-like eggs were measured, and the mean length and width were 114.7 μ m and 61.3 μ m with standard error of 13.4 μ m and 0.72 μ m, respectively. The egg shell outer wall were smooth, 2.5 μ m in thickness with standard error of 0.14 μ m, (Fig. 2A), and the embryo appeared to have 2–4 short appendages with pairs of chitinous hook-like structures visible using fine focus adjustment of the microscope at 400 \times magnification (Fig. 2B). A tentative diagnosis of pseudoparasites, most likely mite eggs, was made, and the owner was advised to closely monitor the dog for evidence of coprophagy, scavenging, or consumption of prey. Two weeks post-treatment with ponazuril (Marquis®, Merial) for *Sarcocystis* spp. infection, a fresh fecal sample was submitted for fecal examination. The same arthropod-like eggs were still detected in a moderate to high number in addition to a low number of *Sarcocystis* spp. sporocysts. The owner verified that the dog did not display any feeding habits which might contaminate the feces with spurious or pseudoparasites. Based on the morphology of the arthropod-like eggs and history of the dog being rescued and imported from Ethiopia, a pentastomid parasite, *Linguatula serrata*, infection was suspected.

The arthropod-like eggs were concentrated by centrifugal fecal flotation using Sheather's sugar solution, washed with deionized water, and collected by sedimentation for DNA extraction and PCR analysis. Pentastomid specific PCR targeting the 18S ribosomal RNA gene (Brookins et al., 2009) was performed on extracted DNA. Nucleic acid sequence of the PCR product showed 100% homology to *L. serrata*, a parasite of dogs, and *L. arctica*, a parasite of Norwegian reindeers (NCBI Ref# MH071351).

In December 2017, the dog was brought to the Boren Veterinary Medical Hospital (BVMH), CVHS, OSU for rhinoscopy and head CT scan

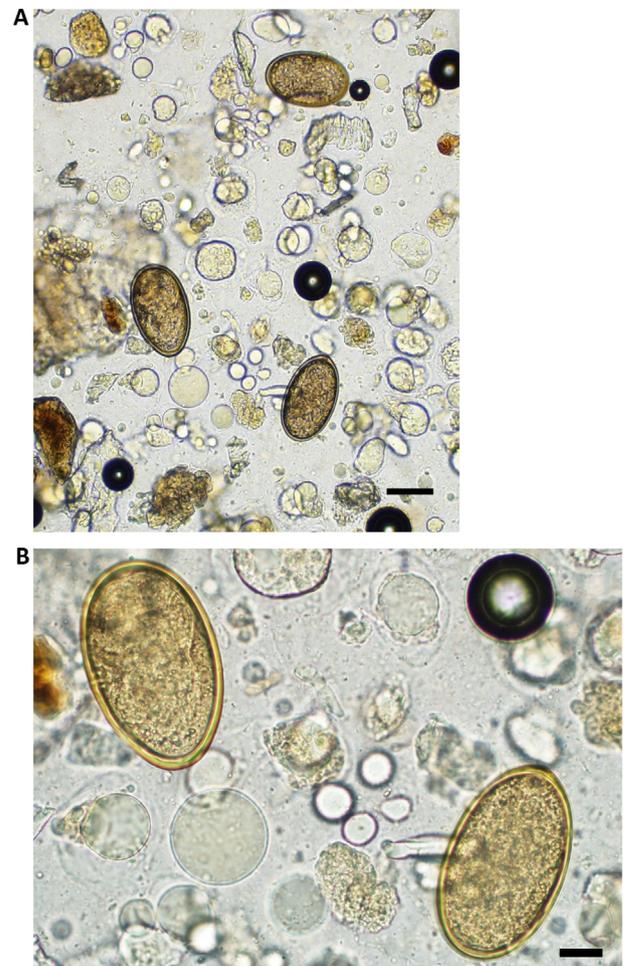


Fig. 1. Eggs of *Linguatula serrata*; they were large (114.7 \times 61.3 μ m), oval-shaped, amber-colored with smooth, semi-thickened (2.5 μ m) outer wall. (A) Scale bar = 50 μ m. (B) Scale bar = 20 μ m. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

to visualize the adult pentastomid and to evaluate possible pathological changes. Physical and imaging examinations did not reveal any abnormalities, and parasites were not detected in the patient during rhinoscopy. Testing of a fecal sample, collected on the same day, also did not reveal any parasitic eggs or parasites. Due to the possibility of juvenile stages of the parasite still migrating between the gastrointestinal tract and nasal airways in the dog, a systemic insecticide and acaricide, fluralaner (Bravecto®, Merck), was administered the next day and continuing treatment recommended for at least 6 months. A fecal examination conducted again in January 2018 did not reveal any parasites or eggs.

3. Discussion

A tentative diagnosis of pseudoparasite, most likely mite eggs, was not unreasonable considering the unresolved phylogenetic and taxonomic relationships of pentastomids to annelids and arthropods. The presence of appendages with hooks in the larval stage is suggestive of a juvenile mite (Mullen and O'Connor, 2009). DNA analysis of the partial 18S rRNA gene verified the eggs as a *Linguatula* species with 100% homology to *L. serrata* and *L. arctica*. Gjerde (2013) also reported 99.9% identity between these species with the 18S rRNA gene sequences but found differences in the sequences of the mitochondrial cytochrome c oxidase subunit I (*cox1*) with 90.2% identity between the two species. *Linguatula arctica* is a common pentastomid parasite found in the nasal

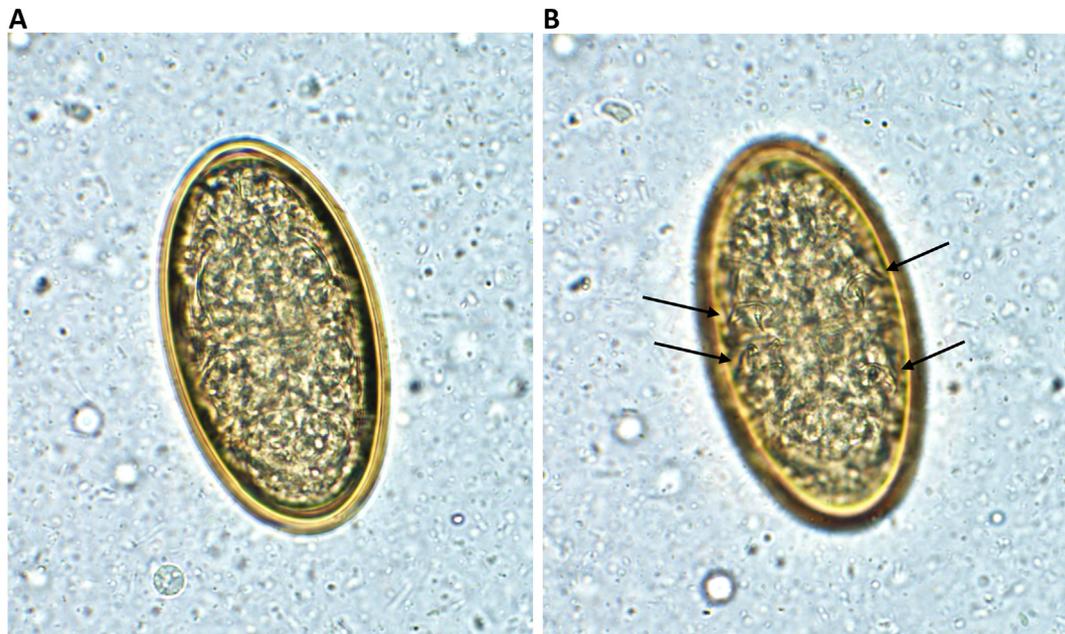


Fig. 2. Egg of *Linguatula serrata*. (A) Focused on the outer egg shell wall. (B) Focused on internal structure. A fully developed larva with 4 irregularly arranged chitinous claws (arrow) inside the egg. In order to make the claws visible, you will need to observe closely and change fine focuses.

cavities of reindeers and is distributed only in northern Norway. *Linguatula arctica* is a unique pentastomid by having ungulates as a definitive host and a direct life cycle (Riley et al., 1987; Nikander and Saari, 2006; Gjerde, 2013). *Linguatula arctica* was historically reported as *L. serrata* (Chapin, 1926; Riley et al., 1987); however, differences in life cycles, definitive hosts, and morphologies of adult parasites resulted in separating them as distinct species (Riley et al., 1987; Nikander and Saari, 2006; Gjerde, 2013). Although our nucleic acid sequence in this report showed 100% homology to *L. serrata* and *L. arctica*, a diagnosis of *L. serrata* was based on the fact that our patient was a dog that had no known travel history to northern Norway. *Linguatula serrata* is a known parasite of canids that has a high prevalence in African canids, making *L. serrata* the probable parasite present in the dog of this case report.

Since the prepatent period of *L. serrata* is approximately 6–7 months (Acha and Szyfres, 2003; Rezaei et al., 2016), this dog had most likely acquired *L. serrata* in Ethiopia between February–March 2017, which would explain why the eggs were not seen by fecal examinations until the test in September. In previous publications, eggs of *L. serrata* are described as oval, large, with a thick chitinous shell and containing a fully developed embryo with 2 pairs of irregularly arranged chitinous claws. The size of eggs reported is somewhat variable, 90–133 × 54–88 μm (Ehrenford and Newberne, 1981; Soulsby, 1982; Blagburn et al., 1983; Rezaei et al., 2016; Shamsi et al., 2017). Mean size of eggs recovered from fecal samples in this report was 114.7 × 61.3 μm (range: 100–155 μm in length and 60–62.5 μm in width) with multiple layers of egg shell wall (Fig. 3A and B) and an embryo showing 2–4 chitinous claws inside, depending on the position of the egg on a slide (Fig. 4). A fully developed first stage larva had 4 leg-like appendages, a chitinous stylet with spines at the anterior end, and tail-like structure with spines at the posterior end (Fig. 5). Canine linguatuliiasis has rarely been reported in the United States with very few scientific publications available (Ehrenford and Newberne, 1981; Blagburn et al., 1983).

Currently there is no known labeled treatment against *L. serrata* in dogs even though systemic ivermectin at 0.2 mg/kg was historically recommended in previous cases (Nilssen et al., 2002; Villedieu et al., 2017). In more recent cases, milbemycin oxime was used to successfully treat a dog that was infected with *L. serrata* (Gjerde, 2013; Mitchell et al., 2016; Springer et al., 2018; Thomas, 2018). Springer et al. (2018)

indicated that multiple doses of praziquantel/pyrantel/fenbendazole (Cestal Plus®, Ceva Santé Animal Romania), spot-on fipronil (Fypryst®, KRKA Romania S.R.L.), and praziquantel/fenbendazole (Caniquantel Plus®, IDT Biologika GmbH) did not clear adult forms of *L. serrata* in a dog. It is still unclear why adult *L. serrata* were not detected through imaging or rhinoscopic examinations nor were eggs detected on fecal examinations in December and January. However, the dog was on a continuing low dose of ivermectin (6 μg/kg) as a monthly heartworm preventative since August, which most likely helped to eliminate the pentastomid from the dog. It is also possible the dog had expelled the adult parasites through sneezing or vomiting.

Linguatula serrata is a zoonotic parasite that can cause visceral, nasopharyngeal, and ocular linguatuliiasis in humans (Gardiner et al., 1984; Baird et al., 1988; Abadi et al., 1996; Acha and Szyfres, 2003). Because eggs dispersed in the feces or nasal discharges are immediately infective to intermediate hosts, humans can easily become exposed to this parasite by handling infected animals and by consuming water and vegetables contaminated with eggs (Drabick, 1987; Acha and Szyfres, 2003). Additionally, humans become infected by ingestion of nymphal stages of *L. serrata* with the raw or undercooked liver or lymph nodes of infected intermediate hosts, commonly sheep and goats (Acha and Szyfres, 2003). Six autochthonous human linguatuliiasis cases have been reported in the United States (Hunter and Higgins, 1960; Rendtorff et al., 1962; Mendeloff, 1965; Gardiner et al., 1984; Baird et al., 1988; Abadi et al., 1996). Two cases reported migrating nymphs in the anterior chamber of the eye (Hunter and Higgins, 1960; Rendtorff et al., 1962), three cases described hepatic granulomas caused by encysted nymphs (Mendeloff, 1965; Gardiner et al., 1984; Baird et al., 1988), and one case diagnosed migrating nymphs in the pericardial sac and epicardium (Abadi et al., 1996). In endemic areas, human cases are generally considered to be infrequent and sporadic compared to high prevalence of *L. serrata* infection in dogs; however, it is difficult to estimate the true occurrence of infection in humans because most cases do not cause any clinical signs, and infections are usually accidental findings during surgery, radiographical examination, and autopsy (Acha and Szyfres, 2003). Linguatuliiasis can be prevented by performing proper hygiene, avoiding consumption of raw or undercooked viscera, limiting contact with infected dogs, and providing regular veterinary cares for pets (Acha and Szyfres, 2003; Tappe and Buttner,

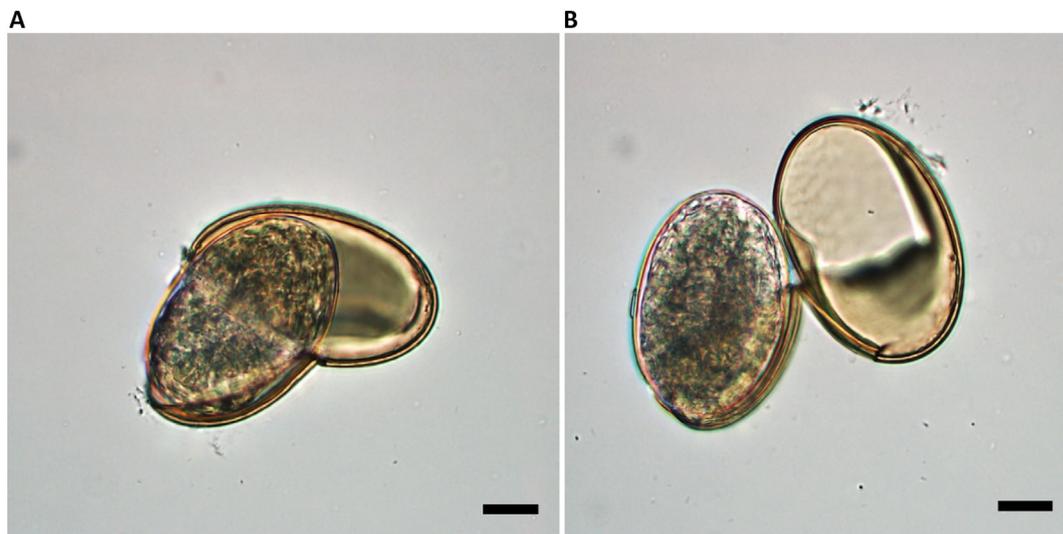


Fig. 3. A & B Egg of *Linguatula serrata*. Egg was inactivated with 10% formalin and broken with a scalpel blade. An embryo was enclosed by multiple layers of egg shell wall. (A) The outer wall was broken. (B) The outer wall was removed. The first stage larva was still enclosed by inner layer.



Fig. 4. Egg of *Linguatula serrata*. Depending on the position of the egg on a slide, only 2 claws may be visible (arrow head). Most eggs observed in this case showed only 2 claws.

2009).

People and animals travel all over the world, and they may unexpectedly transport exotic diseases. Veterinarians, physicians, and the general public need to be aware of the risk of spreading zoonotic pathogens through importing/exporting dogs and other animals. Regional epidemiologic studies are important to identify exotic parasitic infections that can be transported through infected people and animals to non-endemic areas, especially when animals are infected with zoonotic parasites. Lastly it is important to be knowledgeable of uncommon/foreign parasites and include them in the differential diagnosis list especially when treating patients with a history of travel to or originating from regions known to harbor uncommon parasites or other pathogens. In the present case, the dog owner and other members of family and friends were potentially exposed to this parasite for a period of 1–2 month(s). The dog was also a source of infection to other non-human intermediate hosts in this area. This is a case report of rare zoonotic parasite, *Linguatula serrata*, infection in a dog from Africa imported to the United States.

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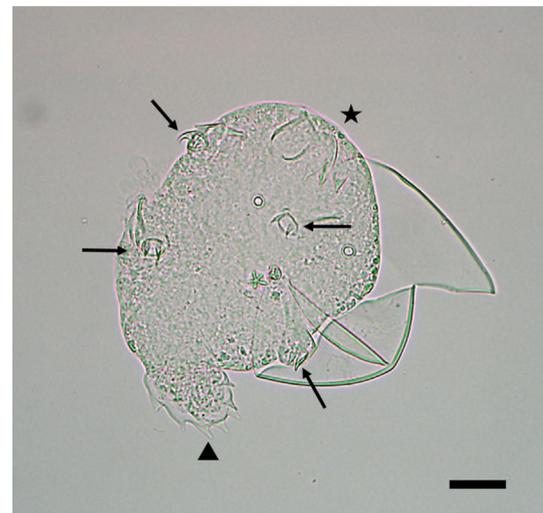


Fig. 5. First stage larva of *Linguatula serrata*. Egg was inactivated with 10% formalin and broken with a scalpel blade, and the first stage larva was recovered. The larva had 4 leg-like appendages (arrow), a chitinous stylet with spines at the anterior end (star), and tail-like structure with spines at the posterior end (arrow head). Scale bar = 20 μ m.

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Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical statement

This article does not contain any experimental studies with human or animal subjects performed by any of the authors.

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