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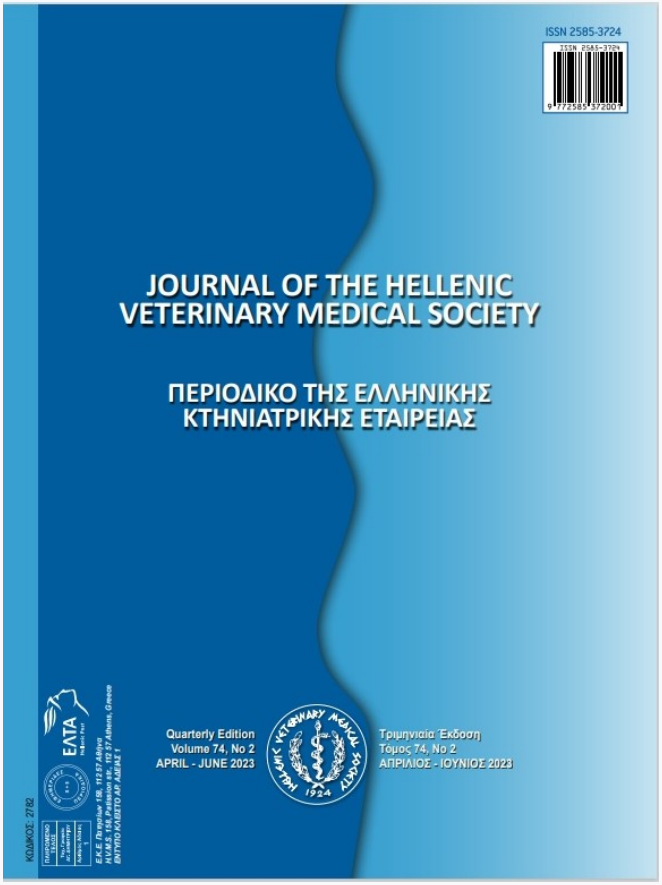

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Case report
Κλινικό περιστατικό

Management of *Linguatula serrata* infestation in a dog from Greece: a case report

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ABSTRACT: Linguatuliasis is caused by the arthropod *Linguatula serrata* (family: Linguatulidae) a parasite with worldwide distribution and zoonotic importance. Dogs, cats, foxes, jackals, and other carnivores are the definitive hosts of this arthropod while any mammal can be a potential intermediate host for this parasite. A two year old female neutered dog cross-breed, weighing 29 kg and living in a semi-free form in a mountain village called Sella (Achaia Prefecture, Greece) was presented to a veterinary clinic in Patra with history of progressive coughing and sneezing, that had started about 2 weeks earlier. Along with the dog, the owner brought a parasite that was expelled from the dog's nose on the same day during the severe coughing and sneezing. Macroscopic morphological aspects of the parasite, in the laboratory under stereoscope, revealed an arthropod 3.8cm long arthropod identified as *L. serrata,* also commonly called tongue worm. The dog was treated with Macrocyclice lactones (Milbemycin oxime 0.5mg/kg PO, Milpro® 12.5mg/125mg, Virbac) and lavage of nasal cavities. Human infection occurs either by direct contact with infected animals (risk of visceral linguatuliasis) or indirectly, by consuming contaminated raw or poorly cooked food. This is the first report of nasopharyngeal linguatuliasis in a dog in Greece.

Key words: *Linguatula serrata*, Pentastomida, zoonosis, dog, Milbemycin oxime

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INTRODUCTION

Linguatula serrata, first described and named by Josef Aloys Frohlich (1789), is an arthropod parasite (Pentastomida) and is the etiological agent of linguatuluasis. It is of zoonotic importance and has global distribution (Hajipour et al., 2018; Shamsi et al., 2020), especially in warm subtropical and temperate regions. Adult *L. serrata* parasites are whitish in color, have elongate shape, with a tongue-like appearance. They have a rounded anterior and pointed posterior end, ventrally flattened. The length of males reaches 20mm, while adult females reach the length of 40 to 85mm or more (Oryan et al., 2008). The cuticle presents with rings, while on the anterior, ventral side there are noticeable curved hooks with sharp tips around the central mouth (Soulsby, 1982; Hendrix and Mosby, 1998; Marquardt et al., 2000; Taylor et al., 2007; Mehlhorn, 2007). Adult and young *L. serrata* live in the nasopharynxl and lower respiratory system of vertebrates. Dogs, cats, foxes, jackals, and other carnivores are the final hosts of this arthropod. Although any mammal could be a potential intermediate host of this parasite (Schmidt et al., 2013), herbivores such as ruminants are the main intermediate hosts (Alcala-Canto et al., 2007). Adult *L. serrata* embed their forebody into the mucosa of nasal pharyngeal cavities, (Acha and Szyfres 2003) and feed on blood and fluids. The life span of adult females is about 2 years and egg production begins about six to seven months after the infection (Mehlhorn, 2007). Millions of embryonated eggs are discharged from the host in nasal secretions or, if ingested, with feces (Shamsi et al., 2020). When the eggs of the parasite are ingested by an intermediate host, the four-legged larvae hatch in the small intestine, penetrate the intestinal wall, and lodge in tissues, particularly in lungs, liver, and lymph nodes, where the nymphal stage develops (Shamsi et al., 2020). The life cycle continues when the infective nymphs are consumed by a definitive host, and attach themselves in the upper digestive tract or quickly travel there from the stomach, reaching the nasopharyngeal cavity (Christoffersen and De Assis, 2013).

Linguatuliasis is a zoonotic disease and humans can become infected in two ways. The first involves the consumption of food contaminated with *L. serrata* embryonated eggs and results in the so called "visceral linguatuliasis", where humans become intermediate hosts. The ingested embryonated eggs hatch in the intestines with the resulting larvae burrowing their way into the visceral cavity of the body and forming eventually granulomatic cysts, typically in the liver or the lymph nodes. This results in the obstruction or compression of biliary, gastrointestinal or respiratory tracts, meninges, brain or in the anterior chamber of the eye, causing iritis or secondary glaucoma (David et al., 2006; Tappe and Buther, 2009; Warrel, 2013). In the second, humans become accidental final hosts usually as a consequence of ingestion of raw or poorly cooked meat and viscera of an infected intermediate host, causing "nasopharyngeal linguatuliasis" (Yagi et al., 1996; Siavashi et al., 2002; David et al., 2006). After ingestion in the stomach, the larvae become liberated from their cysts within a couple of hours and then crawl up the oesophagus and establish themselves in the nose, pharynx or lungs. Symptoms involve headaches, dysphagia, dyspnea, face edema, difficulty in speaking and most importantly nasal discharges, coughing, sneezing, vomiting, lacrimation, hemoptysis, which obviously help in spreading the infection (Khalil and Schacher, 1965; Yagi et al., 1996; David et al., 2006; Mehlhorn, 2007; Schmidt et al., 2013).

There is a number of cases of nasopharyngeal linguatuliasis in dogs being reported in the past few years in UK (Thomas, 2018; Macrelli and Mackintosh, 2022), Germany (Springer et al., 2018; Berberich et al., 2022), Finland (Sievänen et al, 2021) and Romania (Ionita and Mitrea, 2016). The parasite has also been reported in a wolf from Greece (Diakou et al., 2014), a grey wolf from Central Balkans (Pavlović et al., 2017) and in brown hares (Lepus europaeus) in Greece with a remarkable prevalence of almost 20% (Diakou et al., 2014). However, linguatuliasis has not been found in ruminants in Greece (Kantzoura et al., 2013; Kouam et al., 2014) and it has been over three decades that it was reported in dogs (Haralambidis et al., 1988). Moreover, there are very scarce up to date information on the prevalence of *L. serrata* in wild and domestic animals in Europe, with the exception of a Romanian study that reported 17.5% prevalence in wolves, 1.73% in Golden jackals, 1.69% in foxes and one case in roe deer (Barton et al., 2022). In other parts of the world data for the prevalence of *L. serrata* have been updated through studies conducted in the past two decades. Esmaeilnejad et al. (2017) report 26.30% prevalence in goats, 18.32% in sheep, 14.30% in cattle and 13.9% in buffaloes from Iran. A study from South India reported 21% prevalence in goats, 19% in cattle and 8% in buffaloes (Ravindran et al., 2008), while the parasite was found in 17.30% of domestic rabbits in Mosul/Iraq (Al-Moula, 2005) and in almost 60% of stray dogs of Shahrekord, Iran

(Meshgi and Asgarian, 2003). An overall prevalence of 37.45% in client-owned dogs from an area in Nigeria was also reported (Oluwasina et al., 2014). With regard to human cases, there is only one reported case of linguatuliasis in Greece, in a 39 year old woman many years ago (Papadakis and Hourmouziadis, 1958). There are several cases reported from different regions such as North (Gardiner et al., 1984; Baird et al., 1988) and South America (Lazo et al., 1999), Germany (Tappe et al., 2006) and Austria (Koehsler et al., 2011), but mainly from Middle East (Siavashi et al., 2002; Janbakhsh et al., 2015) and Sudan (Yagi et al., 1996). The disease is known as "Halzoun syndrome" in Middle East (Siavashi et al., 2002) and "Marrara" in Sudan (Yagi et al., 1996).

The purpose of this study is to present a case of linguatuliasis in a dog from Greece and to provide information about the treatment of the disease.

CASE REPORT

A two year old female neutered, cross-breed dog, weighing 29 kg and living in a semi-free form in a mountain village called Sella (Achaia Prefecture, Greece) was presented with a history of severe coughing and sneezing, which started about 2 weeks earlier. Along with the dog, the owner brought a parasite that was expelled from the dog's nose on the same day during a severe episode of coughing and sneezing, together with other viscous secretions. Blood examination was normal with profound eosinophilia (Table 1.) and the owner signed the consent form for the animal to be anesthetized (Dexmedetomidine 12.5mcg/kg im, Propofol 1mg/kg iv, Isoflurane for inhalation anesthesia) in order to examine the nasal and pharyngeal cavities, mouth and trachea. During the examination the only pathological finding recorded was local inflammation and mild irritation, presented as mild local swelling and redness, of the left nasal cavity. After consultation with the Laboratory of Microbiology and Parasitology, faculty of Veterinary Medicine, University of Thessaly, the animal was subjected to lavage of the nasal and pharyngeal cavities with normal saline solution in order to remove any remaining parasites. A fecal sample was also collected. Macrocyclic lactones and specifically Milbemycine oxime: 0.5mg/kg, PO (Milpro® 12.5mg/125mg, Virbac) were administered after the animal recovered from anesthesia. The parasite was photographed and placed in a glass container with 10% formaldehyde solution and together with the nasopharyngeal lavage material and fecal sample, were sent to the laboratory mentioned above. There, macroscopic morphological aspects of the parasite were revealed under stereoscope and

Table 1. Complete blood count and basic biochemical profile.

LABORATORY TESTS							
COMPLETE BLOOD COUNT				BASIC BIOCHEMICAL PANEL			
Parameters	Observed value	Units	Reference range	Parameters	Observed value	Units	Reference range
☑ HCT	48.6	%	36.0-56.0	☑ UREA	26.0	mg/dL	14.0-58.0
☑ RBC	6.73	M/μl	5.00-8.00	☑ CREA	0.62	mg/dL	0.30-1.40
☑ Hemoglob	15.7	gr/dL	12.00-18.5	☑ TP	6.3	g/dL	5.5-7.5
☑ WBC	7.0	K/μl	5.5-15.0	☑ ALB	2.9	g/dL	2.2-3.8
☑ MCV	72.2	fl	62.0-78.0	☐ GLOB			
☑ MCH	23.3	pg	21.0-27.0	☐ A-G ratio			
☑ MCHC	32.3	gr/dL	31.0-36.0	☐ GLU			
☐ RETICUL				☐ CHOL			
☑ PLT	218	K/μl	180-500	☑ ALP	27	U/L	10.0-70.0
☑ NEUT #	4.3	K/μl		☑ AST	44.0	U/L	10.0-50.0
☑ NEUT %	60.8	%		☑ ALT	33.0	U/L	15.0-75.0
☐ BAND N#				☑ gGT	4.0	U/L	2.0-9.0
☐ BAND %				☐ TBIL			
☑ LYMPH #	1.3	K/μl		☐ ind BIL			
☑ LYMPH %	19.2	%	5.0-30.0	☐ dir BIL			
☑ MONO #	0.4	K/μl		☐ CK			
☑ MONO %	6.2	%		☐ LDH			
☑ EOS #	1	K/μl		☐ PHOS			
☑ EOS %	**13.7**	%	0.0-4.0	☐ CA+			
☑ BASO #	0.0	K/μl		☐ AMYL			
☑ BASO %	0.1	%		☐ LIPA			

the 3.8cm long arthropod (Figure 1.) was identified as *L. serrata*. It is also commonly known as tongue worm, due to its characteristic transparent tongue-like shaped, slightly convex, and ventrally flattened body structure (Blagburn et al., 1983). Fecal examination was carried out using the flotation technique with zinc sulfate solution. The same technique was also used for the lavage material. (Basset et al., 2022) Microscopic examinations of these were negative for eggs of *L.serrata*. After treatment the dog showed no clinical symptoms from the upper respiratory system and the prophylactic oral administration of the same Macrocyclic lactones (Milbemycine oxime: 0.5mg/kg, PO, Milpro® 12.5mg/125mg, Virbac) per month was suggested for 5 months.

DISCUSSION

Infestations with *L. serrata* in dogs in European territories were once considered rare, however there is an increasing number of cases being reported through the last years (Springer et al., 2018; Thomas M., 2018; Macrelli and Mackintosh, 2022). Nasopharyngeal form presents with unremarkable clinical signs, usually of the upper respiratory system, that include episodes of coughing and sneezing, sometimes accompanied by vomit and topical nasal irritation. Diagnosis comes with a thorough clinical examination, but may be tricky to unsuspected practitioners. Treatment of nasopharyngeal linguatuliasis in dogs, however, remains somewhat empirical and based on recent cases, where it is reported that monthly administration of Milbemycin oxime 0.5mg/kg PO was successful in treating the infestation (Springer et al., 2018; Thomas 2018; Macrelli and Mackintosh, 2022).

Despite the scarcity of cases, nasopharyngeal linguatuliasis should be included in the differential diagnosis whenever a dog is presented with consisted symptoms. Unfortunately, previously exotic diseases may soon stop being so exotic and sparse for a number of reasons. Most recent cases involve dogs that were adopted from European countries with known high prevalence of *L. serrata* in stray dogs (Thomas M., 2018; Macrelli and Mackintosh, 2022). This does not impose risk only on the introduction of parasitosis like linguatualiasis, but on other infectious diseases

Figure 1. *L. serrata* expelled from the dog. Whitish transparent in color, dorsoventrally flattened with elongate shape like a vertebrate tongue. The anterior end is rounded and the posterior pointed. The cuticle presents with rings, while on the anterior, ventral side there are noticeable curved hooks with sharp tips around a central mouth.

as well. Even cases from human medicine involve people who migrated from areas where the parasite is more abundant in nature (Tappe et al., 2006). Moreover, modern human behaviors that could contribute in the spreading of zoonotic diseases should not be overlooked. Nowadays there is an increasing number of people that travel in foreign countries for vacation or in pursuit of a better future, taking their pets, and their parasites, with them. Also, the adaption of primitive feeding habits, which involve raw or insufficiently prepared food, viscera included, in the diets of humans and pets has gained a significant number of fans all around the world. Unfortunately, recommended practices of good hygiene and proper food preparation are often overlooked by the same groups of people, jeopardizing not only the health of their beloved pets but theirs too. The risk for bacterial food poisoning is quite obvious for the scientific world, but the same applies for the spread of parasitic diseases as well. Therefore, it is of supreme importance to constantly update the current databases with the humanly possible more recent information on the prevalence of infectious agents, parasites included, in order to reinforce the surveillance systems. Additionally, the guidance on correct practices of food management and preparation, whether it is for human or pet consumption, should be constant in the view of One Health. To the same direction, public should be properly informed about local or regional risks concerning infectious diseases, when relocating or just visiting foreign countries, in order to adopt appropriate preventative measures.

The aim of this report was to present a parasitic infestation that is a truly, to the best of the authors' knowledge, rare case in Greek veterinary practice and to raise awareness and concerns about diseases that are still treated as rare, even though living conditions, human habits and even climate have changed.

In conclusion linguatuliasis is a worldwide parasitosis with zoonotic importance that is also present in Greece Dogs that have the ability for free roaming and access to infected animal offal have a higher risk for *L. serrata* infestation. The use of macrocyclic lactone compounds in combination with the removal of parasites from the nasopharyngeal region is the suggested treatment for this parasitosis of veterinary importance and public health concern.

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