

Mixed infection with *Dirofilaria immitis*, *Linguatula serrata*, and *Toxocara canis* in a free-roaming dog: Three parasites with zoonotic potential

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Summary

This report describes mixed parasitic infestation with *Dirofilaria immitis*, *Linguatula serrata* and *Toxocara canis* in a free-roaming dog that was subsequently adopted. On physical examination no clinical manifestations were observed except for underweight. Hematoparasitologic examination revealed the presence of microfilariae, and the SNAP® 4Dx® Plus test was positive for circulating *D. immitis* antigens. Coproparasitological examination revealed the presence of *T. canis* eggs. The dog was treated for heartworm disease according to the recommendations of the European Society of Dirofilariosis and Angiostrongylosis (ESDA). Seven days after the initiation of treatment, the animal expelled an adult *L. serrata* worm, which led to the final diagnosis of mixed infection involving three parasites with zoonotic potential. The animal was fully recovered and put up for adoption. This report highlights the risk that free-roaming dogs pose to public and veterinary health.

Keywords: *Dirofilaria immitis*, *Linguatula serrata*, *Toxocara canis*, free-roaming dog, mixed infection

Since ancient times, dogs (*Canis lupus familiaris*) have played a complex role in human society. They are the most widespread pets worldwide (17). They have a positive impact on human psychology and health and are used as companions and helpers in a wide range of activities (hunting, defense, herding farm animals, transportation, etc.) (20). In addition to benefits from the presence of dogs in the proximity of humans, there are also some disadvantages related to the risk of zoonotic diseases, such as those with parasitic etiology. In general, dogs are exposed to a wide range of endoparasites, and stray dogs are at higher risk because they have access to natural areas where they interact with wildlife (3, 19). Dogs can serve as reservoirs for a large number of zoonotic parasites (1, 19), which requires continuous vigilance from physicians and veterinarians in their protection of global public health. The prevalence of parasitic infections in dogs varies widely in different regions of the world, depending on a number of factors, such as climate, lifestyle and behavioral habits, application or absence of prophylactic measures (1, 3, 19). Clinical manifestations, as

well, vary widely from asymptomatic to severe, and their occurrence is influenced by the parasite species, the degree of infestation, the animal's health status, and the presence of co-infections (19). A frequently encountered phenomenon is polyparasitism (17). The aim of this study was to present a case of mixed infection involving three parasites with zoonotic potential in a free-roaming dog without clinical signs of disease.

Case history

In March 2023, an intact mixed-breed female, approximately 4 years old, was presented to a private veterinarian's office for a general health screening. The history revealed that the dog was found wandering at the edge of a forest, approximately 15 km from the town of Târgoviște (44°55'27"N, 25°27'24"E), jud. Dâmbovița, România. No microchip, which would have helped identify the owners, was found. As the dog was friendly, the person who presented it expressed the wish to adopt it for his children.

The animal was clinically examined by general examination methods. At the time of the physical examination, it was in a stable clinical condition with no signs of disease, but it was underweight, with a body condition score of 3/9

and a muscle condition score of 2/4 (moderate muscle loss) (6). Apparent mucous membranes showed normal color, there was no discharge, and peripheral lymph nodes were within normal limits. Rectal temperature was 37.5°C, heart rate was 98 beats per minute (bpm), and respiratory rate was 37 breaths per minute (mpm).

Blood samples were collected for routine complete blood count (CBC) and biochemical analysis, as well as for parasite testing, in order to check for hemoparasites. The hematologic examination revealed anemia, with a decreased erythrocyte count (RBC: $3.96 \times 10^{12}/L$; reference interval (RI): $5.5\text{-}8.5 \times 10^{12}/L$), low hematocrit (26.9%), low hemoglobin (92 g/L; RI: 110-190 g/L), and thrombocytosis (PLT: $606 \times 10^9/L$; RI: $117\text{-}460 \times 10^9/L$). Biochemistry showed increased alanine aminotransferase activity (ALT: 256 U/L, RI: 0-13 U/L) and alkaline phosphatase (ALP; RI: 0-132 U/L). The other parameters were within reference limits.

The hemoparasitological examination included the modified Knott's test for the detection of microfilariae and the SNAP® 4Dx® Plus test (IDEXX Laboratories) for the qualitative detection of antigens and antibodies of various arthropod-borne pathogens (*D. immitis* antigens, *Anaplasma phagocytophilum*/*A. platys* antibodies, *Borellia burgdorferi* antibodies, *Ehrlichia canis*/*E. ewingii* antibodies). In addition, Giemsa-stained blood smears were performed for the microscopic detection of intraerythrocytic pyroplasmas (*Babesia* spp.). Rectal fecal samples were also collected for coproparasitological examinations.

Circulating microfilariae were detected in a blood sample (Fig. 1), and the SNAP® 4Dx® Plus test was positive for circulating *D. immitis* antigens and negative for the other parasites. There were no intraerythrocytic pyroplasmas. Fecal flotation examination revealed *T. canis* (Fig. 2).

In addition to the clinical examination, the patient was referred to an Imaging Center for chest radiography and ultrasonography of the heart. Chest control radiography from dorsoventral and lateral projections revealed no deviations, except for a slight bronchointerstitial pattern of the lung parenchyma, particularly in the caudal lung lobes. The echocardiographic assessment revealed no visible adult forms of *D. immitis* in the heart or pulmonary arteries. A slight regurgitant jet at the level of the mitral valve was identified, which did not appear to be associated with a significant hemodynamic compromise or structural abnormalities at the time of evaluation. No additional valvular dysfunctions, chamber dilation, or signs of myocardial pathology were observed during the imaging study.

On the basis of the history and the results of blood tests, supplemented by imaging investigations, the patient was diagnosed with a mixed parasitic infection, caused by *D. immitis*

and *T. canis*. Given the presence of *D. immitis* infestation, a broad-spectrum antiparasitic treatment was applied in accordance with ESDA (European Society of Dirofilariosis

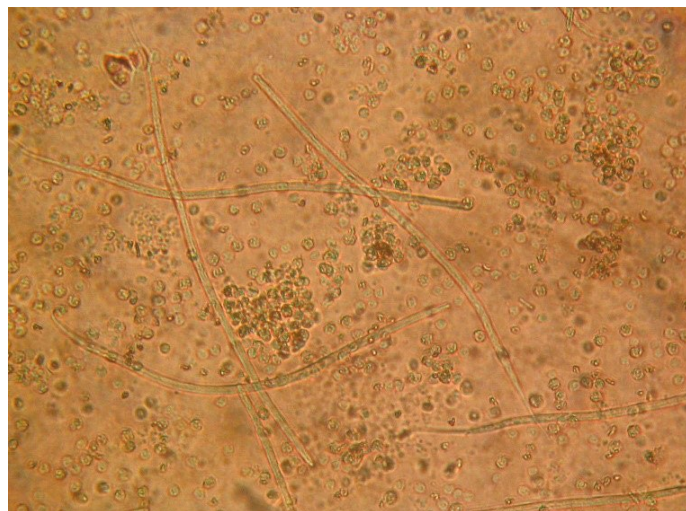


Fig. 1. *D. immitis* microfilariae after concentration with Knott's test, $\times 20$

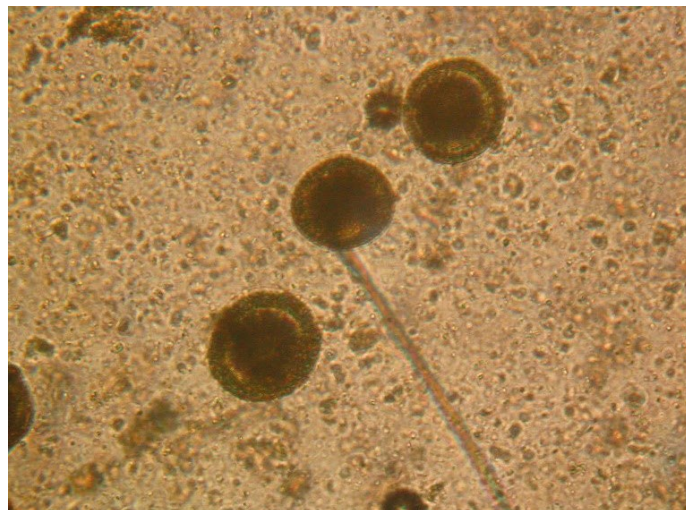


Fig. 2. *Toxocara canis* eggs, $\times 20$

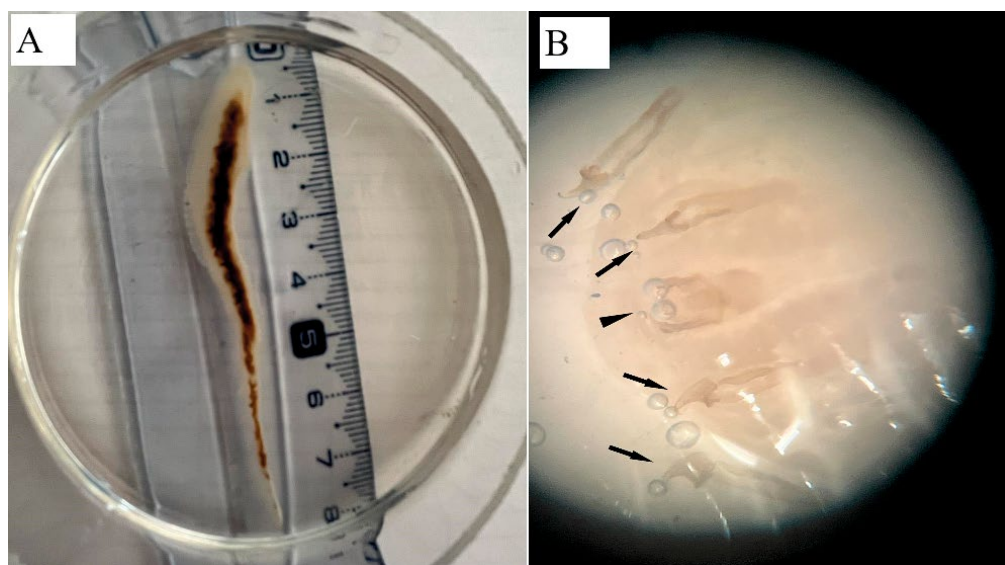


Fig. 3. A) Adult female *Linguatula serrata* expelled by sneezing; B) Ventral side of the anterior end, showing two pairs of hooks (arrows) located on either side of the mouth opening (arrowhead)

and Angiostrongylosis) recommendations. In brief, the treatment regimen included ivermectin (Ivermectin FP, Pasteur SA) 10 µg/kg body weight weekly for 6 months, combined with doxycycline (Ronaxan[®], Boehringer Ingelheim) 10 mg/kg daily for 30 days, with adjuvant medication of Silimarin (Silimarin, Biofarm) 100 mg/day for 2 months. In addition, prednisone (Prednicortone[®], Dechra) was administered at a dose of 0.5 mg/kg twice a day for the first week, 0.5 mg/kg/day for the second week, and 0.5 mg/kg every other day in the third and fourth weeks to reduce the inflammatory process in the lung. The animal was kept under constant observation and placed in a dog playpen to prevent it from coming into contact with other animals or people.

Seven days after the treatment started, following a severe sneezing episode, the dog expelled a worm-like parasite, which was collected in a saline (0.9% NaCl) container and sent for identification. Based on its main morphological characteristics, the parasite was identified as *L. serrata* (2, 11). It presented a whitish-transparent body with a characteristic tongue-like shape, slightly convex dorsally and flattened ventrally. The body length was 7.8 cm, with the anterior end rounded and a sharply pointed posterior end (Fig. 3A). Stereomicroscopic examination revealed the presence of a transversely striate cuticle on the dorsal side of the body, while on the ventral view of the anterior extremity, a subterminally arranged mouth opening was evident, flanked by two pairs of prominent hooks, one pair on each side of the mouth (Fig. 3B). As a result, the initial diagnosis of polyparasitism was completed by adding *L. serrata* infestation.

The animal was clinically re-evaluated, and no other changes were noted. It was recommended that blood and fecal examinations be repeated weekly for the first month and then monthly for 6 months. Fourteen days after the initiation of treatment, coproparasitological tests were negative for *T. canis* and *L. serrata*. After 30 days, the modified Knott's test was negative for circulating microfilariae, and the SNAP[®] 4Dx[®] Plus test was negative after 60 days. Furthermore, it remained negative after 90 days. A continued treatment with a monthly dose of milbemycin was recommended.

Discussion

This report presents an interesting case of polyparasitism in a free-roaming dog (abandoned, lost, or runaway) that was to be adopted by a family with children. The characteristic feature of this clinical case was the involvement of three parasites with zoonotic potential, although the animal showed no clinical signs of disease except for underweight.

The actual provenance of the dog could not be established, but given its temperament and the way it reacted to the people who found it, it may have previously had a social relationship with humans. It had either been abandoned or had wandered off and failed to find its way home, having to roam and search for food in the forest. As a result, interaction with wildlife and vectors was unavoidable, exposing the dog to infestation with various parasites, including *D. immitis*, *L. serrata*, and

T. canis. All these parasites are of significant importance to public and veterinary health.

D. immitis is a filarial nematode transmitted by different species of mosquitoes, implicated in the production of heartworm disease in dogs worldwide (21), including Romania (22). In addition, this nematode can affect other domestic and wild carnivores, as well as the human population (21). In dogs, the course of the disease is usually chronic, and clinical manifestations develop gradually, ranging from asymptomatic to severe, sometimes even fatal, depending on the parasite load, individual reactivity, and intensity of the effort to which the animal is subjected (21, 23). As it emerged from the anamnesis, the patient in the present study was found in a region endemic for *D. immitis*, in which previous studies determined a prevalence of 12.63% (22). In addition, other zoonotic filarioids have been identified in this region, such as *D. repens* (22) and *Onchocerca lupi* (24), which demonstrates the existence of favorable climatic conditions to the presence and distribution of the specific vectors. The clinical examination revealed no clinical signs compatible with cardiopulmonary dirofilariasis, except for a diffuse bronchointerstitial pattern in the lung parenchyma, detected radiographically. Given the duration of the nematode's life cycle and factors that may influence the course of clinical manifestations, these findings may be attributed to a low parasite load or recent infection (21, 23). The echocardiographic results, which did not reveal the presence of worms in the visual field, support this hypothesis. In the absence of adulticide medication (melarsomine dihydrochloride) on the domestic market, a combination of ivermectin and doxycycline was administered. According to previous studies, the use of a combination of ivermectin with doxycycline results in the elimination of circulating microfilariae as well as the "sterilization" of worms due to the combined action of the two drugs, although the cause of their adulticidal effect is not clear (4). Thus, the use of this combination results in the destruction of both immature and mature embryonic stages of *D. immitis*, as well as the depletion of the bacterial endosymbiont *Wolbachia* in all life forms of the parasitic nematode (4, 10). Indeed, previous experimental studies have shown favorable results after a longer period of time compared to the result recorded here, probably due to a higher parasite load, as well as the treatment protocol applied (10).

The presence of canine dirofilariasis poses a risk to the human population living in endemic areas, although only a small number of human cases of *D. immitis* have been recorded in Europe to date (7). Most of them showed pulmonary location, often with an asymptomatic clinical course, but in some cases severe signs were recorded, with chest pain, cough, low fever, hemoptysis and malaise, etc. (7). Ocular and cutaneous locations have also been reported (5).

D. immitis is becoming an increased public health concern in Europe, with seroprevalence rates in humans of 9.3% in Spain, 7.8% in Italy, 6.1% in Portugal, 3.8% in Greece, 2.6% in Serbia (7), 7.4% in Romania, and 14.8% in Moldova (5).

L. serrata is a pentastomid, phylogenetically related to crustaceans, which in the adult stage populates the nasal passages and paranasal sinuses of domestic and wild carnivores (dogs and wolves) considered as definitive hosts (2, 11). Infested animals contaminate the external environment with eggs via nasopharyngeal secretions or, if secretions are swallowed, via feces, contributing to parasite dispersal. Domestic and wild herbivores (cattle, sheep, goats, hares), which are the natural intermediate hosts, become contaminated by the consumption of grass infested with eggs shed by the definitive hosts (18). In Romania, Negrea et al. (18) reported a prevalence of 4.9% for *L. serrata* in dogs, although the true prevalence is likely to be underestimated, as the disease often evolves asymptotically (15). In addition, recent studies in different European countries have reported cases of linguatulosi in adopted stray dogs from Romania (15). On the other hand, the presence of this parasite in Romania has been reported in wild animals, in both intermediate hosts (hares) (11) and definitive hosts (golden jackals, gray wolves, and foxes) (2). Given that the patient was a free-roaming dog living at the edge of a forest, away from human settlements, it is very likely that it became infected by consuming organs from wild animals with linguatulosi, such as wild rabbits, which would concur with the results of other studies. In the case reported here, neither nasal secretions nor eggs of *L. serrata* were observed in the feces, making it difficult to detect this parasite at the initial stage of patient evaluation. The absence of eggs in the feces at the time of examination may be a consequence of the fact that the embryonated eggs of *L. serrata* are predominantly excreted into the external environment through nasal secretions and only a small fraction is swallowed and excreted in the feces. In addition, the elimination of eggs through feces is an intermittent phenomenon (15). In the current case, elimination of the parasite may be attributed to the administration of treatment for heartworm disease (ivermectin), which appears to have some efficacy against *L. serrata* (15), although a specific treatment against this pentastomid has not yet been developed. Despite the lack of nasal secretions and eggs of *L. serrata* in feces, and the fact that only one parasite was detected in the present case, the potential zoonotic risk should not be ignored, especially since canine linguatulosi often evolves asymptotically (15). Humans can contract the disease by consuming food contaminated with nasal secretions or feces of permanently infested hosts, thus becoming intermediate hosts. The result is "visceral linguatulosi", characterized by the formation of parasitic granulomas in the liver, spleen, lungs, eyes or lymph nodes as a result of the migration of

L. serrata larvae to these organs (11, 15). However, they can also become definitive hosts by ingesting raw or undercooked meat or viscera from infested intermediate hosts (11, 15). After ingestion of infested food, the larvae of *L. serrata* are released from the cysts and migrate from the stomach into the esophagus and from there into the pharynx, nose, or lungs, where they remain, causing "nasopharyngeal linguatulosi", also known as Marrara syndrome or Halzoun syndrome. This form of the disease is characterized by headaches, rhinorrhea, epiphora, dyspnea, cough, sneezing, dysphagia, facial edema, vomiting, or hemoptysis (15). In Europe, human linguatulosi is rare, with only a few cases reported in Bulgaria (16), Austria, Portugal (12), and Italy (15).

T. canis is a widespread nematode throughout the world and is considered one of the most common zoonotic parasites found in dogs (1, 9, 20), but it can also affect wild canids, such as wolves, foxes, and jackals (14). It affects dogs of all ages, which can contract the disease by ingestion of embryonated eggs from the external environment or larvae via paratenic hosts (e.g. rodents) (1). As already mentioned, the dog presented here was found wandering at the edge of a forest, so the possibility of *T. canis* infestation was very high. In agreement with previous studies, access to the outdoors and geographical territory are the main factors favoring parasitic infection, especially in free-roaming dogs (19). Typically, a contaminated animal sheds eggs in feces, which can become infestive under favorable environmental conditions. Their presence in the external environment poses a major zoonotic risk, as they can also be accidentally ingested by humans, leading to toxocariasis (14). Humans can contract the disease by consuming infested water or raw vegetables, or by touching contaminated soil or fur of diseased animals and failing to wash hands afterwards (hence the name "dirty hands disease") (20). Although humans are not the definitive host for *T. canis*, infesting larvae migrate through the body, producing *larva migrans visceralis*, *larva migrans ocularis*, neurotoxocariasis, and covert toxocariasis (14). The most common form is *larva migrans visceralis* expressed by cough, wheezing, myalgia, pruritus, eczema, lymphadenopathy, hepatosplenomegaly, or nodular myocarditis. *Larva migrans ocularis* is characterized by chronic endophthalmitis, retinitis, posterior/peripheral granulomas, and blindness, whereas neurotoxocariasis is expressed by headache, epilepsy, fever, seizures, schizophrenia, and cognitive deficits. Covert toxocariasis is associated with a wide range of mostly nonspecific signs, such as weakness, pruritus, rash, pulmonary dysfunction, pulmonary insufficiency, abdominal pain in adult patients, whereas fever, anorexia, headache, nausea, abdominal pain, vomiting, wheezing, lethargy, somnolence, behavior disturbances, etc. are seen in children (20). Thus, the clinical signs vary widely depending on the location of the infesting larvae, and many cases

remain undiagnosed due to asymptomatic, mild, or non-specific course of infection, which makes this disease underdiagnosed in humans (14). The prevalence rate of serum anti-*Toxocara* antibodies in the human population in Europe averages 10.5% (8.5-12.8%), with estimated seroprevalence rates of 2.4% in Denmark, 6.3% in Austria, 7% in Sweden (20), 6.1% in Portugal (8), 3.6% in the Czech Republic, and 8% in Italy (13). In Romania, recent studies have established a prevalence of 22.64% in the western part of the country, which is higher than seroprevalence rates found by other studies in Europe (13).

According to previous studies, the prevalence of parasites with zoonotic potential risk has typically been high in pets, even more so in free-roaming dogs (19), and the close proximity in which dogs and humans live increases the risk of parasite transmission to humans (17). Dogs free roaming over large territories are especially prone to parasite infection as a result of predation and consumption of intermediate or paratenic hosts of different parasites, as well as lack of treatment (19). In addition, it has previously been shown that dogs can be carriers of zoonotic parasites even in the absence of clinical signs (19), as in the case reported here. This may increase the risk of the spread of parasitic diseases, including those with zoonotic potential, such as heartworm disease, linguatulus, and toxocarosis. Permanent awareness-raising campaigns are needed to increase the awareness of the risk that free-roaming dogs pose to public health, especially since the problem of stray dogs remains an unresolved issue in Romania. Dog owners or persons wishing to adopt dogs should be made aware of the importance of taking their dogs to the veterinarian for regular check-ups and appropriate treatment to prevent the spread of zoonotic parasitic diseases. Careful monitoring of animals can thus maintain their health, as well as the health of humans and the environment, in line with the One Health concept.

This report highlights the risk that free-roaming dogs pose to public and veterinary health and the need for rigorous examination before they are adopted (introduced into the community). Contact with wildlife and movement over large territories put free-roaming dogs at increased risk of mixed infection with parasites, such as *D. immitis*, *L. serrata*, and *T. canis*, which are etiological agents with proven zoonotic potential.

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