

## Case Report

# Clinical case of life-threatening co-infection due to *Dirofilaria immitis* and *Aelurostrongylus abstrusus* in a cat: First report of feline heartworm disease in Bulgaria

A. S. TONEV<sup>1</sup>, Z. KIRKOVA<sup>1</sup>, P. T. ILIEV<sup>1,\*</sup>, A. ROUSSENOV<sup>2</sup>, T. CHAPRAZOV<sup>3</sup>, R. ROYDEV<sup>3</sup>, N. PIROVSKI<sup>4</sup>

<sup>1</sup>Department of Veterinary Microbiology, Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria, E-mail: \*[petyo\\_todorov@abv.bg](mailto:petyo_todorov@abv.bg), [3333tonev@gmail.com](mailto:3333tonev@gmail.com), [z.t.kirkova@abv.bg](mailto:z.t.kirkova@abv.bg); <sup>2</sup>Department of Internal Noninfectious Diseases, Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria, E-mail: [vetrousseov@abv.bg](mailto:vetrousseov@abv.bg);

<sup>3</sup>Department of Veterinary Surgery, Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria, E-mail: [tz\\_chaprazov@yahoo.com](mailto:tz_chaprazov@yahoo.com), [rumen\\_tanev@abv.bg](mailto:rumen_tanev@abv.bg); <sup>4</sup>Department of Anatomy, Medical Faculty, Trakia University, 6000 Stara Zagora, Bulgaria, E-mail: [pirovski@abv.bg](mailto:pirovski@abv.bg)

### Article info

Received May 18, 2020  
Accepted September 1, 2020

### Summary

The present report describes the first clinically manifested and serologically proven case of *Dirofilaria immitis* infection in a cat in Bulgaria. A 10-year-old intact male cat was referred to the Small Animal Clinic, Trakia University with a history of anorexia, weight loss, intermittent coughing and itching skin lesions on the head and neck. Physical examination revealed abnormal heart sounds and respiration, cyanosis of the mucous membranes, and generalized enlargement of the lymph nodes. Mild infestation with hard ticks and fleas was also detected during the initial skin inspection. In addition, adult *Otodectes cynotis* mites were observed in the skin lesions. The fecal sample was positive for larvae of *Aelurostrongylus abstrusus* and eggs of *Toxocara cati*. Blood serology revealed antigens of *D. immitis* as well as antibodies against both feline immunodeficiency virus and *D. immitis*. Thoracic radiographic findings included a pronounced generalized reticular interstitial pattern; alveolar and bronchial shades with multiple nodular thickenings throughout the lungs. Electrocardiography demonstrated a sinus tachycardia, a peaked P-wave (P-pulmonale) and an abnormally low ST-segment. The clinical signs disappeared after treatment with selamectin, doxycycline and corticosteroids. Despite the improvement in general health condition, the cat suddenly died several months later.

**Keywords:** Feline heartworm disease; Dirofilariosis; cat; *Aelurostrongylus abstrusus*; *Dirofilaria immitis*; Bulgaria

### Introduction

Cats are one of the most common companion animals establishing more frequent and closer contact with humans than any other pets. Outdoor lifestyle is a common occurrence in cats raised in rural areas in Bulgaria and, therefore, the animals may acquire infections with various parasite species, including the mosquito-borne filaroid *Dirofilaria immitis*. This helminth is the causative agent of Heartworm disease (HWD), which is currently categorized as a severe and potentially fatal cardio-pulmonary disease, primarily

affecting dogs, and also other carnivores, including domestic and wild felids (Pana *et al.*, 2020). The infection in cats is widely reported in regions of several European countries including Spain, Italy, France, Greece and Portugal, with an increasing frequency in the areas where the disease is endemic in dogs (Morchon *et al.*, 2012; Montoya-Alonso *et al.*, 2014; Traversa & Di Cesare, 2014; Montoya-Alonso *et al.*, 2017; Diakou *et al.*, 2018). No results of single cases or epidemiological studies have been reported in Bulgaria to reveal the presence and spread of feline HWD. It is now generally accepted that HWD may occur in cats in any area where dogs are

\* – corresponding author

infected, with prevalence rate of infection in cats between 5 % and 20 % of that for dogs in the same geographic area (Montoya-Alonso *et al.*, 2014).

The cats are considered as imperfect hosts for *D. immitis*, and the following differences may be observed when compared to dogs: most juvenile worms die shortly after arriving in the pulmonary arteries, initiating an inflammatory response; relatively low adult worm burden (2 to 4 worms being the usual burden), prolonged pre-patent period (7 – 8 months), lack or short duration of microfilaremia; about 20 % of infected cats are microfilaremic; approximately one third of adult *D. immitis* are of the same sex; short life span of adult worms (Venco *et al.*, 2008; Montoya-Alonso *et al.*, 2014; Venco *et al.*, 2015). Another indications defining the cat as an imperfect host of *D. immitis* is that the infection usually aborts when develops in cat (dead-end host); aberrant migration occurs more frequently in cats than in dogs, involving body cavities, systemic arteries and the central nervous system; possibility of spontaneous self-cure due to the short life span of parasites; reduced size of adult worms, etc (McCall *et al.*, 2008; Venco *et al.*, 2015; Pennisi *et al.*, 2020).

The clinical manifestations of HWD in cats substantially differ when compared with dogs, and comprise two clinical syndromes: heartworm-associated respiratory disease (HARD) and adult HWD (Garrity *et al.*, 2019). HARD may be induced by the immature worms as soon as they reach the caudal pulmonary arteries. Most of these worms die about 3 – 4 months post-infection, inducing an intense and profound vascular and parenchymal inflammatory response (endo-mesoarteritis with occlusive hypertrophy). This acute reaction is considered more severe in cats compared with dogs due to the increased activity of pulmonary intravascular macrophages in cats that are absent in dogs (Dillon *et al.*, 2008; Pennisi *et al.*, 2020). Clinically, HARD may be manifested by a wide range of non-specific signs e.g. intermittent coughing, dyspnea and vomiting. Once the initial acute inflammatory processes partially subside, a few live immature worms (usually 2 – 4) continue their development and turn into adults in the heart. It is believed that heartworms may secrete a product that downregulates the activity of the pulmonary intravascular macrophages and thus modulates vascular responses, resulting in an anti-inflammatory effect that minimizes clinical signs in infected cats (Lee & Atkins, 2010). In this regard, most cats are usually symptomless or with only mild symptoms, including persistent dyspnea, increased respiratory effort, tachypnea, intermittent coughing, intermittent vomiting unrelated to eating, anorexia and weight loss (chronic course of the infection). Once the adult worm dies, life-threatening complications appear, including very severe pulmonary inflammation, acute anaphylactic reaction, and thromboembolism. These forms of HWD may cause sudden death, which is not a rare event and may occur with or without previous clinical signs, and with infections by as few as one worm (Litster & Atwell, 2008; Venco *et al.*, 2015). These circumstances regarding the clinical course of the infection are very likely to be the main reasons of misdiagnosis of

the disease; therefore, several diagnostic tests should be carried out. The infection with *D. immitis* in dogs is usually diagnosed by detection of the microfilariae in the peripheral blood and antigen tests (Pereira *et al.*, 2018). In contrast, the most efficient approach to the diagnosis of feline HWD is based upon a synergic association of the following tests: thoracic radiography, serum antibody and antigen tests, and echocardiography (Venco *et al.*, 2015; Diakou *et al.*, 2018).

The metastrongyloid *Aelurostrongylus abstrusus* is regarded as one of the most important feline lungworm causing parasite-induced respiratory infection (verminous pneumonia) in felids (Giannelli *et al.*, 2017). The adult nematodes reside in the bronchioles, alveolar ducts and alveoli, where they cause bronchiolitis, interstitial or bronchopneumonia and thicken the pulmonary blood vessels (Giannelli *et al.*, 2017; Traversa & Di Cesare, 2014; Pennisi *et al.*, 2015). Clinical manifestations of the infection considerably vary from asymptomatic to very severe respiratory signs, including a chronic cough and gradually increasing dyspnea accompanied with wheezing, sneezing and nasal discharge (Schnyder *et al.*, 2014).

The purpose of the current case report is to present data on the clinical manifestation and laboratory diagnostic approaches in a cat with co-infection due to *D. immitis* and *A. abstrusus*, as well as to record the first case of feline HWD in Bulgaria.

## Materials and Methods

### Case presentation

This case concerns a 10-year-old intact tomcat of mixed breed, weighing 3.800 kg, referred to the Small Animals Clinic, Trakia University, Bulgaria in February 2019, for examination and treatment. The cat originated from a rural outskirts of Stara Zagora city (42°25'N, 25°38'E). The animal lived indoor but a long period of roaming with several other cats has been also allowed. The cat was not vaccinated and the deworming has not been applied regularly. A month ago, the owner noticed wounds on the head and on the neck, and the attempted treatment with polyvidone-iodine has been unsuccessful. As recalled by the owner, during the last few days before visiting the Small Animal Clinic, the cat demonstrated intermittent coughing, anorexia and weight loss.

### Blood examinations

A blood sample was collected by venipuncture of *V. cephalica antebrachii* into vacutainer tubes, containing Lithium heparin or K<sub>2</sub>EDTA, and was submitted for routine hematological examination using an automatic cell counter (Shenzhen Mindray Bio-Medical Electronics Co., Ltd., China). Blood chemistry was performed by means of automatic biochemical analyzers (Reflotron Plus, Germany and Mindray BS-120, China).

Post-centrifugation plasma was assayed using three rapid tests for detection of *D. immitis* antigens (SNAP Heartworm RT Test, IDEXX lab., USA and SNAP 4Dx Plus Test, IDEXX lab., USA) and

host-specific antibodies (Anigen Rapid FHW Ag/Ab Test, BION-OTE Co., Ltd, South Korea). The cat was also tested for FIV and FeLV infections (VETSCAN FeLV/FIV Rapid Test, Abaxis Inc., USA). These tests were performed following the manufacturer's instructions. A blood sample was also processed by Knott's technique (Zajac & Conboy, 2012) for detection of microfilariae.

#### Fecal examination

A fecal sample was examined by following methods: direct smear for detection of motile trophozoites or cysts of protozoa; flotation method using saturated sodium chloride (sp. gr. 1.20) for extraction of lighter helminth eggs and coccidian oocysts or sporocysts; Baermann's technique for detection of lungworm first stage larvae (Zajac & Conboy, 2012).

#### Skin examination

The skin was initially inspected for presence of visible arthropods. For mycological culturing, a sample was collected from affected areas by brushing the skin using sterilized toothbrush (McKinsey's toothbrush technique) according to Hnilika & Paterson (2017). The sample was then cultivated aerobically on Mycosel agar (Merck, Becton Dickinson, USA), at temperature of 26°C for 3 weeks. Swab samples from both ear canals and deep and superficial scraping from the skin lesions were collected and were mixed with lactic acid, mounted on glass slides and coverslipped for microscopic examination. Isolated ectoparasites were identified by morphometrical characteristics of the adult stages described by Zajac & Conboy (2012).

#### Radiography

Thoracic radiography was performed in LL and VD projections using Philips Super 50 CP-D (Philips Diagnostic TH, Germany). Digital processing was achieved by iQ-view/Pro User Manual version 2.7.0 Int., EN200R – compatible with DICOM.

#### Electrocardiography

Cardiac electrocardiography was recorded by an EDAN VET ECG Model-VE-300 (Germany) using the three standard bipolar and the three unipolar electrodes as well as one central electrode. The bandwidth was 25 mm/s and the calibration voltage was 1 mV=10 mm. The contact between the skin of the animal and the apparatus was made with crocodile clips and TOP-RANK Electrodes Conductive Gel.

#### Ethical Approval and/or Informed Consent

The authors declare that all examinations performed were within the scope of a veterinary examination.

#### Results

##### Clinical findings

The cat was in poor body condition, apathetic, flaccid, with reduced skin elasticity, cyanosis of visible mucous membranes, and delayed reflexes. There was a severe inflammation of the skin with vague shaped and uneven lesions on the head and the neck (Fig. 1). Similar changes were observed on the skin of the ear

Table 1. Hematological parameters of a cat with *Dirofilaria immitis* and *Aelurostrongylus abstrusus* co-infection.

Parameters	Results and interpretation*	Reference ranges
White blood cell count (WBC), 10 <sup>9</sup> /L	8.33	5.50 – 19.5
Neutrophils, 10 <sup>9</sup> /L	7.24	3.12 – 12.58
Neutrophils, %	86.9 ↑	38.0 – 80
Lymphocytes, 10 <sup>9</sup> /L	0.59 ↓	0.73 – 7.86
Lymphocytes, %	7.1 ↓	12.0 – 45
Eosinophils, 10 <sup>9</sup> /L	0.36	0.06 – 1.93
Eosinophils, %	4.3	1.0 – 11.0
Basophils, 10 <sup>9</sup> /L	-	0 – 0.12
Basophils, %	-	0.0 – 1.2
Monocytes, 10 <sup>9</sup> /L	0.14	0.07 – 1.36
Monocytes, %	1.7	1.0 – 8.0
Haemoglobin, g/L	113	85 – 153
Haematocrit, %	37.8	30 – 50
Red blood cell count (RBC), 10 <sup>12</sup> /L	6.98	4.60 – 10.20
Mean corpuscular volume (MCV), fL	54.1	38.0 – 54.0
Mean corpuscular hemoglobin (MCH), pg	16.2	11.8 – 18.0
Mean corpuscular hemoglobin concentration (MCHC), g/L	299	290 – 360
Red cell distribution width (RDW-SD), fL	40.4	26.4 – 43.1
Platelets, 10 <sup>9</sup> /L	330	200 – 500
Mean platelet volume (MPV), fL	12.4	9.9 – 16.3

\* ↓ - decreased value; ↑ - increased value



Fig. 1. A large skin lesion on the head.

canals. Purulent discharges were visible from the eyes and nose. Mandibular, retropharyngeal, scapular, and inferior lymph nodes were enlarged without adhesions and painless. An infestation with several *Ixodes* spp. ticks and the fleas *Ctenocephalides felis* was noted. The cat was afebrile (37.6°C), pulse rate - 140 /min, respiratory rate - 64 /min. The capillary refill time was approximately 3 – 4 seconds. Heart auscultation revealed muffled heart tones and protosystolic heart murmur 3/6 on the left and 4/6 on the right. There was neither enlargement of jugular veins nor retrograde venous pulse. Breathing was rapid, shallow and difficult in both phases.

During the auscultation, wet wheezes were detected throughout the lung fields. Examination of the digestive system revealed anorexia, presence of broken teeth and stomatitis, reduced intestinal peristalsis, and soft and painless abdomen. Lack of defecation and urination for the last 24 hours was also noted. The possibility of developing cardiopulmonary collapse was avoided by administering dexamethasone (Dexamethasone, Alfasan International BV, Netherlands) 0.5 mg/kg, intramuscularly.

#### Blood examinations

Complete blood cell count revealed only mild relative neutrophilia and relative lymphopenia (Table 1). Blood biochemical analysis showed hyponatremia, hypophosphatemia, bilirubinemia, and low urea levels (Table 2).



Fig. 2. Results of serologic assays demonstrating positive reactions for FIV and *Dirofilaria immitis* infections

Table 2. Serum biochemical parameters of a cat with *Dirofilaria immitis* and *Aelurostrongylus abstrusus* co-infection.

Parameters	Results and interpretation*	Reference ranges
Potassium, mmol/l	4.20	4 – 5.30
Sodium, mmol/l	147.8 ↓	151 – 158
Phosphorous, mmol/l	1.22 ↓	1.4 – 2.0
Calcium, mmol/l	2.03	1.6 – 3.0
Total protein, g/l	57.2	57 – 80
Albumin, g/l	20.2	20.0 – 39
Total bilirubin, μmol/l	12.5 ↑	up to 8.5
Blood urea nitrogen (BUN), mmol/l	2.4 ↓	6.0 – 10.0
Creatinine, mmol/l	86	60 – 160
Alanine aminotransferase (ALAT), IU/l	45	20 – 100
Gamma-glutamyl transferase (GGT), IU/l	13	0 – 27
Aspartate aminotransferase (ASAT), IU/l	28	12 – 70

\* ↓ - decreased value; ↑ - increased value

Circulating *D. immitis* microfilariae were not detected but the serological examination showed positive reactions for both *D. immitis* antigens and host-specific antibodies (Fig. 2). In addition, the cat was negative for antibodies against *Anaplasma platys*/*Anaplasma phagocytophilum*, *Ehrlichia canis*/*Ehrlichia ewingii*, and *Borrelia burgdorferi*. Serological screening also showed no presence of FeLV antigens but detected antibodies against FIV (Fig. 2).

#### Fecal examination

The coprological examination revealed the presence of helminth eggs and larvae. Based on the morphometric characters, *T. cati* and *A. abstrusus* were detected (Fig. 3). Intensity of infection with *A. abstrusus* was 15,100 larvae per gram of feces (LPG).

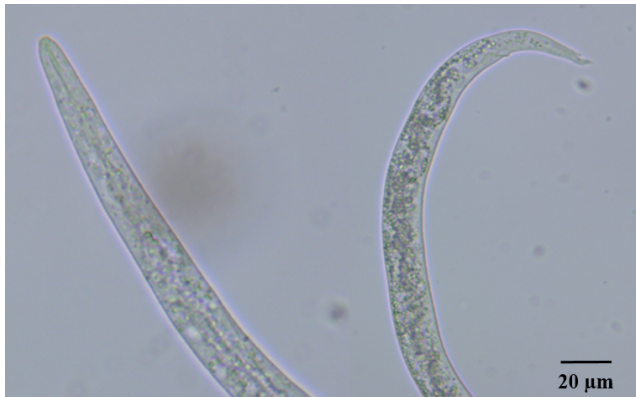


Fig. 3. Anterior extremity and tail of *Aelurostrongylus abstrusus* first stage larva

#### Skin examination

Swab sample and the material from the skin scraping were positive for *Otodectes cynotis* (Fig. 4). Numerous colourless oval-shaped eggs were also found. The mycological examination showed absence of dermatophytes after 14 days of cultivation.

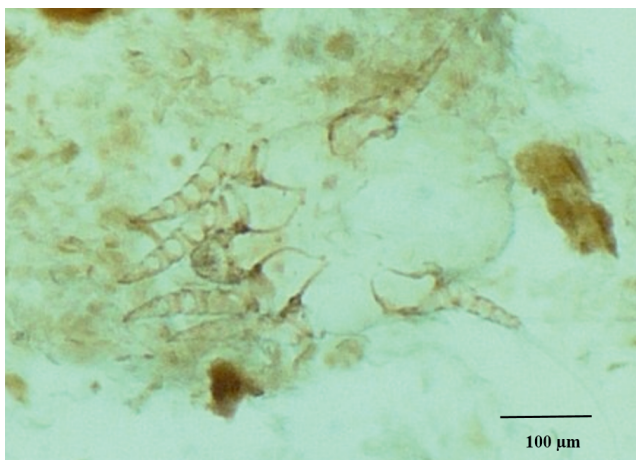


Fig. 4. Adult *Otodectes cynotis*

#### Thoracic radiography

The trachea was well visualized and slightly elongated with no change in its position; rounded cranial and caudal borders of cardiac shadow; vaguely outlined cardiofrenal triangle; caudally displaced cardiac tip; increased contact of cardiac shadow with the sternum; enlarged pulmonary arteries in the hilus and middle part of the lungs; pronounced reticular interstitial pattern; alveolar and bronchial shades with multiple nodular thickenings throughout the lungs (Fig. 5 and Fig. 6).

#### Cardiac electrocardiography

Electrocardiography demonstrated cardiac changes including high-frequency sinus rhythm (HR-166 bpm), increased P-wave amplitude ( $2.68 \pm 0.07$  mV) in 2<sup>nd</sup> bipolar evacuation, deep ST-segment depression ( $-0.26 \pm 0.02$  mV), strongly negative (inverted) T-wave amplitude ( $-0.56 \pm 0.02$  mV) and no deviation in the middle electrical axis of the heart ( $+39$  degrees) (Fig. 7).

#### Treatment and follow-up

The cat was hospitalized in the Small Animal Clinic's Isolation ward. The supportive therapy included butaphosphan and Vitamin B12 (Catosal 10 %, Bayer Animal Health, Germany); Vitamins A, D3 and E (AD3E, Vetprom, Bulgaria) and Vitamin C (Vitamin C, Biovet, Bulgaria). A systemic antimicrobial therapy was performed with amoxicillin/clavulanic acid (Synulox, Zoetis, USA) at a dose of 8.75 mg/kg body weight, subcutaneously, once daily for 5 days. Topical selamectin (Stronghold, Zoetis, USA) was also applied because of its high efficiency against *T. cati*, *A. abstrusus*, larvae of *D. immitis*, and numerous arthropods (fleas, ticks, mites).

At the 5<sup>th</sup> day of treatment, Synulox administration was discontinued and was replaced with oral Doxycyclin (STADA, Stada Arzneimittel, Germany) at a dose of 10 mg/kg body weight, for one month. After finishing the doxycyclin therapy, at the 30<sup>th</sup> day, new physical, laboratory and imaging examinations were performed. The cat demonstrated no clinical signs and an improvement in the general health condition. The skin scraping revealed no presence of *O. cynotis* mites. Also, the fecal examination showed the lack of *T. cati* eggs but *A. abstrusus* larvae (200 LPG) were still being detected. Blood biochemistry and morphology was normal. Radiographic and electrocardiographic tests also showed an improvement in cardiac and lung function. Meanwhile, further treatment with topical selamectin was performed. After another month, the cat was treated for third time with selamectin because few larvae of *A. abstrusus* were detected. Shortly before the next scheduled examination (day 90), the owner informed us that the animal had suddenly died and refused an autopsy.

#### Discussion

Dogs and other canids are recognized as specific final hosts and natural reservoirs of the filarial helminth *D. immitis* causing canine HWD, a very serious and life-threatening infection. However, the

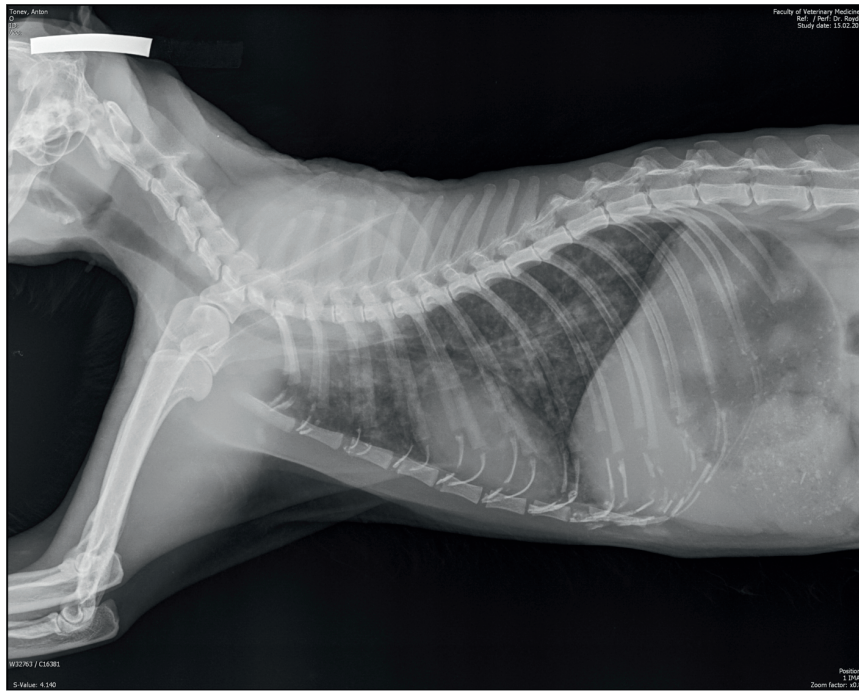


Fig. 5. Radiography demonstrating generalized reticular interstitial pattern, alveolar and bronchial shades with multiple nodular thickenings in a cat with *Dirofilaria immitis* and *Aelurostrongylus abstrusus* co-infection (lateral view)

cats are also considered susceptible but not specific (relatively resistant) hosts. The course of HWD in cats significantly differs than that in dogs, making diagnosis more difficult and complicated. Generally, diagnosis depends on a comprehensive evaluation incorporating history, clinical signs, detection of microfilariae in peripheral blood, antigen and antibody serology, diagnostic imaging, and various other ancillary tests (Garrity *et al.*, 2019). The patient involved in this study demonstrated intermittent coughing, anorexia, weight loss, cyanosis of the mucous membranes and abnormal respiration that seems to be in general agreement with recently published case report by Pana *et al.* (2020). Chronic vomiting and cachexia have been also reported and can be the most obvious clinical manifestations (Dillon *et al.*, 2000; Pennisi *et al.*, 2020). In this regard, Atkins *et al.* (2000) found that more than half of infected cats (64 %) showed respiratory abnormalities (e.g. dyspnea and cough), whereas 34 % showed vomiting as well.

In the present case, heart auscultation revealed muffled heart tones and protosystolic heart murmur; however, electrocardiography demonstrated cardiac changes including tachycardia, increased P-wave amplitude, deep ST-segment depression and inverted T-wave amplitude. These findings may indicate a variety of cardiac and/or respiratory syndromes, including right atrial enlargement and pulmonary thromboembolism. According to Venco *et al.* (2015), feline HWD does not involve right cardiac chambers and therefore the results of electrocardiography are not of primary importance. Although referred as less efficient and not capable of giving useful information, we believe that electrocardiography might provide valuable findings useful for the diagnosis of feline

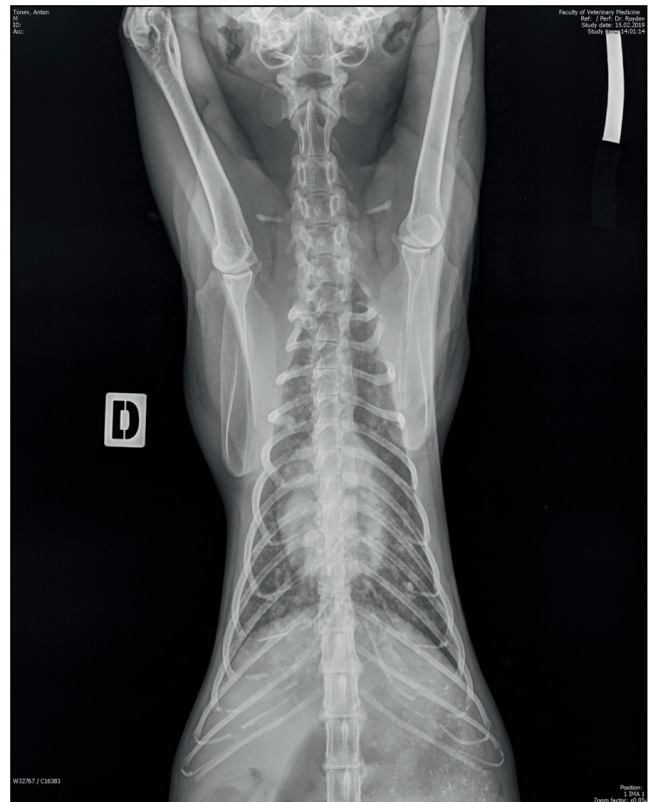


Fig. 6. Radiography demonstrating generalized reticular interstitial pattern, alveolar and bronchial shades with multiple nodular thickenings in a cat with *Dirofilaria immitis* and *Aelurostrongylus abstrusus* co-infection (ventrodorsal view)

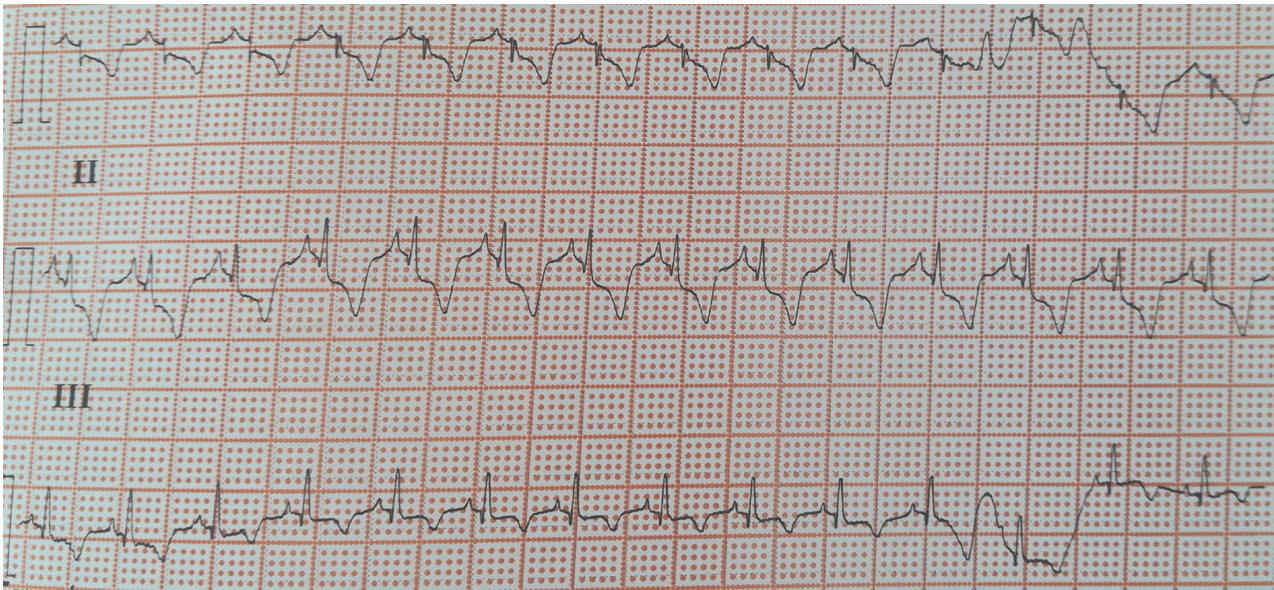


Fig. 7. Cardiac electrocardiography showing an increased P-wave amplitude, deep ST-segment depression and strongly negative (inverted) T-wave amplitude in a cat with *Dirofilaria immitis* and *Aelurostrongylus abstrusus* co-infection

HWD, especially in cases when cardiac failure has already appeared. Unlike dogs, right cardiac chambers in cats well bear pulmonary hypertension and right cardiac failure is an unusual finding (Dillon *et al.*, 1995; Atkins *et al.*, 1995; Atkins *et al.*, 2000). Heartworm infection in cats is basically pulmonary in nature; however, some affected cats very rarely present a systolic heart murmur when worms reside in the right atrioventricular junction and interfere with tricuspid valvular function (Venco *et al.*, 2015). These severe changes in cardiac function of diseased cats may be attributed to adult HWD clinical syndrome occurring after death of mature *D. immitis* (natural or after treatment) and subsequent fragmentation of the parasitic bodies causing thromboembolism. In this case, electrocardiography may serve as an important and valuable tool for diagnosis of HWD. Cardiac disorders of our patient were additionally evaluated following thoracic radiography. We found rounded borders of cardiac shadow, caudally displaced cardiac tip, and increased contact of cardiac shadow with the sternum. These results are in agreement with reports by Litster *et al.* (2005) and Venco *et al.* (2008), who revealed that the cardiac silhouette seldom appears enlarged. Regarding pulmonary radiography, we found abnormalities, including enlarged pulmonary arteries in the hilus and middle part of the lungs; pronounced reticular interstitial pattern; alveolar and bronchial shades with multiple nodular thickenings throughout the lungs. A recently published case report also showed pulmonary alterations consisting of a diffuse bronchointerstitial pattern of the pulmonary parenchyma more evident in the caudal lung lobes, as well as enlarged caudal lobar pulmonary arteries (Pana *et al.*, 2020). Diffuse or focal bronchointerstitial parenchymal patterns and vascular abnormalities in infected cats have been widely reported (Atkins *et al.*, 2000; Litster & Atwell, 2008; Venco *et al.*, 2008; Garrity *et al.*, 2019; Pennisi *et al.*, 2020).

Importantly, in some cases of feline HWD, thoracic radiographs provide no evidence of infection (Venco *et al.*, 2008). In addition, diffuse patterns are also characteristic of other respiratory diseases in the cat, such as asthma or aelurostrongylosis (Venco *et al.*, 2008), and discriminating between these diseases is mandatory (Garrity *et al.*, 2019). According to Genchi *et al.* (2014), the disorders caused by *A. abstrusus* are mainly associated with inflammation of the alveoli, bronchioles and arteries leading to very similar lung and vascular abnormalities.

Considering the above, the clinical presentation, electrocardiographic and radiographic findings were still insufficient for the definitive and specific diagnosis, therefore, blood tests for first stage larvae (L1s, microfilariae), adult antigens, as well as host-specific antibodies were also performed in our patient. The results showed no presence of microfilaremia; however, positive reactions for *D. immitis* antigens and host-specific antibodies were obtained. The lack of L1s in the blood of our patient is not surprising despite the positive antigen and antibody tests. That may be related to several reasons including light worm burden (typically 2 to 4 worms); infections represented only by male worms (single sex infections are common event); infections only due to immature worms (prepatent period); reduced lifespan of adults. In this regard, fewer than 20 % of infected cats are microfilaremic as well as the microfilaremia is transient and persists only 1 – 2 months, at which time the cat immune system eliminates L1s and suppresses further embryogenesis (Nelson, 2008; Lee & Atkins, 2010; Garrity *et al.*, 2019). According to Atkins *et al.* (2000), only 1 of 8 infected cats has had circulating L1s. In contrast, the microfilaremia is considered to be 100 % in infected dogs. Given these features, the use of techniques for recovering blood L1s in cats is regarded as virtually unreliable and is not recommended (Garrity *et al.*, 2019); however,

when present, microfilaraemia provides a definitive diagnosis (Venco *et al.*, 2015; Pereira *et al.*, 2018). Otherwise, antigen and antibody serology should be included in testing procedures. Antigen tests are the most commonly used tools in diagnosing HWD in dogs over the years. These tests detect the antigens from mature females and, therefore, they are indicative of active adult (patent) infection. Although considered as “gold standard”, a negative antigen test may not be sufficient to rule out the infection because of several reasons discussed above, including infections with males *D. immitis* or single female parasite, and due to immature worms. Importantly, the blood circulating antigens become detectable at about 5.5 – 8 months after the initial transmission and when the patent infection is represented by at least 2 (or more) mature female worms (McCall, 1998; Venco *et al.*, 2015). However, the rate of false-positive results with antigen serology is low, and a positive result generally indicates a current infection (Litster & Atwell, 2008). Regarding antibody test, it is very promising and mandatory for diagnosis of feline HWD because it detects 93 % to 100 % of heartworm infections, including late L4 larvae and juveniles (HARD syndrome) as well as adult worms (Nelson, 2008). The test is also capable of disclosing previous exposure to *D. immitis* in cats. It should be also taken into account that in cats, antibodies against *D. immitis* are detectable from 2 months after the infection (Venco *et al.*, 2015). Combining the results of serum antigen and antibody tests may achieve higher sensitivity and specificity than by using either test alone (Snyder *et al.*, 2000; Litster & Atwell, 2008). Treatment protocols in cats considerably differ when compared with dogs. Adulticidal therapy with arsenical compounds including thiacetarsamide and melarsomine is not safe for cats and may trigger very severe and life-threatening conditions (pulmonary thromboembolism and anaphylactic reactions), leading to sudden death in the posttreatment period (Pennisi *et al.*, 2020). For these reasons and because heartworm infection in cats is often self-limiting, infected cats exhibiting clinical signs should be managed only with supportive treatment (corticosteroids, bronchodilators, and anti-emetics) (Litster & Atwell, 2008). The best approach to feline HWD is the prevention by using chemoprophylactic regimens with macrocyclic lactones including ivermectin (monthly dose 24 mg/kg given orally); milbemycin oxime (monthly dose 2.0 mg/kg given orally); topical moxidectin (monthly dose 1.0 mg/kg); topical selamectin (monthly dose 6 mg/kg) and topical eprinomectin (monthly dose 0.48 mg/kg) (Venco *et al.*, 2015). These products are a safe and convenient option for cats living in areas where canine heartworm disease is considered endemic (Litster & Atwell, 2008). Additionally, administration of macrocyclic lactones protects cats from infections with various nematode species (e.g. *A. abstrusus* and *T. cati*) and parasitic arthropods (e.g. fleas, mites and hard ticks).

## Conclusion

Given the data mentioned above, we could conclude that the dis-

eased cats should be subjected to testing for different cardiopulmonary nematodes, including these animals showing no clinical presentation of cardiovascular or respiratory disease. Also, the development of regimens for treatment and prevention of feline HWD is essential.

## Conflict of Interest

Authors state no conflict of interest.

## References

- ATKINS, C.E., ATWELL, R.B., DILLON, A.R., GENCHI, C., HAYASAKY, M., HOLMES, R.A., KNIGHT, D.H., LUKOFF, D.K., MCCALL, J.W., SLOCOMBE, J.O. (1995): Guidelines for the diagnosis, treatment, and prevention of heartworm (*Dirofilaria immitis*) infection in cats. In: SOLL, M.D., KNIGHT, D.H. (Eds) *Proceedings of the heartworm symposium '95*. American Heartworm Society, Batavia, IL, pp. 309 – 312
- ATKINS, C.E., DEFRANCESCO, T.C., COATS, J.R., SIDLEY, J.A., KEENE, B.W. (2000): Heartworm infection in cats: 50 cases (1985 – 1997). *J. Am. Vet. Med. Assoc.*, 217(3): 355 – 358. DOI: 10.2460/javma.2000.217.355
- DIAKOU, A., SOUBASIS, N., CHOCHLIOS, T., OIKONOMIDIS, I. L., TSELEKIS, D., KOUTINAS, C., KARAIOSIF, R., PSARALEXI, E., TSOULOUFI, T. K., BRELLOU, G., KRITSEPI-KONSTANTINOU, M., RALLIS, T. (2018): Canine and feline dirofilariosis in a highly enzootic area: first report of feline dirofilariosis in Greece. *Parasitol. Res.*, 118(2): 677 – 682. DOI: 10.1007/s00436-018-6135-9
- DILLON, R., WARNER, A.E., MOLINA, R.M. (1995): Pulmonary parenchymal changes in dogs and cats after experimental transplantation of dead *Dirofilaria immitis*. In: Soll, M. D., Knight, D. H. (Eds) *Proceedings of the heartworm symposium '95*. American Heartworm Society, Batavia, IL, pp. 97 – 101
- DILLON, A.R., BRAUNER JR, A.R., ROBERTSON-POLOUCH, C.K., GUERREIRO, J. (2000): Feline heartworm disease: correlations of clinical signs, serology, and other diagnostic results of a multicentre study. *Vet. Ther.*, 1(3): 176 – 182.
- DILLON, A.R., WARNER, A.E., BRAUNER, W., HUDSON, J., TILLSON, M. (2008): Activity of pulmonary intravascular macrophages in cats and dogs with and without *Dirofilaria immitis*. *Vet. Parasitol.*, 158: 171 – 176. DOI: 10.1016/j.vetpar.2008.09.004
- GARRITY, S., LEE-FOWLER, T., REINERO, C. (2019): Feline asthma and heartworm disease: Clinical features, diagnostics and therapeutics. *J. Feline Med. Surg.*, 21(9): 825 – 834. DOI: 10.1177/1098612X18823348
- GENCHI, M., FERRARI, N., FONTI, P., DE FRANCESCO, I., PIAZZA, C., VIGLIETTI, A. (2014): Relation between *Aelurostrongylus abstrusus* larvae excretion, respiratory and radiographic signs in naturally infected cats. *Vet. Parasitol.*, 206(3-4): 182 – 187. DOI: 10.1016/j.vetpar.2014.10.030
- GIANNELLI, A., CAPELLI, G., JOACHIM, A., HINNEY, B., LOSSON, B., KIRKOVA, Z., RENE-MARTELLET, M., PAPADOPOULOS, E., FARKAS, R., NAPOLI,



- E., BRIANTI, E., TAMPONI, C., VARCASIA, A., ALHO, A. M., CARVALHO, L. M., CARDOSO, L., MAIA, C., MIRCEAN, V., MIHALCA, A.D., MIRO, G., SCHNYDER, M., CANTACESSI, C., COLELLA, V., CAVALERA, M., LATROFA, M.S., ANNOSCIA, G., KNAUS, M., HALOS, L., BEUGNET, F., OTRANTO, D. (2017): Lungworms and gastrointestinal parasites of domestic cats: a European perspective. *Int. J. Parasitol.*, 47(9): 517 – 528. DOI: 10.1016/j.ijpara.2017.02.003
- HNILICA, K., PATTERSON, A. (2017): *Small Animal Dermatology*. 4<sup>th</sup> Edition, St. Louis, Missouri, USA, Elsevier, pp. 110
- LITSTER, A., ATKINS, C., ATWELL, R., BUCHANAN, J. (2005): Radiographic cardiac size in cats and dogs with heartworm disease compared with reference values using the vertebral heart scale method: 53 cases. *J. Vet. Cardiol.*, 7(1): 33 – 40. DOI: 10.1016/j.jvc.2005.02.002
- LITSTER, A.L., ATWELL, R.B. (2008): Feline heartworm disease: a clinical review. *J. Feline Med. Surg.*, 10(2): 137 – 144. DOI: 10.1016/j.jfms.2007.09.007
- LEE, A.C., ATKINS, C.E. (2010): Understanding feline heartworm infection: disease, diagnosis, and treatment. *Top. Companion Anim. Med.*, 25(4): 224 – 230. DOI: 10.1053/j.tcam.2010.09.003
- MCCALL, J.W. (1998): Dirofilariasis in the domestic ferret. *Clin. Tech. Small Anim. Pract.*, 13(2): 109 – 112. DOI: 10.1016/S1096-2867(98)80015-7
- MCCALL, J.W., GENCHI, C., KRAMER, L.H., GUERRERO, J., VENCO, L. (2008): Heartworm disease in animals and humans. *Adv. Parasitol.*, 66: 193 – 285. DOI: 10.1016/S0065-308X(08)00204-2
- MONTOYA-ALONSO, J.A., CARRETON, E., GARCIA-GUASCH, L., EXPOSITO, J., ARMARIO, B., MORCHON, R., SIMON, F. (2014): First epidemiological report of feline heartworm infection in the Barcelona metropolitan area (Spain). *Parasite Vectors*, 7: 506. DOI: 10.1186/s13071-014-0506-6
- MONTOYA-ALONSO, J.A., MORCHON, R., FALCON-CORDON, Y., FALCON-CORDON, S., SIMON, F., CARRETON, E. (2017): Prevalence of heartworm in dogs and cats of Madrid, Spain. *Parasite Vectors*, 10(1): 354. DOI: 10.1186/s13071-017-2299-x
- MORCHON, R., CARRETON, E., GONZALEZ-MIGUEL, J., MELLADO-HERNANDEZ, I. (2012): Heartworm disease (*Dirofilaria immitis*) and their vectors in Europe – new distribution trends. *Front. Physiol.*, 3: 196. DOI: 10.3389/fphys.2012.00196
- NELSON, C.T. (2008): *Dirofilaria immitis* in cats: diagnosis and management. *Compend. Contin. Educ. Vet.*, 30(7): 393 – 400
- PANA, D., RADULESCU, A., MITREA, I.L., IONITA, M. (2020): First report on clinical feline heartworm (*Dirofilaria immitis*) infection in Romania. *Helminthologia*, 57(1): 49 – 56. DOI: 10.2478/helm-2020-0009
- PENNISI, M.G., HARTMANN, K., ADDIE, D.D., BOUCRAUT-BARALON, C., EGGERINK, H., FRYMUS, T., GRUFFYDD-JONES, T., HORZINEK, M.C., HOSIE, M.J., LLORET, A., LUTZ, H., MARSILIO, F., RADFORD, A.D., THIRY, E., TRUYEN, U., MOSTL, K. (2015): Lungworm disease in cats: ABCD guidelines on prevention and management. *J. Feline Med. Surg.*, 17(7): 626 – 636. DOI: 10.1177/1098612X15588455
- PENNISI, M.G., TASKER, S., HARTMANN, K., BELAK, S., ADDIE, D., BOUCRAUT-BARALON, C., EGGERINK, H., FRYMUS, T., HOFMANN-LEHMANN, R., HOSIE, M., LLORET, A., MARSILIO, F., THIRY, E., TRUYEN, U., MOSTL, K. (2020): Dirofilarioses in cats: European guidelines from the ABCD on prevention and management. *J. Feline Med. Surg.*, 22(5): 442 – 451. DOI: 10.1177/1098612X20917601
- PEREIRA, B.B., BASTOS, B.F., KEIDEL, L., LELES, D., BRENER, B. (2018): Feline heartworm (*Dirofilaria immitis*) infection: first case report of serological diagnosis in Brazil, confirmed by molecular assay. *An. Acad. Bras. Cienc.*, 90(2): 2293 – 2297. DOI: 10.1590/0001-3765201820170063
- SCHNYDER, M., DI CESARE, A., BASSO, W., GUSCETTI, F., RIOND, B., GLAUS, T., CRISI, P., DEPLAZES, P. (2014): Clinical, laboratory and pathological findings in cats experimentally infected with *Aelurostrongylus abstrusus*. *Parasitol. Res.*, 113: 1425 – 1433. DOI: 10.1007/s00436-014-3783-2
- SNYDER, P., LEVY, J., SALUTE, M.E., GORMAN, S.P., KUBILIS, P.S., SMAIL, P.W., GEORGE, L.L. (2000): Performance of serologic tests used to detect heartworm infection in cats. *J. Am. Vet. Med. Assoc.*, 216(5): 693 – 700. DOI: 10.2460/javma.2000.216.693
- TRAVERSA, D., DI CESARE, A. (2014): Cardio-pulmonary parasitic nematodes affecting cats in Europe: unraveling the past, depicting the present, and predicting the future. *Front. Vet. Sci.*, 1: 11. DOI: 10.3389/fvets.2014.00011
- VENCO, L., GENCHI, C., GENCHI, M., GRANDI, G., KRAMER, L. H. (2008): Clinical evolution and radiographic findings of feline heartworm infection in asymptomatic cats. *Vet. Parasitol.*, 158(3): 232 – 237. DOI: 10.1016/j.vetpar.2008.09.011
- VENCO, L., MARCHESOTTI, F., MANZOCCHI, S. (2015): Feline heartworm disease: a 'Rubik's-cube-like' diagnostic and therapeutic challenge. *J. Vet. Cardiol.*, 17(1): 190 – 201. DOI: 10.1016/j.jvc.2015.08.004
- ZAJAC, A., CONBOY, G. (2012): *Veterinary Clinical Parasitology*. 8<sup>th</sup> Edition, Chichester, UK, Wiley-Blackwell, pp. 14 – 87