DOI 10.2478/helm-2020-0009

HELMINTHOLOGIA, 57, 1: 49 – 56, 2020

Case Report

First report on clinical feline heartworm (*Dirofilaria immitis*) infection in Romania

D. PANĂ, A. RĂDULESCU, I. L. MITREA*, M. IONITA

Department of Parasitology and Parasitic Diseases & Animal Biology, Faculty of Veterinary Medicine, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 105 Splaiul Independentei, 5th district, 050097, Bucharest, Romania, *E-mail: *liviumitrea@yahoo.com*

Article info

Received October 9, 2019
Accepted December 16, 2019

Summary

Dirofilaria immitis (Nematoda: Filarioidea) is the causative agent of heartworm disease (HWD), a severe and potentially fatal condition in dogs. Although cats are considered more resistant to infection than dogs, they are also susceptible to heartworm infection. Moreover, the clinical importance of feline dirofilariosis has increased in recent years, especially in heartworm endemic areas. In contrast to dog, definitive antemortem diagnosis of heartworm infection in cat is difficult to achieve and a combination of testing methods must be used for clinical confirmation. Here we describe a clinical case of heartworm infection in a 12-year-old male mixed breed cat, originated from Southern Romania, which was referred to a veterinary clinic with a history of vomiting and clinical signs of respiratory distress. The thoracic radiograph showed a diffuse bronchointerstitial pattern of the pulmonary parenchyma. The serological test for *D. immitis* circulating antibody was positive and heartworms were visualized by echocardiography in the main pulmonary artery. In conclusion, the present study clearly shows that cats are at risk for *D. immitis* infection in heartworm-endemic areas in Romania. Additionally, the findings highlight the urgent need for increased awareness among veterinary practitioners of the existence of feline heartworm diseases and for adequate prophylactic measures to be applied. To our knowledge, this is the first report on clinical evolution and radiographic and echocardiographic features of a naturally heartworm-infected cat in Romania.

Keywords: *Dirofilaria immitis* infection; respiratory distress; thoracic imaging; cat; Romania

Introduction

Heartworm disease (HWD) is a severe and potentially fatal cardio-pulmonary disease caused by the mosquito-borne filariod *Dirofilaria immitis*, primarily infecting dogs, but also other carnivores, including domestic and wild felids. Adult worms are located in the pulmonary arteries and occasionally in the right heart. The dog is considered the main reservoir, although *D. immitis* can also affect cats and other mammal species, and in rare cases, humans (McCall *et al.,* 2008). HWD is endemic in Mediterranean countries but currently an increase in the geographical range and its spreading towards central and eastern European areas are reported (Genchi *et al.,* 2011).

Cats are also susceptible to heartworm infection, although they are considered more resistant to infection than are dogs. There are some indications based on which the cat is considered an imperfect or not ideal host for heartworms, such as: low adult worm burden (1 – 6 worms) (Dillon *et al.,* 2007), their short life span (2 – 3 years) (Genchi *et al.,* 2008), lack or short duration of microfilaremia, and a prolonged pre-patent period (8 months) (Dillon *et*

* – corresponding author

al., 2007; Atkins *et al.*, 2008). Therefore, most cats seem to tolerate heartworm infection for prolonged period without or only with transient clinical signs (González-Miguel *et al.,* 2010).
Moreover, clinical signs associated with HWD in cats are usually nonspecific, comprising predominantly respiratory sings (intermittent coughing, dyspnea, tachypnea, ataxia) but also gastrointestinal disorders (e.g. vomiting, diarrhea, weight loss, anorexia), as well as the frequent asymptomatic course of feline dirofilariosis in which the sudden death of the animal is sometimes the only clinical sign shown (Dillon, 1984; McCall *et al.,* 1994; Lee & Atkins 2010). Some spontaneously self-cure as a result of natural death of parasites in infected cats without showing clinical signs or a suddenly acute respiratory syndrome may arise without warning (Atkins *et al.,* 2000; McCall *et al.,* 2008). All these facts make the diagnosis of HWD in cats more difficult and underestimated (Lee & Atkins 2010).
Dirofilariosis is usually diagnosed by detection of the circulating microfilariae in the peripheral blood, immunological tests (ESDA, 2017) and molecular approaches (Liu *et al.,* 2005; Nuchprayoon *et al.,* 2006). Because of the specific features that characterize HWD in cats, reflected by the relatively low adult worm burden, low intensity and transitory microfilaraemia, low circulating antigenemia, the diagnostic approach to feline heartworm infection must incorporate a combination of testing methods (Lee and Atkins, 2010). Of these, heartworm serology (antigen test, antibody test), thoracic radiography and echocardiography are considered the most useful tools of clinical confirmation (Atkins *et al.,* 2000; ESDA, 2017).
Though microfilaraemia is seldom present in infected cats, because patency is short, and performing a modified Knott test for detecting circulating microfilariae is rarely successful, when present, microfilaraemia is considered a definitive diagnosis (ESDA, 2017). However, serological tests are typically the initial screening tool for feline dirofilariosis and have been used for large-scale studies (Kramer & Genchi, 2002) and also to assess clinical infections (Atkins *et al.,* 2000). Currently, commercially available antigen tests, that target a protein in the reproductive tract of adult females, are considered highly specific and sensitive (100 % and 98.9 %, respectively) methods for infection screening or for confirming a clinical suspicion (Genchi *et al.,* 2018). However, sensitivity can be lower in case of low burden or single worm/sex infection (ESDA, 2017). Antibody tests are able to detect infection by both male and female worms and are the only tests that can be positive as early as 2 months post infection (Morchon *et al.,* 2004) but do not confirm a current infection or the presence of adult worms (Snyder *et al.,* 2000).
In cats with either immature or adult heartworms, significant parenchymal and airway disease is manifested radiographically and histologically (Atkins *et al.,* 2000; Dillon *et al.,* 2007). Therefore, additionally, and independent of serologic test results, thoracic radiography may provide strong evidence of feline HWD and is valuable for assessing the severity of disease and its monitoring (ESDA, 2017). Also, echocardiography is a useful adjunctive test in cats with clinical suspicion of HWD and, in almost all cases, is the only definitive way to diagnose infection antemortem (Atkins *et al.,* 2008).
During the last years, several different Polymerase-Chain-Reaction (PCR)-based protocols have been developed for single species or simultaneous detection of different *Dirofilaria* species in cats using nested and multiplex PCR and PCR-RFLP (Restriction Fragment Length Polymorphism), respectively assays, as useful diagnostic tools to detect the *D. immitis* low infection in feline HWD (Liu *et al.,* 2005; Nuchprayoon *et al.,* 2006).
The clinical importance of HWD in cats has increased in recent years, especially in heartworm endemic areas, mainly do to increased awareness of this disease, as even a small number of worms are potentially life-threatening (McCall *et al.,* 1994).
In the last decade the dynamics of canine HWD in Romania has been changed from sporadic to emerging (Ionita *et al.,* 2012) and currently an alarming increased prevalence is reported, with endemic foci in South-Eastern areas (Mircean *et al.,* 2012; Girdan *et al.,* 2015; Pana *et al.,* 2018). However, to our knowledge, there are no reports about feline heartworm disease in Romania. Here we describe a clinical case of natural heartworm infection in a cat in Southern Romania.

Material and Methods

Case presentation
A twelve year-old male mixed breed cat was referred, in October 2018, to a veterinary clinic in Bucharest with a history of vomiting and difficult breathing. The cat originated from a rural area from Giurgiu county (44°24'54"N 25°49'28"E) (Southern Romania), at about 30 km far by Bucharest. The cat, with a mixed indoor and outdoor life style, lived with other 2 cats and 24 dogs in the same household and she had not received heartworm prevention prior to the visit, as the owner stated. The history of cat, as recalled by the owner, did not include any travel outside the home city limits. Cat spent most of the time outdoors.
Laboratory investigations
Cat blood samples were collected and submitted for routine hematological and biochemical investigations and for parasitological testing.
In order to asses the cause and the severity of the disease, the cat was also subjected for imagistic investigations consisting of thoracic radiography (by using a Roentgen Examion Maxivet DR apparatus) and cardiac echocardiography (by using a General Electric Logiq 9 ultrasound system).
Parasitological evaluation included a modified Knott test (Knott, 1939), for detecting circulating microfilariae of *D. immitis* and two commercial immuno-chromatographic rapid tests for detecting circulating *D. immitis* antigen (Ag) (SNAP®4DX® Plus, Idexx) and antibody (Ab) (FeliCheck-3, VetExpert), respectively.
The modified Knott test was applied for the concentration, detec-

tion and identification of microfilariae, as briefly described here: 1.0 ml of EDTA venous blood was mixed in a 15 ml conical centrifuge tube with 9.0 ml of 2 % buffered formalin and centrifuged for 5 minutes at 1500 rotation per minute (rpm). The supernatant was decanted, and a drop of 0.1 % methylene blue was added to the sediment and stained for 2 minutes. A drop of the sediment was transferred to a glass slide and covered with a cover slip. The slide was examined under the microscope at 10x to detect the presence of microfilariae and then at 40x to observe the morphological features. The whole sediment was analyzed.

The SNAP®4DX® Plus test is a point-of-care ELISA-based immunochromatographic commercial kit for the detection of *D. immitis* antigen; additionally, it is able to simultaneous detect antibodies against *Anaplasma phagocytophilum/Anaplasma platys*, *Ehrlichia canis, Ehrlichia ewingii*, and *Borrelia burgdorferi* (IDEXX Laboratories, Westbrook, ME).
The FeliCheck-3 Ab test used is a chromatographic immunoassay for the simultaneously qualitative detection of feline heartworm Ab (FHW Ab), feline immunodeficiency virus antibody (FIV Ab), and feline leukemia virus antigen (FeLV Ag).

Ethical Approval and/or Informed Consent

All applicable national and institutional guidelines for the care and use of animals were followed.

Results

Clinical and laboratory findings
On physical and clinical examinations, the cat was in a good body condition but showed, intermittent coughing, cyanotic mucous membranes and dyspnea, without murmur and arrhythmia.
The hematological examination revealed increased number of the white blood cells (WBC 25.99 K/µL) characterized by lymphocytosis (13.6 K/µL), mild neutrophilia (NEU 11.09 K/µL) and monocytosis (0.54 K/µL). The biochemical analysis showed increased hepatic enzymes (alaninaminotranspherase 67 UI/L; alkaline phosphatase 118 UI/L; total protein 8.7 g/dl) (Table 1).

Table 1. Hematological and serum biochemical parameters of a 12-year old mixed breed cat with heartworm infection.

Parameter	Measure unit	Reference value	Results (interpretation*)
White blood cells (WBC)	K/µL	5.1 – 16.2	25.99 (↑)
Hematocrit (HCT)	%	27.7 – 46.8	38.9
Hemoglobin (HGB)	g/dL	8.1 – 14.2	13.1
Neutrophils (NEU)	K/µL	2.3 – 10.7	11.09 (↑)
Lymphocytes (LYM)	K/µL	1.2 – 6.8	13.6 (↑)
Monocytes (MON)	K/µL	0.1 – 0.4	0.54 (↑)
Platels (PLT)	K/µL	156 – 626	216
Blood Uruine Nitrogen (BUN)	mg/dL	15 – 35	27
Glucose (GLU)	mg/dL	61 – 120	162 (↑)
Alkaline phosphatase (ALKP)	UI/L	12 – 65	118 (↑)
Total protein (TP)	g/dL	5.4 – 7.9	8.7 (↑)
Alaninaminotranspherase (ALT)	UI/L	8 – 53	67 (↑)
Creatinine (CRE)	mg/dL	0.5 – 2	1.5
Amylase (AMYL)	UI/L	370 – 1200	894
Total bilirubin (TBIL)	µmol/L/dL	0.15 – 0.5	0.3

* ↑: increased

Subsequently to the parasitological testing, circulating *D. immitis* microfilariae and Ag were not detected, but the serology was positive for feline heartworm Ab, as well as for FIV Ab (Fig. 1).
The thoracic radiograph showed a diffuse bronchointerstitial pattern of the pulmonary parenchyma (more evident in the caudal lung lobes) (Fig. 2) and enlarged caudal lobar pulmonary arteries (Fig. 3).
During of cardiac ultrasonographic examination, adult heartworms were detected in the main pulmonary artery. The heartworm appeared as short, segmented strongly echogenic parallel lines (Fig. 4).

Treatment and follow-up
After diagnostic, a treatment protocol was designed for the infected

Fig. 1. Serologic (FeliCheck-3) test showing positive reaction for feline immunodeficiency virus (FIV) and feline heartworm (FHW) infection

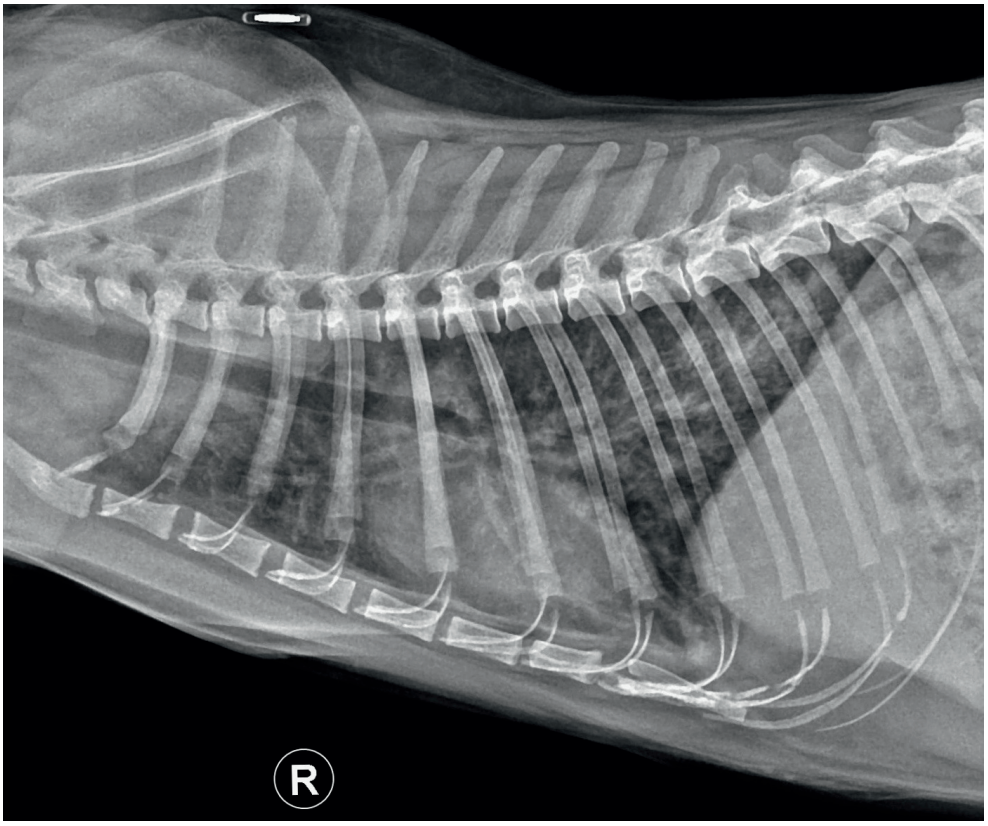

Fig. 2. Thoracic radiograph showing a diffuse bronchointerstitial pattern in a 12-year old cat with heartworm infection (lateral view)

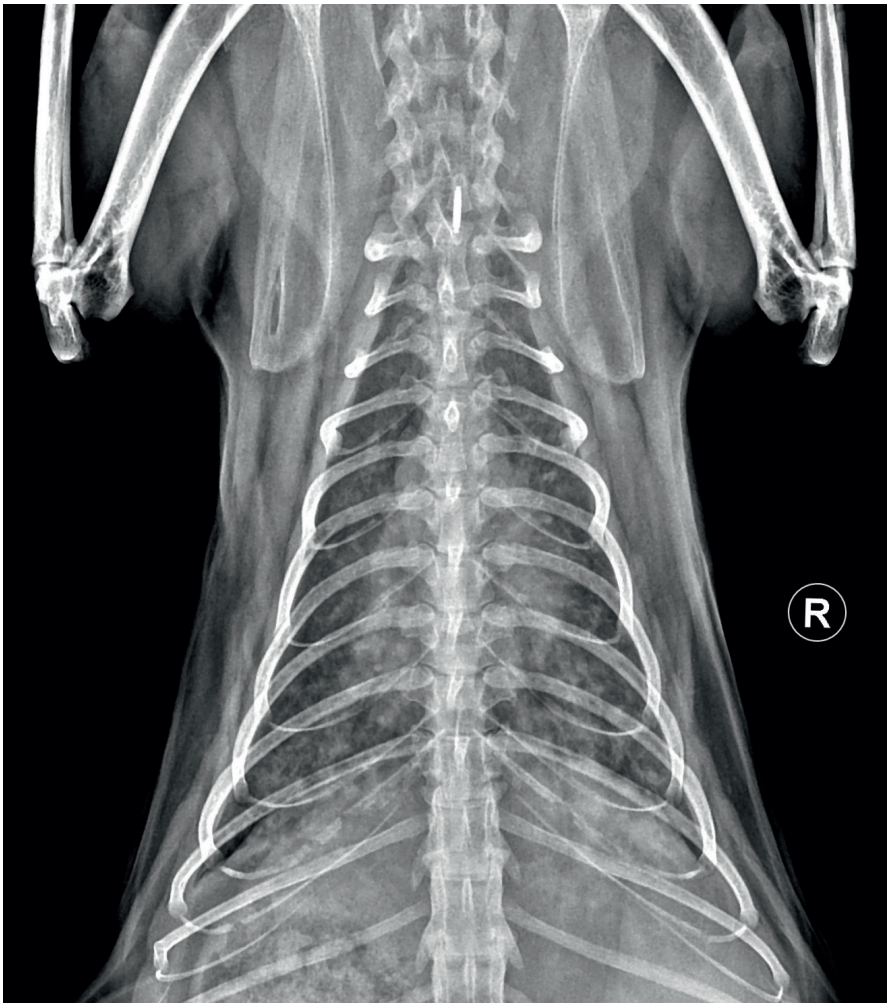

Fig. 3. Thoracic radiograph showing enlarged caudal lobar pulmonary arteries in a 12-year old cat with heartworm infection (dorsoventral view)

cat aiming firstly to stabilize the respiratory signs and to stop the progression of cardiac disease. A symptomatic treatment for the pulmonary hypertension (sildenafil, 1.0 mg/kg, every 12 hours, 30 days) was commenced. Additionally, supportive hepatic and immuno-stimulating therapy was recommended, for 30 and 60 days, respectively. After few days, the cat stopped vomiting, showing normal breathing and displayed no other suspicious clinical signs. Further heartworm chemoprophylaxis with monthly doses of either milbemycin oxime orally, or topical selamectin was recommended for the cat to avoid repeat infection (Litster & Atwell 2008), and serologic retesting at 6 – 12-month intervals for the purpose of monitoring infection status (ESDA, 2017).

As the positive cat lived in a house with other at risks' animals, 2 cats and 24 dogs, we recommended to the owner to test all the co-habiting animals. The 2 cats were tested by serologic (Felicheck 3, VetExpert) for and by Knot tests, for circulating *D. immitis* Ab and microfilariae, respectively, and both were negative.

The 24 co-habiting dogs were tested for circulating *D. immitis* Ag, by using the Ag test (Snap 4Dx) and microfilariae, by the modified Knott test. Of them, five dogs (5/24; 20.8 %) were positive for the both, circulating *D. immitis* Ag and microfilariae.

For all the owned-cats and dogs, heartworm chemoprophylaxis was also recommended.

Discussion

Over the recent decades feline dirofilariosis, caused by *D. immitis* heartworm, has gained the attention of the scientific community which recognized significant differences between feline and the classical, better known, canine heartworm disease, in all aspects: host response, patho-physiology, and clinical presentation (Lee & Atkins 2010; ESDA, 2017). Therefore, a conscious awareness of clinicians of its existence is critical.

Here we describe the first survey carried out on the clinical evolution and radiographic and echocardiographic features of a naturally heartworm-infected cat in Southeastern Romania. To the authors'

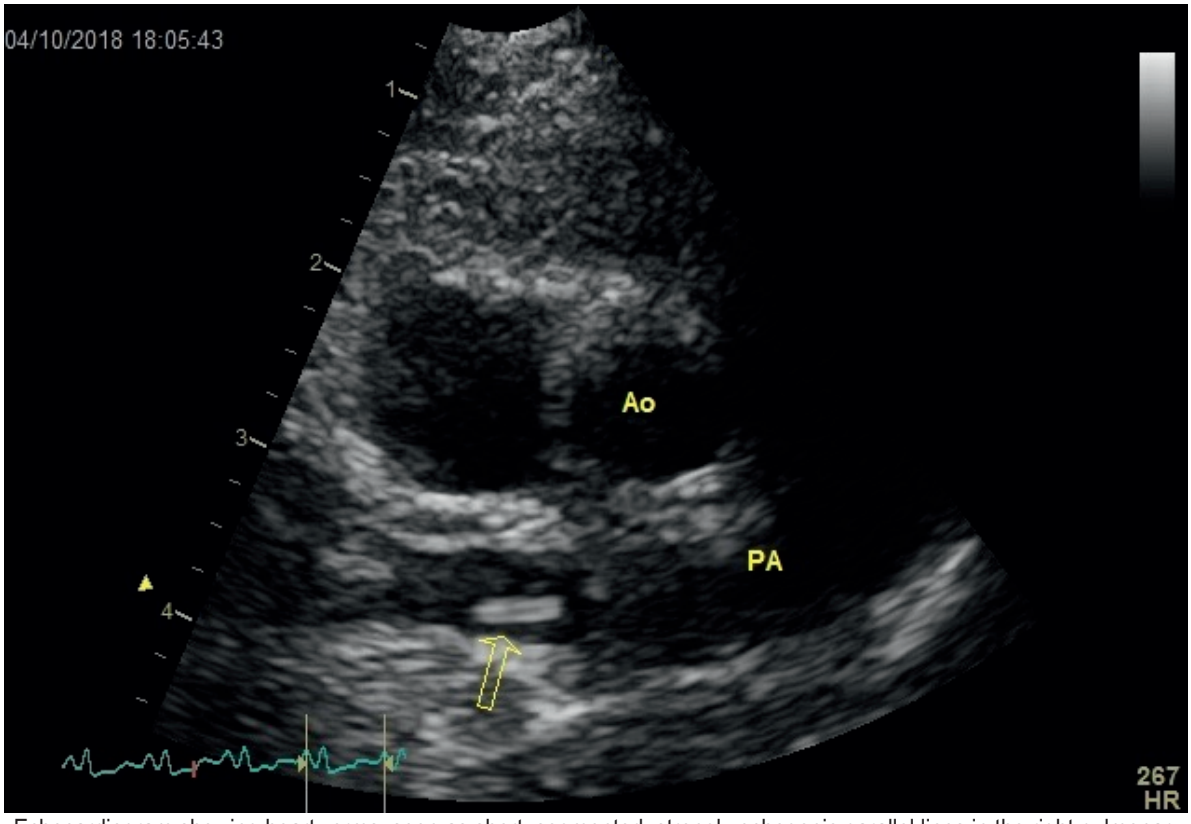

Fig. 4. Echocardiogram showing heartworms, seen as short, segmented, strongly echogenic parallel lines in the right pulmonary artery (arrow) of a 12-year naturally infected cat (Ao: aorta; PA: pulmonary artery) (right parasternal short axis view at the heart's base)

knowledge, no epidemiological surveys were carried out to investigate the exposure of cats to *D. immitis* infection in Romania.

In contrast to heartworm infection in dogs, the prevalence of feline heartworm infection is less well defined because definitive ante-mortem diagnosis is difficult to achieve. Additionally, according to the current opinion, most feline HW infections are asymptomatic, which support the idea that the true prevalence of HW infection in cats in HW-endemic areas is underestimated (Venco *et al.,* 2008). However, it is generally estimated to be 5 % to 20 % that of the canine population in a particular area (Hermesmeyer *et al.,* 2000). This estimation is supported by recent field surveys, such as epidemiological surveys in Italian endemic areas and the metropolitan area of Barcelona (Spain), that reported a prevalence of feline HW infection of approximately 10 % and 10.4 %, respectively of that of dogs in the same area (Venco *et al.,* 2011; Montoya-Alonso *et al.,* 2014).

For Romania, recently, several studies have been reported on existing endemic foci for canine HWD in Southeastern Romania, the infection rate values varying from 13.4 % to 23.0 % (Ionita *et al.,* 2012; Girdan *et al.,* 2015; Anghel *et. al.,* 2016; Pană *et al.,* 2018). The infected cat in the present study originated from such heartworm-endemic area in Southeastern Romania, Moreover, the cat was living with dogs of which 20.8 % were confirmed to be heartworm infected. These demonstrate that cats from that area are at high risk for HW infection and it is very likely that many clinicians are not aware about the real risks of cats for HWD. Further epidemiological surveys will elucidate the current status of feline dirofilariosis in Romania.

Moreover, it is suggested that cats that are living in the urban areas are also at risk to be infected with heartworm. Building construction and human activity increase the density of potential hosts and develop suitable environments for the proliferation of mosquitoe populations due to an increase in the provision of water sources and vegetation (Harrus & Baneth, 2005; Petríc *et al*., 2014).

The outdoor lifestyle of the infected cats in the present study appears as a risk factor for HW infection. It is states that outdoor lifestyle increases cat's exposure to mosquito vectors in HW-endemic areas (Genchi *et al.,* 2008). However, different studies showed that cats living strictly indoors are not fully protect against HW infection, as between 19 % and 27 % of confirmed HW-infected cats were indoor only (Atkins *et al.,* 2000).

The observed patho-physiology and clinical picture including history of vomiting, intermittent coughing, and increased respiratory efforts, are of the most common clinical signs heartworm-infection associated in cats (Venco *et al.,* 2015). The echocardiography has proved the clinical suspicion of heartworm disease in the cat with positive serology (Ab test), but the Ag test for circulating *D. immitis* microfilariae was negative. These findings clearly support the re-

commendation that multiple test diagnosis must be used for clinical confirmation of HWD in cats, some of which may need to be repeated, especially in endemic areas characterized by high index of suspicion (ESDA, 2017).

Recent evidence-based knowledge acquired from various field studies on the detrimental effects of the both larval and adult stages of *D. immitis* that can potentially have of any exposed cat, clearly demonstrate that prevention against infection in cats is the best approach to feline heartworm disease (Venco *et al.,* 2015). For this, there are several highly effective heartworm preventive products commercially available, all from the macrocyclic lactone group. Therefore, a monthly feline heartworm chemoprophylaxis with either ivermectin (24 µg/kg) or milbemycin oxime (2.0 mg/kg) orally, or topical selamectin (6 – 12 mg/kg), moxidectin (1.0 mg/kg), or eprinomectin (0.48 mg/kg) is recommended at least during of the heartworm transmission season (Lee & Atkins, 2010; ESDA, 2017).

Conclusion

The present study shows that cats are at risk for *D. immitis* infection in heartworm-endemic areas in Romania. Additionally, this study highlights the urgent need for increased awareness among veterinary practitioners of the existence of feline heartworm disease and for adequate prophylactic measures to be applied.

Conflict of Interest

Authors state no conflict of interest.

References

Anghel, R.G., Mitrea. I.L., Ionită, M. (2016): Retrospective study on prevalence of canine vector borne diseases in Bucharest area. *Rev Rom Med Vet*., 26(4): 27 – 32

Atkins, C.E., Arther, R.G., Ciszewski, D.K., Davis, W.L., Ensley, S.M., Guity, P.S., Chopade, H., Hoss, H., Settje, T.L. (2008): Echocardiographic quantification of *Dirofilaria immitis* in experimentally infected cats. *Vet Parasitol*., 158 (3): 164 – 170. doi.org/10.1016/j.vetpar.2008.09.003

Atkins, C.E., Defrancesco, T.C., Coats, J.R., Sidleym 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

Dillon, A.R. (1984): Feline dirofilariasis. *Vet Clin North Am Small Anim Prac.,* 14(6): 1185 – 1199. DOI: 10.1016/s0195-5616(84)50153-3

Dillon, A.R., Blagburn, B.L., Tillson, D.M., Brawner, W.R., Welles, B., Johnson, C., Spenser, J., Kaltenboeck, B., Rynders, P.E. (2007): Immature heartworm infection produces pulmonary parenchymal, airway, and vascular disease in cats. *J Am Vet Int Med*., 21(3): 608 – 609. WOS:000246435200163

ESDA: European Society Of Dirofilariosis And Angyiostrongylosis (2017): Guidelines for clinical management of feline heartworm disease. Retrieved July, 2019 from https://www.esda.vet.

Genchi, C., Venco, L., Ferrari, N. (2008): Feline heartworm *(Dirofilaria immitis)* infection: a statistical elaboration of the duration of the infection and the life expectancy in asymptomatic cats. *Vet Parasitol*., 158(3): 177 – 182. DOI: 10.1016/j.vetpar.2008.09.005

Genchi, C., Mortarino, M., Rinaldi, L., Cringoli, G., Traldi, G., Genchi, M. (2011): Changing climate and changing vector-borne disease distribution, the example of *Dirofilaria* in Europe. *Vet Parasitol*., 176(4): 295 – 299. DOI: 10.1016/j.vetpar.2011.01.012

Genchi, M. Mangia, C., Ferrari, N., Loukeri, S. (2018): Evaluation of a rapid immunochromatographic test for the detection of low burden *Dirofilaria immitis* (heartworm) in dogs and cats. *Parasitol Res*., 117(1): 31 – 34. DOI: 10.1007/s00436-017-5709-2

Girdan, G., Anghel, R.G., Ioniță, M., Mitrea, I.L. (2015): Data on canine heartworm (*Dirofilaria immitis*) infection and other vector-borne pathogens in dogs in Bucharest area, Romania. *Scientif Works C Series Vet Med*., 61: 146 – 151

González-Miguel, J., Morchón, R., Siles-Luca, M., Oleaga, A., Simón, F. (2010): Identification of *Dirofilaria immitis* immunoreactive proteins recognized by sera from infected cats using two-dimensional electrophoresis and mass spectrometry. *Mol Biochem Parasitol*., 174(1): 78 – 82. DOI: 10.1016/j.molbiopara.2010.06.013

Harrus, S., Baneth, G. (2005): Drivers for the emergence and re-emergence of vector-borne protozoal and bacterial diseases. *Int. J. Parasitol*., 35 (11 – 12): 1309 – 1318. DOI: 10.1016/j.ijpara.2005.06.005

Hermesmeyer, M., Limberg-Child, R.K., Murphy, A.J., Mansfield, L.S. (2000): Prevalence of *Dirofilaria immitis* infection among shelter cats. *Am Vet Med Assoc*., 217(2): 211 – 212. DOI: 10.2460/javma.2000.217.211

Ionita, M., Enachescu, V., Mitrea, I.L. (2012): Preliminary data on serological survey of exposure to arthropod-borne pathogens in stray dogs from Bucharest, Romania. *Scientif Works C Series Vet Med*., 58(4): 220 – 224

Knott, J. (1939): A method for making microfilarial surveys on day blood. *T Roy Soc Trop Med H*., 33: 191 – 196

Kramer, L., Genchi, C. (2002): Feline heartworm infection: serological survey of asymptomatic cats living in northern Italy. *Vet Parasitol*., 104(1): 43 – 50. DOI: 10.1016/s0304-4017(01)00602-1

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

Litster, A.L., Atwell, R.B. (2009): Feline heartworm disease: a clinical review. *J Feline Med Surg*., 10(2): 137 – 144. DOI: 10.1016/j.jfms.2007.09.007

Liu, J., Song, K.H., Lee, S.E., Lee, J.Y., Lee, J.I., Hayasaki, M., You, M.J., Kim, D.H. (2005): Serological and molecular survey of *Dirofilaria immitis* infection in stray cats in Gyunggi province, South Korea. *Vet Parasitol*., 130(1 – 2): 125 – 129. DOI: 10.1016/j.vetpar.2005.03.026

McCall, J.W., Calvert, C.A., Rawlings, C.A. (1994): Heartworm infection in cats: a life-threatening disease. *Vet Met*., 89(7): 639 – 648

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

Mircean, V., Dumitrache, M., Györke, A., Pantchev, N., Jodies, R., Mihalca, A., Cozma, V. (2012): Seroprevalence and geographic distribution of *Dirofilaria immitis* and tick-borne infections (*Anaplasma phagocytophilum, Borrelia burgdorferi* sensu lato, and *Erlichia canis*) in dogs from Romania. *Vector Borne Zoonotic Dis*., 12(7): 595 – 604. DOI: 10.1089/vbz.2011.0915

Montoya-Alonso, J.A., Carretón, E., García-Guasch, L., Expósito, J., Armario, B., Morchón, R., Simón, F. (2014): First epidemiological report of feline heartworm infection in the Barcelona metropolitan area (Spain). *Parasit Vectors*, 7: 506. DOI: 10.1186/s13071-014-0506-6

Morchón, R., Ferreira, A.C., Martín-Pacho, J.R., Montoya-Alonso, J.A., Mortarino, M., Genchi, C., Simón, F. (2004): Specific IgG antibody response against antigens of *Dirofilaria immitis* and its *Wolbachia* endosymbiont bacterium in cats with natural and experimental infections. *Vet Parasitol*., 125(3 – 4): 313 – 321. DOI: 10.1016/j.vetpar.2004.08.003

Nuchprayoon, S., Junpee, A., Nithiuthai, S., Chungpivat, S., Suvannadabba, S., Poovorawan, Y. (2006): Detection of filarial parasites in domestic cats by PCR-RFLP of ITS1. *Vet Parasitol*., 140(3 – 4): 366 – 372. DOI: 10.1016/j.vetpar.2006.04.003

Pana, D., Mitrea, I.L., Ionita, M. (2018): Parasitological screening on canine dirofilariosis, Southern Romania: preliminary data. *Sci Parasitol*., 19(1 – 2): 45 – 51

Petric, D., Bellini, R., Scholte, E.J., Rakotoarivony, L.M., Schaffner, F. (2014): Monitoring population and environmental parameters of invasive mosquito species in Europe. *Parasit Vectors*, 7: 187. DOI: 10.1186/1756-3305-7-187

Snyder, P.S., Levy, J.K., 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: 693 – 700. DOI: 10.2460/javma.2000.216.693

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., Genchi, M., Genchi, C., Gatti, D., Kramer, L. (2011): Can heartworm prevalence in dogs be used as provisional data for assessing the prevalence of the infection in cats? *Vet Parasitol*., 176(4): 300 – 303. DOI: 10.1016/j.vetpar.2011.01.013

Venco, L., Marchesotti, F., Manzocchi, S. (2015): Feline heartworm disease: A'Rubik's-cube- like' diagnostic and therapeutic challenge. *J Vet Cardiol*., (17): S190 – S201. DOI: 10.1016/j.jvc.2015.08.004