Clinical-parasitological screening for respiratory capillariosis in cats in urban environments

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Article info

Summary

Respiratory capillariosis is a widely distributed zoonotic parasitic disease caused by the nematode *Capillaria aerophila* (Trichocephalida, Trichuridae) that commonly infects wild carnivores but also cats and dogs. This retrospective study aims to describe cases of respiratory capillariosis in cats from the city of Belgrade, Serbia. Between 2015 and 2019, a total of 155 pet cats with or without respiratory symptoms were submitted to physical examination and parasitological examination of the feces. All cats lived indoor but had free access to outdoor. In suburban settlements, wild carnivores commonly share their living environments with owned cats and dogs. It can be assumed that more intense urbanization spreading into the natural habitats of will carnivores creates the opportunity for closer and more frequent contacts between the population of cats and feral carnivores which might increase the risk of feline contamination. The findings confirm the existence of capillaries in cats in urban areas of the city of Belgrade, contribute to a better understanding of the epidemiology of this nematode and warn that, because of close contacts between cats of pets and humans, capillaries can cause human infection.
Keywords: *Capillaria aerophila;* cat*; coprology; physical examination;* Belgrade

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Introduction

Respiratory capillariosis caused by the nematode *Capillaria aerophila* (Trichocephalida, Trichuridae) is a parasitic disease common in wild carnivores including foxes (Sreter *et al*., 2003; Lalošević *et al*., 2013; Čabrilo *et al*., 2018), coyotes (Gompper *et al*, 2003), wolves (Pence & Bryant, 2002), raccoons (Schuster *et al*., 2001) and bears (Crum *et al*., 1978; Paoletti *et al*., 2017). The nematode may also infect dogs (Traversa *et al*., 2009), cats (Madeira de Carvalho *et al*., 2009), and sporadically humans (Lalošević *et al*., 2008; Di Cesare *et al*., 2012).

Capillaria aerophila has a worldwide distribution and is considered as the second most common respiratory helminth of domestic cats in the Northern Hemisphere after *Aelurostrongylus*

abstrusus (Knaus *et al*., 2015). Adult stages of the parasite live on the surface of bronchioles, bronchi, and trachea, where they are completely or partly fixated to the mucosa, causing injury to the epithelium and lung parenchyma. Although less frequently, they can also infest the nasal mucosa and nasal and frontal sinuses (Barrs *et al*., 2000).

Capillaria aerophila infection is usually subclinical and reported sporadically in cats. However, in the past three decades, the parasite has been increasingly detected in cats in Europe (Traversa *et al*., 2010), Asia (Matayoshi *et al*., 1996; Bowman *et al*., 2008), Australia (Milstein & Goldsmid, 1997), New Zealand (Langham & Charleston, 1990), North Africa (Kohart *et al*., 2014) and North and South America (Echeverry *et al*., 2012).

Knowledge of epidemiological data (e.g. range of hosts and geo-

graphic distribution) of C. aerophila in Europe is fragmentary, thus at the moment it is difficult to assess to what degree this parasite may be spreading or what influence global warming or other factors may have on the current distribution. Indeed, although climate changes seem to play a key role in the epidemiological variations for pet heart-worms and lungworms, other possible factors can not be ruled out (Traversa et al., 2010). In Europe, C. aerophila is widely distributed as supported by the published feline case reports in Hungary (Capári et al., 2013; Rehbein et al., 2014), Belgium (Thienpoint et al., 1981), France (Elhamiani Khatat et al., 2016), Germany (Krone et al., 2008), Serbia (Ilić et al., 2009), Greece (Haralampides, 1978), Italy (Di Cesare et al., 2010; Spada et al., 2013), Albania (Knaus et al., 2014), Portugal (Waap et al., 2014), Romania (Mircean et al., 2010), Austria, Bulgaria, Latvia and Lithuania (Rehbein et al., 2014. Recently, different genetic haplotypes of C. aerophila have been shown to coexist in foxes, martens, cats, and dogs in European countries, confirming a common pattern of transmission of this parasite between wild animals and pets (Di Cesare et al., 2014).

In feline medicine, respiratory parasitic infections represent a particular challenge for clinicians due to a large number of conditions with similar clinical manifestations. Knowledge of the prevalence and clinical expression of the disease in cats such as malaise, cough, sneezing, mucopurulent nasal discharge, and "air hunger" should encourage the veterinarian to consider pulmonary capillariosis in the differential diagnosis of these clinical manifestations (Traversa et al., 2010). Furthermore, given the prevalence and zoonotic potential of C. aerophila, it is recommended to include it in the differential diagnosis of cardio-respiratory diseases in dogs and cats.

Therefore, this paper aims to determine the prevalence of C. aerophila infection in cats through the parasitological examination of the feces in the city of Belgrade, Serbia, and its suburban settlements and to describe the clinical signs and complains associated with this infection.

Material and Methods

Research period and area
This retrospective study is based on the record protocols of the University Clinic for Small Animals, and the Department of Parasitology, Faculty of Veterinary Medicine, University of Belgrade (Serbia) which included all cats that had any respiratory problems in the period 2015 – 2019 (155). The cats enrolled were from the city of Belgrade including its suburban settlements (44°49'NG and 20°27' IGD). The city covers an area of 322,268 ha, of which 35,996 belongs to the inner city area.

Studied population
A total of 155 files of pet cats were searched. Out of the total number of examined cats, 61 were male and 94 female. The cats were 2 – 9 years old. From the files, we selected cats that live indoors but also have free access from the outside, including nearby parks and forests.

Clinical examination
For each cat, the following information was recorded including vaccination status, clinical difficulties observed by the owner especially respiratory symptoms, the duration of symptoms, previous consultation for the same difficulties, previous treatments, and the evolution with previous treatments. Each cat was then submitted to a physical examination which involved auscultation, and thermometry. Also, we found five cats with serious respiratory problems who have complete blood cell count, chest x-rays, and only for the one dead cat also cytology from the tracheobronchial lavage. Necropsy was performed on one deceased cat. After the diagnosis was made and the degree of infection was determined, antiparasitics were recommended, and owners were advised to treat cats twice at intervals of 21 days.

Parasitological examination of the feces
The fecal samples were examined using the standard procedure of gravitational flotation with a saturated aqueous solution of NaCl (specific weight 1,200 at 20°C) and the saturated aqueous solution of ZnSO4 (specific weight 1,350 at 20°C) (Sloss et al., 1994). Using light microscopy, C. aerophila barrel-like eggs (length 74,50 ± 1,50 µm, width 32.45 ± 3.40 µm) were detected, with the asymmetry of bipolar plugs and walls with a network of anastomosing ridges (Traversa et al., 2011). The microscopical identification of other fecal endoparasites (*Isospora* spp., *Toxocara mystax*, *Toxascaris leonina*, Ancylostomatidae) was also performed at the same time, using qualitative methods of coprological diagnostic with the concentration of parasitic elements. By way of macroscopic feces examination, the proglottids of *Dipylidium caninum* were found, and after mechanical disintegration, the eggs of this cestode microscopically were found as well.

Quantification of the obtained results was made using the McMaster method (Kochanowski et al., 2013) and less than 50 eggs/g was diagnosed in the samples. Since the threshold of the method is 50 eggs/g, the degree of infection was determined approximately and helminth eggs were calculated according to the semi-quantitative fecal egg count (FEC) scale (Pittman et al., 2010). Using this scale, eggs can be calculated in 5 – 10 g of a fecal sample for an approximate determination of the three levels of the infection degree - low: 1 – 10 (+), medium: 11 – 49 (++) and high: ≥ 50 (+++) of eggs calculated per a covering). The method semi-quantitative FEC is based on the principle of the fecal gravitation flotation. It is necessary to examine the whole fecal suspension in two microscopic slides and make the assessment of the egg count after that.

Necropsy in one cat
During the parasitological dissection of the respiratory tract, the trachea was isolated with the larynx until the bifurcation, was opened full length along the front and observed under the binocu-
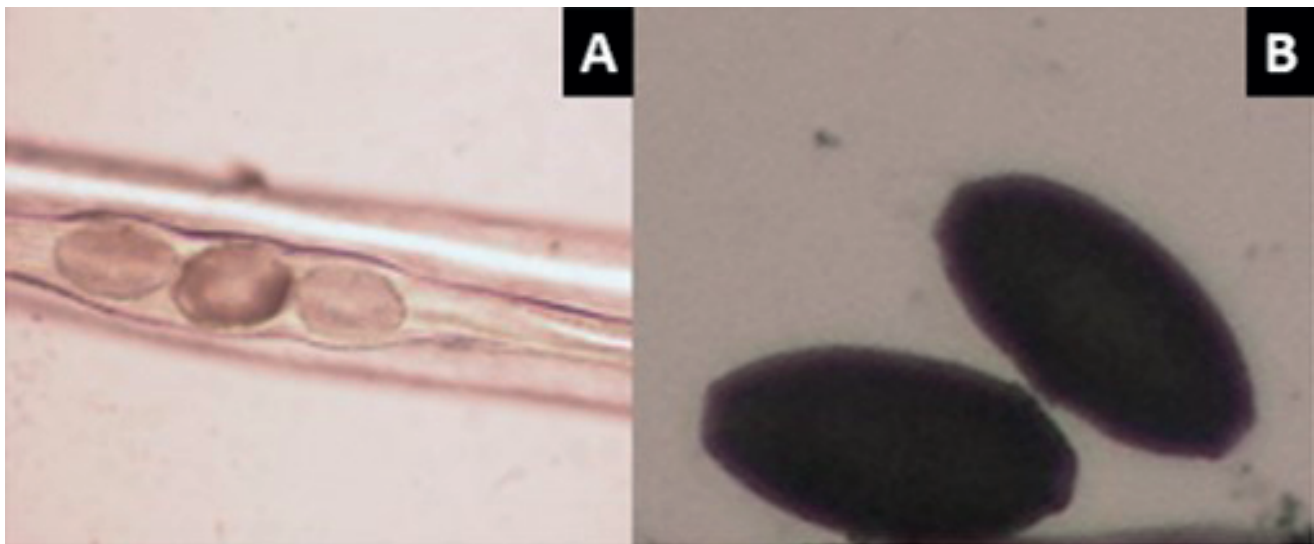
Fig. 1. Necropsy in one cat, light microscopy. A) Female *C. aerophila* with eggs embedded in the remnant uterus in the trachea of a cat, native preparation (100x magnification); B) Eggs of *C. aerophila* detected in microscopic examination of the tracheal lavage, native preparation (400x magnification)

lar magnifying glass, at a 50 times magnification. The macroscopical examination of samples from the trachea, bronchi, and lungs was also performed. We don't find a need for histologic evaluation in this case. Also, the tracheal lavage was cytologically evaluated. A sample of the pulmonary parenchyma (25g) was examined by the Baerman technique. The material was examined fresh, after which adult capillaries were identified and then preserved in 30 % ethyl alcohol.

Morphological identification of parasites from the necropsy
Morphological identification of the collected parasites was performed at the Laboratory for Parasitology at the Department of Parasitology at the Faculty of Veterinary Medicine, University of Belgrade. After recovering, the parasite samples were washed in phosphate buffer saline (PBS), and clearing the nematodes lactophenol solution was used. Identification of nematodes was carried out by using a stereomicroscope (Carl Zeiss, Jena) and the

nematodes were classified into species according to the standard taxonomic key (Anderson *et al*., 2009). Based on the diagnosed trichurid-type eggs and adult forms of the parasite, the finding of *C. aerophila* nematode was confirmed.

Treatment
The cats hospitalized at the Small Animal Clinic at the Faculty of Veterinary Medicine, University of Belgrade, received mostly fluid therapy, amoxicillin with clavulanic acid (8.75 mg/kg/24h/SC/divided into 2 or 3 doses), prednisolone (0.5 mg/kg PO/24h), fenbendazole (50 mg/kg PO/24h), theophylline (1.4 mg/kg PO/12h) and nebulization therapy in animal cages. Serious cases were managed in the intensive care unit.
Cats that had already been treated in other clinics and that had febrile and respiratory problems were hospitalized. Fenbendazole was given to all cats with respiratory symptoms in whom the presence of *C. aerophila* and other endoparasites was determined

Fig. 2. Coprology examination, light microscopy. A) Eggs of *C. aerophila* (100x magnification); B) Egg of *C. aerophila* (400x magnification); C) Egg polar plugs of *C. aerophila* (1000x magnification). Missing thickening at the base of the bipolar plug (arrow).

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Table 1. Prevalence of endoparasites diagnosed in the feces of examined cats
N =155 (number of cats tested); N°= case number of infection; % = infection prevalence rate;

ENDOPARASITES	Total number of cats tested		In Monoinfection*	
	N°	N%	N°	N%
Toxocara mystax	29	18.71	13	8.39
Capillaria aerophila	23	14.84	16	10.32
Isospora spp.	17	10.97	6	3.87
Ancylostomatidae	12	7.74	5	3.22
Dipylidium caninum	5	3.22	2	1.29
Toxascaris leonina	3	1.93	3	1.93

TOTAL: (67 positive cats of endoparasites) N% 43,22

*(45 positive cats with monoinfection) N% 29,03

by coprological examination. The owners were referred to the deworming protocol and were introduced to its importance and currently available preparations on the local market. Twenty-one days after the first treatment, control coprology examinations of cats were performed, and in those that were still positive, the treatment was repeated (for the cats staying mostly outside).

Ethical Approval and/or Informed Consent for research

Research showing standard clinical/laboratory practices or the advancement of standard practices.

Results

Parasitological examination

By parasitological examination of 155 cats, we found 23 that were positive for *C. aerophila*, while 44 were positive for other endoparasites (Table 1 and 2). In one cat, the diagnosis was confirmed by coprological examination and targeted partial necropsy, and in the others 22 cats by coprological examination. Parasitological examination of 55 cats with registered respiratory symptoms developmental forms of *C. aerophila* were diagnosed in 23 individuals (eggs in 23 cats and adult parasites in 1 cat). Adult stages of *C. aerophila* were detected in the trachea and large bronchi during

Table 2. Prevalence of mixed endoparasites infections diagnosed in the feces of examined cats
N =155 (total number of cats tested); N°= case number of infection; % = rate in coinfection;

ENDOPARASITES	N°	N%	In Double infections	N°	N%
Toxocara mystax	16	10.32	*Toxocara mystax* *Capillaria aerophila*	4	2.58
Capillaria aerophila	7	4.52	*Isospora* spp. *Capillaria aerophila*	3	1.93
Isospora spp.	11	7.10	*Isospora* spp. *Toxocara mystax*	5	3.22
Ancylostomatidae	7	4.51	*Isospora* spp. *Ancylostomatidae*	3	1.93
Dipylidium caninum	3	1.93	*Toxocara mystax* *Ancylostomatidae*	4	2.58
Toxascaris leonina	/	/	*Toxocara mystax* *Dipylidium caninum*	3	1.93

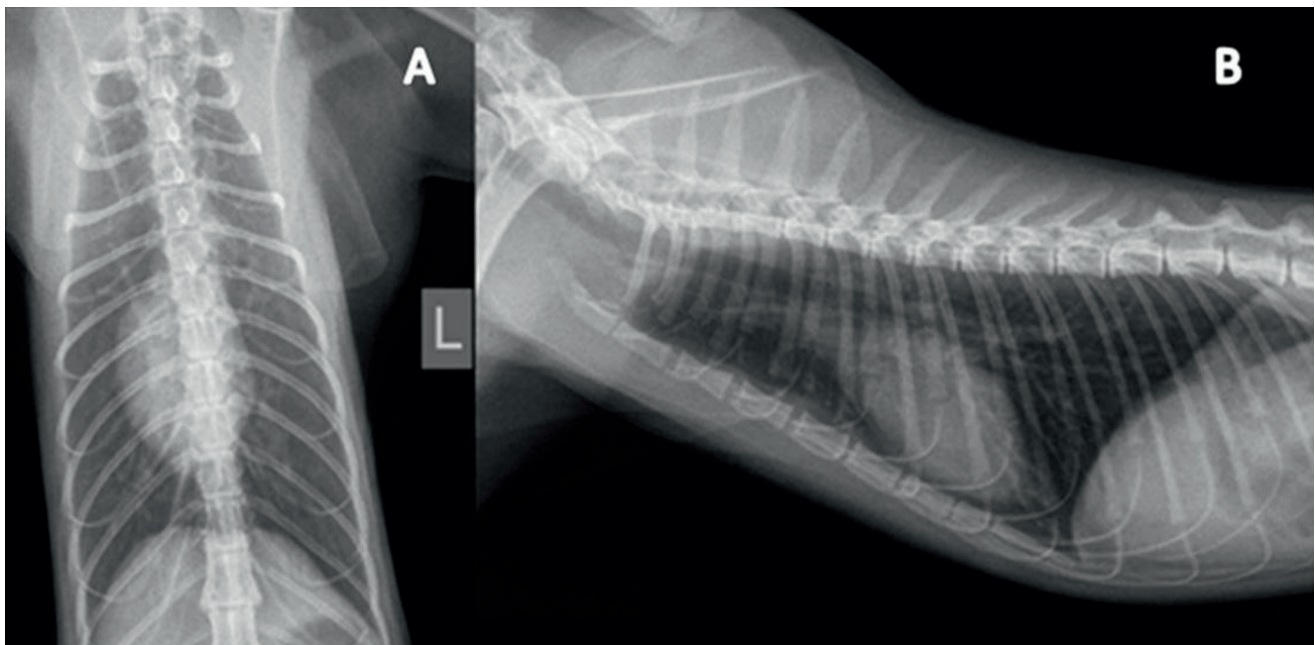TOTAL: (22 positive cats of double infections) N% 14.19

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Fig. 3. (A) ventrodorsal and (B) right lateral thoracic X-rays of a two-year-old male cat with *Capillaria aerophila* infection. A complex lung pattern is visible across the pulmonary fields, characterized by bronchial and alveolar changes and mild reticular interstitial pattern, with the symptoms of the respiratory signs due to; tachypnoea, dyspnoea with abdominal breathing, and end-inspiratory crackles upon auscultation.

necropsy of this cat (Fig. 1A). The eggs of the parasite were identified by the examination of the tracheal washing sample from the deceased cat (Fig. 1B) and in the feces of this and all other cats (Fig. 2A, 2B, and 2C).

Parasitological examination of the feces enabled the detection of 44 (28.38 %) positive cats for endoparasites other than *C. aerophila*. The identified gastrointestinal parasites were *Isospora* spp. oocysts, *T. mystax*, *T. leonina*, Ancylostomatidae eggs, and *D. caninum* proglottids. The total prevalence of endoparasitic infections was 43.22 % (67/155), with the prevalence of single and mixed infections reaching 29.03 % (45/155) and 14.19 % (22/155), respectively. The highest prevalence of single infection was recorded for *Toxocara mystax* infection (18.71 %) while the most frequent mixte infections were with *Isospora* spp and *T. mystax* (3.22 %). Mixed infections with *C. aerophila* and *Isospora* spp were found in 1.93 % (3/155) and with *C. aerophila* and *T. mystax* in 2.58 % (4/155) (Table 1 and 2).

Modifications of the physical examination
Among the 155 cats included in the study, 55 (35.48 %) displayed respiratory signs on physical examination including tachypnea (75 – 80 inspirations per minute) and cough that was usually dry and chronic but occasionally evolved into wet and productive, especially in the presence of secondary bacterial infection. Other abnormalities on physical examination of the 55 cats included tachycardia (sometimes over 200 beats per minute) and fever with a body temperature up to 40°C recorded. (Table 3).
The prevalence of *C. aerophila* infection detected by parasitologi-

cal examination of the feces was 14.84 % and 41.81 % in the total population studied (155 cats) and the cats displaying respiratory signs (55 cats), respectively.

All cats positive for *C. aerophila* belonged to the group of cats with respiratory signs at physical examination. In 90.9 % (50/55) of cats, clinical signs were moderate, which was determined by low (1 – 10 epc) or medium (11 – 49 epc) intensity infections. No statistical analysis has been performed to determine if there is a positive relationship between these two parameters. In this group, one cat (1.81 %) was presented in a serious clinical condition with severe cyanosis and dyspnea. Unfortunately, the animal died after placing him under oxygen in a respiratory box. The severe *C. aerophila* infection (≥ 50 epc) detected in this cat led to airway obstruction and collapse.

Thoracic auscultation revealed difficult breathing and bilateral crackles. Also, increased respiratory sounds were detected during tracheal auscultation and increased sensitivity of tracheal sensitivity upon artificial cough induction.

In all 22 cats which were treated and survived, a weight loss was reported.

Blood count results
Bacterial complications are one of the characteristics of parasitic infections, so Table 1 was formed to show only basic hematological results as (WBC), (Er), and (Eo). Leucocytes were between 5.00 to 23.00 x 10^9/L, erythrocytes, between 4.5 – 9.5 x 10^{12} g/ and the eosinophils 2 – 6 %. Referent values and the results are in (Table 3).

Table 3. Modifications from physiological parameters find on a general and special examination for 23 cats positive for *C. aerophila*

Cat N°	Sex	Age	TM (Kg)	Animal Condition Score	Fever (°C) Ower 38 - 39.5	Assessing Dehydration %	Pulse 140-220	Heart Murmurs	Resp.Rate 20-30	Dyspnea and cyanosis	Crackles	Coffin	WBC 10⁹/L 3.8-19.5	Er 10¹² g/L 5.0-10.5	Eo % 2-12%	RTG pic No:	Degre of infection Coprology	Diagnose	The outcome of treated cats
1	M	3.5	3.2	Critical	35.5	(O) 8-10	225	M 2/6	80	+++	++	++	23.000	4.5	6	-	+++	BP	DV, EX
2	M	5	5.0	Excellent	39.0	(A)	130	-	55	-	-	-	17.000	9.5	2	-	+	GIP	DV
3	M	2	3.8	Fair	(SF) 40	(M) 5-8	210	M 2/6 T 3/6	75	++	+	+	21.000	5.5	3	3 A, B	+	BP	DV
4	M	7	5.2	Good	38.5	(A)	156	-	50	-	-	-	17.500	7.5	2	-	+	GIP	DV
5	F	6	4.2	Fair	(WI) 40.3	(M) 5-8	196	-	75	++	+	++	22.000	5.0	4	3	++	BP	DV
6	F	9	3.5	Poor	36.5	(O) 8-10	240	-	80	++	+	++	6.000	5.0	2	4	+	DCa	DV, EX
7	F	4.5	8.0	Fair	38.5	(A)	150	-	50	-	-	-	15.000	6.0	2	-	+	GIP	DV
8	F	6.5	4.0	Fair	38.6	(A)	145	-	42	-	-	-	16.000	6.5	2	-	+	GIP	DV
9	F	5.5	4,6	Good	38.0	(A)	140	-	40	-	-	-	18.000	8.0	2	-	+	GIP	DV
10	F	3	3.6	Fair	38.7	(A)	140	-	36	+	-	+	17.000	8.0	2	-	+	GIP	DV
11	M	7.5	5.0	Fair	(WI) 40.2	(M) 5-8	230	M 3/6	80	++	+	+	21.500	6.0	5	5	++	PT	S, DV
12	F	6	4.6	Good	38.2	(A)	130	-	30	-	-	-	18.000	8.5	2	-	+	GIP	DV
13	F	6.5	5	Fair	38.4	(A)	136	-	32	-	-	-	16.500	8.0	2	-	+	GIP	DV
14	F	7	3.8	Fair	38.0	(A)	140	-	36	-	-	-	17.500	8.5	2	-	+	GIP	DV
15	M	8	5.3	Good	(WI) 40.1	(A)	195	A 2/6	70	+	-	+	26.000	5.5	3	-	++	GIP	DV
16	M	8,5	9.2	Fair	38.0	(A)	150	M 3/6	55	-	-	-	18.000	6.5	2	2	+	GIP,HP	DV

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17	F	9	3.3	Poor	37.2	(M) 5-8	165	P 1/6 T 2/6	60	-	-	++	7.000	6.0	4	1	++	HP, CT, BP, CHF	DV
18	M	3	3.5	Poor	(WI) 40.5	(M) 5-8	170	P 4/6 M 3/6	65	+	++	++	5.000	6.5	5	6	++	BaCa, BP	DV, EX
19	F	3.5	4.2	Good	38.0	(A)	130	-	30	-	-	-	18.600	7.0	4	-	+	GIP	DV
20	F	5	4.3	Good	38.3	(A)	135	-	35	-	-	-	16.000	6.5	3	-	+	GIP,	DV
21	M	7.5	5.5	Fair	38.1	(A)	126	-	28	-	-	-	14.500	7.0	4	-	+	GIP	DV
22	F	6	4.1	Fair	38.0	(A)	140	-	30	-	-	-	16.400	7.5	3	-	+	GI	DV
23	M	5	4	Fair	38.6	(A)	140	-	32	-	-	-	14.400	6.5	3	5	+	PT, CHF, GIP, DV	

Abbreviations: Used medical abbreviations to describe findings include:

1. **RAVS Animal Condition Score:** Excellent, Good, Fair, Poor, and Critical
2. **Fever (°C) / According to the degree of increase:** (SF) sub febrile increase of 0.1 - 0.5 °C, (WI) weak increase by 1 °C (Duration: *febris ephemera*; 1-2 days)
3. **Assessing Dehydration:** (A) 0-5% - Adequate- No abnormalities were seen, (M) 5-8% - Marginal - CRT (2 sec), eyes slightly sunken in sockets, mucous membranes slightly dry or tacky, (O) 8-10% -Obvious delay - increased CRT (2- 2.5 sec), eyes sunken in sockets, mucous membranes dry, slightly tacky, (5-10 seconds) in skin returning to normal position.
4. **Auscultation: Dyspnea, cyanosis, "crackles" and coffin:** Negative finding / (-), Low / (+), Medium / (++), High / (+++).
5. **Heart Murmurs, location and Evaluating:** Usually, the valve area over which the murmur is loudest = (A) Aortic / (M) Mitral / (T) Tricuspid / (P) Pulmonic. **Grade: 1/6** – Can only be heard in a quiet room after several minutes of listening, **2/6** – Can be heard immediately, but is very soft, **3/6** – Low to moderately intense, **4/6** – Loud, but without a palpable thrill, **5/6** – Loud, with a palpable thrill, **6/6** – Can be heard with the stethoscope slightly off the thoracic wall
6. **Diagnosis/outcome of treated cats:** HP - Hydroperikard, CT - colapsus tracheae, CHF - congestive heart failure, BP - Bacterial bronchopneumonia, PT - Pneumothorax, VBP - Virus bronchopneumonia. DCM - Dilated cardiomyopathy. OP - Oedema pulmonum, DCa - Disseminated carcinoma, BaCa - Bronchoalveolar localization of pulmonary carcinoma, DV - dewormed, GIP - gastro-intestinal parasitosis, S - suction, EX - exitus.

Degree of infection / Coprology (semi-quantitative FEC scale by Pittman et al., 2010): Low (+): 1-10 eggs; Medium (++): 11-49 eggs; High (+++): ≥ 50 eggs (number of eggs calculated per a covering - epc)
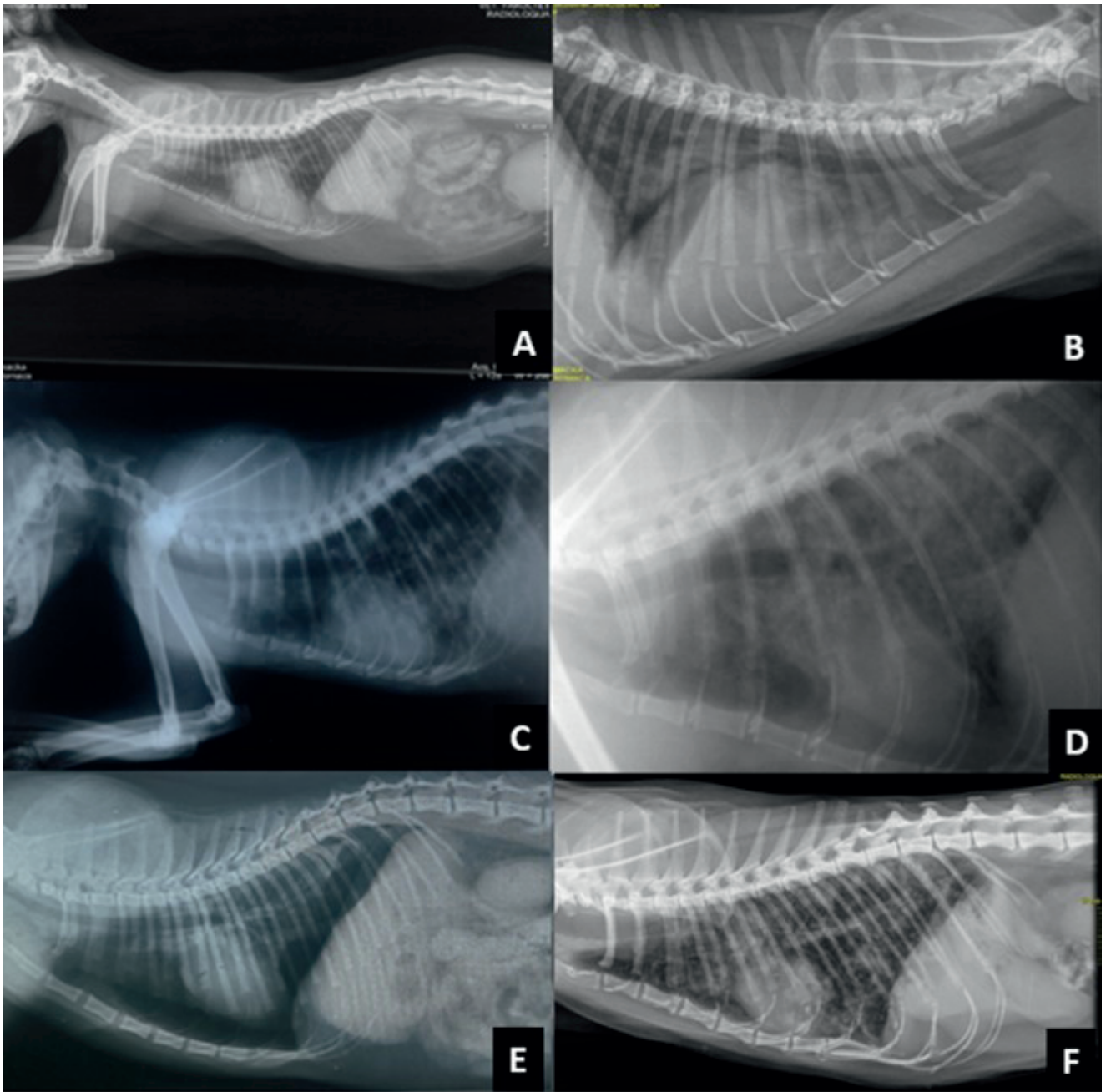
Fig. 4 (A, C, D, E, and F) right lateral thoracic and (B) left lateral thoracic X-rays of the cats with *Capillaria aerophila* infection.

(A) Right lateral thoracic X-ray a 9 year - old female infected with *C. aerophila*. A complex lung pattern is visible across the pulmonary fields, characterized by bronchial and alveolar changes and mild reticular interstitial pattern and there is an enlargement of the cardiac silhouette with collapses tracheae and ascites.

(B) Left lateral thoracic X-ray an 8,5 year - old male infected with *C. aerophila*. An oedema pulmonum is present and there is a large enlargement of the cardiac silhouette due to the dilated cardiomyopathy with hydropericardium present with bronchial wall thickening and increased interstitial opacity.

(C) Right lateral thoracic X-ray a 6 year - old female infected with *C. aerophila*. Bacterial bronchopneumonia with complex lung pattern, characterized by bronchial and alveolar changes and emphysema and atelectasis of the diaphragmatic lobes.

(D) Right lateral thoracic X-ray a 9 year - old female infected with *C. aerophila*. The virus caused bronchopneumonia with secondary bacterial complications and complex lung pattern, characterized by bronchial and alveolar changes with atelectasis of caudal pulmonary lobes and increased interstitial opacity.

(E) Right lateral thoracic X-ray a 7,5 year -old male infected with *C. aerophila*. Bacterial bronchopneumonia complicated with pneumothorax and pulmonary hypertension - there is an enlargement of the pulmonary artery of the caudal lobar pulmonary artery.

(F) Right lateral thoracic X-ray a 7,5 year -old male infected with *C. aerophila*. Bronchoalveolar localization of Ca with oedema pulmonum characterized by bronchial and alveolar changes and very difficult respiratory signs due to; tachypnoea, dyspnoea with abdominal breathing, and end-inspiratory crackles upon auscultation.

Thoracic radiography results

X-rays of the cats with *Capillaria aerophila* infection have a typical mild lung pattern across the pulmonary fields and is characterized by mild bronchial and alveolar changes and a mild reticular interstitial picture (Fig. 3) We present radiograms of the ventrodorsal and right lateral thoracic X-rays of 7 cats with *C. aerophila* infection. Four of them had bronchopneumonia with a mild reticular interstitial pattern or with complex lung pattern which was visible across the pulmonary fields, characterized by bronchial and alveolar changes and increased interstitial opacity due to bacterial bronchopneumonia, or viral bronchopneumonia complicated with the secondary bacterial infection, pneumothorax, edema pulmonum, bronchial wall thickening, emphysema, atelectasis, tracheal collapse, an enlargement of the pulmonary artery and pulmonary hypertension. Those changes were followed by respiratory clinical signs due to: tachypnea, dyspnea, abdominal breathing, and end-inspiratory crackles upon auscultation (Fig. 4).

Four cats (7.27 %; 4/55) had severe mixed dyspnea upon auscultation, with the hematology panel indicating eosinophilia and bronchitis on chest x-ray (Fig. 3A, 3B), based on which eosinophilic granulomatous pneumonia was suspected. In 9.1 % (5/55) of cats, dyspnea accompanied by wheezing and crepitations were detected during pulmonary auscultation and were suspected to be due to bronchopneumonia. Hematology panel of these animals showed leukocytosis and eosinophilia. Infection of the lower portions of the airways in diseased cats was accompanied by hyperthermia in 21,7 % (5/23) of cats.

Cytological evaluation of the tracheal lavage of one dead cat

In most cats with respiratory symptoms, bronchial lavage reveals the presence of cellular exudate, foam, and mucus, while in some cats adult *C. aerophila* stages were present in the lumen of the bronchi. Cytology of the bronchoalveolar fluid can demonstrate moderate cellular density, large amounts of degenerated neutrophils (>90 %), and intracellular bacteria (Coccobacilli), suggesting the presence of suppurative bronchitis. There were no deviations from physiological parameters in the performed cytological analyzes of one dead cat.

Domestic cats brought by the owners had constant access to the open space. For 55 cats, we found a history of respiratory symptoms lasting from 2 weeks to 4 months. All the examined cats had been previously treated in some private veterinary clinic, even for as long as several weeks, for various respiratory conditions: snoring, dysphonia, and initially nonproductive cough, which a short time after had usually turned into a productive cough. During the treatment, cats were treated with corticosteroids, one or several antibiotics, including enrofloxacin, doxycyclin, and cephalosporins, and some of them were also on nebulization therapy. In general, the therapy did not produce improvements.

Discussion

In carnivores, *C. aerophila* infection usually causes chronic bronchitis (Traversa *et al*., 2010). However, it can follow different clinical courses, from mild subclinical infection to life-threatening acute respiratory distress (Traversa *et al*., 2010).

Rehbein *et al*. (2014) have described a prevalence of 6.6 % of *Capillaria* spp. eggs in the the feces of cats in seven European countries (Albania - 31,6 %, Austria - 3,8 %, Bulgaria - 4,3 %, Germany - 1,6 %, Hungary - 5,0 %, Latvia - 4,1 %, Lithuania - 2,6 %). The total number of infection varied among the regions probably because of differences in the living behavior of the studied cats (rural, suburban, or urban areas).

As the most common helminths, gastrointestinal parasites have been described in cats worldwide: ascarids (*Toxocara cati* and with a significantly lower prevalence of *Toxascaris leonina*), hookworms (mainly *Ancylostoma tubaeforme* and depending on climate, occasionally *Uncinaria stenocephala*), cestodes from family Dilepididae and Taenidae (predominantly *Taenia taeniaeformis*, and less frequently *Echinococcus multilocularis* in endemic areas) (Rehbein *et al*., 2014).

In this study of gastrointestinal parasitosis, the highest prevalence of toxocariasis (18.83 %) was diagnosed, followed by *Isospora* spp (10.97 %) and ancylostomatids (7.74 %). Of the mixed infections, only double infections were identified. The most common infection was *Isospora* spp. and *T. mystax* (3.22 %), and an identical prevalence (2.58 %) of coinfection of this ascarid with *C. aerophila* and ancylostomatids were found. The factors such as cat category (e.g., stray cats, cats from animal shelters, pets kept under controlled conditions), habitat (urban or rural) or access to open spaces can have a significant impact on the variation of prevalence of these endoparasitoses (Mircean *et al*., 2010; Capari *et al*., 2013).

The first studies of respiratory capillariosis in cats in Serbia were conducted in the area of Belgrade in the period 2003 – 2007 when the prevalence of *C. aerophila* was determined to be 8.03 % (Ilić *et al*., 2009). In the last decade, the prevalence of this nematode in cats in Belgrade has almost doubled. The established growth trend can be attributed to the increase in the number of foxes as a reservoir of infection for pets (Traversa & Di Cesare, 2016). This claim is supported by the results of research, according to which an extremely high prevalence of *C. aerophila* was found in foxes in Serbia (Lalošević *et al*., 2013; Ilić *et al*., 2016).

Several studies from different countries have confirmed that in the past two decades the number of cases of respiratory capillariasis has grown both in cats and humans (Barrs *et al*., 2000; Di Cesare *et al*., 2012; Cross, 1998; Lalošević *et al*., 2008). Human infections have been described in Ukraine, Russia, France, Morocco, Iran (Di Cesare *et al*., 2012), and Serbia as well (Lalošević *et al*., 2008). Some recent molecular-based studies have shown that different

haplotypes of *C. aerophila* may circulate between domestic and wild carnivores (Di Cesare *et al*., 2012). Indeed, genetic studies demonstrated that some *C. aerophila* haplotypes may infect both pet and wild animals from the same locations suggesting that wild animals may have an impact on the transmission to and the propagation of respiratory capillariosis in the feline population (Traversa & Di Cesare, 2016). In Europe, fifteen different haplotypes have been identified, out of which five are identical between pet and wild animals in Italy (Di Cesare *et al*., 2014). Three genetic subpopulations infecting cats and foxes were identified in Serbia (haplotype I and II) and one each in Romania (haplotype I) and Portugal (haplotype VII) (Di Cesare *et al*., 2014).

Literature data also suggests that cats can be infected with the nematodes from the *Trichuris* genus (*T. felis, T. campanula,* and *T. serrata*). Although these species are very rarely diagnosed in cats and only in limited geographical areas (Traversa *et al*., 2010; Traversa, 2011), the finding of their eggs in the feces additionally complicates the interpretation of coprology results and precise diagnosis (Traversa & Di Cesare, 2016; Giannelli *et al*., 2017).

The predominant clinical signs associated with respiratory capillarisosis are productive cough accompanied by mucopurulent nasal discharge, tachypnea, dyspnea with evident abdominal breathing, and end-inspiration crackling during auscultation. In more severe cases, respiratory failure leads to mucosal cyanosis and respiratory acidosis (Traversa *et al*., 2012). In 14.84 % of the studied cats, increased broncho-vesicular respiratory sounds, sneezing, wheezing and dry cough were the most frequent clinical signs detected. These clinical findings are the result of *C. aerophila* adult worms which are capable of inducing lesions in the trachea and lung parenchyma. In a few cases, the cough became productive probably because of a more advanced disease phase associated with secondary bacterial bronchopneumonia. Fever was the consequence of secondary bacterial bronchopneumonia.

Furthermore, the achievement of an accurate diagnosis is further complicated because some cats infected with respiratory parasites can display clinical improvement after unspecific treatment including corticosteroids and bronchodilators (Traversa & Di Cesare, 2016). This apparent improvement can delay the definitive diagnosis for several weeks (Traversa & Di Cesare, 2016).

The differential diagnosis may include all diseases causing upper and/or lower respiratory signs including other parasitic infections (dirofilariasis, pulmonary toxoplasmosis, aelurostrongylosis, troglostrongylosis) but also tracheobronchitis, tracheal stenosis, pneumonia (of bacterial, viral or fungal etiology), airway obstructions caused by foreign bodies, nasopharyngeal polyps, tumors, pulmonary granulomatosis, allergies, and chronic bronchial diseases affecting cats (asthma) (Spada *et al*., 2013; Ilić *et al*., 2015). All the before mentioned diseases are not characterized by pathognomonic symptoms which complicates the clinical diagnosis.

Complications associated with a fatal outcome in infected animals are interstitial emphysema, lung edema, and secondary bacterial infections (Traversa *et al*. 2012). In some cases, these infec-

tions may be complicated by pleural effusions or pneumothorax (Traversa *et al*., 2009). An additional complication is an absence or the non-pathognomonic abnormalities detect on thoracic radiographs (Traversa *et al*. 2012) The most common finding is an alveolar lung pattern, possibly associated with the bronchial and interstitial pattern, with bronchial wall thickening and reduced interstitial transparency (Traversa *et al*. 2012). In chronic or mixed infections, right-side cardiomegaly may develop, associated with eccentric hypertrophy, occurring secondary to pulmonary hypertension. This condition is the consequence of severe bronchopneumonia caused by a secondary bacterial infection or mixed infection with *A. abstrusus* or *Dirofilaria immitis* (Dirven *et al*., 2012).

Secondary bacterial infections contribute to the severity of parasitic infections of the lungs; that is the reason why broad-spectrum antibiotics combined with corticosteroids should always be administered (at anti-inflammatory doses) to all cats with bronchopneumonia symptoms (Foster, 2004). Pleural effusion and pneumothorax require immediate thoracocentesis (pleural tap) (Pechman, 1984). The care of a cat with respiratory failure should include the management in an intensive care unit with mandatory use of oxygen therapy and nebulization (Foster, 2004).

All the cats included in this study reached a complete improvement (absence of clinical signs and negative coprology results) after treatment with fenbendazole. However, in some of these cats, clinical signs were present for even up to six weeks after anthelminthic treatment, as shown by positive coprology results for *C. aerophila*. This could be explained by either reinfection due to living conditions (households with many cats and free access to open spaces) or by a partial response to fenbendazole treatment. Several protocols have been reported in the literature for the treatment of respiratory capillariosis in cats, such as the combinations of fenbendazole paste, milbemycin-oxime/praziquantel, various spot-on formulations, abamectin, ivermectin, and levamisole (Brianti *et al*., 2013; Traversa & Di Cesare, 2013). Oral administration of fenbendazole with different doses and duration of treatment has been suggested by several authors (from 20 mg/kg in 5 days to 50 mg/kg in 15 days) as effective treatment (Grandi *et al*., 2005). Although positive results have been reported with ivermectin, there is a prevailing opinion that these agents should not be used in cats, especially in kittens, due to toxicity concerns (Brianti *et al*., 2013; Traversa & Di Cesare, 2013). In fieldwork, individual administration of the formulation imidacloprid 10 % / moxidectin 1 % in naturally infected cats has been reported to be safe and effective. To improve clinical signs, supportive therapy is recommended to help respiratory function, eliminate secondary infection, and stimulate the appetite of affected cats (Gianelli *et al*., 2014).

Environmental changes resulting from global warming in addition to oral vaccination against rabies have significantly increased the population of wild carnivores, especially foxes (Ilić *et al*., 2016a). The population of foxes has to be considered as a constant reservoir of infection for domestic carnivores in suburban areas. therefore, data on the way of living and the territory of owned cats are

very important to collect when taking anamnesis. Similarly, canine and feline stray populations have increased in both urban and sub-urban environments in Serbia (Đurić *et al*., 2011; Lažetić *et al*., 2012). Consequently, the increase in both populations of will and stray carnivores has contributed to significantly raise the population of possible reservoirs for pets, creating favorable conditions for the constant maintenance and spread of zoonotic parasites (Obrenovic *et al*., 2003; Nikolić *et al*., 2008; Ilić *et al*., 2017; Ilić *et al*., 2017a) including *C. aerophila*.

Because of the direct life cycle and zoonotic potential of *C. aerophila*, sanitary measures are necessary to control the disease, especially in living environments with larger groups of animals (Brianti *et al*., 2012; Gianelli *et al*., 2014; Brianeri *et al*., 2014).

Conclusion

Considering the zoonotic potential of *C. aerophila* and the spreading tendency of parasitic diseases caused by nematodes, more effort should be managed to achieve an accurate diagnosis of the causal organism. This study stressed out the importance of including parasitic diseases in the differential diagnosis of respiratory conditions in cats. Although allergic bronchitis is usually considered the most frequent cause of chronic cough in cats, it is recommended to delay the steroid therapy until a potential infection is excluded.

In humans, the disease is characterized by a non-specific clinical picture and is usually detected accidentally, which is the reason why prevention measures are essential in the fight against this parasitosis of cats. Finally, this study contributes to a better understanding of the epidemiology of respiratory capillariosis in the city of Belgrade and highlights the possibility of human infection due to the close contact between infected pet cats and their owners.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgments

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