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Endoparasites of wildcats in Croatia

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ABSTRACT

Reports on the parasitic fauna of wildcats (*Felis silvestris silvestris*) are rare and often based on a small sample size, therefore the goal of this research was to investigate the prevalence of endoparasites in wildcats in Croatia. Necropsy was conducted on 34 adult wildcats killed in traffic or provided by hunters following regular hunting operations. All animals tested negative for rabies. The contents of the stomach and intestine were examined under a microscope. Feces from the rectum were analyzed using flotation with a saturated $ZnSO_4$ solution, while the diaphragm was examined using artificial digestion. Direct immunofluorescence was used for the first time to detect *Giardia* sp. cysts in wildcats. All animals were infected with at least one species of parasites, while the most diverse infestation included six different species of parasites in a single animal. The following parasite species were found (% of prevalence of adult parasites and their developmental stages in all analyzed samples): *Taenia taeniaeformis* (55.9%), *Capillaria* sp. (50%), *Toxocara cati* (50%), *Isospora* sp. (29.4%), *Strongyloides* sp. (23.5%), *Giardia* sp. (17.6%), *Ancylostoma tubaeformae* (14.7%), *Physaloptera* sp. (11.8%), Hymenolepididae (8.8%), *Alaria alata* (5.9%), *Aelurostrongylus abstrusus* (5.9%), *Toxascaris leonina* (5.9%), *Trichinella* sp. (5.9%), *Mesocestoides lineatus* (5.9%), Anoplocephalidae (2.9%), *Dipylidium caninum* (2.9%), *Trichuris* sp. (2.9%), *Isospora felis* (2.9%), *Eimeria* sp. (2.9%) and *Sarcocystis* sp. (2.9%). Among

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those, *Eimeria* sp., *Trichuris* sp. eggs, anoplocephalid and hymenolepidid type eggs are spurious parasites, coming from ingested prey. Four of the identified species have never been previously reported in wildcats - *Giardia* sp., *Strongyloides* sp., *Sarcocystis* sp. and *Dipylidium caninum*.

Key words: parasites, wildcat, *Felis silvestris silvestris*, Croatia

Introduction

The wildcat (*Felis silvestris silvestris*) is the most common and widely-distributed wild felid, found throughout most of Africa, Europe, southwest and central Asia. In Europe the species is native to most countries, except Fennoscandia, but today the species has a sporadic distribution throughout the continent (YAMAGUCHI et al., 2015). In Croatia the wildcat is present throughout the country, except on the islands (JANICKI et al., 2007). Unfortunately, detailed distribution maps are not available for Croatia, and also there are no scientific data about population size and trends, demographic dynamics, ecology, and diseases.

The effects of pathogens and diseases on the conservation of carnivores are of major concern, but knowledge of their parasitic fauna is scarce (FUNK et al., 2001). Comprehensive parasitological studies depend on the ability to collect a large number of samples. Carcasses are the best source for research of gastrointestinal fauna, while copromicroscopic surveys may be a useful alternative (NAPOLI et al., 2016). In protected species (like the wildcat in most European countries, including Croatia since 2013) it may be difficult to obtain carcasses, as they are rarely found in nature (apart from road kills). Studying wildcat gastrointestinal parasites from feces is also challenging because, due to the low population densities and their solitary way of life, it may be challenging to find fresh samples. As a result, studies on parasites of wildcats are not only rare, but also based on a limited sample size (BURT et al., 1980; SCHUSTER et al., 1993; PAPADOPOULOS et al., 1997; RODRIGUEZ and CARBONELL, 1998; KRONE et al., 2008; KIRKOVA et al., 2011; NAPOLI et al., 2016; VERONESI et al., 2016).

In Croatia, there are no data about parasites in wildcats. Therefore, the aim of this survey was to investigate the prevalence of gastrointestinal and *Trichinella* parasites in wildcats in Croatia.

Materials and methods

Samples. A total of 34 entire carcasses or intestines with diaphragm of adult wildcats killed in traffic accidents or culled during regular game management activities (prior to the hunting ban in 2013) were collected and stored at -20 °C. DNA analysis confirmed all the samples were from wildcats and that there were no domestic cats in the sampling (SINDIČIĆ et al., 2016).

Study area. The origin of samples collected is presented on Fig. 1.

Fig. 1. Location of origin of wildcat samples. The size of the circle corresponds to the number of samples from each location.

Necropsy and parasitological examination. Before necropsy, each animal was tested for rabies and all the tests were negative. Necropsies were conducted at the Faculty of Veterinary Medicine, University of Zagreb, according to a standard veterinary protocol. The gastrointestinal tract (stomach and intestines) was removed from the animal, stored in separated plastic containers, opened along its length and flushed with water, while its mucosa was scraped and separated in another plastic container. From some wildcats only the intestines and diaphragms were available.

The contents of the stomach and intestines were examined in Petri dishes on a dark background under a stereomicroscope, while the scraped mucosa was checked for parasites under the microscope. Parasites were then washed out with saline to remove the gastrointestinal contents in order to facilitate definition to the genus or species level, depending on the condition.

Copromicroscopic examination. Feces was removed from the rectum and separated into two parts of 5 g each. One part was analyzed using the flotation method with a saturated $ZnSO_4$ solution (S.G. = 1.3), and the other was used to detect *Giardia* sp. cysts. Fecal samples were first prepared using the flotation technique with sucrose, and then analyzed by the direct immunofluorescence method (Meridian Bioscience, Inc., Merifluor® *Cryptosporidium/Giardia*).

Artificial digestion. The presence of *Trichinella* sp. parasites was analyzed using diaphragm samples (10g) by the reference method for artificial digestion of *Trichinella* in meat (The European Community Regulation (EC) No. 2075/2005).

Parasite determination. The parasite identifications and staining were based on the available descriptions (MEHLHORN et al., 1993; HRČKOVA et al., 2011; BRIANTI et al., 2012; SCHMÄSCHKE, 2013). Briefly, adult parasites were stained with lactophenol (nematodes,

tapeworms) or *lactic acid-carmine stain (tapeworms), if needed. N*ematodes were identified by anterior end morphology (*Toxocara canis, Toxascaris leonina, Ancylostoma tubaeformae, Physaloptera* sp.*, Strongyloides* sp.*)*, cestodes by hooks size, hermaphrodite and gravid proglottid morphology (*Taenia taeniaeformis, Mesocestoides lineatus, Dipylidium caninum*), and developmental parasite stages (larvae, eggs, oocysts) by size (length, width), the thickness and smoothness of the egg shell, or specific morphological characteristics such as egg protuberance morphology (*Trichuris* sp., *Capillaria* sp.), operculum presence (*Alaria alata*), larval tail morphology (*Aelurostrongylus abstrusus*), or stichocyte presence (*Trichinella* sp.). Some of the parasites were microphotographed and edited with QuickPhoto Micro 2.3 software (Fig. 2 - Fig. 4). Due to significant degradation, identification to species level was not possible for some samples.

Common parasite species were defined as those whose prevalence was at least 20% (WAID and PENCE, 1988).

Results

All animals were infected with at least one parasite species. The most diverse infection, including six different species of parasites, was found in three animals (8.82%), while mono infections dominated in eight examined samples (23.53%) (Tables 1 and 3).

Table 1. Number of parasite species per wildcat

No. animals/No of parasite species per animal	Prevalence (%) n = 34
3/6	8.82
5/5	14.71
6/4	17.65
6/3	17.65
6/2	17.65
8/1	23.53

Parasites from 19 different genera and at least 20 different species were identified (Tables 2 and 3). The most prevalent parasites in this survey were *T. cati* (Fig. 2a), *T. taeniaeformis* (Fig. 2b, Fig. 2c), *Capillaria* sp., *Isospora* sp. and *Strongyloides* sp. (Fig. 3e, Fig. 3f). Other parasites, such as *T. leonina* (Fig. 2a), *M. lineatus* (Fig. 3a, Fig. 3b)*, D. caninum* (Fig. 3c, Fig. 3d)*, A. tubaeformae* (Fig. 4a, Fig. 4b)*, A. abstrusus* (Fig. 4c, Fig. 4d)*, A. alata* (Fig. 4e)*, Giardia* sp. (Fig. 4f), *Physaloptera* sp., *Isospora felis*, *Sarcocystis* sp., *Eimeria* sp., *Trichuris* sp., Hymenolepididae and Anoplocephalidae, were less prevalent (Table 2). *Trichinella* sp. larvae were found in only two wildcat samples (Table 3).

Table 2. Prevalence of gastrointestinal helminth and protozoan parasites in wildcats (n = 34)

Species	(NPE)	P (%)	(NCE)	P (%)	Total (NPE+NCE)	P (%)
Taenia taeniaeformis	18	52.9	4	11.8	19	55.9
Mesocestoides lineatus	1	2.9	1[a]	2.9	2	5. 9
Hymenolepidid eggs[s]	2[e]	5.9	2	5.9	3	8.8
Anoplocephalid eggs[s]	0	0.0	1	2.9	1	2.9
Dipylidium caninum	1	2.9	0	0.0	1	2.9
Alaria alata	1[e]	2.9	2	5. 9	2	5. 9
Ancylostoma tubaeforme	2	5.9	5	14.7	5	14.7
Aelurostrongylus abstrusus	1[l]	2.9	2	5. 9	2	5.9
Toxocara cati	16	47.1	15	44.1	17	50.0
Toxascaris leonina	2	5.9	2	5.9	2	5. 9
Trichuris sp.[s]	0	0.0	1	2.9	1	2.9
Capillaria sp.	7[e]	20.6	17	50.0	17	50.0
Physaloptera sp.	4	11.8	1	2.9	4	11.9
Strongyloides sp.	7	20.6	1	2.9	8	23.5
Isospora felis	0	0.0	1	2.9	1	2.9
Isospora sp.	0	0.0	10	29.4	10	29.4
Eimeria sp.[s]	0	0.0	1	2.9	1	2.9
Giardia sp.	0	0.0	6	17.6	6	17.6
Sarcocystis sp.	0	0.0	1	2.9	1	2.9

NPE: number of parasitologically positive intestines containing adult parasites if not differently indicated; NCE: number of positive fecal samples containing eggs, oocysts or larvae if not differently indicated; NPE+NCE: number of positive intestines and fecal samples; P: prevalence; [e]: only eggs were detected; [l]: only larvae were detected; [a]: only adults were detected; [s]: spurious parasites.

Table 3a. Parasite species per wildcat sample (samples WC1-WC11)

Sample No	WC1	WC2	WC3	WC4	WC5	WC6	WC7	WC8	WC9	WC10	WC11
Species	PE/CE	PE/CE	PE/CE	PE/CE	PE/CE	PE/CE	PE/CE	PE/CE	PE/CE	PE/CE	PE/CE
T. taeniaeformis	+/-	+/-	-/-	+/-	-/-	-/-	-/-	+/-	-/-	-/-	+/-
M. lineatus	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	+/-	-/-
Hymenolepidids	-/-	-/-	-/-	-/-	+^{e}/+	+^{e}/-	-/-	-/-	-/-	-/-	-/-
Anoplocephalids	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/+	-/-	-/-	-/-
D. caninum	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
A. alata	-/-	-/-	-/-	-/-	-/-	-/-	-/-	+^{e}/+	-/-	-/-	-/-
A. tubaeforme	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
A. abstrusus	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
T. cati	+/+	-/-	+/+	+/+	+/+	+/+	-/-	+/+	-/-	-/-	+/+
T. leonina	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Trichuris sp.	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Capillaria sp.	-/-	-/-	-/-	-/+	-/-	-/-	+^{e}/+	+^{e}/+	-/-	+^{e}/+	-/+
Physaloptera sp.	-/-	-/-	-/-	+/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Strongyloides sp.	-/-	-/-	-/-	-/-	-/-	-/-	-/-	+/-	-/-	-/+	+/-
I. felis	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Isospora sp.	-/-	-/-	-/+	-/+	-/+	-/+	-/-	-/+	-/+	-/-	-/+
Eimeria sp.	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Giardia sp.	-/-	-/-	-/+	-/+	-/+	-/+	-/-	-/-	-/-	-/-	-/-
Sarcocystis sp.	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Trichinella sp. ^{ad}	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-

WC: wildcat; PE: Parasitological examination of intestines containing adult parasites if not differently indicated; CE: Coprological examination containing eggs, oocysts or larval stages if not differently indicated; +: positive; -: negative; ^{a}: adults; ^{e}: eggs; ^{l}: larvae; ^{o}: oocysts; ^{ad}: artificial digestion - results obtained with artificial digestion method

Table 3b. Parasite species per wildcat sample (samples WC12-WC22)

Sample No	WC12	WC13	WC14	WC15	WC16	WC17	WC18	WC19	WC20	WC21	WC22
Species	PE/CE	PE/CE	PE/CE	PE/CE	PE/CE	PE/CE	PE/CE	PE/CE	PE/CE	PE/CE	PE/CE
T. taeniaeformis	+/-	-/-	-/-	+/-	-/-	+/-	+/+	+/-	-/-	+/-	+/-
M. lineatus	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Hymenolepidids	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Anoplocephalids	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
D. caninum	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	+/-	-/-	-/-
A. alata	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
A. tubaeforme	-/-	-/-	-/-	-/-	-/-	-/-	-/+	-/-	-/-	-/-	-/+
A. abstrusus	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/+	$+^{l}$/+	-/-	-/-
T. cati	-/-	-/-	+/+	-/-	-/-	+/-	+/-	-/-	-/-	+/+	+/+
T. leonina	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Trichuris sp.	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/+	-/-
Capillaria sp.	-/+	-/-	-/+	-/-	-/-	-/+	$+^{e}$/+	$+^{e}$/+	$+^{e}$/+	-/+	$+^{e}$/+
Physaloptera sp.	-/-	-/-	+/-	-/-	-/-	+/-	-/-	-/-	-/-	-/-	-/-
Strongyloides sp.	-/-	+/-	+/-	-/-	-/-	-/-	+/-	+/-	+/-	-/-	-/-
I. felis	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Isospora sp.	-/-	-/-	-/-	-/-	-/-	-/+	-/-	-/+	-/-	-/-	-/+
Eimeria sp.	-/+	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Giardia sp.	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Sarcocystis sp.	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Trichinella sp. [ad]	-/-	$+^{l}$	-/-	-/-	$+^{l}$	-/-	-/-	-/-	-/-	-/-	-/-

WC: wildcat; PE: Parasitological examination of intestines containing adult parasites if not differently indicated; CE: Coprological examination containing eggs, oocysts or larval stages if not differently indicated; +: positive; -: negative; [a]: adults; [e]: eggs; [l]: larvae; [o]: oocysts; [ad]: artificial digestion - results obtained with artificial digestion method

Table 3c. Parasite species found in individual wildcat sample (samples WC22-WC34)

Sample No	WC23	WC24	WC25	WC26	WC27	WC28	WC29	WC30	WC31	WC32	WC33	WC34
Species	PE/CE	PE/CE	PE/CE	PE/CE	PE/CE	PE/CE	PE/CE	PE/CE	PE/CE	PE/CE	PE/CE	PE/CE
T. taeniaeformis	+/+	+/+	-/-	-/-	+/-	-/-	+/-	-/-	-/+	+/-	+/-	-/-
M. lineatus	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/+[a]	-/-	-/-	-/-
hymenolepidids	-/-	-/-	-/-	-/+	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
anoplocephalids	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
D. caninum	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
A. alata	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/+	-/-	-/-
A. tubaeforme	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	+/+	-/+	+/+	-/-
A. abstrusus	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
T. cati	-/-	-/-	+/+	-/-	+/+	-/-	+/+	-/-	-/-	+/+	-/+	-/-
T. leonina	+/+	+/+	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Trichuris sp.	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Capillaria sp.	-/-	-/-	-/-	-/+	-/-	-/-	-/+	-/+	-/-	-/-	-/+	-/-
Physaloptera sp.	-/-	-/-	-/-	-/-	-/-	+/+	-/-	-/-	-/-	-/-	-/-	-/-
Strongyloides sp.	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
I. felis	-/-	-/-	-/-	-/-	+[o]/+	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Isospora sp.	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Eimeria sp.	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Giardia sp.	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/+	-/+
Sarcocystis sp.	-/-	-/-	-/-	-/-	-/+	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Trichinella sp. [ad]	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-

WC: wildcat; PE: Parasitological examination of intestines containing adult parasites if not differently indicated; CE: Coprological examination containing eggs, oocysts or larval stages if not differently indicated; +: positive; -: negative; [a]: adults; [e]: eggs; [l]: larvae; [o]: oocysts; [ad]: artificial digestion - results obtained with artificial digestion method

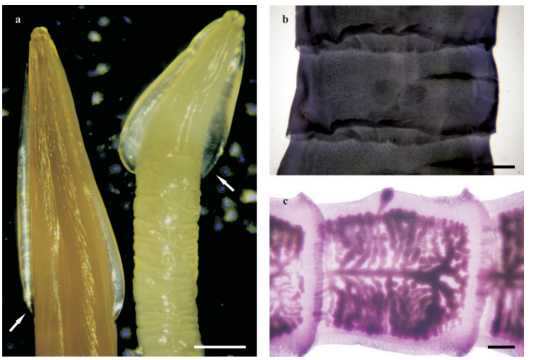

Fig. 2. Parasites of wildcat. (a) Morphological comparison of *Toxascaris leonina* and *Toxocara cati* anterior end. Long and narrow cervical alae in *T. leonina* on the left (arrow) and short and wide cervical alae in *T. cati* on the right (arrow). (b) *Taenia taeniaeformis* hermaphrodite proglottids. (c) *Taenia taeniaeformis* gravide proglottids. Scale bar = 500 μm.

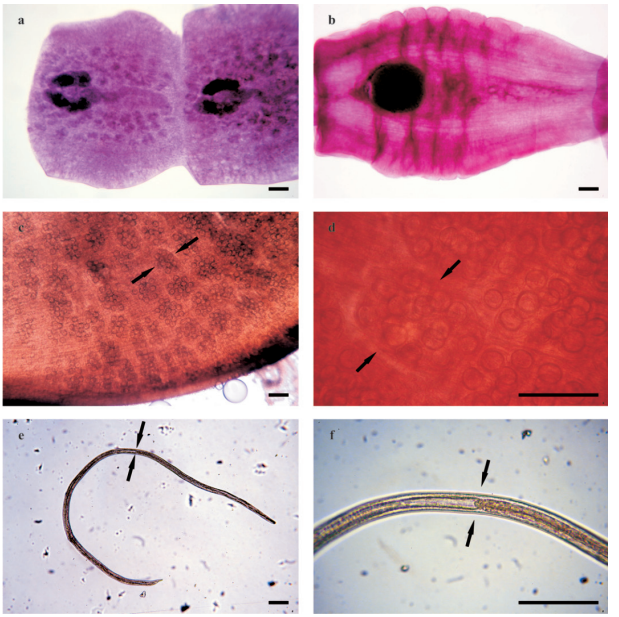

Fig. 3. Parasites of wildcat. (a) *Mesocestoides lineatus* hermaphrodite proglottids. (b) *Mesocestoides lineatus* gravide proglottids. (c) *Dipylidium caninum* gravide proglottid containing cocoons with eggs (in between arrows). (d) *Dipylidium caninum* gravide proglottid containing cocoons with eggs (in between arrows). (e) *Strongyloides* sp. female with long esophagus (arrows indicating esophagus to intestine transition). (f) *Strongyloides* sp. female with long esophagus (arrows indicating esophagus to intestine transition). Scale bar = 100 μm.

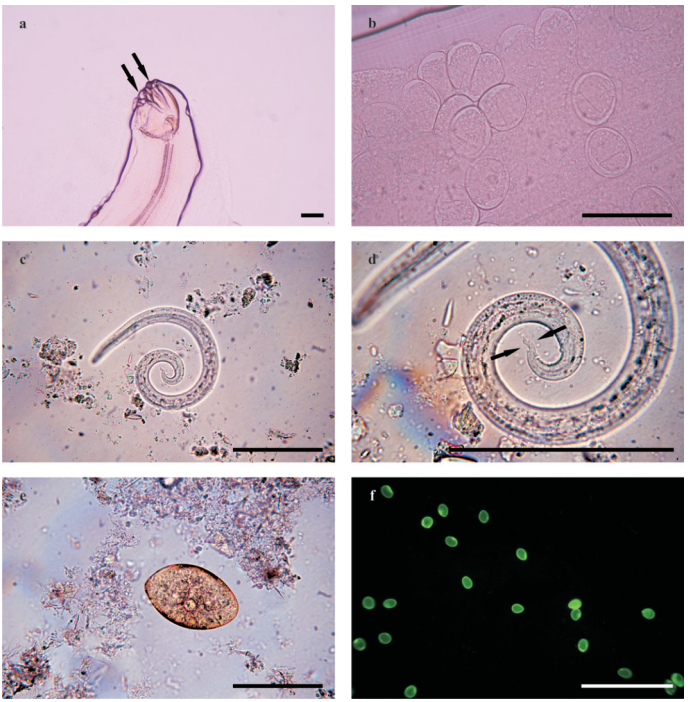

Fig. 4. Parasites of wildcat. (a) Latero-lateral view of *Ancylostoma caninum* anterior end. Overlapping teeth on dorsal margin of buccal capsule (arrows). (b) *Ancylostoma caninum* eggs in female. (c) *Aelurostrongylus abstrusus* larva. (d) *Aelurostrongylus abstrusus* larva with characteristic tail shape (arrow). (e) *Alaria alata* egg. (f) *Giardia* sp. cysts under the fluorescence microscope after performed IFA test. Scale bar = 100 μm.

Discussion

The 100% prevalence of endoparasitic infections in this study was the same as reported previously in wildcats in Greece, Spain and Germany (PAPADOPOULOS et al., 1997; RODRIGUEZ and CARBONELL, 1998; KRONE et al., 2008). Similarly, a high prevalence was also found in Bulgaria (95.5%) (KIRKOVA et al., 2011) and Italy (90.9%) (NAPOLI et al., 2016).

Species diversity in this study was 0.6 (34 animals tested and 20 parasites species identified). The diversity of parasitic fauna and species prevalence differs between studies. In Greece, five different species of endoparasites were identified in four cats (PAPADOPOULOS et al., 1997), in France seven species in 39 samples (LEPLE, 2001), in Germany 8 in 15 wildcats (KRONE et al., 2008), in Slovenia 8 in 12 samples (BRGLEZ and

ŽELEZNIK, 1976), in Italy 10 species in 121 samples (NAPOLI et al., 2016), in Bulgaria 11 in 22 samples (KIRKOVA et al., 2011) in Spain 15 in 19 samples (RODRIGUEZ and CARBONELL, 1998); while the present survey had the highest diversity in terms of the different species identified (20 from 34 samples).

Among all parasites detected, the common parasites, with a prevalence of 20% or higher (WAID and PENCE, 1988) were *T. taeniaeformis, T. cati, Capillaria* sp., *Isospora* sp., and *Strongyloides* sp. *Taenia taeniaeformis, M. lineatus, T. cati, T. leonina, Capillaria* sp*., A. tubaeformae, A. abstrusus, Physaloptera* sp., *Trichinella* sp., *I. felis*, *Isospora* sp. have been commonly found in wildcat endoparasite surveys across Europe with differing prevalence, and the findings of this survey are in accordance with other studies. Four types of spurious parasites were found by copromicroscopic analysis: *Eimeria* sp., *Trichuris* sp. eggs, anoplocephalid and hymenolepidid type eggs. It may be assumed that they all originate from prey, rodents or lagomorphs, as previously reported by RODRIGUEZ and CARBONELL (1998) and KIRKOVA et al. (2011).

In this study, *Trichinella* spp. was found in two out of 34 analyzed samples (5.88%). In other studies conducted in different European countries, *Trichinella* was found in a higher prevalence, with the highest prevalence in Bulgaria (BRGLEZ and ŽELEZNIK, 1976; KIRKOVA et al., 2011; BLAGA et al., 2009; BADAGLIACCA et al., 2016). Infection of wildlife with *Trichinella* spp. is widespread throughout Europe. There are records of *T. britovi* and *T. spiralis* infection in wildcats, where *T. britovi* infection prevails by 73% vs. 27% (POZIO et al., 2009). In Croatia, *Trichinella* sp. infection in wildcats has not been detected until now, but is already known in other wild carnivores and omnivores including wolves, badgers, wild boars, bear, and lynx (BECK et al., 2009).

Four parasite species were identified which to our knowledge have previously never been reported in free-living wildcats: *D. caninum, Sarcocystis* sp., *Strongyloides* sp., and *Giardia* sp.

Strongyloides sp. was detected in eight wildcat samples (23.5%) in the current study and this is the first finding of this parasite in *Felis silvestris silvestris*. *Strongyloides* species are known, but rare parasites of felines worldwide, and up to now several different species have been described (THAMSBORG et al., 2016). There are a few reports about the low prevalence of *Strongyloides* sp. infection in domestic cats in Europe (TAKEUCHI-STORM at al., 2015a; MIRCEAN et al., 2010). Also *S. stercoralis* was found in one sample in *Lynx pardinus* in Spain (RODRIGUEZ and CARBONELL, 1998).

According to SPEARE and TINSLEY (1987), when fewer feces are used in routine diagnostics, the chances of finding *Strongyloides* sp. are slight. This problem can be avoided by using the Baerman test and greater quantities of feces. The females present in the intestine are very thin, and can be found only by dissecting the mucosa, a procedure not done consistently in any former studies. In this study, adults were found in seven

intestines, with rhabditid larvae in only one separate fecal smear (Table 3a-Table 3c). The reason for the low *Strongyloides* sp. prevalence within fecal samples, besides the preservation state of samples, could also be due to the amount of feces used. It could be concluded that *Strongyloides* sp. parasites are present in Croatian wildcats in a higher prevalence than was detected by fecal examination. In the future, more attention should be paid to avoiding false negative results, especially if only examination of feces is used for monitoring the parasites.

A. alata eggs were found in feces, which is a species previously reported but it is rare in wildcats (TAKACS et al., 2011). The species has been reported in domestic cats in several publications - adult flukes were identified in Uruguay (CASTRO et al. 2009), eggs in upstate New York (LUCIO-FORSTER and BOWMAN, 2011) and on Gran Canaria island (RODRÍGUEZ-PONCE et al., 2016); while mesocercariae were identified in domestic cats in Denmark (TAKEUCHI-STORM et al., 2015b). Recently, among all the surveys conducted on wildcats (BURT et al., 1980; SCHUSTER et al., 1993; PAPADOPOULOS et al., 1997; RODRIGUEZ and CARBONELL, 1998; KRONE et al., 2008; KIRKOVA et al., 2011; NAPOLI et al., 2016), only TAKACS et al. (2011) recorded *A. alata.* Adult flukes were found in the duodenum and eggs in intestinal scrapings in one male wildcat. *Alaria alata* was previously described in Croatia in other species: the presence of *A. alata* mesocercariae was described in wild boar (JAKŠIĆ et al., 2002), while adult flukes were recorded in foxes (RAJKOVIĆ-JANJE et al., 2002) and golden jackals (SINDIČIĆ et al., 2017).

It has never been proven that felids can act as definitive hosts for *A. alata* in Europe, and it is still uncertain if cats can act as paratenic or final hosts of *A. alata*, as they can host *Alaria marcinae*. In *A. marcinae* infection, both cat sexes are final hosts; while lactating females are primarily paratenic hosts, and mesocercarie can be transmitted through milk to their offspring (SHOOP and CORKUM, 1987). Unfortunately, adult flukes were not found in wildcat intestines in this investigation. Among animals analyzed in this study, there were also male cats infected with *A. alata*, indicating the possibility that felids can act as final hosts for *A. alata*. Additionally, reports from TAKACS et al. (2011), TAKEUCHI-STORM et al. (2015b) and RODRÍGUEZ-PONCE et al. (2016) and the present findings indicate that *A. alata* could behave in the same way as *A. marcinae* in feline hosts. Certainly, further investigation is needed to elucidate if wildcats can be definitive hosts for *A. alata* or not.

Both *D. caninum* and *Sarcocystis* sp. were found in only one sample (2.94%). To the authors' knowledge these species have not been previously reported in wildcat studies, but both of these parasites are a common finding in domestic cats, reaching up to 83.3% for *D. caninum* and 0.8-1% for *Sarcocystis* sp. in some surveys (KNAUS et al., 2011; LUCIO-FORSTER and BOWMAN, 2011; MIRCEAN et al., 2010). In general, the life cycle of *Sarcocystis* sp. exclusively includes intermediate and final hosts, where the former

behaves as a prey and the latter as a predator. Development to mature stages and the patent period of this parasite in cats are usually transient as most of the oocysts are shed during an approximately two-week period (ECKERT et al., 2005). It could be assumed that this short patent period is the reason why the parasite has never been identified in previous studies, because the sample has to be collected exactly during this short period when the parasite is present in the intestines.

Giardia sp. is a common parasite of domestic cats in Europe. During the 2001 - 2014 period, 29 surveys on domestic cats were conducted with prevalence ranging from 0% - 37%, depending on study area and method used (BOUZID et al., 2015). The current survey results showed the presence of *Giardia* sp. cysts in six wildcat samples (17.6%); while to the authors' knowledge no other studies conducted on wildcats detected the presence of *Giardia* sp. Infection with *Giardia* sp. in wild carnivores in Croatia, other than wildcats, was described by BECK et al. (2011a) and ranged from 4.5% in foxes, 10% in wolves, and up to 12.5% in jackals. Also, there is a report of *Giardia* sp. infection in captive felines from the Croatian Zagreb ZOO (BECK et al., 2011b) and in domestic cats (CACCIÒ et al., 2010). The low prevalence of *Giardia* sp. infection in this study could indicate that wildcats ingest *Giardia* sp. cysts by feeding on rodents. Rodents carry *Giardia muris, Giardia microti,* or *Giardia duodenalis* assemblage G, which may be unable to establish infection in wildcats, as was described for foxes (BECK et al., 2011a). Still, the true origin of *Giardia* sp. in wildcats remains to be discovered.

The results of the intestine examination coincide with the copromicroscopic examination in the majority of samples (Table 2, Table 3). There are some exceptions, e.g. *T. taeniaeformis* eggs were less prevalent in feces than the adults in intestine. This result was expected because the sensitivity of the flotation method is only 60-80% when more than 1000 eggs/g of feces are present. With lower fecal egg content, the results could be a false negative (ECKERT et al., 2005). Other exceptions (Table 2, Table 3) are due to the sample preservation state. Namely, some wildcats were collected as road kill, then frozen until examined, and thawed. As previously reported (RODRIGUEZ and CARBONELL, 1998; KIRKOVA et al., 2011), carcass degradation caused by environmental conditions could considerably influence the results, as some parasites (e. g. tapeworms, nematodes, nematode larvae, oocysts) could deteriorate over time and could be difficult to identify.

To the authors' knowledge this is the first report of the wildcat helminth and protozoan fauna in Croatia. Also, the free-living wildcat is reported as a host for *Giardia* sp., *Sarcocystis* sp., *Strongyloides* sp. and *D. caninum* for the first time.

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SAŽETAK

Znanstveni radovi o parazitima divljih mačaka (*Felis silvestris silvestris*) rijetki su i najčešće rađeni na malom broju uzoraka te je stoga naš cilj bio istražiti učestalost endoparazita divljih mačaka u Hrvatskoj. Pregledali smo lešine 34 divlje mačke, stradale u prometu ili u sklopu provedbe lovnogospodarskih osnova. Sve su životinje bile negativne na bjesnoću. Sadržaj želudca i crijeva pregledali smo pod mikroskopom. Izmet iz debelog crijeva pregledan je metodom flotacije pomoću zasićene otopine $ZnSO_4$, dok je dijafragma pregledana pomoću umjetne probave. Upotrijebili smo izravnu imunofluorescenciju za utvrđivanje cista *Giardia* sp., što je prvo takvo istraživanje kod divljih mačaka. Sve pregledane životinje bile su invadirane barem jednom vrstom parazita, dok je najraznolikija invazija uključivala šest različitih vrsta parazita utvrđenih kod jedne životinje. Sljedeće su vrste parazita identificirane (% prevalencije odraslih parazita i razvojnih stadija u svim analiziranim uzorcima): *Taenia taeniaeformis* (55,9 %), *Capillaria* sp. (50 %), *Toxocara cati* (50 %), *Isospora* sp. (29,4 %), *Strongyloides* sp. (23,5 %), *Giardia* sp. (17,6 %), *Ancylostoma tubaeformae* (14,7 %), *Physaloptera* sp. (11,8 %), *Hymenolepididae* (8,8 %), *Alaria alata* (5,9 %), *Aelurostrongylus abstrusus* (5,9 %), *Toxascaris leonina* (5,9 %), *Trichinella* sp. (5,9 %), *Mesocestoides lineatus* (5,9 %), *Anoplocephalidae* (2,9 %), *Dipylidium caninum* (2,9 %), *Trichuris* sp. (2,9 %), *Isospora felis* (2,9 %), *Eimeria* sp. (2,9 %) i *Sarcocystis* sp. (2,9 %). Među njima, *Eimeria* sp., *Trichuris* sp., jaja anoplocefalida i himenolepidida su pseudoparaziti, koji potječu od plijena. Četiri roda koje smo identificirali do sada nisu nikada opisani kod divljih mačaka - *Giardia* sp., *Strongyloides* sp., *Sarcocystis* sp. i *Dipylidium caninum*.

Ključne riječi: paraziti, divlja mačka, *Felis silvestris silvestris*, Hrvatska