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Parasites and zoonotic bacteria in the feces of cats and dogs from animal shelters in Carinthia, Austria

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ABSTRACT

Due to their close associations with humans, dogs and cats can be important reservoirs for zoonotic pathogens. In the current study 200 fecal samples of dogs ($n = 70$ samples) and cats ($n = 130$ samples) from animal shelters in Carinthia, southern Austria, were examined for the presence of parasites (fecal flotation and larval migration assay) and selected bacteria. Overall, 17.1% of the canine and 38.5% of the feline samples were positive for parasites ($p < 0.001$), most commonly *Giardia duodenalis* (dogs and cats), including potentially zoonotic genotypes revealed by multilocus genotyping, and *Toxocara cati* (cats). *Cryptosporidium (C. felis)*, *Cystoisospora* spp. (dogs and cats), hookworms (dog), *Trichuris* (dog) *Capillaria hepatica* (cats), taeniids (cat), and *Aelurostrongylus abstrusus* (cat) were also found. Zoonotic bacteria were detected in 10.5% of the samples, *Salmonella enterica* (dogs), *Campylobacter jejuni* (dogs and cats) and *Yersinia enterocolitica* (cat) and were significantly associated with parasite infections in cats but not in dogs. Samples that were positive for several pathogens were common; especially *G. duodenalis* and *T. cati* were frequently found in association with each other, other parasites or bacteria. The spectrum of detected pathogens is comparable to that of other dog and cat populations in central Europe. However, since animals from shelters are frequently rehomed, diagnostic measures, appropriate hygiene and therapy as well as training of shelter staff are recommended to prevent zoonotic transmission of enteropathogens to staff or new owners. The presence of heteroxenic parasites, i.e. *Aelurostrongylus abstrusus* and *Taenia taeniaeformis*, and spurious excretion of Ca. *hepatica* in cats, indicates that these animals preyed on intermediate hosts, and that biosafety measures in pet shelters need to be evaluated for their efficacy in the prevention of pathogen transmission.

1. Introduction

Due to their lifestyle in close contact with humans, domestic cats and dogs can be reservoirs for zoonotic pathogens including parasites, bacteria, fungi and viruses (Bauernfeind et al., 2013; Baneth et al., 2016). The risk of infection (and pathogen transmission to humans) is linked to various factors, including veterinary care (e.g. deworming and vaccination), feeding and contact to other animals. Animal shelters are responsible for abandoned or lost animals or animals confiscated from illegal puppy trades and can be confronted with various challenges concerning the health of their animals. This includes infectious agents that are transmissible among animals in close contact or zoonotic pathogens. It is generally assumed that parasitic infections are more common in animal shelters than in households (Ortuño and Castella, 2011; Raza et al., 2018), first because of the close and changing contact between different individuals, second because at least a proportion of the animals in shelters is likely to have received sub-standard veterinary care before submission and are therefore more likely to harbor parasites than other animals upon submission to the shelter. This may also be true for other pathogens like enteropathogenic bacteria that may pose a risk for both animal and public health (Verma et al., 2021; Leahy et al., 2016, 2017; Sanchez et al., 2002; Stehr-Green and Schantz, 1987).

The current study aimed to determine the status of selected endoparasites and enteric bacteria in dogs and cats in animal shelters in the

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south of Austria, to determine their zoonotic potential.

2. Materials and methods

2.1. Locations, samples and background information on animals

This study was conducted as a collaborative project between the University of Veterinary Medicine Vienna (www.vetmeduni.ac.at) and the Provincial Government of Carinthia. The province of Carinthia is the most southern province of Austria, bordering Italy and Slovenia (https://en.wikipedia.org/wiki/Carinthia). It has four registered animal shelters (https://www.hundepartei.at/tierheime-oesterreich/#was_leistet_ein_tierheim) that all contributed with samples using sample collection kits provided by the University of Veterinary Medicine containing disposable plastic containers (150 ml) and single-use spatulas as well as a form for documentation of the sampled animals. All samples were collected on site and transferred to the laboratory for examination within 48 h. In total 200 fecal samples (70 canine and 130 feline samples) were provided as individual samples either from animals being housed individually or in groups or as samples from animal groups (Table 1). Individual samples from animal groups were not pooled but examined separately but they could not be attributed to the individual animals of the group.

Information on anthelmintic treatment was available for 41 dogs (56.9%) and 14 cats (10.8%). Dogs were treated with a combination of praziquantel, pyrantel and febantel every three months ($n = 29$) or milbemycin oxime + praziquantel ($n = 12$) at unknown intervals. Cats were treated with pyrantel ($n = 3$) with a combination of praziquantel, pyrantel and febantel (n = 1) or milbemycin oxime + praziquantel ($n = 10$) before sampling. The last dewormings were carried out one to six months before sampling.

2.2. Parasitological examinations

All fecal samples were examined by fecal flotation using zinc sulfate solution (specific density: 1.3) with centrifugation (300 x*g* for 8 min) and a larval migration assay using the Baerman funnel method overnight (Deplazes et al., 2020). In addition, all samples were examined by coproantigen tests (IDEXX SNAP© Giardia; IDEXX; Vienna, Austria, and FASTest® CRYPTO Strip, Megacor, Hörbranz, Austria) according to the manufacturer's instructions.

Twelve fresh fecal samples from cats kept in groups (three from shelter A, nine from shelter B, 26 from shelter D) were examined for *Tritrichomonas foetus* using the Feline InPouch® system (Biomed Diagnostics, Inc., White City, OR, USA).

2.3. Molecular analyses of parasite-positive samples

PCRs of parasite-positive samples were conducted in an Eppendorf Mastercycler® Pro, (Eppendorf, Hamburg, Germany). For this, DNA was extracted from each sample using the NucleoSpin® Soil kit (Macherey-Nagel GmbH, Düren, Germany). Samples positive by PCR were subjected to amplicon sequencing on both strands (Microsynth AG, Balgach, Switzerland).

Samples that were positive for *G. duodenalis* (either by flotation or coproantigen test) were analyzed by nested PCRs targeting glutamate dehydrogenase (gdh; Cacciò et al., 2008), triose phosphate isomerase (tpi; Sulaiman et al., 2003) β-giardin (bg; Tseng et al., 2014). *Cryptosporidium*-positive samples (coproantigen testing) were amplified at the 18S locus (Ryan et al., 2003) as well as the the glycoprotein 60 (gp 60) locus (de Oliveira et al., 2021; Jiang et al., 2020) in a nested PCR. Details on the PCR conditions are given in Table 2. After amplification, samples were cooled to 15 °C until removal from the machine.

Samples that were positive for hookworm eggs were analyzed by PCR of the cytochrome oxidase I (CO I; Fuehrer et al., 2015) in a total volume of 25 µl of master mix containing 10 pmol each of primers COI_Nema_fw (GAAAGTTCTAATCATAARGATATTGG) and COI_Nema_rv (ACCTCAGGATGACCAAAAAAYCAA), 1× GreenTaq reaction buffer (Promega, Wisconsin, USA), 25 mM dNTPs each, 5 U Taq-Polymerase (Promega, Fitchburg, WI, USA), and 5 µl DNA template extracted as described above. Initial denaturation (95 °C, 2 min) was followed by 35 cycles of denaturation (95 °C, 1 min), annealing (50 °C, 1 min) and elongation (72 °C, 1 min, final elongation 5 min). The expected product size was 660–710 bp. Both hookworm-positive samples were examined after direct extraction of DNA from feces and after flotation of eggs from feces before DNA extraction using the NucleoSpin® Soil kit (Macherey-Nagel). Samples that were positive for taeniid eggs were prepared as for hookworm egg differentiation and subjected to PCR targeting the CO I of Neodermata (Folmer et al., 1994) with primers COI_Neod_Fw (TTTACTTTGGATCATAAGCG) and COI_Neod_Rv (CCAAAAAACCAAAACATATGTTGAA) that target a product of 683–686 bp. Annealing temperature was 48 °C, otherwise the cycling conditions were the same as for the nematodes.

2.4. Bacteriological examinations

For bacteriological examination swabs taken from feces samples were plated onto MacConkey II agar (for the isolation and differentiation of *Enterobacteriaceae* including *E. coli* and *Salmonella* spp.), CIN (Cefsulodin-Irgasan-Novobiocin) agar (for the selective isolation of *Y. enterocolitica*), and *Campylobacter* blood-free agar (for the isolation of *Campylobacter* spp. including *C. jejuni*) (all BD Diagnostics, Vienna, Austria), using the three-phase streaking method. Plates were incubated aerobically at 37 °C (MacConkey II agar), 28 °C (CIN agar), or microaerobically at 42 °C (*Campylobacter* blood-free agar) for 48–72 h. Microbial growth was semi-quantitatively graded as light, moderate or heavy depending on the number of isolated colonies in streaking sections. Colonies were identified to species level by matrix-assisted laser desorption ionization – time of flight mass spectrometry (MALDI-TOF MS) as previously described (Spergser et al., 2018). For enrichment and selective isolation of *Salmonella*, swabs were also incubated in Buffered Peptone Water (BD Diagnostics, Vienna, Austria) at 37 °C in ambient air for 24 h. After incubation, 100 µl of cultures were transferred to Selenite and Rappaport-Vassiliadis R10 broth (both BD Diagnostics, Vienna,

Table 1
Examined samples according to animal shelter, animal species and group or individual samples. Individual: samples that could be assigned to individual animals, group: anonymous individual samples from animal groups.

Samples [n] Shelter	Samples from dogs			Samples from cats			Total		
	Individual	Group	Total	Individual	Group	Total	Individual	Group	Total
A	0	13	13	3	33	36	3	46	49
B	27	2	29	41	21	62	68	23	91
C	0	14	14	5	15	20	5	29	34
D	12	2	14	7	5	12	19	7	26
Total	**39***	**31**	**70**	**56**	**74**	**130**	**95**	**105**	**200**

* 16 samples from dogs kept in groups could be assigned individually.

Table 2
Details on the nested PCRs for molecular typing of *Giardia* (A) and *Cryptosporidium* (B) from feces. R.: round (in nested PCR).

A: *Giardia* gene loci			
Locus	Glutamate dehydrogenase (gdh)	Triose phosphate isomerase (tpi)	β-giardin (bg)
Primers 1st r., 5′ to 3`ends	gdh_1F: TTCCGTRTYCAGTACAACTC gdh_2R: ACCTCGTYCTGRGTGGCGCA	AL3543-for: AAATIATGCCTGCTCGTCG AL3546-rev: CAAACCTTITCCGCAAACC	G7: AAGCCCGACGACCTCACCCGCAGTGC G759: GAGGCCGCCCTGGATCTTCGAGACGAC
Primers 2nd r., 5′ to 3`ends	gdh_3F: ATGACYGAGCTYCAGAGGCACGT gdh_4R: GTGGCGCARGGCATGATGCA	AL3544-for: CCCTTCATCGGIGGTAACTT AL3545-rev: GTGGCCACCACICCCGTGCC For assemblage D: TPIDF: CCGTTCATAGGTGGCAACTT TPIDR: GTAGCCACTACACCAGTTCC	β-GIAR2-F: GAACGAGATCGAGGTCCG β-GIAR2-R: CTCGACGAGCTTCGTGTT
Master Mix [all containing 1× Green Taq reaction buffer; all reagents: Promega]			
Primer	10 pmol each	50 pmol each	10 pmol each
Additional $MgCl_2$	/	3 mM	/
dNTPs	0.2 mM each	0.2 mM each	0.2 mM each
Taq polymerase	0.5 U	1.25 U	0.5 U
DNA template	5 µl	1 µl	5 µl
Total reaction volume	25 µl	50 µl	25 µl
Initial denaturation (94 °C)	2 min	2 min	5 min
Cycles: number	35	35	35
denaturation	94 °C, 30 s	94 °C, 45 s	95 °C, 30 s
annealing	60 °C (1st r.)/ 58 °C (2nd r.), 30 s	50 °C, 45 s (1st and 2nd r,)	65 °C (1st r.)/ 55 °C (2nd r.), 30 s
elongation	72 °C, 60 s (final el.: 7 min)	72 °C, 60 s (final el.: 10 min)	72 °C, 60 s (final el.: 7 min)
Amplicon size	754/530 bp	605/530 bp	753/511 pb
B: *Cryptosporidium* gene loci			
Locus	Glycoprotein 60 (gp60) for *C. parvum*	18 ribosomal DNA (18S)	Glycoprotein 60 (gp60) for *C. felis*
Primers 1st round, 5′ to 3`ends	AL 3531_fw: ATAGTCTCCGCTGTATTC AL 3535_rev: GGAAGGAACGATGTATCT	18SiCF2: GAC ATA TCA TTC AAG TTT CTG ACC 18SiCR2: CTG AAG GAG TAA GGA ACA ACC	GP60-Felis-F1: TTT CCG TTA TTG TTG CAG TTG CA GP60-Felis-R1: ATC GGA ATC CCA CCA TCG AAC
Primers 2nd round, 5′ to 3`ends	AL-3532_fw: TCCGCTGTATTCTCAGCC AL-3534_rev: GCAGAGGAACCAGCATC	18SiCF1: CCT ATC AGC TTT AGA CGG TAG G 18SiCR1: TCT AAG AAT TTC ACC TCT GAC TG	GP60-Felis-F2: GGG CGT TCT GAA GGA TGT AA GP60-Felis-R2: CGG TGG TCT CCT CAG TCT TC
Master Mix [all containing 1× Green Taq reaction buffer; all reagents: Promega]			
Primer	10 pmol each	12.5 pmol each	6 pmol each (1st r.), 12 pmol each (2nd r.)
Additional $MgCl_2$	6 mM	1.5 mM	2.5 mM
dNTPs	0.2 mM each	0.2 mM each	0.2 mM each
Taq polymerase	1.25 U	0.5 U	1.5 U
DNA template	3 µl	5 µl	1 µl
Total reaction volume	50 µl	25 µl	50 µl
Initial denaturation (94 °C)	5 min	5 min	5 min
Cycles: number	35	45	35
denaturation	94 °C, 45 s	94 °C, 30 s	94 °C, 45 s
annealing	59 °C (1st r.)/ 50 °C (2nd r.), 45 s	58 °C 30 s (1st and 2nd r.)	52 °C, 45 s (1st and 2nd r.)
elongation	72 °C, 1 min (final el.: 10 min)	72 °C, 30 s (final el.: 10 min)	72 °C, 80 s (final el.: 10 min)
Amplicon size	879/846 bp	763/587 bp	1.200/900 bp

Austria), incubated at 42 °C for 24 h, and subsequently sub-cultured onto XLD (Xylose-Lysine-Desoxycholate) agar, incubated aerobically at 37 °C for 24–48 h. Presumed *Salmonella* colonies were identified by MALDI-TOF MS and serotyped at the National Reference Center for Salmonella (AGES) in Graz, Austria. *E. coli* isolates were tested for Shiga toxin genes (*stx1*, *stx2*) using PCR as described previously (Müller et al., 2007).

2.5. Statistical analyses

Testing for significance was done on a 95% CI with Pearson's X^2 test (nominal data) and the Mann–Whitney-*U* test (metric data). Correlations were calculated using Spearman's test. All statistical analyses were performed using IBM SPSS Statistics 24 (IBM GmbH, Ehningen, Germany). A p-value <0.05 was considered as significant.

3. Results

3.1. Parasitological examination and genotyping

Out of the 200 fecal samples, 62 (31.0%) were positive for at least one parasite, 12 samples from dogs (17,1%) and 50 samples from cats (38.5%). Seven of the 12 positive canine samples were positive for *G. duodenalis* (one each from shelters A and B, five from shelter C) two (shelter A) for hookworms, one (shelter A) for whipworms, two (shelter B) for *Cystoisospora*.

Four of the 12 positive canine samples originated from shelter A (Table 3) that kept their dogs exclusively in groups. Two of these contained hookworm eggs, one whipworm eggs, and one was positive for *G. duodenalis*. The nematode-positive dogs were aged 4–6 years. In shelter B, three dog samples were positive, one 10 weeks old puppy shed coccidia, one 13 years old dog and one dog of unknown age were positive for *Cystoisospora*. In shelter C, five dogs aged 2–13 years were positive for *G. duodenalis*, they all lived in groups. The age of positive dogs was known for 10 dogs and ranged from 10 weeks to 13 years *[note that when the age is not given it was not known]*.

The positive feline samples originated from all four shelters, they were positive for *G. duodenalis* (19 samples) and/or *Cryptosporidium* spp. (8 samples) in rapid tests, *Toxocara cati* (33 samples), *Capillaria hepatica* (two samples), *Cystoisospora* spp. (four samples), taeniid eggs (one sample) and/or *Aelurostrongylus abstrusus* (two samples) (Table 5). The age of the positive cats was known for nine animals and ranged from two months to 11 years.

The PCR analysis of the 26 *G. duodenalis*-positive samples returned sequences in 13 cases, 12 from cats and one from a dog. The dog sample (shelter B) contained *G. duodenalis* assemblage C (syn. *Giardia canis*); 10 cat samples (three from shelter A, three from shelter B and two each from shelters B and D) contained assemblage F (syn. *G. cati*) and two cat

Table 3
Samples positive for at least one parasite by host species, housing type and shelter.

Samples [n]	Dog samples			Cat samples			Total		
Shelter	Individual	Groups	Total	Individual	Groups	Total	Individual	Groups	Total
A	0	4	4	1	15	16	1	19	20
B	2	1	3	11	8	19	13	9	22
C	0	5	5	2	8	10	2	13	15
D	0	0	0	3	2	5	3	2	5
TOTAL	2	10	12	17	33	50	19	43	62

samples from shelter A contained assemblage A (*G. duodenalis* s.l.). Sequencing of the latter two revealed subassemblages A1 at the GDH-, A1 at the ß-giardin-, and A4 at the TPI locus (Table 6). The two cat samples for the same shelter were identical and clustered with Swedish *G. duodenalis* samples (subassemblage AI) from different hosts including humans (Fig. 1).

Genotyping of *Cryptosporidium* (nine feline samples from shelters A-three samples, two from subadult cats, B-four samples, one from a subadult cat, and C-two samples) revealed two *Cryptosporidium felis*-positive samples. The similarity to *C. felis* from cat was 99.4% (Genbank® accession no. MN394123.1; www.ncbi.nlm.nih.gov/genbank/) resp. 94.9% (OL615020.1); the similarity to a *C. felis* isolate from

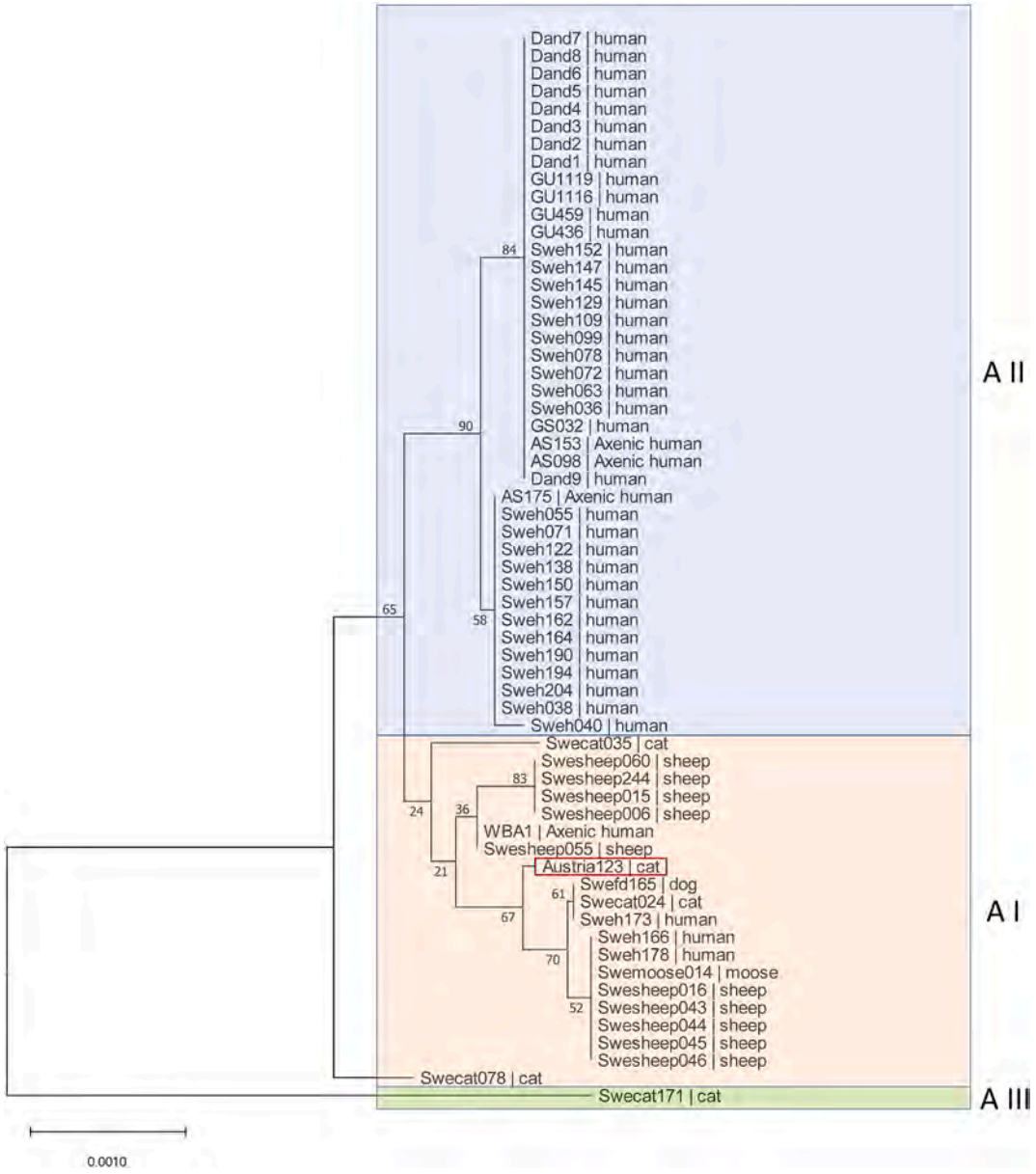

Fig. 1. Phylogenetic relationships of *Giardia duodenalis* assemblage A isolates "Austria cat 123" (see Table 6) with related sequences (ref. Ankarklev et al., 2018; Klotz et al., 2022). The tree was inferred by neighbor-joining analysis of concatenated BG, GDH and TPI sequences (blue frame: subassemblage AII-group, red frame: subassemblage AI-group). The sequences from the Swedish isolate Cat171 (subassemblage AIII; green frame) were used to create the outgroup. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

humans (MN174093.1) was 99.4% resp. 94.6%. Subtyping for *C. felis* at the gp60 locus was only successful for one of the mentioned two samples and revealed subtype XIXa, clustered with other sequences from this subtype and showed a 98.7% similarity to a feline *C. felis* isolate from China (ON160043.1).

PCR for *C. parvum* or hookworms was unsuccessful.

The PCR of one sample containing taeniid eggs returned an amplicon, and sequence comparison of the amplified cytochrome oxidase 1 gene sequence revealed high similarities with *Taenia taeniaeformis* sequences in the BOLD database (www.boldsystems.org/; BPHA033–18, BPHA023–18, BPHA024–18; all 100% similarity) and in Genbank® (JQ663994.1; 99.84%).

No *Tr. foetus* could be detected in the InPouch®-analyzed samples.

In relation to treatment, helminth infections were detected in three cats (all positive for *T. cati*, one additionally for lungworms) that all had received milbemycin oxime + praziquantel 40–95 days before sampling.

3.2. Bacteriological examination and mixed infections with bacteria and parasites

In total, 21 samples (10.5%) were positive for zoonotic enteric bacteria, seven from dogs (10%) and 14 (10.8%) from cats. Four samples from adult dogs (3.5–12 years, shelter D) were positive for *Salmonella enterica* serovar Coeln (antigenic formula 4,5,12, y: 1,2). Three canine samples (two from shelter A, one from shelter B; the latter a puppy of 10 weeks) and 13 feline samples (two from shelter A, two from shelter B, seven from shelter C, and two from shelter D; age range three months to two years) were positive for *Campylobacter jejuni* and one sample from a cat at shelter A was positive for *Yersinia enterocolitica* (Table 4). Mixed zoonotic bacterial infections were not found. *E. coli* was isolated from 193 feces samples (96.5%). All *E. coli* isolates ($n = 286$, representing different colony morphotypes per sample) were tested negative for Shiga toxin genes *(stx1, stx2)* using PCR.

3.3. Mixed infections

Half of these cat samples were positive for mixed bacterial-parasitic infections: the one positive for *Yersinia enterocolitica* (shelter A) was also positive for *Cryptosporidium* + *T. cati*. Of the *Campylobacter jejuni* positive cats, one was co-infected with *G. duodenalis* (shelter A, age: two years), two with *T. cati* (one each from shelters A and C), one with *Cystoisospora* (shelter D, 4 months), two with *G. duodenalis* and *Cryptosporidium* sp. (one from shelter B aged 4 months, one from shelter C), one with *T. cati* and taeniids and one with *T. cati* and *Cystoisospora* spp. (both from shelter C). One cat from shelter C was coinfected with *G. duodenalis*, *Cryptosporidium* sp., *Cystoisospora* spp. and *T. cati*.

3.4. Statistical evaluation

Possible effects of shelter, host species or host age and correlations between parasite excretion and detection of bacterial pathogens were evaluated.

Table 4
Zoonotic enteric bacteria isolated from dog and cat samples in four Carinthian shelters.

Samples [n]	Dog samples		Cat samples	
Shelter	Individual	Groups	Individual	Groups
A	0	*C. jejuni* (2)	0	*Y. enterocolitica* (1) *C. jejuni* (2)
B	*C. jejuni* (1)	0	*C. jejuni* (1)	*C. jejuni* (1)
C	0	0	*C. jejuni* (1)	*C. jejuni* (6)
D	*S. enterica* (2)	*S. enterica* (2)	0	*C. jejuni* (2)
TOTAL	*C. jejuni* (1) *S. enterica* (2)	*C. jejuni* (2) *S. enterica* (2)	*C. jejuni* (2)	*Y. enterocolitica* (1) *C. jejuni* (11)

Table 5
Details on the parasitological findings in feline samples.

Shelter [n pos. Samples/all samples; %]	Positive samples
A [7/36; 19.4%]	*Giardia* [1]
	Toxocara [4]
	Cryptosporidium + *Toxocara* [2]
B [19/62; 30.6%]	*Giardia* [2]
	Cystoisospora [1]
	Toxocara [9]
	Aelurostrongylus [1]
	Giardia + *Cryptosporidium* [4]
	Giardia + *Toxocara* [1]
	Toxocara + *Capillaria* [1]
C [10/20; 50%]	*Giardia* [2]
	Toxocara [4]
	Giardia + *Cryptosporidium* [1]
	Cystoisospora + *Toxocara* [1]
	Toxocara + Taeniidae [1]
	Giardia + *Cryptosporidium* + *Toxocara* + *Cystoisospora* [1]
D [5/11; 41.7%]	*Cystoisospora* [1]
	Toxocara [1]
	Giardia + *Toxocara* [1]
	Toxocara + *Aelurostrongylus* [1]
	Giardia + *Toxocara* + *Capillaria* [1]

While there was no effect of the shelter ($p > 0.05$) or individual keeping vs. animal groups ($p > 0.05$) for dogs or cats, cat samples were significantly more often positive for parasites (39%) than dog samples (17%) ($p < 0.001$), and cat samples positive for bacteria were significantly more often positive for parasites; 42% of the animals that shed bacteria but only 20% of those that did not excreted bacteria were positive for parasites ($p = 0.006$), while this was not observed for dog samples ($p > 0.05$). For those animals where the age was known ($n = 94$), it was negatively correlated with parasite shedding when dogs and cats were evaluated jointly, with the highest rates in animals less than one year (33%) compared to the mean rate of 17% (Spearman's Rho = −0,247; $p = 0.008$), but this effect was not observed for the separate host species ($p > 0.05$).

4. Discussion

In the present study, 200 fecal samples from dogs and cats in animal shelters in Carinthia, Austria, were examined for parasites and zoonotic enteric bacteria. Samples were derived from animals kept individually or in groups, so infection rates based on the numbers of animals could not be calculated. In addition, only single samples were taken so excretion of pathogens at another time points could not be considered. Therefore, only the occurrence of the detected parasites but not their frequency will be discussed here in the context of the existing literature.

Although data regarding the origin of the examined animals were not routinely available or could not be provided for reasons of data protection legislation (e.g., for confiscated animals) it can be assumed that the southern border of Carinthia is a regular entry for animals originating from the Mediterranean or the Balkan areas or returning from travel to these regions and may also be part of the shelter populations investigated here.

Overall, one third of the samples, mostly from cats, were positive for parasites. The most frequent parasite genera detected were *Giardia*, *Cryptosporidium* and *Toxocara*.

The rate of *T. cati* positive samples in the different shelters ranged from 17 to 35%, being considerably higher than previously reported from Austrian owned cats. In a study involving 10 European countries including Austria with 80 samples, the occurrence of gastrointestinal and respiratory parasites in 1990 cats with outdoor access was assessed by coproscopy, and 30.8% of them (Austria: 11.3%) were infected with at least one parasite, most prevalently by ascarids (16.5%; Austria:

Table 6
Results of genotyping *Giardia duodenalis* at three different loci (assemblages according to the literature cited in the Methods section). Age of animals is given where known in months [m] or years [y]. Only samples that yielded at least one positive result are listed. Empty field: no sequence obtained.

Host [age]	Shelter	Subassemblage*	MLG**	Genotypes [reference sequence Genbank®]		
				gdh	tpi	bg
Dog	B				C	C
Cat	A	AI	novel	A1 [KJ027433]	A4 [GQ329677]	A [MK862315]
Cat	A	AI	novel	A1 [KJ027433]	A4 [GQ329677]	
Cat [6 y]	A			F	F	F
Cat [2 y]	A			F	F	F
Cat [13 y]	A			F	F	F
Cat	B			F	F	
Cat	B			F	F	F
Cat	B			F	F	
Cat	C			F	F	F
Cat	C			F	F	F
Cat	D			F	F	
Cat	D			F	F	F

*according to phylogenetic analysis of concatenated sequences. **multilocus genotyping according to Cacciò et al., 2008; only for assemblage A genotypes.

2.5%), but also lungworms (10.6%; Austria: 0%), most commonly *A. abstrusus*, coccidia (6.5%; Austria: 3.8%), hookworms (4.5%; Austria: 0%) and taeniids (1.1%, Austria: 2.5%) were common. *Giardia duodenalis* cysts were detected in 0.7% of all and 2.5% of the Austrian samples (Giannelli et al., 2017) which was also lower than in the present study with an intra-shelter rate of 3–30% positive feline samples.

Only two out of nine feline samples positive for *Cryptosporidium* could be typed to species level and were identified as *C. felis*. *Cryptosporidium felis* is a potentially zoonotic species that can cause diarrheal disease in both the animal and the human host and is usually isolated from immunocompromised humans (Pedraza-Díaz et al., 2001; Cacciò et al., 2002; Beser et al., 2015). Subtyping revealed subtype family XIXa which has previously been isolated from humans as well as cats (Jiang et al., 2020). *Cryptosporidium parvum*, a more common zoonotic species, usually associated with calves and also present in Austria (Lichtmannsperger et al., 2020), was not detected.

Of the *G. duodenalis*-positive samples, the majority could be genotyped, and revealed mostly host-specific genotypes (assemblage C in the dog sample and assemblage F in 10 of the 12 cat samples). Two cat samples contained putatively zoonotic genotypes. However, the detected combination could not be assigned to a multi-locus-genotype according to the proposal of Cacciò et al. (2008). However, phylogenetic analysis of concatenated sequences allowed to allocate this sequence to the potentially zoonotic subassemblage AI. A recent study suggested a limited zoonotic transmission of subassemblage AI (which contained all animal- and few human-derived isolates examined) and an absence of animal-derived isolates in subassemblage AII (Klotz et al., 2022). Based on these results, potentially zoonotic genotypes must be assumed to be present in these two cat samples. This is in accordance with a previous study where we predominantly detected assemblage F but also assemblage A in four samples of cats in Austria (Hinney et al., 2015). Although *G. duodenalis*-infections in humans in Austria are usually travel-related and not zoonotic (Lee et al., 2017), focal transmission of zoonotic genotypes by cats is conceivable for people at risk, e.g. animal caretakers.

Toxocara cati, the feline roundworm, is a common zoonotic nematode and can induce *larva migrans* syndrome affecting different organs (Fisher, 2003; Jimenez Castro and Sapp, 2020; Maciag et al., 2022). In contrast to *G. duodenalis* and *Cryptosporidium*, which are immediately infectious after excretion, humans become infected by *Toxocara* via ingestion of larvated eggs from the environment – eggs are excreted non-larvated and require a temperature dependent period of maturation in the environment of several weeks, therefore contact with fresh cat feces, e.g. during cleaning of the shelter facilities, is not a risk factor for toxocariasis. However, in light of the infections of a number of cats in the enrolled shelters, maintenance of the transmission cycle appears to be present, and this may also include the possibility of zoonotic infections (Raza et al., 2018). *Tocoxara cati* is a common parasite both in shelter and owned cats in Europe as determined by copromicroscopy (Beugnet et al., 2014; Kostopoulou et al., 2017; Symeonidou et al., 2018; Sauda et al., 2019; Zottler et al., 2019; Genchi et al., 2021). It was also the most prominent parasite detected in a study from Denmark, 84.8% of 900 necropsied cats with outdoor access harbored adults of this nematode (Takeuchi-Storm et al., 2015). Du to the high environmental resistance of *Toxocara* eggs, transmission must be considered to be effective in cat populations with subsequent risk of human infections upon ingestion of larvated eggs, too (Fisher, 2003).

Capillaria hepatica (syn. *Calodium hepaticum, Hepaticola hepatica*), was found in the feces of two cats from the same shelter. This roundworm species has a peculiar lifestyle: Adults dwell in hepatic parenchyma and the deposited eggs are not shed into the environment by the infected host (most commonly lagomorphs and rodents, rarely other mammals including humans) but are excreted in feces of predators after ingestion of infected prey and subsequent digestion of liver tissue and release of eggs (Fuehrer, 2014a, 2014b. Consequently, the presence of eggs in cat feces does not indicate infection of the cat but predation of infected animals and is independent of regular deworming. Since humans can become infected upon ingestions of eggs (Fuehrer et al., 2011) the presence of *C. hepatica* in the premises of this shelter indicate a zoonotic risk.

Other, non-zoonotic parasites detected in the samples include *Cystosiospora* spp., the host-specific intestinal coccidia of dogs and cats, and *Tr. vulpis*, the canine whipworm, that were not further typed to the species level, as well as the feline lungworm *A. abstrusus* and taeniid eggs identified as *Taenia taeniaeformis* in genotyping. The latter two species are transmitted via intermediate (and in case of *A. abstrusus*, paratenic) rodent hosts, and it is noteworthy that the circulation of heteroxenous parasites obviously cannot be prevented completely under the highly controlled conditions of an animal shelter where dogs and cats are regularly dewormed. Interestingly, the cat that shed *A. abstrusus* was located in the same shelter that housed the cats that shed *C. hepatica* eggs, further supporting the impression that rodent control in this place was not sufficient.

The predominance of *G. duodenalis* and the absence of *T. canis* in dogs is in line with a recent study on parasites of dogs from different districts of the Austrian capital Vienna and its surrounding area, where up to 11% of samples where *G. duodenalis*-positive while a surprisingly low prevalence of *Toxocara canis* (up to 2%) was detected in owned dogs (Hinney et al., 2017).

In diagnostic fecal samples submitted to a veterinary diagnostic laboratory 2003–2010, *Giardia* (18.6% in dogs, 12.6% in cats), *Toxocara* (6.1% in dogs, 4.7% in cats), hookworms (2.2% in dogs, 0.2% in cats) and coccidia (5.6% in dogs, 6.0% in cats) were the most common parasites, and juvenile and subadult animals showed significantly higher infection rates with *Giardia*, *Toxocara* and coccidia for both species

(Barutzki and Schaper, 2011). This was in agreement with a previous study form the same authors for the period of 1999–2002 when *Giardia* was the most prominent parasite in dogs (16.6%) and cats (12.6%) followed by *Toxocara* (7.2% in dogs, 6.4% in cats), coccidia (7.0% in dogs, 5.4% in cats) and hookworms in dogs (2.8%) but not in cats (<0.5%) (Barutzki and Schaper, 2003). A more detailed evaluation by the same authors (Barutzki and Schaper, 2013) later revealed that within the first year of life, *Toxocara*, *Giardia* and coccidia are most common; they first occur from the third to the fifth week of life and peak around that time, and coinfections are common up to six months. *Giardia* occurred in different assemblages including the zoonotic assemblages A (*G. duodenalis)* and B (*G. enterica)* in owned cats and dogs in Germany (Pallant et al., 2015).

In a study comparing parasites of shelter dogs more globally (Raza et al., 2018) *Giardia* was described as the most prevalent parasite of shelter dogs from Rome with 55.3% of the samples but the authors of this review put this down at least in part to the high sensitivity of the used ELISA technique. This was also debated for *Cryptosporidium* (37.9%) in Romania. In Spain high prevalences were detected by PCR for *Giardia* (Ortuño et al., 2014), so prevalence rates cannot be compared well between studies due to analytical differences.

Overall, it therefore appears that the parasite spectrum of the shelter animals was similar to that of owned dogs or cats with or without outdoor access, however, compared to previous studies (Barutzki and Schaper, 2003, 2011, 2013) an age relation was not seen in all cases – infections with *Cystoisospora* were detected in animals less than six months of age, while infections with *Giardia* and *Toxocara* occurred in cats younger than one year but also frequently in adult and even in older cats. The uneven age distribution of the study population with a much higher rate of young cats vs. young dogs, however, must be considered as a possible confounder in this study. Infection in older animals signifies the capability of directly transmitted parasites to remain in a contaminated environment and re-infect animals continuously. It must therefore be assumed that on premises with a turnover of animals from different age groups, like in a shelter, cleaning and disinfection as part of the hygiene regime is not sufficient to prevent reinfections, and that regular treatment, as recommended by ESCCAP (2021; https://www.esccap.org/guidelines/gl1/), must also be part of the routine to prevent transmission among sheltered animals, in the present case especially of cats.

In this study samples of shelter animals were also examined for zoonotic enteric bacteria focusing on the four most common causes of zoonoses in the European Union, namely *C. jejuni S. enterica*, *Y. enterocolitica*, and Shiga toxin-producing *E. coli* (STEC) (EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), 2022). Although foodborne exposure is the most common route of human transmission, these zoonotic bacterial pathogens can also be transmitted by direct contact with the feces of infected animals, and dogs and cats have been identified as possible source of human infection (Hetem et al., 2013; Mughini Gras et al., 2013; Hoelzer et al., 2011; Damborg et al., 2004; Wolfs et al., 2001). Overall, 21 (10.5%) feces samples were positive for zoonotic enteric bacteria in our study indicating that apparently healthy shelter animals may serve as reservoirs for these bacteria and could pose a hazard for future owners and an increased occupational risk for animal caretakers. In fact, intermittent excretion of zoonotic enteric bacteria in asymptomatic carriers and detection of these bacteria in pooled samples from animals kept in groups suggest prevalence rates even higher than the rates of occurrence determined in our study. Interestingly, there was a strong correlation between the presence of bacteria and parasites in feline samples. While the life history of the individual cats could not be obtained, it can be speculated that these animals had a simultaneous high exposition to environmental stages of both pathogen groups, and possibly also a high disposition for enteral infections, showing that individual risks also play a role in infection and excretion dynamics.

Campylobacter jejuni was the most prevalent zoonotic bacterial pathogen isolated from 3 (4.3%) dog and 13 (10%) cat samples. Estimates of *Campylobacter* ssp. (including *C. upsaliensis*, *C. helveticus*, and *C. jejuni*) carriage among dogs and cats varied widely in previous work, depending on the age of the animals, housing conditions (shelter versus household pets), the absence / presence of intestinal disease, season, diet, and the detection method utilized (Acke, 2018). Studies performed in the last ten years in Europe and North America revealed prevalence rates of *C. jejuni* in healthy dogs ranging from 2.2 to 13.6% whereas in healthy cats *C. jejuni* carriage was between 4.4 and 8.5% (Lemos et al., 2021; Verma et al., 2021; LaLonde-Paul et al., 2019; Pölzler et al., 2018; Leahy et al., 2017; Giacomelli et al., 2015; Procter et al., 2014; Andrzejewska et al., 2013). In some of these studies, risk factors for *C. jejuni* infection have been identified including intensive housing (shelter) of dogs in the USA and Italy (Leahy et al., 2017; Giacomelli et al., 2015) and younger age of Austrian cats substantiated by significantly higher prevalence rates in juvenile cats <1 year of age (14.3%) than in adult cats (3.8%) (Pölzler et al., 2018). An age-dependent carriage of *C. jejuni* in cats has also been noted in our study since all samples positive for *C. jejuni* originated from animals aged between three months and two years.

Salmonella enterica was the second most prevalent zoonotic bacterial pathogen isolated from four (5.7%) of 70 dog samples which was within the prevalence rates of 1.9 and 8.3% previously reported for shelter dogs in the USA (Leahy et al., 2016, Tupler et al., 2012). All *Salmonella* isolates in the presented study were identified as *S. enterica* serovar Coeln and only occurred at the very end of the study period in shelter D (in two dogs kept in two different groups and in two dogs individually housed) indicating point source transmission during shelter stay via environmental contamination rather than dog-to-dog transmission. Estimates of fecal *Salmonella* shedding in healthy pet dogs varied considerably across previous studies, depending on the dog population, geographic location, housing conditions, feeding habits, and time of sampling, ranging from approximately 1 to 36% (Wei et al., 2020; Kiflu et al., 2017; Reimschuessel et al., 2017; Leahy et al., 2016; Procter et al., 2014; Leonard et al., 2011; Sanchez et al., 2002). Risk factors identified for *Salmonella* carriage among dogs in Canada included consumption of raw meat and contact to livestock (Leonard et al., 2011; Lefebvre et al., 2008), however, none of these risk factors applied for the dogs tested positive for *Salmonella* in our study. Detection of a single *Salmonella* serovar (S. Coeln) is at variance with the high degree of serovar diversity found among dogs in previous studies. Serovars most commonly isolated from dogs in the USA, China, and Canada included *S*. serovar Typhimurium, *S*. serovar Kentucky, *S*. serovar Indiana, *S*. serovar Heidelberg, *S*. serovar Newport, and *S*. serovar Javiana (Wei et al., 2020; Reimschuessel et al., 2017; Leahy et al., 2016; Leonard et al., 2011; Lefebvre et al., 2008). To our knowledge, *Salmonella* Coeln has never been reported to occur in dogs and has only been scarcely associated with human salmonellosis (Vestrheim et al., 2016; Haeghebaert et al., 2001). However, S. Coeln has been isolated in high frequency (21.8%) in wild boars in northern Italy (Chiari et al., 2013), adjacent to Carinthia where our study has been performed. *Salmonella* was not detected in any of the cat samples examined which is in accordance with significantly lower carriage rates (0.6 to 1.8%) in cats than those determined for dogs in previous studies (Wei et al., 2020; Reimschuessel et al., 2017).

In the presented study, *Y. enterocolitica* could only be isolated from one fecal sample originating from two cats that were kept together. This low prevalence of *Y. enterocolitica* in cats agrees with two European studies revealing *Y. enterocolitica* in 0.3 and 3% of samples from healthy cats, respectively (Stamm et al., 2013; Bucher et al., 2008). In healthy dogs, however, much higher *Y. enterocolitica* carriage rates ranging from 4.6% to 9.8% (Europe, China) have been described (Liang et al., 2015; Stamm et al., 2013; Bucher et al., 2008) which contradicts the *Y. enterocolitica* negative results for dog samples in our study. None of the *E. coli* isolates ($n = 296$) recovered from dog and cat samples tested positive for the Shiga toxin genes *stx1* and *stx2*. Comparatively little is known about STEC carriage in dogs and cats. However, two studies from Austria and Germany revealed STEC prevalence rates of 2.2 and 4.8% in

healthy dogs, and 0.7 and 13.8% in healthy cats, respectively (Franiek et al., 2012, Beutin et al., 1993.

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Ethical statement

Samples and animal data relevant to this study were provided by the participating animal shelters as legal caretakes of the animals. All experiments were performed in accordance with relevant institutional and national guidelines and regulations.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The sponsor of this study, the Carinthian provincial government, has not taken part in the design, conduct or analysis of the study or the compilation of this manuscript.

Data availability

All data from this study are included in the manuscript.

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References

Acke, E., 2018. Campylobacteriosis in dogs and cats: a review. N. Z. Vet. J. 66 (5), 221–228. https://doi.org/10.1080/00480169.2018.1475268.

Andrzejewska, M., Szczepańska, B., Klawe, J.J., Spica, D., Chudzińska, M., 2013. Prevalence of *Campylobacter jejuni* and *Campylobacter coli* species in cats and dogs from Bydgoszcz (Poland) region. Pol. J. Vet. Sci. 16 (1), 115–120. https://doi.org/10.2478/pjvs-2013-0016.

Ankarklev, J., Lebbad, M., Einarsson, E., Franzén, O., Ahola, H., Troell, K., Svärd, S.G., 2018. A novel high-resolution multilocus sequence typing of *Giardia intestinalis* assemblage a isolates reveals zoonotic transmission, clonal outbreaks and recombination. Infect. Genet. Evol. 60, 7–16. https://doi.org/10.1016/j.meegid.2018.02.012.

Baneth, G., Thamsborg, S.M., Otranto, D., Guillot, J., Blaga, R., Deplazes, P., Solano-Gallego, L., 2016. Major parasitic zoonoses associated with dogs and cats in Europe. J. Comp. Pathol. 55, 54–74. https://doi.org/10.1016/j.jcpa.2015.10.179.

Barutzki, D., Schaper, R., 2003. Endoparasites in dogs and cats in Germany 1999-2002. Parasitol. Res. 90 (3), S148–S150. https://doi.org/10.1007/s00436-003-0922-6.

Barutzki, D., Schaper, R., 2011. Results of parasitological examinations of faecal samples from cats and dogs in Germany between 2003 and 2010. Parasitol. Res. 109 (1), S45–S60. https://doi.org/10.1007/s00436-011-2402-8.

Barutzki, D., Schaper, R., 2013. Age-dependant prevalence of endoparasites in young dogs and cats up to one year of age. Parasitol. Res. 112 (1), S119–S131. https://doi.org/10.1007/s00436-013-3286-6.

Bauernfeind, R., Kimmig, P., Schiefer, H.G., Schwarz, T., Slenczka, W., Zahner, H., 2013. Zoonosen – Zwischen Tier Und Mensch übertragbare Krankheiten, fourth ed. Deutscher Ärzteverlag Köln, Germany.

Beser, J., Toresson, L., Eitrem, R., Troell, K., Winiecka-Krusnell, J., Lebbad, M., 2015. Possible zoonotic transmission of *Cryptosporidium felis* in a household. Infect. Ecol. Epidemiol. 6 (5), 28463. https://doi.org/10.3402/iee.v5.28463.

Beugnet, F., Bourdeau, P., Chalvet-Monfray, K., Cozma, V., Farkas, R., Guillot, J., Halos, L., Joachim, A., Losson, B., Miró, G., Otranto, D., Renaud, M., Rinaldi, L., 2014. Parasites of domestic owned cats in Europe: co-infestations and risk factors. Parasit. Vectors 7, 291. https://doi.org/10.1186/1756-3305-7-291.

Beutin, L., Geier, D., Steinrück, H., Zimmermann, S., Scheutz, F., 1993. Prevalence and some properties of verotoxin (Shiga-like toxin)-producing *Escherichia coli* in seven different species of healthy domestic animals. J. Clin. Microbiol. 31 (9), 2483–2488. https://doi.org/10.1128/jcm.31.9.2483-2488.1993.

Bucher, M., Meyer, C., Grötzbach, B., Wacheck, S., Stolle, A., Fredriksson-Ahomaa, M., 2008. Epidemiological data on pathogenic *Yersinia enterocolitica* in Southern Germany during 2000-2006. Foodborne Pathog. Dis. 5 (3), 273–280. https://doi.org/10.1089/fpd.2007.0076.

Cacciò, S., Pinter, E., Fantini, R., Mezzaroma, I., Pozio, E., 2002. Human infection with *Cryptosporidium felis*: case report and literature review. Emerg. Infect. Dis. 8 (1), 85–86.

Cacciò, S.M., Beck, R., Lalle, M., Marinculic, A., Pozio, E., 2008. Multilocus genotyping of *Giardia duodenalis* reveals striking differences between assemblages A and B. Int. J. Parasitol. 38, 1523–1531. https://doi.org/10.1016/j.ijpara.2008.04.008.

Chiari, M., Zanoni, M., Tagliabue, S., Lavazza, A., Alborali, L.G., 2013. *Salmonella* serotypes in wild boars (*Sus scrofa*) hunted in northern Italy. Acta Vet. Scand. 55 (1), 42. https://doi.org/10.1186/1751-0147-55-42.

Damborg, P., Olsen, K.E., Moller Nielsen, E., Guardabassi, L., 2004. Occurrence of *Campylobacter jejuni* in pets living with human patients infected with *C. jejuni*. J. Clin. Microbiol. 42, 1363–1364. https://doi.org/10.1128/JCM.42.3.1363-1364.2004.

de Oliveira, A.G.L., Sudré, A.P., Bergamo do Bomfim, T.C., HLC, Santos, 2021. Molecular characterization of *Cryptosporidium* spp. in dogs and cats in the city of Rio de Janeiro, Brazil, reveals potentially zoonotic species and genotype. PLoS One 16. https://doi.org/10.1371/journal.pone.0255087 e0255087.

Deplazes, P., Joachim, A., Mathis, A., Strube, C., Taubert, A., von Samson-Himmelsterna, G., Zahner, H., 2020. Parasitologie für die Tiermedizin, fourth ed. Thieme, Stuttgart, Germany. 978-3-13-242138-7 (ISBN).

EFSA (European Food Safety Authority) & ECDC (European Centre for Disease Prevention and Control), 2022. The European Union one health 2021 Zoonoses report. EFSA J. 20 (12), 7666. https://doi.org/10.2903/j.efsa.2022.7666.

ESCCAP. (2021). Wirm Control in Dogs and Cats. Guideline 1, 6th edition May 2021. ISBN: 978-1-913757-18-2. Available under: https://www.esccap.org/guidelines/gl1/.

Fisher, M., 2003. *Toxocara cati*: an underestimated zoonotic agent. Trends Parasitol. 19 (4), 167–170. https://doi.org/10.1016/s1471-4922(03)00027-8.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3, 294–299. https://pubmed.ncbi.nlm.nih.gov/7881515/.

Franiek, N., Orth, D., Grif, K., Ewers, C., Wieler, L.H., Thalhammer, J.G., Würzner, R., 2012. ESBL-produzierende *E. coli* und EHEC bei Hunden und Katzen in Tirol als mögliche Quelle für humane Infektionen. Berl. Münch. Tierärztl. Wochenschr. 125 (11–12), 469–475. https://pubmed.ncbi.nlm.nih.gov/23227764/.

Fuehrer, H.P., 2014a. An overview of the host spectrum and distribution of *Calodium hepaticum* (syn. *Capillaria hepatica*): part 1-Muroidea. Parasitol. Res. 113 (2), 619–640. https://doi.org/10.1007/s00436-013-3691-x.

Fuehrer, H.P., 2014b. An overview of the host spectrum and distribution of *Calodium hepaticum* (syn. *Capillaria hepatica*): part 2-Mammalia (excluding Muroidea). Parasitol. Res. 113 (2), 641–651. https://doi.org/10.1007/s00436-013-3692-9.

Fuehrer, H.P., Igel, P., Auer, H., 2011. *Capillaria hepatica* in man–an overview of hepatic capillariosis and spurious infections. Parasitol. Res. 109 (4), 969–979. https://doi.org/10.1007/s00436-011-2494-1.

Fuehrer, H.P., Silbermayr, K., Glawischnig, W., Joachim, A., 2015. Barcoding parasitischer Würmer – eine Sammlung ungeliebter Tiere? Das Fallbeispiel *Onchocerca jakutensis*. Acta ZooBot Austria 152, 173–178. https://www.zobodat.at/pdf/VZBG_152_0173-0178.pdf.

Genchi, M., Vismarra, A., Zanet, S., Morelli, S., Galuppi, R., Cringoli, G., Lia, R., Diaferia, M., Frangipane di Regalbono, A., Venegoni, G., Solari Basano, F., Varcasia, A., Perrucci, S., Musella, V., Brianti, E., Gazzonis, A., Drigo, M., Colombo, L., Kramer, L., 2021. Prevalence and risk factors associated with cat parasites in Italy: a multicenter study. Parasit. Vectors 14 (1), 475. https://doi.org/10.1186/s13071-021-04981-2.

Giacomelli, M., Follador, N., Coppola, L.M., Martini, M., Piccirillo, A., 2015. Survey of *Campylobacter* spp. in owned and unowned dogs and cats in northern Italy. Vet. J. 204 (3), 333–337. https://doi.org/10.1016/j.tvjl.2015.03.017.

Giannelli, A., Capelli, G., Joachim, A., Hinney, B., Losson, B., Kirkova, Z., René-Martellet, M., Papadopoulos, E., Farkas, R., Napoli, E., Brianti, E., Tamponi, C., Varcasia, A., Margarida Alho, A., Madeira de Carvalho, L., Cardoso, L., Maia, C., Mircean, V., Mihalca, A.D., Miró, G., Schnyder, M., Cantacessi, C., Colella, V., Cavalera, M.A., Latrofa, M.S., Annoscia, G., Knaus, M., Halos, L., Beugnet, F., Otranto, D., 2017. Lungworms and gastrointestinal parasites of domestic cats: a European perspective. Int. J. Parasitol. 47 (9), 517–528. https://doi.org/10.1016/j.ijpara.2017.02.003.

Haeghebaert, S., Duché, L., Gilles, C., Masini, B., Dubreuil, M., Minet, J.C., Bouvet, P., Grimont, F., Delarocque Astagneau, E., Vaillant, V., 2001. Minced beef and human salmonellosis: review of the investigation of three outbreaks in France. Euro Surveill. 6 (2), 21–26. https://doi.org/10.2807/esm.06.02.00223-en.

Hetem, D.J., Pekelharing, M., Thijsen, S.F., 2013. Probable transmission of *Yersinia enterocolitica* from a pet dog with diarrhoea to a 1-year-old infant. BMJ Case Rep. https://doi.org/10.1136/bcr-2013-200046 bcr2013200046.

Hinney, B., Ederer, C., Stengl, C., Wilding, K., Štrkolcová, G., Harl, J., Flechl, E., Fuehrer, H.P., Joachim, A., 2015. Enteric protozoa of cats and their zoonotic

potential-a field study from Austria. Parasitol. Res. 114 (5), 2003–2006. https://doi.org/10.1007/s00436-015-4408-0.
Hinney, B., Gottwald, M., Moser, J., Reicher, B., Schäfer, B.J., Schaper, R., Joachim, A., Künzel, F., 2017. Examination of anonymous canine faecal samples provides data on endoparasite prevalence rates in dogs for comparative studies. Vet. Parasitol. 15 (245), 106–115. https://doi.org/10.1016/j.vetpar.2017.08.016.
Hoelzer, K., Moreno Switt, A.I., Wiedmann, M., 2011. Animal contact as a source of human non-typhoidal salmonellosis. Vet. Res. 42 (1), 34. https://doi.org/10.1186/1297-9716-42-34.
Jiang, W., Roellig, D.M., Lebbad, M., Beser, J., Troell, K., Guo, Y., Li, N., Xiao, L., Feng, Y., 2020. Subtype distribution of zoonotic pathogen *Cryptosporidium felis* in humans and animals in several countries. Emerg. Microbes Infect. 9 (1), 2446–2454. https://doi.org/10.1080/22221751.2020.1840312.
Jimenez Castro, P.D., Sapp, S.G., 2020. Role of cats in human toxocarosis. Companion Anim. 26 (1), 1–8. https://doi.org/10.12968/coan.2020.0104.
Kiflu, B., Alemayehu, H., Abdurahaman, M., Negash, Y., Eguale, T., 2017. *Salmonella* serotypes and their antimicrobial susceptibility in apparently healthy dogs in Addis Ababa, Ethiopia. BMC Vet. Res. 13 (1), 134. https://doi.org/10.1186/s12917-017-1055-y.
Klotz, C., Sannella, A.R., Weisz, F., Chaudhry, U., Sroka, J., Tůmová, P., Nohýnková, E., Ignatius, R., Aebischer, T., Betson, M., Troell, K., Cacciò, S.M., 2022. Extensive testing of a multi-locus sequence typing scheme for *Giardia duodenalis* assemblage A confirms its good discriminatory power. Parasit. Vectors 15 (1), 489. https://doi.org/10.1186/s13071-022-05615-x.
Kostopoulou, D., Claerebout, E., Arvanitis, D., Ligda, P., Voutzourakis, N., Casaert, S., Sotiraki, S., 2017. Abundance, zoonotic potential and risk factors of intestinal parasitism amongst dog and cat populations: the scenario of Crete, Greece. Parasit. Vectors 10 (1), 43. https://doi.org/10.1186/s13071-017-1989-8.
LaLonde-Paul, D., Cummings, K.J., Rodriguez-Rivera, L.D., Wu, J., Lawhon, S.D., 2019. Ciprofloxacin resistance among *Campylobacter jejuni* isolates obtained from shelter dogs in Texas. Zoonoses Public Health 66 (3), 337–342. https://doi.org/10.1111/zph.12544.
Leahy, AM, Cummings, KJ, Rodriguez-Rivera, LD, Rankin, SC, Hamer, SA, 2016. Evaluation of Faecal Salmonella Shedding Among Dogs at Seven Animal Shelters across Texas. Zoonoses Public Health 63 (7), 515–521. https://doi.org/10.1111/zph.12257.
Leahy, A.M., Cummings, K.J., Rodriguez-Rivera, L.D., Rankin, S.C., Hamer, S.A., 2016. Evaluation of faecal *Salmonella* shedding among dogs at seven animal shelters across Texas. Zoonoses Public Health 63 (7), 515–521. https://doi.org/10.1111/zph.12257.
Leahy, A.M., Cummings, K.J., Rodriguez-Rivera, L.D., Hamer, S.A., Lawhon, S.D., 2017. Faecal *Campylobacter* shedding among dogs in animal shelters across Texas. Zoonoses Public Health 64 (8), 623–627. https://doi.org/10.1111/zph.12356.
Lee, M.F., Auer, H., Lindo, J.F., Walochnik, J., 2017. Multilocus sequence analysis of *Giardia* spp. isolated from patients with diarrhea in Austria. Parasitol. Res. 116 (2), 477–481. https://doi.org/10.1007/s00436-016-5306-9.
Lefebvre, S.L., Reid-Smith, R., Boerlin, P., Weese, J.S., 2008. Evaluation of the risks of shedding salmonellae and other potential pathogens by therapy dogs fed raw diets in Ontario and Alberta. Zoonoses Public Health 55 (8–10), 470–480. https://doi.org/10.1111/j.1863-2378.2008.01145.x.
Lemos, M.L., Nunes, A., Ancora, M., Cammà, C., Costa, P.M.D., Oleastro, M., 2021. *Campylobacter jejuni* in different canine populations: characteristics and zoonotic potential. Microorganisms 9 (11), 2231. https://doi.org/10.3390/microorganisms9112231.
Leonard, E.K., Pearl, D.L., Finley, R.L., Janecko, N., Peregrine, A.S., Reid-Smith, R.J., Weese, J.S., 2011. Evaluation of pet-related management factors and the risk of *Salmonella* spp. carriage in pet dogs from volunteer households in Ontario (2005-2006). Zoonoses Public Health 58 (2), 140–149. https://doi.org/10.1111/j.1863-2378.2009.01320.x.
Liang, J., Duan, R., Xia, S., Hao, Q., Yang, J., Xiao, Y., Qiu, H., Shi, G., Wang, S., Gu, W., Wang, C., Wang, M., Tian, K., Luo, L., Yang, M., Tian, H., Wang, J., Jing, H., Wang, X., 2015. Ecology and geographic distribution of *Yersinia enterocolitica* among livestock and wildlife in China. Vet. Microbiol. 178 (1–2), 125–131. https://doi.org/10.1016/j.vetmic.2015.05.006.
Lichtmannsperger, K., Harl, J., Freudenthaler, K., Hinney, B., Wittek, T., Joachim, A., 2020. *Cryptosporidium parvum, Cryptosporidium ryanae,* and *Cryptosporidium bovis* in samples from calves in Austria. Parasitol. Res. 2119 (12), 4291–4295. https://doi.org/10.1007/s00436-020-06928-5.
Maciag, L., Morgan, E.R., Holland, C., 2022. *Toxocara*: time to let cati 'out of the bag'. Trends Parasitol. 38 (4), 280–289. https://doi.org/10.1016/j.pt.2021.12.006.
Mughini Gras, L., Smid, J.H., Wagenaar, J.A., Koene, M.G., Havelaar, A.H., Friesema, I. H., French, N.P., Flemming, C., Galson, J.D., Graziani, C., Busani, L., Van Pelt, W., 2013. Increased risk for *Campylobacter jejuni* and *C. coli* infection of pet origin in dog owners and evidence for genetic association between strains causing infection in humans and their pets. Epidemiol. Infect. 141, 2526–2535. https://doi.org/10.1017/S0950268813000356.
Müller, D, Greune, L, Heusipp, G, Karch, H, Fruth, A, Tschäpe, H, Schmidt, MA, 2007. Identification of unconventional intestinal pathogenic Escherichia coli isolates expressing intermediate virulence factor profiles by using a novel single-step multiplex PCR. Appl Environ Microbiol 73 (10), 3380–3890. https://doi.org/10.1128/AEM.02855-06.
Ortuño, A., Castella, J., 2011. Intestinal parasites in shelter dogs and risk factors associated with the facility and its management. Israel J. Vet. Med. 66, 1–5. http://www.ijvm.org.il/sites/default/files/shelter_dogs_from_september_book_en_250911-10.pdf.
Ortuño, A., Scorza, V., Castellà, J., Lappin, M., 2014. Prevalence of intestinal parasites in shelter and hunting dogs in Catalonia, northeastern Spain. Vet. J. 199, 465–467. https://doi.org/10.1016/j.tvjl.2013.11.022.
Pallant, L., Barutzki, D., Schaper, R., Thompson, R.C.A., 2015. The epidemiology of infections with *Giardia* species and genotypes in well cared for dogs and cats in Germany. Parasit. Vectors 8, 2. https://doi.org/10.1186/s13071-014-0615-2.
Pedraza-Díaz, S., Amar, C., Iversen, A.M., Stanley, P.J., McLauchlin, J., 2001. Unusual *Cryptosporidium* species recovered from human faeces: first description of *Cryptosporidium felis* and *Cryptosporidium* 'dog type' from patients in England. J. Med. Microbiol. 50 (3), 293–296. https://doi.org/10.1099/0022-1317-50-3-293.
Pölzler, T., Stüger, H.P., Lassnig, H., 2018. Prevalence of most common human pathogenic *Campylobacter* spp. in dogs and cats in Styria, Austria. Vet. Med. Sci. 4 (2), 115–125. https://doi.org/10.1002/vms3.93.
Procter, T.D., Pearl, D.L., Finley, R.L., Leonard, E.K., Janecko, N., Reid-Smith, R.J., Weese, J.S., Peregrine, A.S., Sargeant, J.M., 2014. A cross-sectional study examining *Campylobacter* and other zoonotic enteric pathogens in dogs that frequent dog parks in three cities in South-Western Ontario and risk factors for shedding of *Campylobacter* spp. Zoonoses Public Health 61 (3), 208–218. https://doi.org/10.1111/zph.12062.
Raza, A., Rand, J., Quamar, A.G., Jabbar, A., Kopp, S., 2018. Gastrointestinal parasites in shelter dogs: occurrence, pathology, treatment and risk to shelter workers. Animals 8, 108. https://doi.org/10.3390/ani8070108.
Reimschuessel, R., Grabenstein, M., Guag, J., Nemser, S.M., Song, K., Qiu, J., Clothier, K. A., Byrne, B.A., Marks, S.L., Cadmus, K., Pabilonia, K., Sanchez, S., Rajeev, S., Ensley, S., Frana, T.S., Jergens, A.E., Chappell, K.H., Thakur, S., Byrum, B., Cui, J., Zhang, Y., Erdman, M.M., Rankin, S.C., Daly, R., Das, S., Ruesch, L., Lawhon, S.D., Zhang, S., Baszler, T., Diaz-Campos, D., Hartmann, F., Okwumabua, O., 2017. Multilaboratory survey to evaluate *Salmonella* prevalence in diarrheic and nondiarrheic dogs and cats in the United States between 2012 and 2014. J. Clin. Microbiol. 55 (5), 1350–1368. https://doi.org/10.1128/JCM.02137-16.
Ryan, U., Xiao, L., Read, C., Zhou, L., Lal, A.A., Pavlasek, I., 2003. Identification of novel *Cryptosporidium* genotypes from the Czech Republic. Appl. Environ. Microbiol. 69 (7), 4302–4307. https://doi.org/10.1128/AEM.69.7.4302-4307.2003.
Sanchez, S., Hofacre, C.L., Lee, M.D., Maurer, J.J., Doyle, M.P., 2002. Animal sources of salmonellosis in humans. J. Am. Vet. Med. Assoc. 221, 492–497. https://doi.org/10.2460/javma.2002.221.492.
Sauda, F., Malandrucco, L., De Liberato, C., Perrucci, S., 2019. Gastrointestinal parasites in shelter cats of Central Italy. Vet. Parasitol. Reg. Stud. Rep. 100321 https://doi.org/10.1016/j.vprsr.2019.100321.
Spergser, J., Loncaric, I., Tichy, A., Fritz, J., Scope, A., 2018. The cultivable autochthonous microbiota of the critically endangered northern bald ibis (*Geronticus eremita*). PLoS One 13, e0195255. https://doi.org/10.1371/journal.pone.0195255.
Stamm, I., Hailer, M., Depner, B., Kopp, P.A., Rau, J., 2013. *Yersinia enterocolitica* in diagnostic fecal samples from European dogs and cats: identification by fourier transform infrared spectroscopy and matrix-assisted laser desorption ionization-time of flight mass spectrometry. J. Clin. Microbiol. 51 (3), 887–893. https://doi.org/10.1128/JCM.02506-12.
Stehr-Green, J.K., Schantz, P.M., 1987. The impact of zoonotic diseases transmitted by pets on human health and the economy. Vet. Clin. North Am. Small Anim. Pract. 17, 1–15. https://doi.org/10.1016/s0195-5616(87)50601-5.
Sulaiman, I.M., Fayer, R., Bern, C., Gilman, R.H., Trout, J.M., Schantz, P.M., Das, P., Lalle, M., Xiao, L., 2003. Triosephosphate isomerase gene characterization and potential zoonotic transmission of *Giardia duodenalis*. Emerg. Infect. Dis. 9, 1444–1452. https://doi.org/10.3201/eid0911.030084.
Symeonidou, I., Gelasakis, A.I., Arsenopoulos, K., Angelou, A., Beugnet, F., Papadopoulos, E., 2018. Feline gastrointestinal parasitism in Greece: emergent zoonotic species and associated risk factors. Parasit. Vectors 11 (1), 227. https://doi.org/10.1186/s13071-018-2812-x.
Takeuchi-Storm, N., Mejer, H., Al-Sabi, M.N.S., Olsen, C.S., Thamsborg, S.M., Enemark, H.L., 2015. Gastrointestinal parasites of cats in Denmark assessed by necropsy and concentration McMaster technique. Vet. Parasitol. 214, 327–332. https://doi.org/10.1016/j.vetpar.2015.06.033.
Tseng, Y.C., Ho, G.D., Chen, T., Huang, B.F., Cheng, P.C., Chen, J.L., Peng, S.Y., 2014. Prevalence and genotype of *Giardia duodenalis* from faecal samples of stray dogs in Hualien city of eastern Taiwan. Trop. Biomed. 31, 305–311. https://www.msptm.org/files/305_-_311_Peng_SY.pdf.
Tupler, T., Levy, J.K., Sabshin, S.J., Tucker, S.J., Greiner, E.C., Leutenegger, C.M., 2012. Enteropathogens identified in dogs entering a Florida animal shelter with normal feces or diarrhea. J. Am. Vet. Med. Assoc. 241 (3), 338–343. https://doi.org/10.2460/javma.241.3.338.
Verma, A., Carney, K., Taylor, M., Amsler, K., Morgan, J., Gruszynski, K., Erol, E., Carter, C., Locke, S., Callipare, A., Shah, D.H., 2021. Occurrence of potentially zoonotic and cephalosporin resistant enteric bacteria among shelter dogs in the central and south-central Appalachia. BMC Vet. Res. 17, 313. https://doi.org/10.1186/s12917-021-03025-2.
Vestrheim, D.F., Lange, H., Nygård, K., Borgen, K., Wester, A.L., Kvarme, M.L., Vold, L., 2016. Are ready-to-eat salads ready to eat? An outbreak of *Salmonella* Coeln linked to imported, mixed, pre-washed and bagged salad, Norway, November 2013. Epidemiol. Infect. 144 (8), 1756–1760. https://doi.org/10.1017/S0950268815002769.

Wei, L., Yang, C., Shao, W., Sun, T., Wang, J., Zhou, Z., Chen, C., Zhu, A., Pan, Z., 2020. Prevalence and drug resistance of *Salmonella* in dogs and cats in Xuzhou, China. J. Vet. Res. 64 (2), 263–268. https://doi.org/10.2478/jvetres-2020-0032.

Wolfs, T.F., Duim, B., Geelen, S.P., Rigter, A., Thomson-Carter, F., Fleer, A., Wagenaar, J. A., 2001. Neonatal sepsis by *Campylobacter jejuni*: genetically proven transmission from a household puppy. Clin. Infect. Dis. 32 (5), E97–E99. https://doi.org/10.1086/319224.

Zottler, E.M., Bieri, M., Basso, W., Schnyder, M., 2019. Intestinal parasites and lungworms in stray, shelter and privately owned cats of Switzerland. Parasitol. Int. 69, 75–81. https://doi.org/10.1016/j.parint.2018.12.005.