Veterinary Parasitology: Regional Studies and Reports 16 (2019) 100270

Contents lists available at ScienceDirect

Veterinary Parasitology: Regional Studies and Reports

journal homepage: www.elsevier.com/locate/vprsr

Regional report

Prevalence survey of gastrointestinal and respiratory parasites of shelter cats in northeastern Georgia, USA

Kristen R. Hoggard, Dixie M. Jarriel, Thomas J. Bevelock, Guilherme G. Verocai*,[1]

Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, 501 D.W. Brooks Drive, Athens, GA 30602, USA

ARTICLE INFO

Keywords:
Aelurostrongylus
Baermann
Endoparasites
Hydatigera
North America

ABSTRACT

The goal of this study was to assess the prevalence of gastrointestinal and respiratory parasites of shelter cats from northeast Georgia, thus promoting a more targeted approach in parasite diagnosis and treatment. Fecal samples of cats kept in a shelter located in Lavonia, northeastern Georgia, USA, were processed for the presence of parasites using double centrifugation sugar flotation (n = 103) and Baermann techniques (n = 98). Flotation revealed eggs of *Toxocara cati* (17.5%), *Ancylostoma* sp. (11.7%), Taeniidae (3.9%), *Spirometra mansonoides* (2.9%), *Mesocestoides* sp. (1%), *Dipylidium caninum* (1%), and *Eucoleus aerophilus* (1%), and oocysts of *Cystoisospora felis* (16.5%), and *Cystoisospora rivolta* (8.7%). Baermann diagnosed *Aelurostrongylus abstrusus* larvae in 5 cats (5.1%), while fecal flotation alone identified only 2 of these infections. Taeniidae eggs were identified to species-level by PCR and sequencing targeting the cytochrome oxidase c subunit 1 (*cox1*) of the mitochondrial DNA. All isolates belong to *Hydatigera taeniaeformis sensu stricto*, which is the first unequivocal report of the species in North America. Overall, 45.6% of the cats were infected with at least one parasite. This prevalence of infection is much higher than what is generally reported in client owned animals, highlighting the importance of using appropriate fecal diagnostic techniques to detect gastrointestinal and respiratory parasites on newly adopted cats. Correct diagnosis may direct appropriate treatment and control strategies, which would mitigate the risk of infection of other animals in household, and human exposure to zoonotic parasites.

1. Introduction

In the United States approximately 1.6 million cats are adopted from shelters every year, and cats adopted from shelters or humane societies make up 31% of all owned cats (ASPCA, 2017). Multiple studies have been conducted which demonstrate that shelter cats are several times more likely to be infected with enteric parasites than owned cats (Rembiesa and Richardson, 2003; Hoopes et al., 2015). Many shelter animals originate as free roaming strays and are therefore more likely to ingest parasitic eggs and larvae from either intermediate hosts or a contaminated environment. In addition, incomplete parasite diagnosis and treatment, as well as close contact with other infected animals, help perpetuate infections within shelters. These infected cats introduce a potential risk of parasite transmission to other household cats as well as their owners. Due to their high-volume nature, shelters often use routine fecal diagnostic techniques that may not identify certain parasites, as specific techniques are required to accurately diagnose certain parasites. Accurate diagnosis of enteric parasites is also influenced by the particular flotation technique used. Studies conducted comparing passive flotation and zinc-sulfate centrifugation techniques in fecal examination have demonstrated that using passive flotation alone increases the incidence of false negatives and may result in up to 50% of positive samples being missed (Zajac et al., 2002; Gates and Nolan, 2009). The most commonly reported feline enteric parasites in North America include *Ancylostoma* spp., *Toxocara cati, Cystoisospora* spp., *Dipylidium caninum,* and tapeworms within the family Taeniidae, though there are geographical differences in which species have higher prevalence (Lillis, 1967; Lucio-Forster and Bowman, 2011; Little et al., 2015; Wyrosdick et al., 2017; Nagamori et al., 2018). Knowledge of the most common feline parasites in an area is beneficial for a shelter to both utilize appropriate testing techniques and stock effective parasiticides.

In addition to enteric parasites shelter animals may also harbor lungworms, which are an important cause of respiratory disease in cats. Infected cats may have subclinical lung damage or may show a range of respiratory disease signs, and undiagnosed infections may lead to increased anesthetic complications (Gerdin et al., 2011; Schnyder et al., 2014; Elsheikha et al., 2016). In a study conducted on feline patients of

* Corresponding author.
E-mail address: gverocai@cvm.tamu.edu (G.G. Verocai).
[1] Present address: Department of Veterinary Pathobiology, College of Veterinary Pathobiology and Biomedical Sciences, College Station, TX, 7843–4467, USA.

https://doi.org/10.1016/j.vprsr.2019.100270
Received 28 September 2018; Received in revised form 3 February 2019; Accepted 8 February 2019
Available online 11 February 2019

a veterinary clinic in Italy nearly 50% of cats infected with *Aelurostrongylus abstrusus* showed no respiratory signs, while nearly 90% of infected cats were shown to have at least some radiographic changes within their respiratory tract (Genchi et al., 2014). *Aelurostrongylus abstrusus* infections are difficult to diagnose using the routine fecal flotation methods normally employed by shelter facilities, as larvae are more reliably found when performing the more sensitive Baermann test (Elsheikha et al., 2016). In contrast, the eggs of *Eucoleus* spp., also respiratory parasites, are found upon fecal flotation techniques (Conboy, 2009).

A previous prevalence study on shelter cats conducted in northwestern Georgia using zinc sulfate fecal flotation demonstrated an enteric helminth prevalence of 39.6%, with no reported incidence of *A. abstrusus* (Carleton and Tolbert, 2004). To our knowledge, no feline parasite prevalence study has been performed in northeastern Georgia, and no prevalence study conducted within Georgia has utilized the Baermann technique. The goal of this study is to assess the prevalence of gastrointestinal and respiratory parasites of shelter cats from northeastern Georgia, thus promoting a more targeted approach in parasite diagnosis and treatment of shelter cats.

2. Methods

2.1. Sample acquisition and processing

Fecal samples were collected from cats housed at shelter in Lavonia, Franklin and Hart Counties, northeastern Georgia, USA. The shelter receives strays as well as owner surrenders. A total of 121 samples were sent from the shelter, including and 103 unique samples that were used for prevalence analysis, and 18 repeated samples. Of the unique samples, 50 cats were under 6 months of age, 16 cats were between 6 months and one year of age, and 37 cats were over one year of age, following age classes previously used for similar cat studies (Nagamori et al., 2018). Samples used in this study were collected between the months of September and April from cats that had not been treated with an endoparasiticide since arriving at the shelter. Feces was collected from the litterbox of each individually housed cat, labeled for the age and sex of the cat when available, and subsequently transported to the University of Georgia's Parasitology Diagnostic Laboratory. Each sample was examined for proglottids and processed the day of arrival by a standard Double Centrifugal Sugar Flotation (DCSF) using a Sheather's sugar solution (specific gravity = 1.25) and 2 g of feces (Zajac and Conboy, 2012). Slides were scanned using a compound microscope at 100× magnification within 48 h of preparation for the presence of helminth eggs and coccidian oocysts, which were identified to genus or species based on morphometry at 400×. These samples were not assessed for the presence of *Giardia* sp. cysts or *Cryptosporidium* sp. oocysts. Samples with an adequate amount of feces remaining were also processed by a modified Falcon Baermann using 2 g of feces ($N = 98$). Feces was wrapped within a pouch made of kimwipe and cheesecloth, then submerged at the top of a 50 mL Falcon tube filled with lukewarm water. Tubes were left undisturbed for 18–24 h before removing the sample bag and collecting the bottom 14 mL of sample and transferred to another tube, which was centrifuged for 10 min at 1300 rpm. The supernatant was removed to leave approximately 1 mL of sample, which was then transferred *via* micropipette in 200–500 µL aliquots onto slides to be scanned for *A. abstrusus*. Three 200 µL aliquots were scanned, then the remainder of the sample was scanned using 500 µL aliquots and larval counts were recorded. Five fecal samples (four unique samples, one duplicate) positive for eggs of Taeniidae were stored at −20 °C for subsequent molecular analysis.

2.2. Statistical analysis

We compared the effects of sex and age class on the prevalence of infection by at least one parasite using Chi-square using SAS (SAS Version 9.4, SAS Institute, Cary, NC, US), assuming statistical significance at $\alpha = 0.05$. Data did not allow for comparisons on prevalence for each parasite genus or species.

2.3. Molecular and phylogenetic analyses

The samples positive for Taeniidae eggs were thawed to room temperature. The DCSF technique was performed using Sheather's sugar solution, then the top 2 mL was removed from each tube, resuspended in 12 mL of water, and centrifuged again at 1300 rpm for 10 min. The supernatant was removed, and eggs were isolated from the remainder using a 50 µm mesh sieve then caught on a 20 µm mesh sieve (Überstrainer PluriSelect, Leipzig, Germany). The remaining solution was centrifuged, transferred to a 2 mL Eppendorf, then centrifuged once more at 8000 rpm for 2 min. The supernatant was removed, then the tubes were subjected to a modified thermal shock method described by Hidalgo (2018). Briefly, the samples were placed in a − 80 °C freezer for 10 min, incubated at 90 °C for 30 min, then rapidly cooled on ice. DNA extraction was accomplished using the QIAGEN DNeasy Blood and Tissue Kit (Qiagen, Valencia, California, USA). After the addition of the Buffer ATL and proteinase K solutions, the samples were incubated at 56 °C for 12 h, as per the referenced thermal shock method (Hidalgo et al., 2018). The remainder of the extraction was accomplished as indicated in the DNeasy kit.

DNA templates were subjected to a Polymerase chain reaction (PCR) for targeting a fragment of the cytochrome *c* oxidase subunit 1 (cox1) gene of the mitochondrial DNA (mtDNA) using primers 2575 (5′-TTT TTTGGGCATCCTGAGGTTTAT-3′) and 3021 (5′-TAAAGAAAGAACA TAA7TGAAAATG-3′) (Bowles et al., 1992). PCR was performed in a 30 µL reaction including Go™Taq Green Master Mix (Promega, Madison, WI, USA), 0.5 µmol/L of each primer and 2 µL of DNA template. PCR parameters were as follows: denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 52 °C for 1 min, and 72 °C for 1 min, with a final 7 min extension at 72 °C. Successful amplification was confirmed by visualization of bands using gel electrophoresis. PCR products were purified using the E.Z.N.A. Cycle Pure kit (Omega Bio-Tek, Norcross, GA, USA) and sequenced in both directions using the forward and reserves PCR primers using BigDye Terminator Cycle Sequencing (Applied Biosystems).

The generated cox1 fragments were edited and aligned in MEGA 7 (Kumar et al., 2016). A phylogenetic analysis was performed using MEGA 7 (Kumar et al., 2016) using the Maximum Likelihood method with 1000 bootstrap replicates. The best-fitting evolutionary model for the data set was HKY + G + I. All sequences were trimmed to 336 bp. Outgroups consisted in homologous sequences of other cyclophyllidean and pseudophyllidean cestodes available in GenBank.

3. Results

Of the 103 samples tested, 45.6% ($N = 47$) were positive for at least one parasite species, 20.4% ($N = 21$) were positive for at least 2 parasite species, and 3.8% (N = 4) were positive for 3 parasite species. The most common parasite present in coinfections was *T. cati,* which was present in twelve coinfected samples. Of the 21 samples positive for more than one parasitic species, eight were found to be coinfected with *T. cati* and *Ancylostoma* sp. In the seven samples positive for lungworms, five were coinfected with enteric parasitic species.

Overall, the DCSF revealed eggs of *Toxocara cati* (17.5%), *Ancylostoma* sp. (11.7%), Taeniidae (3.9%), *Spirometra mansonoides* (2.9%), *Mesocestoides* sp. (1%), *Dipylidium caninum* (1%), *Eucoleus aerophilus* (1%), and oocysts of *Cystoisospora felis* (16.5%), and *Cystoisospora rivolta* (8.7%) (Table 1). Additionally, eggs of *Eucoleus boehmi*, a canine respiratory parasite, were observed as a spurious parasite in one cat. Baermann diagnosed *A. abstrusus* larvae in 5 cats (5.1%), and only 2 of these infections could be detected through DCSF.

The prevalence of infection by at least one parasitic species by age

Table 1
Parasite prevalence in shelter cats (n = 103) from northeastern Georgia, USA.

	Prevalence % (N)					
Parasite	Total (n = 103)	< 6 mo (n = 50)	6-12mo (n = 16)	> 12mo (n = 37)	Female (n = 61)	Male (n = 42)
Nematoda						
Toxocara cati	17.5 (18)	12.0 (6)	37.5 (6)	16.2 (6)	14.8 (9)	21.4 (9)
Ancylostoma sp.	11.7 (12)	10.0 (5)	18.8 (3)	10.8 (4)	6.6 (4)	19.0 (8)
Aelurostrongylus abstrusus[a]	5.1 (5)	4.0 (2)	0 (0)	8.1 (3)	8.2 (5)	0 (0)
Eucoleus aerophilus	1.0 (1)	0 (0)	0 (0)	2.7 (1)	0 (0)	2.4 (1)
Cestoda						
Taeniidae[b]	3.9 (4)	0 (0)	6.3 (1)	8.1 (3)	4.9 (3)	2.4 (1)
Spirometra mansonoides	2.9 (3)	2.0 (1)	0 (0)	5.4 (2)	3.3 (2)	2.4 (1)
Dipylidium caninum	1.0 (1)	0 (0)	0 (0)	2.7 (1)	1.6 (1)	0 (0)
Mesocestoides sp.	1.0 (1)	0 (0)	0 (0)	2.7 (1)	0 (0)	2.4 (1)
Protozoa						
Cystoisospora spp.	20.4 (21)	26.0 (13)	31.2 (5)	8.1 (3)	21.3 (13)	19.0 (8)
Cystoisospora felis	16.5 (17)	20.0 (10)	25.0 (4)	8.1 (3)	16.4 (10)	16.7 (7)
Cystoisospora rivolta	8.7 (9)	14.0 (7)	6.3 (1)	2.7 (1)	9.8 (6)	7.1 (3)

[a] Based on 98 samples processed by the Baermann technique.
[b] All four cats, and a repeated sample from one of these, were molecularly identified as *Hydatigera taeniaeformis sensu stricto*.

group was 42% in cats under 6 months of age, 68.8% in cats aged six months to one year, and 40.5% in cats over one year of age. The effect of age class on the prevalence of infection by at least one parasite genus or species was not significant (X^2 = 4.099, P = .128). *Cystoisospora* spp. was the most commonly reported parasite of cats under six months old (26.0%), while *T. cati* was the most common parasite of cats aged six months and older (Table 1). The prevalence of infection by at least one parasitic species by sex was 42.6% in females, and 50% in males, and was also not statistically significant (X^2 = 0.546, P = .460). The most common parasites found in female cats were *Cystoisospora* spp. (21.3%), followed by *T. cati* (14.8%), whereas in males the most common parasite was *T. cati* (21.4%), followed by *Ancylostoma* sp., and *Cystoisospora* spp. (19.0%, each) (Table 1).

Sequencing data and phylogenetic analysis revealed that Taeniidae eggs in all five positive samples belonged to *Hydatigera taeniaeformis* s. s. (GenBank MH938572–76), forming a well-supported clade (100% bootstrap support) with other *H. taeniaeformis* isolates (Fig. 1).

4. Discussion

The overall gastrointestinal parasite prevalence reported in this study is similar to that reported in northwest Georgia (Carleton and Tolbert, 2004). Our study, however, brings an up-to-date baseline prevalence for shelter cats in the state, and also included prevalence of the often neglected, respiratory helminths. No effect of age class was observed on prevalence of infection by at least one parasite genus or species, however Nagamori et al. (2018) found that cats younger than 6 months are significantly more infected than cats older than 6 months. Similar to Nagamori et al. (2018), no effect of sex on prevalence of infection by parasites was observed.

The nematodes *Ancylostoma* sp. and *T. cati* were found to have prevalence of 11.7% and 17.5% respectively, which are both higher than that reported the prevalence of hookworms and roundworms reported by the Companion Animal Parasite Council (CAPC) for Georgia (Companion Animal Parasite Council, 2018). This is likely due to the larger number of owned cats being included in the CAPC's sample data as compared to the exclusively shelter cats used in this study. *Toxocara cati* was found in a greater percentage of cats sampled in this study as compared to a shelter study conducted in Florida, but lower than that reported in studies conducted in Connecticut, New Jersey, New York, and Oklahoma (Lillis, 1967; Rembiesa and Richardson, 2003; Lucio-Forster and Bowman, 2011; Wyrosdick et al., 2017; Nagamori et al., 2018). Disparities noted between prevalence studies of different shelters may be attributed to variations in backgrounds and previous treatment history of the sampled cats. The shelter used in this study has a high percentage of surrendered animals, while other shelters may have a higher number of strays. In addition, there is geographic variability in each parasite's hosts and environmental viability.

The most commonly found cestode in this study were members of the family Taeniidae, similar to the recent findings of Nagamori et al. (2018). All isolates were molecularly confirmed to be *H. taeniaeformis* s. s., constituting the first unequivocal record of the parasite in North America (MH938572–76) (Fig. 1). Isolates belonging to *Hydatigera kamiyai* and an undescribed species have been reported from Alberta and Saskatchewan, Canada (Hoopes et al., 2015; Lavikainen et al., 2016). *Spirometra mansoides* and *Mesocestoides* sp. were found in 2.9% and 1.0% of samples respectively, which suggests that these parasites are occasionally found within Georgia. These two tapeworms were also found in a Florida shelter, with *S. mansonoides* having a higher prevalence than reported here (Wyrosdick et al., 2017). Presence of *H. taeniaeformis* s. s., *S. mansonoides*, and *Mesocestoides*, tapeworms that use vertebrate animals as intermediate or paratenic hosts, highlights that stray and outdoor cats are relying on predation and, therefore, are at higher risk of infection by these parasites when compared to indoor cats. *Dipylidium caninum* was found in 1.0% of samples, a prevalence lower than reported across North America (Rembiesa and Richardson, 2003). This was expected but likely underestimated, as *D. caninum* is most often diagnosed by finding proglottids in the feces, and is not reliably diagnosed through the use of fecal flotation alone (Little et al., 2015). In addition, several previous prevalence reports included infections found upon necropsy (Lillis, 1967; Power, 1971). The shelter used for this study did not treat their cats for fleas and have historically had issues with flea infestations. These samples were collected during winter months, which may contribute to the lower prevalence, though there most likely were undiagnosed infections.

When considered together, members of the genus *Cystoisospora* were found to have a combined prevalence of 20%, higher than any other parasite. This prevalence corroborates the findings of Nagamori et al. (2018) for cats under 12 months of age. This high prevalence of infection is likely due to a number of factors, including the environmental persistence of infective oocysts and their capability of being transmitted both directly and through facultative intermediate hosts (Bowman, 2013). The high stress, high capacity shelter environment likely assists in the spread of infection. The prevalence of other common parasitic protozoa such as *Giardia* and *Cryptosporidium* were not assessed in this study, as the diagnostic methods used here are not sensitive for their identification.

The prevalence of *A. abstrusus* found in this study (5.1%) is significantly lower than reported in Alabama (18.5%), similar to the findings of Lucio-Forster and Bowman. (2011) in shelter cats in New

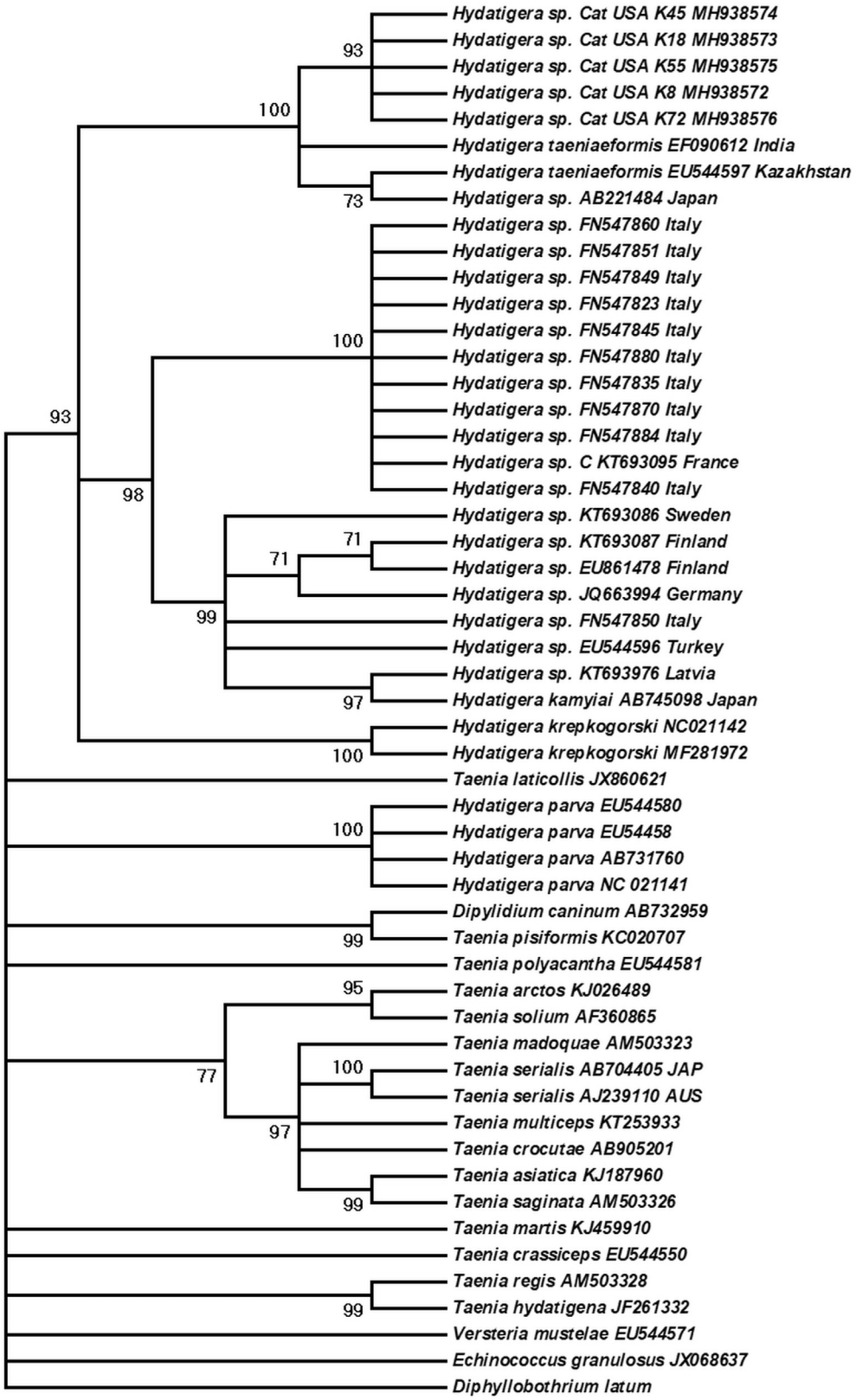

Fig. 1. Maximum Likelihood tree depicting phylogenetic relationships among species of *Hydatigera* and other cestodes inferred from cytochrome *c* oxidase subunit 1 (*cox*1) mitochondrial gene. Branches with < 70% bootstrap support were collapsed. Bootstrap support shown besides branches are based on 1000 replicates. Sequences of *Hydatigera taeniaeformis sensu stricto* that were produced have been accessioned at GenBank (MH938572–76). Sequences of other *Hydatigera* species within the *H. taeniaeformis* species complex are labeled with their country of origin.

York (6.2%), and higher than that reported in the more northern states of Connecticut (0.2%) and New Jersey (0.1%) (Lillis, 1967; Willard et al., 1988; Rembiesa and Richardson, 2003). These discrepancies may be attributed to the intermittent shedding of infected cats as well as possible differences in histories of the cats sampled, as the shelter used for this study receives animals from both owners and as strays. As discussed previously, stray cats are more likely to prey upon the intermediate hosts of parasites, including those that may transmit *A. abstrusus*, and shelter cats are more likely to harbor parasites transmitted by prey animals (Traversa et al., 2008). In addition, these samples were collected in the cooler months of the year and temperature may have an effect on the parasite's gastropod intermediate hosts and in turn the incidence of newly infected cats. Finally, while there are several older studies that demonstrate the prevalence of this parasite, there are few comprehensive studies across North America that incorporate the Baermann technique and can give accurate prevalence reports. A recent prevalence study conducted in Europe using the Baermann method has found that feline lungworm prevalence is higher than historically reported, which may indicate that current prevalence reports of *A. abstrusus* within the United States may be inaccurate (Giannelli et al., 2017). In addition, further investigation into lungworm lifecycles have revealed that transmission of infective larvae may occur outside of traditional intermediate host predation. For example, larvae may be transmitted through water contaminated by the death of infected gastropods as well as by paratenic hosts that are frequently preyed upon by cats (Giannelli et al., 2015; Falsone et al., 2017). Due to these advancements in understanding lungworm life cycles and transmission rates, it is important to gather updated information on infection prevalence and perform appropriate diagnostic methods in order to accurate identify infections. It is important to note that the flotation method alone identified less than half of the cats that tested positive for *A. abstrusus* larvae using the Baermann technique. Meanwhile, eggs of *Eucoleus* spp. were found on flotation, which provides support that although they are less common, they are at least sporadically present, and therefore diagnosis of parasitic respiratory infections should include both a DCSF and Baermann to identify multiple respiratory parasites (Bowman, 2013). *Eucoleus aerophilus* is commonly reported from cats, dogs and wild canids in North America and Europe (Conboy, 2009; Hoopes et al., 2015; Giannelli et al., 2017; Nagamori et al., 2018).).

Nearly half of all the cats sampled in this study were found to be infected with at least one parasite. The overall parasite prevalence found in this study's feline population is much higher than what is generally reported in client owned cats, which highlights the importance of performing fecal examinations to detect both gastrointestinal and respiratory parasites on newly adopted cats. Correct diagnosis of parasitic infections may direct appropriate treatment and control strategies, which would mitigate the risk of infection of other animals in household, and human exposure to zoonotic parasites.

Conflict of interest statement

The authors declare no conflict of interest.

Ethical statement

No animal experimentation was performed for this case report. The use of a diagnostic sample for research is covered by the UGA IACUC (AUP # A2017 05-014-Y1-A0).

Acknowledgements

Tammie Jourdanais and the shelter personnel, Rob Miller and the UGA CVM Class of 2019 for facilitating sample collections, and Janet Yoder (UGA) for diagnostic assistance. We would like to thank Dr. Kelsey Paras (UGA) for revising the manuscript prior to submission. TJB was supported by the Georgia Veterinary Scholar Summer Program (GVSP), UGA CVM, funded by Boehringer-Ingelheim.

References

ASPCA, 2017. Pet Statistics. https://www.aspca.org/animal-homelessness/shelter-intake-and-surrender/pet-statistics.
Bowles, J., Blair, D., McManus, D.P., 1992. Genetic variants within the genus *Echinococcus* identified by mitochondrial DNA sequencing. Mol. Biochem. Parasit. 54, 165–173.
Bowman, D.D., 2013. Georgis' Parasitology for Veterinarians, 10th edition. Elsevier Saunders, St. Louis, MO (477 pp).
Carleton, R.E., Tolbert, M.K., 2004. Prevalence of *Dirofilaria immitis* and gastrointestinal helminths in cats euthanized at animal control agencies in northwest Georgia. Vet. Parasitol. 119, 319–326.
Companion Animal Parasite Council, 2018. Roundworm prevalence map. https://www.capcvet.org/maps/#2018/all/roundworm/cat/united-states/georgia/.
Conboy, G., 2009. Helminth parasites of the canine and feline respiratory tract. Vet. Clin. North Am. Small Anim. Pract. 39, 1109–1126.
Elsheikha, H.M., Schnyder, M., Traversa, D., Di Cesare, A., Wright, I., Lacher, D.W., 2016. Updates on feline aelurostrongylosis and research priorities for the next decade. Parasit. Vectors 9, 389.
Falsone, L., Colella, V., Napoli, E., Brianti, E., Otranto, D., 2017. The cockroach *Periplaneta americana* as a potential paratenic host of the lungworm *Aelurostrongylus abstrusus*. Exp. Parasitol. 182, 54–57.
Gates, M.C., Nolan, T.J., 2009. Comparison of passive fecal flotation run by veterinary students to zinc-sulfate centrifugation flotation run in a diagnostic parasitology laboratory. J. Parasitol. 95, 1213–1214.
Genchi, M., Ferrari, N., Fonti, P., De Francesco, I., Piazza, C., Viglietti, A., 2014. Relation between *Aelurostrongylus abstrusus* larvae excretion, respiratory and radiographic signs in naturally infected cats. Vet. Parasitol. 206, 182–187.
Gerdin, J.A., Slater, M.R., Makolinski, K.V., Looney, A.L., Appel, L.D., Martin, N.M., McDonough, S.P., 2011. Post-mortem findings in 54 cases of anesthetic associated death in cats from two spay-neuter programs in New York state. J. Feline Med. Surg. 13, 959–966.
Giannelli, A., Colella, V., Abramo, F., do Nascimento Ramos, R.A., Falsone, L., Brianti, E., Varcasia, A., Dantas-Torres, F., Knaus, M., Fox, M.T., Otranto, D., 2015. Release of lungworm larvae from snails in the environment: potential for alternative transmission pathways. PLoS Negl. Trop. Dis. 9, e0003722.
Giannelli, A., Capelli, G., Joachim, A., Hinney, B., Losson, B., Kirkova, Z., Rene-Martellet, M., Papadopoulos, E., Farkas, R., Napoli, E., Brianti, E., Tamponi, C., Varcasia, A., Margarida Alho, A., Madeira de Carvalho, L., Cardoso, L., Maia, C., Mircean, V., Mihalca, A.D., Miro, 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, 517–528.
Hidalgo, A., Melo, A., Romero, F., Hidalgo, V., Villanueva, J., Fonseca-Salamanca, F., 2018. DNA extraction in *Echinococcus granulosus* and *Taenia* spp. eggs in dogs stool samples applying thermal shock. Exp. Parasitol. 186, 10–16.
Hoopes, J., Hill, J.E., Polley, L., Fernando, C., Wagner, B., Schurer, J., Jenkins, E., 2015. Enteric parasites of free-roaming, owned, and rural cats in prairie regions of Canada. Can. Vet. J. 56, 495–501.
Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874.
Lavikainen, A., Iwaki, T., Haukisalmi, V., Konyaev, S.V., Casiraghi, M., Dokuchaev, N.E., Galimberti, A., Halajian, A., Henttonen, H., Ichikawa-Seki, M., Itagaki, T., Krivopalov, A.V., Meri, S., Morand, S., Nareaho, A., Olsson, G.E., Ribas, A., Terefe, Y., Nakao, M., 2016. Reappraisal of *Hydatigera taeniaeformis* (Batsch, 1786) (Cestoda: Taeniidae) sensu lato with description of *Hydatigera kamiyai* n. Sp. Int. J. Parasitol. 46, 361–374.
Lillis, W.G., 1967. Helminth survey of dogs and cats in New Jersey. J. Parasitol. 53, 1082–1084.
Little, S., Adolph, C., Downie, K., Snider, T., Reichard, M., 2015. High prevalence of covert infection with gastrointestinal helminths in cats. JAAHA 51, 359–364.
Lucio-Forster, A., Bowman, D.D., 2011. Prevalence of fecal-borne parasites detected by centrifugal flotation in feline samples from two shelters in upstate New York. J. Feline Med. Surg. 13, 300–303.
Nagamori, Y., Payton, M.E., Duncan-Decocq, R., Johnson, E.M., 2018. Fecal survey of parasites in free-roaming cats in northcentral Oklahoma, United States. Vet. Parasitol. Reg. Stud. Rep. 14, 50–53.
Power, L.A., 1971. Helminths of cats from the Midwest with a report of *Ancylostoma caninum* in this host. J. Parasitol. 57, 610.
Rembiesa, C., Richardson, D.J., 2003. Helminth parasites of the house cat, *Felis catus*, in Connecticut, U.S.A. Comp. Parasitol. 70, 115–119.
Schnyder, M., Di Cesare, A., Basso, W., Guscetti, F., Riond, B., Glaus, T., Crisi, P., Deplazes, P., 2014. Clinical, laboratory and pathological findings in cats experimentally infected with *Aelurostrongylus abstrusus*. Parasitol. Res. 113, 1425–1433.
Traversa, D., Lia, R.P., Iorio, R., Boari, A., Paradies, P., Capelli, G., Avolio, S., Otranto, D., 2008. Diagnosis and risk factors of *Aelurostrongylus abstrusus* (Nematoda, Strongylida) infection in cats from Italy. Vet. Parasitol. 153, 182–186.
Willard, M.D., Roberts, R.E., Allison, N., Grieve, R.B., 1988. Diagnosis of *Aelurostrongylus abstrusus* and *Dirofilaria immitis* infections in cats from a humane shelter. JAVMA 192, 913–916.
Wyrosdick, H.M., Chapman, A., Martinez, J., Schaefer, J.J., 2017. Parasite prevalence survey in shelter cats in Citrus County, Florida. Vet. Parasitol. Reg. Stud. Rep. 10, 20–24.
Zajac, A.M., Conboy, G., 2012. Vetertinary Clinical Parasitology, 8th ed. vol. 354 Wiley-Blackwell.
Zajac, A.M., Johnson, J., King, S.E., 2002. Evaluation of the importance of centrifugation as a component of zinc sulfate fecal flotation examinations. JAAHA 38, 221–224.