



ORIGINAL ARTICLE

WILEY

Prevalence of zoonotic parasites in feral cats of Central Virginia, USA

Sara J. Taetzsch^{1,2} | Karen R. Gruszynski³ | Andrea S. Bertke¹ | Jitender P. Dubey⁴ | Katelynn A. Monti⁵ | Anne M. Zajac⁵ | David S. Lindsay⁵

¹Department of Population Health Sciences, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, Virginia

²Feral Cat Trap-Neuter-Release Clinics, Mechanicsville, Virginia

³Virginia Department of Health, Office of Epidemiology, Richmond, Virginia

⁴Animal Parasitic Diseases Laboratory, Agricultural Research Service, US Department of Agriculture, Beltsville Agricultural Research Center, Beltsville, Maryland

⁵Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, Virginia

Correspondence

Sara J. Taetzsch, Department of Population Health Sciences, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, VA 24061.

Email: schiaram@vt.edu

Abstract

Felis catus, the domestic cat, is the definitive host for parasites that may result in adverse health outcomes in humans. Prevalence data of zoonotic parasites in feral cats, which are free-roaming domestic cats that are born and live in the wild, are limited. The objective of this study was to assess seroprevalence of *Toxoplasma gondii* antibodies and copro-prevalence of potentially zoonotic parasites in feral cats and to evaluate risk factors for seropositivity and faecal excretion of parasites. In this cross-sectional survey, 275 feral cats at Trap-Neuter-Release clinics in Central Virginia were tested for parasites via faecal flotation, direct immunofluorescence assay (faeces) and modified agglutination testing (serum). *Toxoplasma gondii* seroprevalence was 22.35% (95% CI: 17.47–27.86). Faecal prevalence of *T. gondii*-like oocysts was 1.04% (95% CI: 0.13–3.71), *Toxocara cati* 58.85% (95% CI: 51.54–65.89), *Ancylostoma* spp. 18.75% (95% CI: 13.49–25.00), *Giardia duodenalis* 5.73% (95% CI: 2.89–10.02) and *Cryptosporidium* spp. 3.33% (95% CI: 1.37–7.24). Female cats were more likely than males to excrete faecal *Ancylostoma* spp. eggs (OR 2.88; 95% CI 1.34–6.17). Adults were more likely than immature cats to be seropositive (OR 2.10; 95% CI: 1.11–3.97) and to excrete faecal *Ancylostoma* spp. eggs (OR 2.57; 95% CI: 1.10–5.99). However, immature cats were more likely than adults to excrete *T. cati* eggs (OR 6.79; 95% CI: 3.31–13.90) and to excrete one or more potentially zoonotic species (OR 4.67; 95% CI: 2.28–9.55) in faeces. Results of this study have implications for human and animal health and highlight the importance of collaboration between public health, medical and veterinary communities in preventive efforts.

KEYWORDS

feral cat, parasite, *Toxocara*, *Toxoplasma*, Zoonotic

1 | INTRODUCTION

Cats can serve as reservoirs for parasites that may result in public health consequences, negative economic outcomes and reduced health in animals and humans (Gerhold & Jessup, 2013). Cats that roam outdoors can contaminate the environment with faecal parasite eggs, cysts and oocysts which infect people and other animals, including pets, livestock and wildlife, upon ingestion. Parasites

having zoonotic potential that are excreted by felids include *Toxoplasma gondii*, *Toxocara cati*, *Ancylostoma* spp., *Giardia duodenalis* and *Cryptosporidium* spp.

Toxoplasma gondii is a protozoan parasite that can infect a wide variety of species, including humans, but for which felids are the only definitive host (Elmore et al., 2010). Although most felids in North America, including bobcats (*Lynx rufus*) and cougars (*Puma concolor*), are suitable definitive hosts for *T. gondii*, domestic cats (*Felis catus*)

produce the most oocyst numbers of all felids and are believed to contribute most oocysts contaminating the environment (Dubey, 2010). Toxoplasmosis results after ingestion of infectious *T. gondii* oocysts from an environment contaminated with cat faeces or through ingestion of tissue cysts in raw or undercooked meat of an intermediate host. Toxoplasmosis is characterized by symptoms ranging from mild/flu-like to encephalitis and death; infection during pregnancy can result in miscarriage or developmental foetal abnormalities (Jones, Parise, & Fiore, 2014). Toxoplasmosis is reported to be the second deadliest food-borne disease in the USA, costing an estimated 3.3 billion dollars annually (Scallan et al., 2011; USDA, 2014). Foods that have been implicated as sources of *T. gondii* parasite stages originating from cats include raw produce, undercooked meat, raw seafood and unpasteurized goat's milk (Hussain, Stitt, Szabo, & Nelan, 2017). Wildlife, including game species, may be infected with *T. gondii* from cats, representing a source of food-borne exposure for people who hunt and consume game animals. For example, studies detected *T. gondii* seroprevalences of 56.3% in elk in the Central Appalachians, 58.8% of white-tailed deer in Northeast Ohio and 27.7% of feral pigs in North Carolina (Ballash et al., 2015; Cox et al., 2017; Sandfoss, DePerno, Patton, Flowers, & Kennedy-Stoskopf, 2011). Water may be contaminated with *T. gondii* oocysts shed in the faeces of infected cats. One of the largest documented outbreaks of toxoplasmosis in people was thought to be caused by oocyst run-off from free-roaming domestic and wild cats into a municipal reservoir in British Columbia, Canada (Bowie et al., 1997). Toxoplasmosis has been targeted for public health action as 1 of 5 neglected parasitic infections in the USA by the Centers for Disease Control and Prevention (CDC) (CDC, 2016).

The ascarid, *Toxocara cati*, a zoonotic parasite acquired from cats, causes toxocariasis after ingestion of infective *T. cati* eggs excreted in cat faeces, either on contaminated raw produce, soil or water, or (less frequently) through ingestion of encysted larvae in undercooked meat (Woodhall, Eberhard, & Parise, 2014). After ingestion, parasite larvae migrate through the body of an infected individual, damaging organs, such as lungs, liver, eyes and central nervous system, and may result in permanent debilitating disease including blindness (CAPC, 2016a; Woodhall et al., 2014). Toxocariasis is considered a neglected parasitic infection by the CDC (Woodhall et al., 2014). Although thought to be one of the most common parasitic infections in the USA, the true importance of toxocariasis in humans is not known because of underdiagnosis, in part due to lack of commercially available specific immunological tests but also because symptoms can be vague, resulting in undertesting (Woodhall et al., 2014).

Other parasites excreted in cat faeces that may cause disease in humans, depending on parasite species/genotype and the immunocompetence of the infected individual, include *Ancylostoma* spp. (hookworms), *Giardia duodenalis* and *Cryptosporidium* spp. (Rabinowitz & Conti, 2010). Some species of *Ancylostoma* cause cutaneous larva migrans in humans, characterized by a pruritic rash, after infective larvae penetrate and migrate through the skin (Bowman, Montgomery, Zajac, Eberhard, & Kazacos, 2010; CAPC,

Impacts

- We examined the copro-prevalence of potentially zoonotic parasites, seroprevalence of *T. gondii* antibodies and factors predicting parasite excretion and seropositivity in a feral cat population in Central Virginia. Immature stages of these parasites are excreted in cat faeces, persist in the environment and can be inadvertently ingested by humans and other animals, resulting in negative health outcomes.
- Several species of parasites were identified, with more than half of the faecal samples testing positive for *T. cati* eggs and almost one-fourth of the cats testing positive for *T. gondii* antibodies. Immature cats were significantly more likely than adults to excrete *T. cati* and one or more potentially zoonotic parasite species in the faeces ($p < 0.05$).
- Feral cats in Central Virginia, which roam freely outdoors, are a source of environmental contamination with parasites that can negatively affect the health of humans and other animals, thus representing a significant public health risk. Emphasis should be placed on humane reduction of feral cat populations and prevention of free-roaming cats.

2016b). *Giardia duodenalis* cysts and *Cryptosporidium* spp. oocysts are often present in cat faeces; however, because these parasites demonstrate genetic variation and a level of host specificity, it is not clear what role cats play in transmitting them to humans (Feng & Xiao, 2011; Lucio-Forster, Griffiths, Cama, Xiao, & Bowman, 2010). Giardiasis is prevalent in children in developing areas of the world and can result in growth stunting and impaired cognitive development (CDC, 2015b; Feng & Xiao, 2011). Cryptosporidiosis is frequently linked to outbreaks associated with recreational water use in the USA and can cause gastrointestinal disease in people, with potentially severe health outcomes in immunocompromised individuals (CDC, 2015a).

The estimated 60–100 million feral cats which roam outdoors in the USA are a source of environmental contamination with parasites that pose a risk for transmission to humans (Roebing et al., 2014). One cat infected with zoonotic parasites can excrete millions of eggs, cysts or oocysts into the environment, and in many cases, the infective parasite stages are extremely resistant, persisting for months to years in soil and/or water (CAPC, 2016a; Dabritz & Conrad, 2010; Harhay, Horton, & Olliaro, 2010). Cats produce an estimated 1.2 million tons of faeces in the USA each year (Torrey & Yolken, 2013). Feral cats, which are born and live in the wild, do not receive the same level of husbandry and veterinary care as do most owned cats, including routine faecal parasite tests and antiparasitic medications. Furthermore, cats roaming

outdoors are more likely to ingest parasite stages through predation of intermediate or paratenic hosts compared with owned cats living indoors. Consequently, feral cats may experience a relatively high level of parasite infection. Existing prevalence data for zoonotic parasites in cats are highly variable depending on the population sampled and methods used for parasite detection. In Virginia, parasite prevalence in felines is largely based on commercial laboratory analysis of samples submitted by veterinary clinics or small studies of owned cats, but few data concerning parasite prevalence in feral cats are available (Companion Animal Parasite Council, 2017; Hsu, Grant, Zajac, Witonsky, & Lindsay, 2011; Lilly & Wortham, 2013). Considering the lack of data from feral cats, the objective of this study was to assess the prevalence of potentially zoonotic parasites, specifically the seroprevalence of antibodies against *T. gondii* and copro-prevalence of *T. cati* and *Ancylostoma* spp. eggs, *G. duodenalis* cysts, and *Cryptosporidium* spp. and *Toxoplasma*-like oocysts, in feral cats in Virginia. Sex and age were evaluated as risk factors for seropositivity and excretion of faecal parasites. The hypothesis was that the feral cats would have high parasite copro-prevalence and *T. gondii* seroprevalence and that age and sex would predict faecal excretion of parasite stages as well as *T. gondii* seroprevalence.

2 | MATERIALS AND METHODS

2.1 | Cats

Feral cats ($n = 275$; 131 females, 144 males) were sampled from July through November 2016. The cats were humanely trapped in standard metal live traps by the general public and brought to monthly feral Trap-Neuter-Release (TNR) clinics sponsored by Operation Catnip, a nonprofit organization based in Richmond, Virginia. Each cat was given an identification number that was placed on its history chart, faecal sample bag and blood tubes. The cats were anaesthetized, physically examined, treated with the insecticide fipronil/(s)-methoprene (9.8/11.8%; 0.5 ml topically; FRONTLINE®Plus for cats, Merial Inc., Duluth, GA) and antiparasitic ivermectin (0.2 mg/kg subcutaneously; Noromectin® 1% injection [ivermectin], Norbrook® Inc., Overland Park, KS), given rabies vaccines and neutered in accordance with Operation Catnip's standard protocol, based on guidelines recommended by the Association of Shelter Veterinarians (Association of Shelter Veterinarians' Veterinary Task Force to Advance Spay-Neuter et al., 2016). Ivermectin was administered a maximum of 1.5 hr prior to faecal sample collection and was deemed not to affect faecal parasite detection. Cat age was estimated, and cats were classified as immature if <1 year or adult if ≥1 year of age (Table 1).

2.2 | Sample collection

Blood and faecal samples were collected from cats during recovery from surgery. Faecal samples were collected from 192 (69.8%) of the cats, either by digital rectal palpation or directly from the trap

TABLE 1 Age and gender of cats from which serum ($n = 264$) and faeces ($n = 192$) were collected

Cats	Samples collected (%)	
	Serum	Faeces
Male	141 (53.4)	104 (54.2)
Female	123 (46.6)	88 (45.8)
Immature	106 (40.2)	74 (38.5)
Mature	158 (59.8)	118 (61.5)

if available. Faeces were unavailable from 83 (30.2%) of the cats. Faecal samples were placed into individual plastic re-sealable bags and stored in a cooler on ice for transport and then stored at 4°C. All faecal samples were analysed within 48 hr of collection.

Blood samples were collected from 264 (96.0%) of the cats via venipuncture of a jugular or medial saphenous vein. Blood was not collected from 11 (4.0%) of the cats due to inability to readily collect an adequate volume or because rapid recovery from anaesthesia precluded safe handling of the cat. Blood was collected into a syringe, transferred into serum separator tubes and allowed to clot for 30 min at room temperature before placing into a cooler with ice for transport and then centrifuged at 1,500× *g* for 10 min at 4°C. Serum was then transferred into microcentrifuge tubes and stored at 4°C until analysis was performed.

2.3 | Sample analysis

Individuals conducting serological and faecal examinations knew the identification numbers on the samples and that samples were obtained from cats, but were blinded to cat age and sex. Faecal samples were processed by centrifugal flotation in a 33% zinc sulphate solution as previously described (Zajac & Conboy, 2012). Processed samples were examined microscopically for parasites, first scanning using the 10× objective and then using the 20× or 40× objectives if necessary to confirm identification of smaller parasite cysts and oocysts. Parasite identifications were based on published descriptions, and all parasites identified were recorded (Zajac & Conboy, 2012). Faecal parasite stages considered as having zoonotic potential were *T. gondii*-like oocysts, *T. cati* and *Ancylostoma* spp. eggs, *G. duodenalis* cysts and *Cryptosporidium* spp. oocysts. Coccidial oocysts that were 10–12 μm in diameter were considered as *T. gondii*-like (Dubey, 2010).

To evaluate for the presence of *Cryptosporidium* spp. oocysts, fresh faeces remaining after faecal flotation ($n = 180$) were stored at room temperature in 10% formalin until further analysis using a commercially available direct immunofluorescence assay (DFA) kit (MERIFLUOR® *Cryptosporidium/Giardia*, Meridian Bioscience Inc., Cincinnati, OH). Testing procedures and interpretation were performed according to the manufacturer's instructions (Anon, Meridian Bioscience, Inc., 2017). Samples were considered positive if 4–6-μm round-to-oval apple green oocysts were observed using a microscope equipped with epifluorescent and differential interference contrast optics.

Serum was assayed for the presence of IgG antibodies against *T. gondii* using a modified direct agglutination test (MAT) at the USDA Animal Parasitic Disease Laboratory in Beltsville, Maryland, as previously described (Dubey, & Desmonts, 1987). A 1:25 antibody titre was considered positive (Dubey, & Desmonts, 1987).

2.4 | Statistical analysis

Target sample size of $n = 323$ was determined a priori for a 95% CI and alpha of 0.05 based on anticipated seroprevalence (30%–60%) for *T. gondii* antibodies (Elmore et al., 2010; Nutter, Dubey et al., 2004). Epi Info™ 7 (Centers for Disease Control and Prevention, Atlanta, GA) was used to perform unconditional logistic regression to assess age and gender as risk factors for seroprevalence of *T. gondii* and copro-prevalence of zoonotic parasites. Applicable test assumptions were met for all statistical procedures. Statistical findings for which p -values were <0.05 were considered significant.

2.5 | Approval

This study was approved and conducted in accordance with the Virginia Tech Institutional Animal Care and Use Committee (VT IACUC# 16-106).

3 | RESULTS

Microscopic examination of faecal flotation preparations ($n = 192$) detected at least 1 potentially zoonotic parasite species in 64.58% (95% CI: 57.37–71.34) of samples. Specifically, 58.85% (95% CI: 51.54–65.89) of samples were positive for *T. cati* eggs, 18.75% (95% CI: 13.49–25.00) were positive for *Ancylostoma* spp. eggs, 5.73% (95% CI: 2.89–10.02) were positive for *G. duodenalis* cysts, and 1.04% (95% CI: 0.13–3.71) were positive for *Toxoplasma*-like oocysts. Of 180 faecal samples tested by DFA, 3.33% (95% CI: 1.37–7.24), were positive for *Cryptosporidium* spp. oocysts. Antibodies to *T. gondii* were detected in 22.35% (95% CI: 17.47–27.86) of cat sera (Table 2).

3.1 | Risk factors

Age and gender were significant predictors of seropositivity to *T. gondii* and faecal excretion of *T. cati* and *Ancylostoma* spp. (Table 3). Faecal prevalence of *G. duodenalis* cysts, *Cryptosporidium*

spp. oocysts and *T. gondii*-like oocysts was too low to conduct a statistically valid regression analysis. Adult cats were more likely to be seropositive for *T. gondii* (OR 2.10; 95% CI: 1.11–3.97; $p = 0.022$) and to excrete faecal *Ancylostoma* spp. eggs (OR 2.57; 95% CI: 1.10–5.99; $p = 0.029$) compared to immature cats. However, immature cats were more likely to excrete faecal *T. cati* eggs (OR 6.79; 95% CI: 3.31–13.9; $p = 0.000$) and to have 1 or more potentially zoonotic parasite species per faecal sample (OR 4.67; 95% CI: 2.28–9.55; $p = 0.000$) than adults. Female cats were more likely to excrete faecal *Ancylostoma* spp. eggs (OR 2.88; 95% CI: 1.34–6.17; $p = 0.007$) compared to males.

4 | DISCUSSION

Feral cats in Central Virginia excrete parasites into the environment, representing a source of parasite infection for other animal species and humans. Significant associations ($p < 0.05$) were detected between age, gender, faecal parasite excretion and *T. gondii* seropositivity.

Felids are the only definitive hosts for *T. gondii* and thus represent the only source of infective oocysts for humans and other animal species. *Toxoplasma*-like oocysts were identified in 1.04% of faecal samples, which is consistent with findings of other studies (Elmore et al., 2010). Faecal oocysts measuring 10–12 μm in diameter were considered *T. gondii*-like because oocysts of *Hammondia hammondi*, *Neospora caninum* and some *Besnoitia* spp. are morphologically similar, and bioassays, which are time-consuming and expensive, are needed for definitive identification. From a public health viewpoint, these oocysts should be considered *T. gondii* and precautions should

TABLE 2 Copro-prevalence and seroprevalence of parasites with zoonotic potential

Organism	# Positive	% Positive	95% CI
<i>Ancylostoma</i> spp.	36	18.75%	13.49–25.00
<i>Giardia duodenalis</i>	11	5.73%	2.89–10.02
<i>Toxocara cati</i>	113	58.85%	51.54–65.89
<i>Cryptosporidium</i> spp.	6	3.33%	1.37–7.24
<i>Toxoplasma gondii</i> -like oocysts	2	1.04%	0.13–3.71
<i>Toxoplasma gondii</i> IgG	59	22.35%	17.47–27.86

TABLE 3 Significant risk factors for parasite positivity

Organism	Significant predictor	OR	95% CI	p -Value
<i>Ancylostoma</i> spp.	Female	2.8752	1.3408–6.1657	0.0067
	Adult	2.5667	1.0998–5.9903	0.0293
<i>Toxocara cati</i>	Immature	6.7869	3.312–13.9076	0.0000
Faecal zoonotic species	Immature	4.6700	2.2803–9.5471	0.0000
<i>Toxoplasma gondii</i> IgG	Adult	2.1017	1.1119–3.9725	0.0222

be taken while processing cat faeces. The findings of *T. gondii* oocysts in only 1.04% of cats may be an underestimation because of the small amount (1–2 g per sample) of faeces available for testing. Additionally, some cats excrete numbers of oocysts which may be below the threshold (1,000 oocysts/g) of microscopic detection (Dubey, 2010). Furthermore, cats usually excrete *T. gondii* oocysts for about a week and are unlikely to excrete oocysts on subsequent exposures, resulting in only about 1% of cats excreting oocysts at any given time (Dubey, 2010; Elmore et al., 2010).

Serology, a measure of parasite exposure, is superior to faecal flotation as an indicator of *T. gondii* prevalence in cat populations (Dubey, Lappin, & Thulliez, 1995). Due to the short window of faecal excretion, seropositive cats are not likely to also be actively excreting oocysts. Most *T. gondii* seropositive cats previously excreted millions of oocysts which are extremely resistant in the environment, persisting for months to years, making serology a good indicator of environmental contamination and zoonotic potential (Dabritz & Conrad, 2010). Seroprevalence of antibodies against *T. gondii* in this study was 22.35%, which was lower than that expected for a free-roaming, outdoor cat population in a relatively humid region. Reports indicate that feline *T. gondii* seroprevalence ranges from 16% to 80% in the USA, depending on the cat population, geographical region and immunological test used (Dubey, 2010). Seroprevalence of 16% has been documented in drier US climates but is closer to 60% in humid climates (Elmore et al. 2010). Ingestion of *T. gondii* oocysts is relatively noneffective in producing patent infections in cats (Elmore et al., 2010). Rather, cats are infected with *T. gondii* through ingestion of tissue cysts in birds and small mammals that act as intermediate hosts. Therefore, cats which roam outdoors and engage in predation are expected to have high *T. gondii* exposure.

Of note is the high rate of *T. cati* faecal excretion by the cats in this study, 58.85%. Feline copro-prevalence of *T. cati* eggs in the USA varies depending on the region and cat lifestyle (indoor vs. free roaming or feral) as well as testing methodology. A large nationwide study of owned cats in the USA detected *T. cati* eggs in 4.6% to 5.1% of faecal samples tested from 2011 to 2014 (Lucio-Forster, Mizhquiri Barbecho, Mohammed, Kornreich, & Bowman, 2016). In 2017, the Companion Animal Parasite Council (CAPC) reported a 4.97% and 4.29% faecal prevalence in owned cats in the USA and Virginia, respectively (Companion Animal Parasite Council, 2017). Test sensitivity is an unlikely factor in the relatively high *T. cati* copro-prevalence of our study compared to these, as all three studies used similar testing methodologies. Neither CAPC nor Lucio-Forster et al. reported cat age or sex, and it is possible that demographics of the study populations could have accounted for some of the differences in *T. cati* copro-prevalence. However, it is likely that feral cat lifestyle (e.g. lack of veterinary care, roaming outdoors and predatory behaviour) is a major contributing factor to such a comparatively high *T. cati* copro-prevalence in our study population vs. the owned cats reported by CAPC and Lucio-Forster. Recent studies of *T. cati* in shelters found copro-prevalences of 21% in upstate New York (Lucio-Forster & Bowman, 2011) and 11% in Florida (Sabshin et al., 2012). Testing methodology by Lucio-Forster and Bowman

was similar to our methodology, but Sabshin et al. used a less sensitive method of detecting faecal *T. cati* eggs, which may account for the comparatively low copro-prevalence of that study (Zajac & Conboy, 2012). Furthermore, most cats presented to shelters are treated with antiparasitics, which could result in the underestimation of parasite prevalence if egg-producing parasites are removed prior to collection of faecal samples (Sabshin et al., 2012). Sabshin et al. reported that faecal samples were collected within 24 hr of deworming and that 16% of the cats were owned prior to admission to the shelter. Although many cats presented to shelters are stray, some are owner-surrendered and potentially lived indoors and received routine veterinary care, which could also account for lower *T. cati* prevalence in shelter cats compared to feral cats. The large percentage of free-roaming cats excreting *T. cati* eggs in our study represents a significant public health concern. Toxocariasis may be associated with significant morbidity in humans, including blindness and neurological symptoms (Rabinowitz & Conti, 2010; Woodhall et al., 2014). A single infected cat may excrete tens of thousands of environmentally persistent faecal *T. cati* eggs per day (CAPC, 2016a).

The faecal prevalence of *Ancylostoma* spp. in this study population was 18.75%. Again, faecal prevalence in cats varies depending on the population, region sampled and testing methodology. A large survey in the USA detected that only 0.63% of owned cats were excreting *Ancylostoma* spp. eggs (De Santis, 2006), whereas 75% of feral cats sampled in Florida were positive (Anderson, Foster, & Forrester, 2003). The Companion Animal Parasite Council reported a 0.49% copro-prevalence of *Ancylostoma* spp. in Virginia among owned cats in 2017 (Companion Animal Parasite Council, 2017). Testing methodology reported by CAPC was similar to the methodology used in our study; however, De Santis et al. used a less sensitive test (Zajac & Conboy, 2012), and Anderson et al. used a more sensitive method for detecting *Ancylostoma* spp. infection. In addition, the warmer Florida climate is likely more conducive to year-round transmission of *Ancylostoma* spp. compared to Virginia, which may account in part for the comparatively higher prevalence of *Ancylostoma* spp. in the feral cat population evaluated by Anderson et al. Again, lifestyle is the most likely reason for the higher copro-prevalence of *Ancylostoma* spp. in the feral cat population of our study compared to that of the owned Virginia cats of the CAPC study.

Giardia duodenalis cysts were detected in 5.73% of faecal samples by centrifugal faecal flotation. Although giardiasis has traditionally been considered a zoonotic disease, molecular techniques are contributing to new understanding of this protozoan's host specificity. Based on molecular analysis, seven different assemblages of *G. duodenalis* have been identified (Assemblages A-G). Assemblage F is specific to cats, but feline infection can occur with Assemblages A and B, which are the major assemblages that infect humans. For example, a survey of cats infected with *G. duodenalis* in the USA detected that 35.3% were infected with Assemblage A (subgroup AI); although within Assemblage A genotypes, humans are more commonly infected by subgroup AII, infections with subgroup AI have occurred (Feng & Xiao, 2011; Vasilopoulos, Rickard,

Mackin, Pharr, & Huston, 2007). A similar study detected 11.1% of cats with *G. duodenalis* which were infected with Assemblage B (Feng & Xiao, 2011). *Giardia duodenalis* prevalence in cats ranges from 0.6% to 44.4%, depending on the cat population tested and the testing methodology used (Feng & Xiao, 2011). *Giardia* cysts may be excreted on the order of billions in the faeces of an infected individual and are immediately infective upon defecation (Yoder, Gargano, Wallace, & Beach, 2012). These cysts survive for months in the environment (including water), and ingestion of as few as 10 cysts can result in severe diarrhoea, weight loss and dehydration in animals and humans (CDC, 2015b).

Cryptosporidium spp. oocysts were identified in 3.33% of 180 faecal samples tested by DFA, but none were detected by means of faecal flotation. *Cryptosporidium* oocysts are small (4–6 µm) and have thin walls, making them difficult to detect using light microscopy following faecal flotation (Lucio-Forster et al., 2010). Reports of *Cryptosporidium* spp. copro-prevalence in shelter and owned cats in the USA using similar testing methodology ranged from 4.7% to 12%, respectively (Ballweber et al., 2009; Mekaru, Marks, Felley, Chouicha, & Kass, 2007). Cryptosporidiosis in humans is mostly caused by *C. hominis* and *C. parvum*, but *C. felis*, for which the cat is the natural reservoir, may cause disease in immunocompromised humans. Cryptosporidiosis may result in a spectrum of clinical signs in people and animals from mild diarrhoea to death, depending on the immunocompetence of the individual (CDC, 2015a; Greene, 2006). As with *Giardia*, *Cryptosporidium* oocysts are immediately infectious upon defecation, persist in the environment for several months, are resistant to chlorine treatment and may be excreted in large numbers by an infected individual (CDC, 2009).

Age was a significant risk factor for seropositivity to *T. gondii*, consistent with findings of other studies (Opsteegh et al., 2012; Sævik et al., 2015). Given the greater length of time exposed to the environment, it follows that aged cats are more likely to have been exposed to *T. gondii* oocysts and tissue cysts than immature cats. Interestingly, adults were more likely to excrete faecal *Ancylostoma* spp. eggs, but immature cats were more likely to excrete *T. cati* and multiple potentially zoonotic parasite species. This may reflect a generally less robust immune system of kittens compared to adults. In addition, transmammmary transmission of *T. cati* may occur, giving kittens a unique exposure route for this parasite, whereas cats are mainly infected with *Ancylostoma* spp. through ingestion of paratenic hosts (Bowman, 1999; CAPC, 2016b). Sex was a significant predictor of *Ancylostoma* spp. faecal excretion, with females being more likely than males to excrete this parasite. One could hypothesize that female feral cats are immunocompromised compared to male cats, considering that on average, they experience multiple pregnancies per year (Nutter, Levine, & Stoskopf, 2004). Immunosuppression is a feature of late pregnancy, and early lactation may be associated with increased parasite egg excretion (Tizard, 2000).

Feral cats in Central Virginia are a source of parasites that may cause adverse health outcomes in animals and humans. Zoonotic diseases such as toxoplasmosis and toxocarasis in humans can be difficult to diagnose, complicated to treat and may result in permanent

organ damage and death. Vaccines to prevent these infections do not exist. Thus, optimal health outcomes hinge on preventing exposure to parasites. Prevention can be achieved through interdisciplinary collaboration of policy makers and public health, veterinary and human health professionals. Prevention measures should emphasize public education regarding the risks of zoonotic parasites associated with free-roaming cats as well as efforts to humanely reduce feral cat populations.

ACKNOWLEDGEMENTS

The authors thank Dr. Meriam Saleh for assistance with performance and interpretation of faecal flotations, Dr. Thomas Taetzsch for assistance in record-keeping and sample processing, and Dr. Oliver Kwok for performing *Toxoplasma* serology. Also, thank you to Operation Catnip board members and volunteers who made this study possible. The authors declare that there are no conflict of interests and no external funding was received in connection with this study.

ORCID

Sara J. Taetzsch  <http://orcid.org/0000-0002-2274-425X>

REFERENCES

- Anderson, T. C., Foster, G. W., & Forrester, D. J. (2003). Hookworms of feral cats in Florida. *Veterinary Parasitology*, 115(1), 19–24. [https://doi.org/10.1016/S0304-4017\(03\)00162-6](https://doi.org/10.1016/S0304-4017(03)00162-6)
- Anon, Meridian Bioscience, Inc. (2017) Retrieved from <https://www.meridianbioscience.com/>
- Ballash, G. A., Dubey, J. P., Kwok, O. C. H., Shoben, A. B., Robison, T. L., Kraft, T. J., & Dennis, P. M. (2015). Seroprevalence of *Toxoplasma gondii* in white-tailed deer (*Odocoileus virginianus*) and free-roaming cats (*Felis catus*) across a suburban to urban gradient in Northeastern Ohio. *EcoHealth*, 12(2), 359–367. <https://doi.org/10.1007/s10393-014-0975-2>
- Ballweber, L. R., Panuska, C., Huston, C. L., Vasilopoulos, R., Pharr, G. T., & Mackin, A. (2009). Prevalence of and risk factors associated with shedding of *Cryptosporidium felis* in domestic cats of Mississippi and Alabama. *Veterinary Parasitology*, 160(3–4), 306–310. <https://doi.org/10.1016/j.vetpar.2008.11.018>
- Bowie, W. R., King, A. S., Werker, D. H., Isaac-Renton, J. L., Bell, A., Eng, S. B., & Marion, S. A. (1997). Outbreak of toxoplasmosis associated with municipal drinking water. The BC Toxoplasma Investigation Team. *Lancet (London, England)*, 350(9072), 173–177.
- Bowman, D. D. (1999). *Georgis' parasitology for veterinarians* (7th ed.). Philadelphia, PA: W.B. Saunders Company.
- Bowman, D. D., Montgomery, S. P., Zajac, A. M., Eberhard, M. L., & Kazacos, K. R. (2010). Hookworms of dogs and cats as agents of cutaneous larva migrans. *Trends in Parasitology*, 26(4), 162–167. <https://doi.org/10.1016/j.pt.2010.01.005>
- CAPC (2016a). Ascarid (also Roundworm, also Toxocara) | CAPC Vet. Companion Animal Parasite Council. Retrieved from <https://www.capcvet.org/capc-recommendations/ascarid-roundworm>
- CAPC (2016b). Hookworms | CAPC Vet. Companion Animal Parasite Council. Retrieved from <https://www.capcvet.org/capc-recommendations/hookworms>

- CDC (2009). Outbreak of cryptosporidiosis associated with a splash park - Idaho, 2007. *MMWR. Morbidity and Mortality Weekly Report*, 58(22), 615–618.
- CDC (2015a). *Parasites - cryptosporidium | cryptosporidium | parasites | CDC*. Retrieved from <https://www.cdc.gov/parasites/cryptosporidium/>
- CDC (2015b). *Pathogen & environment | giardia | parasites | CDC*. Retrieved from <https://www.cdc.gov/parasites/giardia/pathogen.html>
- CDC (2016). CDC - parasites - neglected parasitic infections (NPIs) in the United States. *Centers for Disease Control*. Retrieved from <https://www.cdc.gov/parasites/npi/>
- Companion Animal Parasite Council (2017). *Parasite prevalence maps | CAPC vet*. Retrieved from <https://www.capcvet.org/parasite-prevalence-maps>
- Cox, J. J., Slabach, B., Hast, J. T., Murphy, S. M., Kwok, O. C. H., & Dubey, J. P. (2017). High seroprevalence of *Toxoplasma gondii* in elk (*Cervus canadensis*) of the central Appalachians, USA. *Parasitology Research*, 116(3), 1079–1083. <https://doi.org/10.1007/s00436-017-5391-4>
- Dabritz, H. A., & Conrad, P. A. (2010). Cats and *Toxoplasma*: Implications for public health. *Zoonoses and Public Health*, 57(1), 34–52.
- DeSantis, A. C., Raghavan, M., Caldano, R. J., Glickman, N. W., Moore, G. E., Lewis, H. B., ... Glickman, L. T. (2006). Estimated prevalence of nematode parasitism among pet cats in the United States. *Journal of the American Veterinary Medical Association*, 228(6), 885–892.
- Dubey, J. P. (2010). *Toxoplasmosis of animals and humans* (2nd ed.). Boca Raton, FL: CRC Press.
- Dubey, J. P., & Desmonts, G. (1987). Serological responses of equids fed *Toxoplasma gondii* oocysts. *Equine Veterinary Journal*, 19, 337–339. <https://doi.org/10.1111/j.2042-3306.1987.tb01426.x>
- Dubey, J. P., Lappin, M. R., & Thulliez, P. (1995). Long-term antibody responses of cats fed *Toxoplasma gondii* tissue cysts. *The Journal of Parasitology*, 81(6), 887–893. <https://doi.org/10.2307/3284035>
- Elmore, S. A., Jones, J. L., Conrad, P. A., Patton, S., Lindsay, D. S., & Dubey, J. P. (2010). *Toxoplasma gondii*: Epidemiology, feline clinical aspects, and prevention. *Trends in Parasitology*, 26(4), 190–196. <https://doi.org/10.1016/j.pt.2010.01.009>
- Feng, Y., & Xiao, L. (2011). Zoonotic potential and molecular epidemiology of giardia species and giardiasis. *Clinical Microbiology Reviews*, 24(1), 110–140. <https://doi.org/10.1128/CMR.00033-10>
- Gerhold, R. W., & Jessup, D. A. (2013). Zoonotic diseases associated with free-roaming cats. *Zoonoses and Public Health*, 60(3), 189–195. <https://doi.org/10.1111/j.1863-2378.2012.01522.x>
- Greene, C. E. (2006). *Infectious diseases of the dog and cat* (3rd ed.). Maryland Heights, MO: Saunders Elsevier.
- Association of Shelter Veterinarians' Veterinary Task Force to Advance Spay-Neuter, Griffin, B., Bushby, P. A., McCobb, E., White, S. C., Rigdon-Brestle, Y. K., & Tyson, K. (2016). The Association of Shelter Veterinarians' 2016 veterinary medical care guidelines for spay-neuter programs. *Journal of the American Veterinary Medical Association*, 249(2), 165–188. <https://doi.org/10.2460/javma.249.2.165>
- Harhay, M. O., Horton, J., & Olliaro, P. L. (2010). Epidemiology and control of human gastrointestinal parasites in children. *Expert Review of Anti-infective Therapy*, 8, 219–234. <https://doi.org/10.1586/eri.09.119>
- Hsu, V., Grant, D. C., Zajac, A. M., Witosky, S. G., & Lindsay, D. S. (2011). Prevalence of IgG antibodies to *Encephalitozoon cuniculi* and *Toxoplasma gondii* in cats with and without chronic kidney disease from Virginia. *Veterinary Parasitology*, 176(1), 23–26. <https://doi.org/10.1016/j.vetpar.2010.10.022>
- Hussain, M. A., Stitt, V., Szabo, E. A., & Nelan, B. (2017). *Toxoplasma gondii* in the food supply. *Pathogens*, 6(2), 21.
- Jones, J. L., Parise, M. E., & Fiore, A. E. (2014). Neglected parasitic infections in the United States: Toxoplasmosis. *American Journal of Tropical Medicine and Hygiene*, 90(5), 794–799. <https://doi.org/10.4269/ajtmh.13-0722>
- Lilly, E. L., & Wortham, C. D. (2013). High prevalence of *Toxoplasma gondii* oocyst shedding in stray and pet cats (*Felis catus*) in Virginia, United States. *Parasites & Vectors*, 6, 266. <https://doi.org/10.1186/1756-3305-6-266>
- 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. *Journal of Feline Medicine and Surgery*, 13(4), 300–303. <https://doi.org/10.1016/j.jfms.2010.12.013>
- Lucio-Forster, A., Griffiths, J. K., Cama, V. A., Xiao, L., & Bowman, D. D. (2010). Minimal zoonotic risk of cryptosporidiosis from pet dogs and cats. *Trends in Parasitology*, 26(4), 174–179. <https://doi.org/10.1016/j.pt.2010.01.004>
- Lucio-Forster, A., Mizhquiri Barbecho, J. S., Mohammed, H. O., Kornreich, B. G., & Bowman, D. D. (2016). Comparison of the prevalence of *Toxocara* egg shedding by pet cats and dogs in the U.S.A., 2011–2014. *Veterinary Parasitology, Regional Studies and Reports*, 5, 1–13.
- Mekaru, S. R., Marks, S. L., Felley, A. J., Chouicha, N., & Kass, P. H. (2007). Comparison of direct immunofluorescence, immunoassays, and fecal flotation for detection of *Cryptosporidium* spp. and *Giardia* spp. in naturally exposed cats in 4 Northern California animal shelters. *Journal of Veterinary Internal Medicine*, 21(5), 959–965.
- Nutter, F. B., Dubey, J. P., Levine, J. F., Breitschwerdt, E. B., Ford, R. B., & Stoskopf, M. K. (2004). Seroprevalences of antibodies against *Bartonella henselae* and *Toxoplasma gondii* and fecal shedding of *Cryptosporidium* spp., *Giardia* spp., and *Toxocara cati* in feral and pet domestic cats. *Journal of the American Veterinary Medical Association*, 225(9), 1394–1398.
- Nutter, F. B., Levine, J. F., & Stoskopf, M. K. (2004). Reproductive capacity of free-roaming domestic cats and kitten survival rate. *Journal of the American Veterinary Medical Association*, 225(9), 1399–1402.
- Opsteegh, M., Haveman, R., Swart, A. N., Mensink-Beerepoot, M. E., Hofhuis, A., Langelaar, M. F., & van der Giessen, J. W. (2012). Seroprevalence and risk factors for *Toxoplasma gondii* infection in domestic cats in The Netherlands. *Preventive Veterinary Medicine*, 104(3–4), 317–326. <https://doi.org/10.1016/j.pvetmed.2012.01.003>
- Rabinowitz, P. M., & Conti, L. A. (2010). *Human-animal medicine: Clinical approaches to zoonoses, toxicants and other shared health risks*. Maryland Heights, MO: Saunders.
- Roebing, A. D., Johnson, D., Blanton, J. D., Levin, M., Slate, D., Fenwick, G., & Rupprecht, C. E. (2014). Rabies prevention and management of cats in the context of trap-neuter-vaccinate-release programmes. *Zoonoses and Public Health*, 61(4), 290–296. <https://doi.org/10.1111/zph.12070>
- Sabshin, S. J., Levy, J. K., Tupler, T., Tucker, S. J., Greiner, E. C., & Leutenegger, C. M. (2012). Enteropathogens identified in cats entering a Florida animal shelter with normal feces or diarrhea. *Journal of the American Veterinary Medical Association*, 241(3), 331–337. <https://doi.org/10.2460/javma.241.3.331>
- Sævik, B. K., Krontveit, R. I., Eggen, K. P., Malmberg, N., Thoresen, S. I., & Prestrud, K. W. (2015). *Toxoplasma gondii* seroprevalence in pet cats in Norway and risk factors for seropositivity. *Journal of Feline Medicine and Surgery*, 17(12), 1049–1056. <https://doi.org/10.1177/1098612X15569616>
- Sandfoss, M., DePerno, C., Patton, S., Flowers, J., & Kennedy-Stoskopf, S. (2011). Prevalence of antibody to *Toxoplasma gondii* and *Trichinella* spp. in feral pigs (*Sus scrofa*) of Eastern North Carolina. *Journal of Wildlife Diseases*, 47(2), 338–343.
- Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M. A., Roy, S. L., ... Griffin, P. M. (2011). Foodborne illness acquired in the United States—major pathogens. *Emerging Infectious Diseases*, 17(1), 7–15. <https://doi.org/10.3201/eid1701.P11101>
- Tizard, I. R. (2000). *Veterinary immunology: An introduction* (6th ed.). Philadelphia, PA: W.B. Saunders Company.

- Torrey, E. F., & Yolken, R. H. (2013). Toxoplasma oocysts as a public health problem. *Trends in Parasitology*, 29(8), 380–384. <https://doi.org/10.1016/j.pt.2013.06.001>
- USDA (2014). *Cost estimates of foodborne illnesses*. Retrieved from <https://ers.usda.gov/data-products/cost-estimates-of-foodborne-illnesses.aspx>
- Vasilopoulos, R. J., Rickard, L. G., Mackin, A. J., Pharr, G. T., & Huston, C. L. (2007). Genotypic analysis of *Giardia duodenalis* in domestic cats. *Journal of Veterinary Internal Medicine*, 21(2), 352–355. [https://doi.org/10.1892/0891-6640\(2007\)21\[352:gaogdi\]2.0.co;2](https://doi.org/10.1892/0891-6640(2007)21[352:gaogdi]2.0.co;2)
- Woodhall, D. M., Eberhard, M. L., & Parise, M. E. (2014). Neglected parasitic infections in the United States: Toxocariasis. *American Journal of Tropical Medicine and Hygiene*, 90(5), 810–813.
- Yoder, J. S., Gargano, J. W., Wallace, R. M., Beach, M. J., & Centers for Disease Control and Prevention (CDC) (2012). Giardiasis surveillance—United States, 2009–2010. *Morbidity and Mortality Weekly Report. Surveillance Summaries* (Washington, D.C.: 2002), 61(5), 13–23.
- Zajac, A. M., & Conboy, G. A. (2012). Chapter 1: Fecal examination for the diagnosis of parasitism. (8th ed.). In A. M. Zajac & G. A. Conboy (Eds.), *Veterinary clinical parasitology* (pp. 3–169). Ames, IA: Wiley-Blackwell.

How to cite this article: Taetzsch SJ, Gruszynski KR, Bertke AS, et al. Prevalence of zoonotic parasites in feral cats of Central Virginia, USA. *Zoonoses Public Health*. 2018;65:728–735. <https://doi.org/10.1111/zph.12488>