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Observed occurrence of *Tritrichomonas foetus* and other enteric parasites in Australian cattery and shelter cats☆

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Cattery-housed pedigree cats, located mostly within the USA, have the highest reported prevalence of *Tritrichomonas foetus* (*T foetus*) to date. This prospective, multi-institutional, cross sectional study examines the occurrence of *T foetus* and other enteric parasites in cattery-housed and shelter cats within Australia, where *T foetus* has only recently been identified. Faecal specimens were collected from 134 cats, including 82 cattery-housed pedigree cats and 52 shelter cats. Faecal examinations performed for most cats included concentration techniques, Snap *Giardia* test, culture in InPouch medium, and polymerase chain reaction (PCR) amplification of *T foetus* ribosomal ribonucleic acid (rRNA) genes using species-specific primers. Observed occurrence of *T foetus*, *Giardia* species, *Isospora* species and *Toxascaris leonina* for cattery-housed cats (and catteries) were 0%, 7.4 (13.8)%, 10.9 (22.6)% and 1.6 (3.2)%, respectively. Observed occurrence of *T foetus*, *Giardia* species, *Isospora* species and hookworms for shelter cats were 0%, 11.5%, 9.8% and 4.9%, respectively. These results suggest the prevalence of *T foetus* in cattery-housed cats is currently much lower in Australia than in the USA, while *Isospora* and *Giardia* species infections are common.

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Tritrichomonas foetus (*T foetus*) is a flagellate protozoan most widely recognised as a venereal pathogen of cattle, and more recently an enteropathogen and emerging infectious disease of cats.[1–3] *T foetus* colonises the ileum, caecum and colon of cats, typically causing large bowel diarrhoea characterized by hematochezia, mucus, tenesmus and faecal dribbling.[1,2,4] In some cats, the diarrhoea may persist for months to years. Young, pedigree cats subjected to high-density housing appear to be the most susceptible to infection.[5,6] Untreated cats can remain persistently infected for years.[4]

Until very recently, feline *T foetus* was not known to exist in Australia and most small animal veterinarians did not specifically test for *T foetus* beyond the level of a direct faecal examination (personal communication with Australian primary care and referral veterinarians). Within the last year, however, *T foetus* has been identified in several different Australian catteries within Victoria and New South Wales (personal communication with Erin Bell (RSPCA, NSW), Kristen

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Deruddere (Chrinside Park Veterinary Clinic, VIC), Steven Ferguson (Macarthur Veterinary Hospital, NSW), Richard Gowan (The Cat Clinic, VIC) and Liz Walker (Gribbles Diagnostic Lab, Clayton, VIC)).[7] The prevalence of *T foetus* for cats with diarrhoea in the UK, Germany and Austria is reported to be 14–20% as determined by polymerase chain reaction (PCR) techniques[6,8] and 5% in Chile determined by light microscopy.[9] The highest prevalence of feline *T foetus* yet reported was 31% for pedigree cats attending an international cat show in the USA.[5] Unfortunately, the prevalence of *T foetus* in Australian cats is currently unknown. Many people assume that *T foetus* has been present, but remained undiagnosed in Australian cats until recently. Due to its relative geographical isolation to the rest of the world, Australia provides a unique opportunity for learning more about the recent apparent rise and geographic extent of feline trichomoniasis.

The prevalence of enteric parasites in general has been previously examined in Australian cats, including a recent large national survey.[10–17] However, none of these studies specifically examined faecal specimens for *T foetus*, and endoparasite data for cattery-housed cats (where protozoal parasitic burdens are expected to be high) is extremely limited. Therefore, the purpose of the present study was to determine the occurrence of enteric parasites in two subpopulations of Australian cats using commonly available diagnostic techniques, including those appropriate for diagnosis of *T foetus*. This was performed in an effort to help guide faecal testing and raise awareness of feline trichomoniasis within Australia.

Materials and methods

Defining the sample population

Between November 2006 and December 2007, fresh voided faecal specimens uncontaminated by litter were prospectively obtained from 134 cats for the purposes of this study. Faecal specimens ($n = 82$) were obtained from pedigree cats residing in 33 breeding catteries within four Australian states (NSW, South Australia, Victoria, and Western Australia). A further 52 faecal specimens were collected from five animal shelters located in the five largest Australian cities (Adelaide, Brisbane, Melbourne, Perth and Sydney). Where possible, the age, gender and breed of cats sampled from catteries was determined by a questionnaire, and the age of shelter cats were estimated to be $\leq$6 months or >6 months old by investigators or shelter employees.

Faecal examinations performed

Five different investigators (one from each state) obtained faecal specimens for the purposes of this study with fresh faecal examinations performed by the investigators themselves ($n = 3$) or by their parasitology laboratory ($n = 2$). Investigators recorded the consistencies of faecal specimens at the time of submission as either liquid (unformed), soft-formed or firm. Faecal examinations performed for the majority of cats included direct microscopic examination of faeces suspended in 0.9% saline or InPouch media (TropBio Pty, James Cook University QLD for BioMed Diagnostics, White City OR) at the time of inoculation to evaluate for the presence of trophozoites ($n = 67$), zinc sulphate centrifugal flotation ($n = 78$) or zinc sulphate ($n = 18$) or sodium nitrate ($n = 9$) gravitational flotation to evaluate for the presence of ova, cysts and oocysts, an enzyme-linked immunosorbent assay (ELISA; Snap *Giardia*, Idexx Laboratories, Westbrook ME) for the detection of *Giardia* species antigen ($n = 120$), and culture of faeces in InPouch media supportive of *T foetus* ($n = 93$). For culture, a peppercorn-sized amount of faeces (approximately 0.05 g) was inoculated into the InPouch and incubated in the dark at room temperature or 37°C. InPouch contents were examined by light microscopy (200–400× total magnification) for the presence of motile trophozoites (as previously described),[18] every 2 days for 6–12 days, or daily for 3–4 days when incubated at room temperature or 37°C, respectively. In addition, 1–2 g of faeces were placed in a 5–10 ml sterile container, filled with 70% isopropyl alcohol, stored at room temperature and couriered to North Carolina State University within 3 months of collection for DNA extraction and PCR amplification of *T foetus* ribosomal ribonucleic acid (rRNA) genes using species-specific primers as described previously ($n = 134$).[19]

Cattery questionnaire

Breeders participating in the study were asked to complete a written questionnaire identifying their cats' age, gender, breed, gastrointestinal and reproductive medical history, the number of cats in the cattery, access to the outdoors, proximity to cattle or pigs and the cattery location.

Results

Of the 82 faecal specimens from 33 catteries, the median number of cats sampled per cattery was 1.5 (range: 1–10 cats). The age and gender of cats sampled from catteries were reported for 56 and 40 cats, respectively. The median age was 2 years (range: 6 weeks to 13 years old), including 39 adults ($\geq$1 year of age) and 17 kittens ($\leq$6 months of age). Twenty-two of the cats were female (18 intact, four spayed) and 18 were male (16 intact, two castrated). Breeds were reported for 68 cats and included 19 different breeds: Burmese and Russian Blue (nine cats each), Devon Rex (seven cats), Burmilla and Ragdoll (six cats each), British Shorthair (five cats), Abyssinian, Australian Mist, Maine Coon and Siamese (four cats each), Cornish Rex and Oriental (two cats each), and Bengal, Birman, Chinchilla, Exotic, Singapura and Somali (one cat each). None of the cats from catteries

had gastrointestinal complaints at the time of specimen submission.

Of the 52 faecal specimens from five animal shelters, the median number of cats sampled per shelter was 10 (range: 9–12 cats). The age was estimated for 42 of the shelter cats; 17 were >6 months of age and 25 were kittens (≤6 months). Gender was not recorded for any of the shelter cats and none of these cats were purebred. Medical histories were largely unknown for the shelter cats.

The observed occurrence of enteric parasites in Australian cattery and shelter cats, determined from the examination of a single faecal specimen, are presented in Table 1. Eleven faecal specimens from NSW and Victoria were positive for *Giardia* species antigen, but trophozoites or cysts were not detected in any of these samples. The 14 faecal specimens positive for *Isospora* species oocysts, ascarid or hookworm ova were identified by faecal concentration methods. Cats with *Isospora* species oocysts were from NSW and Western Australia, the cat with *Toxascaris leonina* was from Western Australia, and the cats with hookworm ova were from a Brisbane shelter. It should be noted, however, that faecal concentration techniques were not performed for any cats from South Australia. The consistencies of faecal samples submitted were determined for 129 cats. None of five (0%) cats with liquid stool, 7/40 (17.5%) cats with soft-formed stool, and 14/84 (17%) cats with firm stool were positive for enteric parasites.

Questionnaire data was complete for 42 cats (10 catteries), incomplete for 31 cats (17 catteries) and unavailable for nine cats (six catteries). Gastrointestinal histories for cats that received both faecal concentration examinations and *Giardia* species antigen tests were available for 10/14 cats positive for enteric parasites and 49/61 cats negative for enteric parasites. Two of 10 (20%) cats positive for enteric parasites, and 3/49 (6%) cats negative for enteric parasites, had a history of diarrhoea within 6 months of faecal sampling. No breeders reported problems with infertility, abortion or kitten deaths within their cattery. The median number of cats domiciled at each of the 10 catteries with data available was 17 (range: 7–80 cats). Nineteen of 47 cats (3/11 catteries) were allowed to roam free outdoors. One of 43 cats (1/9 catteries) were located within 1 km of cattle or swine.

Discussion

Although feline trichomoniasis was recently reported in Australia[7] and several veterinarians from NSW and Victoria have diagnosed *T foetus* infected cats within the last year, results of this study suggest that the prevalence of *T foetus* for the catteries and shelters sampled was very low. Given that none of the 82 cattery and 52 shelter cats of this study were found to be infected with *T foetus* despite the use of a validated and highly sensitive PCR assay, *T foetus* infection appears to be much less common in cattery-housed cats of Australia than the USA. At a cat show within the USA in 2001, 31% of 117 cattery-housed cats tested positive for *T foetus*.[5] It is unclear why such a large difference exists between these two countries. The occurrence of *T foetus* is almost certainly underestimated in this report, as only one faecal sample per cat was obtained, no cats had diarrhoea, and a very small number of cats and catteries were sampled relative to the entire cattery population of Australia. However, our inability to detect *T foetus* despite testing 134 cats, may also support that feline trichomoniasis is truly an emerging disease and may not have been introduced to Australia until recently. This is possible, as Australia is geographically isolated relative to many countries, but domestic cat importations have grown dramatically in recent years (approximately 10% per annum over the last 6 years) (personnel communication with the Australian Quarantine and Inspection Service). Further studies investigating the prevalence of *T foetus* in Australian cats, including those with diarrhoea, are clearly necessary to determine the importance of feline trichomoniasis in this country at the present time.

With regards to the other enteric parasites detected in this report, *Giardia* and *Isospora* species infections

Table 1. The observed occurrence of enteric parasites in two subpopulations of Australian cats

Parasite	Source of cats		
	Cattery cats % (number)	Catteries % (number)	Shelter cats % (number)
T foetus	0 (0/82)	0 (0/32)	0 (0/52)
Giardia species	7.4 (5/68)	13.8 (4/29)	11.5 (6/52)*
Isospora species	10.9 (7/64)	22.6 (7/31)	9.8 (4/41)*
Toxascaris leonina	1.6 (1/64)	3.2 (1/31)	0 (0/41)
Hookworms	0 (0/64)	0 (0/30)	4.9 (2/41)
Total†	20.3 (13/64)	38.7 (12/31)	26.8 (11/41)

*One cat tested positive for *Giardia* species and *Isospora* species.
†Only reported for cats that had both faecal concentration techniques and *Giardia* species antigen testing performed.

were common for both the cattery cats (7.4 and 10.9%, respectively) and shelter cats (11.5 and 9.8%, respectively), while helminth infections were less common (1.6% for cattery cats, 4.9% for shelter cats). Although *Giardia* species cysts were commonly detected by light microscopy historically[12,20], *Giardia* species infection was only detected in the present report by the use of an ELISA assay. The lack of microscopic detection of *Giardia* species cysts and trophozoites in this report is in agreement with recent Australian surveys where *Giardia* species cysts were only identified in 0 to 2.6% of faecal specimens.[10,11] Despite this, a low parasite burden is likely for many cats, as 60−80% of cats from one of these surveys tested positive for *Giardia* species using ELISA and PCR techniques, respectively.[11,21] Although the observed occurrence of *Giardia* species determined by ELISA in the present study was much lower than 60%, *Giardia* and *Isospora* species infections were thought to be common due to the source of the cats. Intensively housed cats are expected to have high parasitic burdens, as environmental contamination is greater due to high-density housing, frequent exposure to other cats for breeding purposes, presence of kittens and young cats, increased litter box use (less outdoor access) and higher levels of stress.

The endoparasite data generated from faecal concentration techniques of this report is less than ideal in that only a single faecal specimen was examined, not all cats had faecal concentration techniques performed and the methodologies used varied. However, the observed occurrence of helminth infections reported here for cattery and shelter cats is still in agreement with other recent studies indicating the prevalence of helminth parasites (*Toxocara cati* in particular) has declined in Australian cats over the last few decades.[10,11] This decline has been mostly attributed to the widespread use of anthelmintics, including monthly heart worm and flea preventatives containing avermectin compounds, and possibly to reduced outdoor access. Although shelter cats typically have a high prevalence of helminth infections,[10] the only metazoan detected in the shelter cats of this study were two hookworm infections from a Brisbane shelter, where prevalence is expected to be greater due to the tropical climate.[22]

This faecal survey is the first to have specifically examined the occurrence of *T foetus* in Australian domestic cats using sensitive diagnostic techniques. As *T foetus* was not detected in any cat of the present report despite testing 134 intensively housed cats considered to be at high risk for infection, the authors consider it possible that Australia has only recently acquired feline trichomoniasis. Reports of feline trichomoniasis within Australia over the last 12 months, however, indicate that local veterinarians and veterinary clinical pathology laboratories should acquire the knowledge and skills necessary to diagnose and treat feline *T foetus* as ongoing transmission between cats seems likely. This study has also demonstrated that enteric protozoan infections are common and currently the preponderant enteric parasites of Australian cattery and shelter cats. Given that enteric protozoa can be difficult to identify, and cattery-housed cats with a recent history of diarrhoea were more often positive for enteric parasites in this study, veterinarians and private laboratories should consider the use of more sensitive faecal diagnostic techniques (eg, centrifugal versus gravitational flotation, InPouch culture, ELISA and PCR techniques) where the cause of feline diarrhoea is not readily apparent.

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