Article

Infectious disease prevalence in a feral cat population on Prince Edward Island, Canada

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Abstract — Ninety-six feral cats from Prince Edward Island were used to determine the prevalence of selected infectious agents. The prevalence rates were 5.2% for feline immunodeficiency virus, 3.1% for feline leukemia virus, 3.1% for *Mycoplasma haemofelis,* 8.4% for *Candidatus* Mycoplasma haemominutum, 2.1% for *Bartonella* spp. and 29.8% for exposure to *Toxoplasma gondii.* Oocysts of *T. gondii* were detected in 1.3% of the fecal samples that were collected. Gender and retroviral status of the cats were significantly correlated with hemoplasma infections. Use of a flea comb showed that 9.6% of the cats had fleas; however, flea infestation was not associated with any of the infectious agents.

Résumé — Prévalence des maladies infectieuses chez une population de chats féraux de l'Île-du-Prince-Édouard, Canada. Quatre-vingt-seize chats féraux de l'Île-du-Prince-Édouard ont été utilisés pour déterminer la prévalence de certains agents infectieux. Les taux de prévalence étaient de 5,2 % pour le virus de l'immunodéficience féline, de 3,1 % pour le virus de la leucose féline, de 3,1 % pour *Mycoplasma haemofelis,* de 8,4 % pour *Candidatus Mycoplasma hæmominutum,* de 2,1 % pour *Bartonella* spp. et de 29,8 % pour l'exposition à *Toxoplasma gondii.* Des ookystes de *T. gondii* ont été détectés dans 1,3 % des échantillons de fèces qui ont été prélevés. Il y avait une corrélation importante entre le sexe et le statut rétroviral des chats avec les infections à hémoplasmes. L'usage d'un peigne à puces a montré que 9,6 % des chats avaient des puces; cependant, l'infestation de puces n'était associée à aucun agent infectieux.

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Introduction

Feral cats can serve as a direct or indirect source of infectious diseases for outdoor pet cats. Feline hemotrophic mycoplasmas are red blood cell pathogens which can cause hemolytic anemia and severe clinical disease in affected cats (1). Three etiologic agents, collectively called hemoplasmas, have been identified: *Mycoplasma haemofelis, Candidatus* Mycoplasma haemominutum, and *Candidatus* Mycoplasma turicensis (2). *M. haemofelis* is associated with hemolytic anemia in cats (2,3). *M. haemominutum* is usually not associated with clinical disease and typically causes anemia in immuno-compromised cats (2–4). A recent case report from Europe identified *Candidatus* Mycoplasma turicensis as a potential cause of disease in an immuno-competent cat (2–4).

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Toxoplasma gondii can cause clinical disease in cats and has zoonotic potential (5,6). Outdoor cats, due to increased chance for predatory behavior, are more likely to contract the pathogen and act as a significant source of the infection for humans (5). The most important sources for human *T. gondii* infection are contaminated inadequately cooked meat and vegetables, and close contact with cat feces containing infectious *T. gondii* oocysts (5,6). Contaminated water was also recently recognized as a potential source for humans (5).

Bartonella spp. include many fastidious organisms which were recently recognized as emerging feline pathogens (7–11). Infection with these pathogens does not always result in clinical disease (9), but isolated reports described cats in which clinical disease resolved after diagnosis and treatment for *Bartonella* (9). Cat-to-cat transmission of *Bartonella henselae* usually occurs via fleas *(Ctenocephalides felis)* (7–11), while the mode of transmission for other feline *Bartonella* organisms is unknown. Cats are also frequently recognized as a primary reservoir for human infection (7–11). The most severe clinical signs are seen in immuno-compromised humans such as those with human immunodeficiency virus (HIV) infection (11).

Feline retroviruses [feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV)] are widespread feline pathogens that can affect all cats; however, adult, outdoor male cats are at higher risk of contracting the pathogens (12). Retroviruses can

be associated with clinical disease in affected cats and can also predispose the affected cats to other diseases including infectious diseases (3).

The goal of the current study was to determine the prevalence of *M. haemofelis, M. haemominutum, Bartonella* spp. and exposure to *T. gondii,* in a feral cat population on Prince Edward Island. Species of *Bartonella* included in the study were feline pathogens, *B. henselae* and *B. clarridgeiae.* The retroviral status of each cat, the presence of fleas, and the presence of *T. gondii* oocysts were also determined. Eggs or segments of other parasitic pathogens such as roundworms, hookworms, coccidia, and tapeworms discovered during fecal examination allowed determination of the prevalence of these parasites. The relationship between the infectious agents under investigation and age, sex, and flea infestation was also determined.

Materials and methods

Feral cats presenting to the Atlantic Veterinary College (AVC) spay and neuter program were enrolled in the study. The feral cats were defined as free-roaming non-owned intact cats exhibiting feral temperament. The study design was approved by the Animal Care Committee of the Atlantic Veterinary College (AVC), University of Prince Edward Island. Cats were humanely trapped the night before presentation to the AVC. Data collection occurred between March and September of 2009.

For each cat, body weight, approximate age, gender, and assigned feral cat colony number were recorded. Cats were classified as either adult or juvenile based on weight and appearance of the teeth. Cats that weighed less than 2 kg and had deciduous teeth were classified as juveniles; all others were classified as adults. Ninety-six cats were enrolled in the study. All cats underwent general anesthesia, at which time venipuncture was performed and a 3-mL blood sample was obtained. A 1.5 mL volume of blood was placed into a potassium EDTA tube. The other 1.5 mL was placed into a glass tube without additives, allowed to clot, then centrifuged for 10 min at 4000 × *g*; serum was harvested. A small amount of blood from the potassium EDTA tube was used for a point of care enzyme-linked immunoabsorbent assay (ELISA) for the detection of FIV antibodies and FeLV p27 antigen (SNAP Combo; IDEXX, Westbrook, Maine, USA) to determine the retroviral status of each cat. The manufacturer reports sensitivity for FeLV antigen and FIV antibody detection of 97.6% and 100%, respectively, and specificities of 99.1% and 99.5%, respectively (Package insert, SNAP Combo, IDEXX). Retrovirus-negative cats were spayed or castrated; retrovirus-positive cats were humanely euthanized after being checked for fleas and after a stool sample was obtained.

The rest of the blood sample was stored at 4°C overnight. The whole blood was sent to a commercial laboratory (Diagnostic Center for Population and Animal Health, Michigan State University, Lansing, Michigan, USA) for polymerase chain reaction (PCR) detection of *Bartonella* spp., *M. haemofelis,* and *M. haemominutum.* Sensitivity and specificity of these PCR assays were not established at this time; however, any positive results were confirmed by an additional PCR assay (Steve Bolin, Diagnostic Center for Population and Animal Health-Michigan State University, Lansing, Michigan, USA, personal communication, 2010). Serum samples from each cat were submitted to the AVC diagnostic laboratory to determine the presence of *T. gondii* IgG antibodies. A serum agglutination test (Toxotest-MT "EIKEN", San Diego, California, USA) was used, and an antibody titer of greater than 1:64 was deemed positive. Sensitivity and specificity of this test was previously reported elsewhere (13). A fecal sample, if available, was obtained digitally from the rectum. Stool samples were placed into sterile containers and stored at 4°C overnight. Stool analysis was performed 2 d later. Each cat was combed with 3 strokes (1 over dorsum and 2 over lateral sides) using a standard flea comb. A sheet of white paper was placed under each cat during combing in order to aid in detection of flea or flea dirt. Any cat that had flea dirt or fleas was classified as positive for fleas.

Fecal analysis was performed by the AVC diagnostic laboratory using a zinc sulfate fecal floatation technique. Samples were positive if eggs or segments of gastrointestinal parasites were visualized, or if *T. gondii* oocysts were present. The detection limit of fecal floatation for *T. gondii* oocysts was previously reported to be 250 oocysts/g of feces (14). Identification of gastrointestinal parasites based on findings of fecal floatation was performed.

After completion of the surgical procedure, each cat was vaccinated against feline viral rhinotracheitis, calicivirus, panleukopenia, and rabies virus. Each cat was permanently identified with a tattoo in the left pinna. After complete recovery from anesthesia each cat was released back into the area where it had been trapped.

Statistical analysis

Study population size was established to achieve a 95% confidence interval (95% CI) and 8% margin of error. Categorical data were analyzed by using a chi-squared or Fisher's exact test. The level of significance was established at $P < 0.05$. Commercially available software (Minitab 15-MINITAB; State College, Pennsylvania, USA) was used for data analysis.

Results

Ninety-six feral cats trapped on Prince Edward Island were enrolled in the study. There were 39 females and 57 males (40.6% and 59.4%, respectively). Twenty-two cats were classified as juveniles (22.9%) and 74 cats were classified as adults (77.1%). The mean weight of juvenile cats was 1.3 kg (range: 0.6 to 1.8 kg), while the mean weight for adult cats was 3.7 kg (range: 2 to 6.2 kg). Three cats (3.1%) were positive for FeLV, while 5 cats (5.2%) were positive for FIV. One cat was positive for both FIV and FeLV (~1%). Twenty-eight cats (29.8%) had a positive titer for *T. gondii,* and data were missing for 2 cats. Three cats were positive for *M. haemofelis* (3.1%), 8 cats were positive for "*Ca* Mycoplasma haemominutum" (8.4%), and data were missing for 1 cat. Two cats (2.1%) were positive for *Bartonella* spp., while data were missing for 1 cat. Fecal samples were available for 78 cats and only 1 sample (1.3%) contained *T. gondii* oocysts. The cat shedding *T. gondii* oocysts had a serum *T. gondii* titer of 1:256. *Toxocara cati* eggs were found in 27 fecal samples (34%), while 11 cats were positive for *Cystoisospora felis*

Table 1. Feline infectious disease prevalence in a population of feral cats on Prince Edward Island

	FeLV	FIV	*T. gondii*	MHA	MHM	BAR	*T. gondii* oocyst	Fleas	*Taenia* sp.	*T. cati*	*C. felis*
Positive (%)	3 (3.1)	5 (5.2)	28 (29.8)	3 (3.1)	8 (8.4)	2 (2.1)	1 (1.3)	8 (9.6)	12 (15)	27 (34)	11 (14)
Negative N	93 96	91 96	66 94 2*	92 95 1*	87 95 1*	93 95 1*	77 78 18*	87 96	66 78 18*	51 78 18*	67 78 18*

FeLV — Feline leukemia virus, FIV — Feline Immunodeficiency Virus, MHA — *Mycoplasma haemofelis,* MHM — Candidatus *Mycoplasma* haemominutum, BAR — *Bartonella* sp., T. cati — *Toxocara cati, C. felis — Cystoisospora felis,* *Information missing, N — Total number of cases investigated, *T. gondii* — IgG serology.

oocysts (14%). *Taenia* spp. segments were identified in 12 fecal samples (15%). No intestinal hookworms (*Ancylostoma* spp.) were detected. Nine cats had fleas or flea dirt (9.6%) at the time of examination.

"*Ca* Mycoplasma haemominutum"-positive cats were significantly more likely (P = 0.01) to be concurrently retrovirus-positive (FIV or FeLV) than were "*Ca* Mycoplasma haemominutum"-negative cats (3/8 and 4/87 respectively). Three out of 11 *Mycoplasma*-positive cats were also retrovirus positive. Gender played a significant role in hemoplasma infection as all the cats positive for *M. haemofelis* or "*Ca* Mycoplasma haemominutum" were male (P = 0.002). Although only 1 cat positive for retroviral infection was female, statistical significance for gender predisposition was not seen for retroviral infection (P = 0.23). There was no gender predisposition for *T. gondii* (P = 0.5) and *Bartonella* spp. infection (P = 0.5). No significance was reached when comparing *T. gondii* and retroviral positive cats (P = 0.6). Shedding of the *T. gondii* oocysts was detected in only 1 serologically positive cat. The age of the cats did not play a significant role in retroviral (P = 0.34), hemoplasma (P = 0.44), *Bartonella* spp. (P = 1), and *T. gondii* (P = 0.06) infections. None of the cats positive for *Bartonella* spp. were positive for a retrovirus, hemoplasma, or fleas. There was no statistically significant correlation between flea infestation and concurrent *T. gondii* (P = 1) serological positivity.

Discussion

This study investigated the prevalence of selected infectious diseases in a feral cat population in Prince Edward Island (Table 1). All investigated pathogens were present in the study population. "*Ca* Mycoplasma haemominutum" was the most prevalent hemoplasma which is contrary to the results of a study investigating stray cats from Ontario (15), but in agreement with studies from the United States investigating prevalence of hemoplasma in blood submitted from unknown cats to a diagnostic laboratory and from a feral cat population (2,16). There were no cats positive for both hemoplasmas. Cats infected with "*Ca.* Mycoplasma haemominutum" were at increased risk for retroviral (FIV or FeLV) infection. The same was true when hemoplasma positive cats were compared with the retroviral positive cats. This finding was consistent with previous reports suggesting that hemoplasma positive cats are at increased risk for concurrent retroviral infection (2,17,18).

Gender played a significant role in hemoplasma infection, as all hemoplasma positive cats were males. This was statistically significant in spite of the study population being slightly biased towards males. This finding is in agreement with previous studies on cats with hemoplasma (2,16). These results cannot be easily explained as the precise mode of transmission of feline hemoplasmas has yet to be determined (19). Fleas, as vectors of transmission, have been suggested in the past; however, hemoplasmas have been found in cats living in areas with low flea prevalence (19). Also, when 6 naïve cats were exposed to hemoplasma-containing fleas, only 1 cat contracted the disease (19). In addition, none of the hemoplasma-positive cats in this study were positive for fleas at the time of sampling. One possible explanation is that male cats are more likely to engage in roaming and fighting behavior which may increase their chance of contracting the disease. This is especially true if a direct mode of transmission is possible.

Although close to 30% of the cats were positive for *T. gondii* on serology, only 1 cat was shedding *T. gondii* oocysts in the feces. This finding is in agreement with a previous study of environmental burden of *T. gondii* oocysts in cats' feces, showing oocyst shedding in 0.9% of the cats (5). The high prevalence of positive *T. gondii* titers most likely indicated a latent infection or previous exposure and not necessarily clinical disease which is usually seen in retroviral-positive cats or those cats treated with immunosuppressive medications (6). This high prevalence is in agreement with previous prevalence studies from Grenada and California (20,21), but lower prevalence was seen in this study when compared to studies from North Carolina (22). Three cats positive for *T. gondii* antibodies were juvenile, raising a question of possible maternal antibody detection rather than exposure to the pathogen. Based on the findings of this study, feral cats did not seem to pose a great zoonotic risk for *T. gondii.* However, it must be emphasized that cats with latent infection can, in times of stress, start shedding the oocysts in the feces again (5,6). *Toxoplasma gondii* infection in humans can cause serious disease; therefore, the zoonotic potential of any feral cat should not be underestimated. In addition, cats seem to pose a considerable zoonotic risk with respect to the shedding of other intestinal parasites in their feces. Most of the fecal samples showed evidence of at least 1 intestinal parasite, while many samples contained evidence of multiple intestinal parasites.

Bartonella spp. (*B. henselae* and *B. clarridgeiae*) had a much lower prevalence in this population of cats compared with previously reported prevalence rates (16,20–23). This discrepancy in the findings may be due to the different study populations, the confined space of Prince Edward Island, and its harsh

winter climate. Most studies that reported higher prevalence of *Bartonella* spp. came from tropical or much warmer climates (16,20,21,23). None of the *Bartonella* spp. positive cats were positive for other investigated pathogens. These results may suggest that *Bartonella* spp. are not associated with any other feline infectious pathogens investigated in this study, but the small number of positive cats precludes any definitive conclusion. Fleas, one of the vectors of this pathogen, were found in 9.6% of cats but the cats positive for *Bartonella* spp. did not have a concurrent flea infestation. This suggests that *Bartonella* spp. positive cats potentially had fleas sometime in the past or that they were exposed to *Bartonella* spp. via other means. A larger study population is required to determine the possible correlation between flea infestation and *Bartonella* spp. infection, due to the low prevalence of the pathogen in this feral cat population on Prince Edward Island.

The estimated age of the cats did not correlate with the prevalence of the infectious diseases. This finding is most likely influenced by the small sample size. The prevalence of retroviral infection was 7.3%, with only 1 cat being positive for both FIV and FeLV. This study showed a decline in the prevalence of retrovirus-positive feral cats when compared with the previously reported retrovirus prevalence in feral cats (12.4%) on Prince Edward Island (24).

Gender did not correlate with the prevalence of retroviral and *T. gondii* infections. The relatively low prevalence of retroviruses in this feral cat population, and a relatively small number of investigated cats most likely underpowered the study with respect to the gender differences for these pathogens.

The results of this study are in agreement with those of most previous studies investigating infections diseases in cats (2,5,15,18). In addition, we found a low prevalence of *Bartonella* spp. and a higher prevalence of "*Ca.* Mycoplasma haemominutum" than of *M. haemofelis.* The clinical significance of this finding is unknown as feral cats rather than owned cats were investigated. More focused studies, investigating larger number of cats, and determining the clinical and hematological status of each cat would be needed to determine the significance of these pathogens. Analysis of fleas for the presence of *Bartonella* spp. and hemoplasmas would determine their importance as vectors of these infections. Since a direct mode of hemoplasma transmission may be possible, analyzing other body fluids such as saliva may shed more light on the mode of transmission of these pathogens.

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