Follow-up of the health conditions of an urban colony of free-roaming cats (*Felis catus* Linnaeus, 1758) in the city of Rio de Janeiro, Brazil

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Abstract

Similar to other urban areas where food and shelter are abundant, the zoological garden of Rio de Janeiro has dealt for years with a colony of feral or semi-feral domestic cats. A survey was conducted during 2002–2004 as a follow-up to a previous study in 2001 of the cat colony to identify pathogens circulating among the population and to annually follow the status of the cats to analyze morbidity coefficients and associations among infections and infestations identified in the colony. During the 3 years of the present study, 75 cats were sampled at least once, including 44 that were caught and examined only once, 14 that were examined twice, and 17 that were examined three times. For each cat that was caught, records were kept regarding sex, age, general health, and the presence of ectoparasites. Each year, a blood sample was taken for hematologic testing, platelet count, hemoparasite detection, antibodies to *Toxoplasma gondii*, and retrovirus detection. Blood counts were within normal range for the majority of cats tested. Feline immunodeficiency virus, fleas, and lice were detected in all years; however, incidence rates for each of these varied significantly throughout the years. Prevalence of *Cytauxzoon* spp., *Mycoplasma* spp., *T. gondii* infections were variable among the 3 years, although differences were not significant. Prevalence of feline leukemia virus increased significantly over the 3 years. *Mycoplasma* spp. and flea infestations were significantly associated, but no other associations among the pathogens were detected. Over the 3 years, the rate of new cat introductions decreased, and the pathogens showed a tendency to disseminate throughout the colony; however, there was virtually no evidence of clinically detectable disease. Therefore, it seems that stabilizing the population by a judicious control program facilitated the distribution of the pathogens throughout the colony, while the general well-being of the cats was not seriously affected.

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1. Introduction

Among the pathogens circulating in domestic cat populations, *Toxoplasma gondii* (Nicole and Manceaux,

1909) is, perhaps, the major public health concern. Toxoplasmosis is one of the zoonoses most frequently related to the presence of cats, which are a common source of oocysts in urban environments. Felines are generally asymptomatic but may develop nonspecific clinical signs, including fever, weight loss, dyspnea, polypnea, jaundice, abdominal discomfort, uveitis, or retinochoroiditis, if the disease manifests as toxoplasmosis (Dubey and Lappin, 2006). *T. gondii* is widely disseminated among mammals, known to be frequent among feral cats (Nutter et al., 2004) and detection of antibodies is an important screening tool to identify which cats have been previously infected because these animals are not expected to re-shed oocysts. Cats normally preserve their immunity and seldom re-shed oocysts (Martins and Viana, 1998; Dubey and Lappin, 2006), although there are isolated reports of cats that were previously seropositive that resumed shedding of oocysts (Dubey, 1995).

Arthropod-borne hemoparasites occur frequently in cats in Brazil (Almosny, 2002). *Mycoplasma haemofelis* (previously *Haemobartonella felis*) is a frequently diagnosed hemoparasite in cats of Rio de Janeiro (Neimark et al., 2001; Souza, 2002; Mendes-de-Almeida et al., 2004) that can cause hemolytic anemia and other nonspecific clinical signs (Tasker, 2001; Harvey, 2006; Tasker and Lappin, 2006). *Cytauxzoon felis*, a natural parasite of wild cats transmitted by ticks, can cause severe disease in domestic cats (Meinkoth, 2001; Bondy et al., 2005; Greene et al., 2006).

Cats are also susceptible to infections by viruses of different families, and persistent infections are often caused by viruses belonging to the family Retroviridae. Controlling transmission of these viruses is difficult because they are transmitted directly, causing both chronic and subclinical infections (Hartmann, 2006; Sellon and Hartmann, 2006). Feline leukemia virus (FeLV) exposes the infected host to highly malignant neoplasias. Detection of the virus may be intermittent, and transmission occurs through prolonged and intimate contact as well as by infected queens to her kittens either in utero or through the milk (Avery, 2001; Mehl, 2001; Hartmann, 2006). Cats infected with feline immunodeficiency virus (FIV) present with various clinical signs, depending on which opportunistic infection is present. The most common infections seen in these cats include calicivirus, herpesvirus, *Chlamydia* spp., *Mycoplasma* spp., and *T. gondii* (Sellon and Hartmann, 2006). Once cats develop detectable antibodies, they tend to remain seropositive.

There are few studies on the prevalence of diseases of free-roaming cat colonies worldwide. A colony of 52 free-ranging cats living on a farm in the United Kingdom had no animals infected with FeLV, *Chlamydia psittaci*, or *Ctenocephalides felis* (Yamaguchi et al., 1996). However, feline parvovirus antibodies were detected in 96% of cats, coronavirus antibodies in 84%, *T. gondii* antibodies in 62%, FIV antibodies in 53%, and poxvirus antibodies in 2%. *M. haemofelis* was detected in 42% of examined blood samples, and eggs of *Toxocara cati* (91%) and *Toxascaris leonina* (82%) were present in the feces. In the United States, a colony of 80 free-roaming cats living on the campus of the University of California, Davis were caught during a capture-neutering-release program. Among these animals, 12% were infected with *Mycoplasma haemominutum*, 8% with *Mycoplasma* spp., 54% by ascarids, 26% by tapeworms, and 13% by coccidia (Levy et al., 1999). During the 2001 study, pathogens shown to infect the free-roaming cats of the zoological garden of the city of Rio de Janeiro (RIOZOO) included piroplasms, *Mycoplasma* spp., and FIV (Mendes-de-Almeida et al., 2004). The only ectoparasite species found was *C. felis*. No FeLV antigens were detected.

Since domestic cats can transport pathogens between cages, which jeopardizes the health and well-being of the zoo animals, RIOZOO has attempted unsuccessfully to eliminate the presence of the free-roaming cat colony. Therefore, the present survey was carried out in an attempt to monitor the circulating pathogens in the cat colony to assist the RIOZOO in the development of adequate management protocols, which includes a program for hysterectomy of captured female cats (Mendes-de-Almeida et al., 2006).

2. Material and methods

2.1. Animals

The present study, conducted from 2002 to 2004, included domestic cats inhabiting the zoological garden of the city of Rio de Janeiro (RIOZOO), which occupies 13.8 ha in a municipal park located at $23°54'$S and $43°13'$W at an altitude of 44 m.

Each year, animals were caught once a week in the morning (06:00–10:00) or in the evening (17:00–20:00), from June to August using three hand nets and three one-door Tomahawk-traps (Mendes-de-Almeida et al., 2004, 2006). On each trapping day, five people operated the hand nets and the traps, attempting to capture as many cats as possible.

2.2. Procedures

The captured animals were sedated with a combination of ketamine (10 mg/kg – Vetaset[®]; Lab. Fort Dodge Saúde Animal Ltda) and xylazine (2 mg/kg – Rompun[®]; Lab. Bayer do Brasil S/A). While sedated, each animal was examined to determine sex, age, general health status, and presence of ectoparasites. Age was estimated based on dentition, considering the permanent incisors and canines eruption (Dyce et al., 1990). Animals less than 24 weeks of age were considered kittens; all the others were considered adults. All cats received annual vaccination against panleucopenia, calicivirosis, rhinotracheitis, chlamydiosis, and rabies (Felocell CVR-C[®]; Lab. Pfizer and Rabisin-I[®]; Lab. Merial Saúde Animal Ltda) as well as treatments for endoparasites and ectoparasites (ivermectin 50 µg/kg – Ivomec[®]; Lab. Merial Saúde Animal Ltda and fipronil – Frontline Spray[®]; Lab. Merial Saúde Animal Ltda or selamectine 6 mg/kg – Revolution[®]; Lab. Pfizer).

Each cat was identified by a microchip (Friendchip[®]; Avid) injected into the deep subcutaneous tissue in the interscapular region. The biological data and microchip number of each animal were recorded on individual forms.

2.3. Collection of samples and laboratory tests

A 3 mL blood sample was collected from each cat by venipuncture (jugular or femoral) each time the cats were captured. An aliquot of each blood sample was retained with anticoagulant (EDTA) for hematology testing and platelet count; the remainder of the sample was allowed to clot and centrifuged for serum. The samples were identified and maintained at $-20\,^{\circ}$C until used (maximum of 4 months). Two capillary blood smears were made for hemoparasite detection. If the volume of blood was insufficient for all planned tests, priority was given to retroviruses examination and hematology.

Hematology tests were performed according to criteria described by Coles (1986) and included determination of hematocrit, hemoglobin, and total and differential white blood cell counts (WBC). Platelet counts were performed in a Neubauer chamber in 2003 and 2004. Animals were said to be anemic when their hematocrit was 24% or less.

Blood smears were stained with Giemsa and examined under an optical microscope at 1000× power. Plasma samples were examined for detection of FIV antibodies and FeLV antigens, using an immunoenzymatic method (Snap Combo[®]; Lab. Idexx).

The presence of antibodies against *T. gondii* was detected in the serum samples by indirect hemaglutination (Hematoxo[®]; Biolab – Mérieux S/A). Samples that hemaglutinated at a dilution of 1:16 were considered reactive.

Following completion of the tests, the animals were transferred to the population control program (Mendes-de-Almeida et al., 2006).

2.4. Data analysis

The data were compared among years by contingency table analysis, with individual comparisons between years by chi square test. Fisher's exact test was used to verify possible associations between infections and infestations. The incidence of cats that had been caught and examined the previous year and shown not to be infected or infested at that sampling but subsequently became positive was calculated.

3. Results

3.1. Hematology

A total of 75 individual animals (feral or semi-feral) were examined during the 3 years (Table 1). In 2002, 44 cats were captured; in 2003, 47 cats were examined, 25 of which were being examined for the first time; and in 2004, 33 cats were captured, including six cats for the first time. Forty-four of the cats were captured and examined only once, 14 were captured twice, and 17 were captured all 3 years; therefore, the total number of samples examined throughout the 3 years was 123. The majority of animals were adults; seven samples were

Table 1

Characteristics of free-roaming domestic cats in a zoo in Rio de Janeiro, Brazil (RIOZOO) examined during 2002–2004

Characteristic	2002	2003	2004
Total cats evaluated	44	47	33
Adults	41	43[a]	32[b]
Kittens (<24 weeks)	3	4	1
Gender			
Females	21	27	22
Males	23	20	11
No. sampled for the first time	44[c]	25	6

[a] Includes two adults listed as a kitten in 2002.

[b] Includes one adult listed as a kitten in 2002.

[c] Eight of these cats were previously sampled in a different study in 2001 (Mendes-de-Almeida et al., 2004) but are considered as first time sampled for this study.

Table 2
Prevalence of infections, ectoparasite infestations, and anemia in a colony of free-roaming domestic cats living in the RIOZOO in Brazil

	Percentage of animals positive (number positive/number sampled)							
Year	Anemia[a]	FIV	FeLV	*Cytauxzoon* spp.	*Mycoplasma* spp.	*Toxoplasma gondii*	*Felicola subrostratus*	*Ctenocephalides felis*
2002	16.2 (6/37)	55.3 (21/38)	2.6[b] (1/38)	15.8[b] (6/38)	15.8[d] (6/38)	92.1[b] (35/38)	11.4[b] (5/44)	38.6[b] (17/44)
2003	40.4 (19/47)	57.4 (27/47)	6.4[b] (3/47)	34[b,c] (16/47)	36.2[b,e] (17/47)	63.8[c] (30/47)	4.3[b] (2/47)	72.3[c] (34/47)
2004	27.3 (9/33)	75.8 (25/33)	39.4[c] (13/33)	48.5[c] (16/33)	75.8[c] (24/33)	60.6[c] (20/33)	39.4[c] (13/33)	42.4[b] (14/33)

FIV: feline immunodeficiency virus; FeLV: feline leukemia virus; values within columns having different superscript numbers are significantly different (b, c: $p < 0.01$; d, e: $p < 0.05$).
[a] Anemia = hematocrit value $\leq 24\%$.

collected from kittens. Two of these kittens were later sampled as adults (Table 1).

In 2002, blood samples from six animals were lost, and hematology data were unavailable for one cat. Various alterations were observed for differential WBC counts in all years of the study. The prevalence of anemic cats among years approached significance ($p < 0.10$). The lowest rate occurred in 2002 (16.2%) and the highest was in 2003 (40.4%) (Table 2).

3.2. Hemoparasites

Mycoplasma spp. was detected in the colony with significant differences in the prevalence rates among all years ($p = 0.001$), and the percentage of infected animals increased significantly each year (Table 2). Six cats that were negative for *Mycoplasma* spp. in 2002 tested positive in 2003, and 10 cats negative in 2003 became positive for *Mycoplasma* spp. in 2004 (Table 3). One cat negative in 2002 became positive in 2004.

Cytauxzoon spp. demonstrated a significant difference for infection rates among years ($p < 0.025$). There was a greater percentage of infected animals each year of the study (Table 2). However, only the difference between 2002 and 2004 was significant ($p < 0.01$). Five cats that were negative for *Cytauxzoon* spp. in 2002 tested positive in 2003, and 10 cats that were negative in

2003 became positive in 2004 (Table 3). One cat positive for *Cytauxzoon* spp. in 2002 was negative at the 2003 sampling, and four cats positive at the 2003 sampling were negative in 2004.

Antibodies against *T. gondii* were detected in the majority of the animals in all years, with significant differences between the studied years ($p = 0.01$). The highest prevalence rate of this protozoan was observed in 2002 (92.1%), and rates were similar between 2003 (63.1%) and 2004 (60.6%) (Table 2). Three cats positive for *T. gondii* antibodies and negative for FIV antibodies seroconverted to *T. gondii* nonreactive and FIV reactive status.

3.3. Retroviruses

FIV increased slightly during the study, but differences among the years were not significant (Table 2). Five animals that tested negative for FIV antibodies in 2002 were positive in 2003, and six animals negative in 2003 tested positive for FIV antibodies in 2004 (Table 3). Two cats negative in 2002 were positive in 2004.

FeLV antigens were detected with significant differences in the prevalence among the years ($p = 0.001$). Differences were significant ($p < 0.01$) between 2004 and the other 2 years; however,

Table 3
Incidence of new infections and infestations for cats in a free-roaming colony living in the RIOZOO in Brazil that tested negative for the given pathogen the previous year(s)

	Percentage of animals positive (number positive/number sampled)							
Year	Anemia[a]	FIV	FeLV	*Cytauxzoon* spp.	*Mycoplasma* spp.	*Toxoplasma gondii*	*Felicola subrostratus*	*Ctenocephalides felis*
2003	ND	62.5 (5/8)	18.8 (3/16)	50.0 (5/10)	42.9 (6/14)	– (0)	4.8 (1/21)	62.5 (10/16)
2004	ND	66.7 (8/12)[b]	50.0 (11/22)[c]	58.8 (10/17)	78.6 (11/14)[c]	11.1 (1/9)[c]	39.1 (9/23)	50.0 (3/6)

ND: not done; FIV: feline immunodeficiency virus; FeLV: feline leukemia virus.
[a] Anemia = hematocrit value $<24\%$.
[b] Includes two cats negative in 2002 and not tested in 2003.
[c] Includes one cat negative in 2002 and not tested in 2003.

prevalence between 2002 and 2003 was similar (Table 2). Only one cat tested positive for FeLV in 2002 (2.6%) and three cats that tested negative that year were positive for FeLV the following year (Table 3). Ten cats negative for FeLV in 2003 became positive in 2004. One cat that was negative in 2002 was positive in 2004. Also, one cat that was positive in 2002 was negative in 2003; however, this test may have been a false negative because the cat was tested again in 2004 and was positive for FeLV at that time.

3.4. Ectoparasites

Fleas (*C. felis*) were the most prevalent ectoparasite identified on the cats, with significant differences in rates among the 3 years ($p < 0.01$) (Table 2). The highest prevalence was in 2003 (72.3%), and rates in 2002 (38.6%) and 2004 (42.4%) were similar to each other. Of the 16 animals negative for fleas in 2002 that were sampled again in 2003, 10 were infested in 2003 (Table 3). Three animals negative for fleas in 2003 were positive in 2004. However, two cats positive for fleas in 2002 were negative in 2003, and 11 positive in 2003 were negative in 2004.

The presence of lice (*Felicola subrostratus*) showed significant differences among years ($p < 0.001$). The prevalence (39.4%) in 2004 was significantly greater than in either 2002 or 2003 (Table 2). Of the 24 cats present in 2002 that were resampled in 2003, one cat negative in 2002 was positive in 2003 (Table 3), and one cat positive for lice in 2002 was negative in 2003. Nine cats negative for lice in 2003 were positive for these ectoparasites in 2004, and one cat positive in 2003 was negative for lice in 2004.

Only one cat was infested with immature forms of ticks (*Rhipicephalus sanguineus*) in 2003. Studying associations among various infections and infestations indicated that *Mycoplasma* spp. and infestation by fleas were significantly associated ($p = 0.001$).

4. Discussion

Following up the health conditions of the cat population living in the RIOZOO during 3 years allowed the observation of the fluctuations in the frequency of the different infections, clinical alterations, and hematologic characteristics.

Although some cats were infected by *Cytauxzoon* spp., *Mycoplasma* spp., FIV, and FeLV, which are generally known to be associated with anemia (Avery, 2001; Meinkoth, 2001), total erythrocyte counts for most animals remained relatively stable and within

normal range for the species. This finding and the clinically healthy status of the cats suggests that there was an equilibrium between the etiologic agents and the hosts during these investigations. However, the increase in the presence of anemia in the population in 2003 may have been a consequence not only of the number of animals infected by *Mycoplasma* spp., but also of the number of animals infested by fleas, given that both of these parasites can lead to anemia (Moriello, 1994).

The increase in the rate of flea infestation observed in 2003 was reflected in a heavy infestation among the wild animals of the RIOZOO from April to June of that year (Rodrigues, D.P., personal communication. Rio de Janeiro: RIOZOO, 2005), especially because cats tend to remove many of these ectoparasites during grooming (Moriello, 1994). Therefore, finding up to 72.3% of the cats infested indicates heavy infestation of the environment. The association observed between fleas infestation and *Mycoplasma* spp. infection reinforces that fleas are important vectors of this bacterial infection, especially because the pre-patent period of the infection is only a few days and the increase was observed in the same year (Tasker, 2001; Harvey, 2006).

The observation of cats infected by *Cytauxzoon* spp. during the entire study may seem awkward since its known vector *Dermacentor variabilis* (Bondy et al., 2005) is not detected in Brazil (Soares, 2001). It has been suggested that other genera of ticks could also be *Cytauxzoon* spp. vectors (Akucewich et al., 2002) and that there is no direct transmission (Bondy et al., 2005). Thus, the infection suggest the existence of competent vectors in Rio de Janeiro, Brazil, possibly *R. sanguineus*, the only tick species found in these cats.

In 2001, FeLV was not detected in cats from this colony (Mendes-de-Almeida et al., 2004). In 2002, one FeLV-infected animal was detected and from then on the infection spread throughout the colony. In 2004, 39.4% of the population was infected, showing that the infection is easily transmitted from cat to cat. Interestingly, although lice were present in the population since the first year of observation, and as FeLV spread throughout the colony, infestations by lice also spread. As the time went by the social organization of the colony became stronger (Mendes-de-Almeida et al., 2006) and the contact of the individuals intimate, therefore, transmission of both FeLV and lice was facilitated. Lice infestations are generally considered a clinical sign of poor health in cats, although no significant association between these two occurrences could be established. Nevertheless, the fact that the number of FeLV-infected and lice-infested cats

increased at similar rates suggests that FeLV was the pathogen with the highest morbidity potential.

FIV infections are persistent and directly transmissible, mainly through fights (Witt et al., 1989; Yamamoto et al., 1989); therefore, population control programs generally suggest removal of infected cats (Gibson et al., 2002; Richards, 2003). Since the members of the cat population roaming in the RIOZOO were asymptomatic, despite being infected with FIV and other transmissible infections, we decided not to interfere with the population dynamics. Therefore, no animal was euthanized or removed. Thus it seems that, as long as the animals show no clinical signs that justify euthanasia, it should be avoided (Levy et al., 2001). On the other hand, the prevalence of FIV infection increased significantly in the year that a higher migration of animals was observed (Mendes-de-Almeida et al., 2006), and may have been due to greater disputes and fights. Therefore, from a disease control perspective, euthanasia could be a solution when migrations cannot be adequately controlled.

Surprisingly, animals negative for FIV were as susceptible to infections by *Cytauxzoon* spp. and *Mycoplasma* spp. as were FIV-infected cats, suggesting that the immunity status does not significantly alter susceptibility to infection by those hemoparasites. However, immunosupression will most likely predispose animals to become clinically ill due to those infections.

At the beginning of the study, the majority of the cats presented antibodies against *T. gondii* and therefore would not shed oocysts in the feces while they were healthy (Dubey and Lappin, 2006). However, in 2003 and 2004, the proportion of cats carrying antibodies decreased significantly from that in 2002. Considering that 16 new animals migrated into the colony in 2003 (Mendes-de-Almeida et al., 2006), this decline in the proportion of seropositive cats was due to migration of nonreactive animals. However, the fact that three cats seropositive for *T. gondii* in 2002 were seronegative when converted to FIV seropositive does bring about the question as to the shedding status of those three cats after retrovirus infection, since Dubey (1996) has reported that, although rare, cats previously found to be seropositive for *T. gondii* antibodies subsequently resumed shedding of oocysts. Feces were not examined for *T. gondii* oocysts in this study, so it cannot be determined whether these two cats or other new introductions into the colony were shedding. The fact that seronegative animals (cats that would most likely be shedding oocysts) were primarily individuals that recently infiltrated the colony sustains the theory that

methods, such as capture, elimination, or relocation of cats where ''repopulation'' cannot be avoided are not indicated if there may be an increased risk of environmental contamination with *T. gondii*.

Observing the general demeanor of the population, the number of new animals joining the colony decreased over time (Mendes-de-Almeida et al., 2006), and the pathogens included in this study tended to proliferate among the individuals. However, the observed infections did not appear to convert into clinically detectable disease. Thus, it seems that the stabilization of the population verified in the population control program facilitated the transmission of the pathogens without interfering with the well-being of the animals.

5. Conclusion

The prevalence of FeLV increased significantly over the 3 years of the study; however, infected cats showed no clinical signs to justify euthanasia. Prevalence of FIV also increased during the study, but not significantly. These infections did not appear to render animals more susceptible to infections by *Mycoplasma* spp. or *Cytauxzoon* spp. FIV, FeLV, *Mycoplasma* spp. and *Cytauxzoon* spp. infections could not be associated with anemia; however, infestation by fleas was associated with *Mycoplasma* spp. infection. *Cytauxzoon* spp. was a frequently found pathogen in these cats. To avoid the spread of *T. gondii* into the environment, when cat migration cannot be controlled in an area, stabilization of the existing group of cats is indicated. In general, it appeared that stabilization of the population facilitated further distribution of many of the pathogens studied, without any apparent effect on the well-being of the animals.

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