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## Survey of infectious and parasitic diseases in stray cats at the Lisbon Metropolitan Area, Portugal

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A survey of infectious and parasitic diseases of stray cats was carried out using biological samples collected from animals captured during a catch-neuter-release programme in four counties of the Lisbon Metropolitan Area. The main objective was to investigate the potential threat of stray cats for animal and public health. Samples of blood, stool, hair and auricular swabs were collected from 231 cats in 27 colonies. Anti-*Toxoplasma gondii* antibodies were detected in 47/194 samples (24.2%); anti-*Leishmania infantum* antibodies in 1/180 cats (0.6%); intestinal parasites in 23/74 samples (*Toxocara cati*, *Isospora felis*, *Ancylostoma tubaeforme*, *Dipylidium caninum*, *Uncinaria stenocephala*, *Toxascaris leonina*) and *Otodectes cynotis* in 4/182 cats (2.2%); dermatophyte fungi were isolated in 40/136 samples (29.4%); feline immunodeficiency virus antibodies were detected in 23/226 samples (10.2%); feline leukaemia virus antigen in 14/198 samples (7.1%); and feline coronavirus RNA in 9/127 samples (7.1%). Our results revealed that zoonotic agents, namely dermatophyte fungi and *Toxocara cati* were present in stray cat colonies in the investigated counties. Overall the low frequency of major pathogens suggests a balanced relationship between host and agents.

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Stray cat populations may play an important role in the transmission of several pathogenic agents, due to their contact with both domestic cats and humans. The importance of controlling the size of these populations and the most appropriate methods to achieve this purpose, is a controversial issue of concern for municipalities and animal protection associations.

Amongst zoonotic agents transmitted by cats, *Toxoplasma gondii* and *Toxocara cati* are among the most important feline gastrointestinal parasites.<sup>1–3</sup> Additionally, in southern European countries, the cat has been identified as a reservoir for *Leishmania infantum*<sup>4,5</sup> and cats may carry dermatophyte fungi, mostly *Microsporum canis* in their hair coat.<sup>6</sup> Many non-zoonotic infectious agents are also important in cats. Parasites such as *Isospora* and *Otodectes* species cause diarrhoea and otitis. Wild and domestic cats can be infected by viruses such as feline immunodeficiency virus (FIV) and feline leukaemia virus (FeLV). FIV is mainly transmitted by bites<sup>7</sup> and causes an immunodeficiency like disease, similar to Acquired

Immunodeficiency Syndrome (AIDS) in humans.<sup>8</sup> Vertical transmission is also possible.<sup>9</sup> FeLV is mainly transmitted by saliva and respiratory secretions and it also induces an immunocompromising syndrome.<sup>10</sup> Feline coronavirus (FCoV) causes enteric disease but a biotype known as feline infectious peritonitis virus (FIPV)<sup>11</sup> is the aetiological agent of feline infectious peritonitis (FIP), a fatal immune-mediated disease.<sup>12</sup>

This study was undertaken to investigate the frequency of major infectious and parasitic agents in stray cats populations in four counties of the Lisbon Metropolitan Area (map <http://www.aml.pt>), taking advantage of biological samples collected during a catch-neuter-release (CNR) programme carried out by CATUS, a non-profit cat rescue association.

### Materials and methods

#### Sampling and data collection

Biological samples and individual animal data were collected by veterinary surgeons at nine veterinary practices participating on the CNR programme

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between November 2003 and July 2005. Samples of whole blood, serum, faeces, hair and auricular swabs were collected, refrigerated and sent to the Faculty of Veterinary Medicine, Technical University of Lisbon (CIISA-FMV) within 1–5 days. A questionnaire was filled in to record gender, weight, age (estimated by teeth observation), breed, fur colour, social behaviour, frequency of contacts between colonies, diet and clinical examination data including general body condition, respiratory and intestinal signs, skin, mouth and ocular lesions and pregnancy status. Gender, age and the frequency of contacts between cats of different colonies are the host disease determinants assessed in this work.

Data from a total of 231 cats were collected and the following biological samples were processed: whole blood samples ( $n = 224$ ), sera ( $n = 198$ ), blood smears ( $n = 186$ ), hair ( $n = 136$ ), and faecal samples ( $n = 74$ ).

### Data analysis

Descriptive and inferential statistics were performed with Microsoft Excel 2002 and EpiDat 3.1. A *t*-test was used to compare means and a Chi-statistic to compare proportions. A significance level of 5% was used on both tests.

### Gastrointestinal parasites

Detection of gastrointestinal parasites, eggs, larval stages and oocysts was performed in stool by sugar and salt saturated flotation techniques.<sup>13</sup> Parasites were observed by light microscopy and identified according to Thienpont et al<sup>14</sup> and Bowman.<sup>15</sup>

### *Toxoplasma gondii* antibodies

Serum samples were treated with 2-mercaptoetanol and detection of anti-*Toxoplasma gondii* IgG antibodies was accomplished using the kit Toxo Screen DA (BioMérieux, Portugal), based on direct agglutination. Samples were classed positive with titres  $\geq 1/80$ .

### *Leishmania* species antibodies

Anti-*Leishmania infantum* antibodies in serum were detected by immunofluorescence with *Leishmania* Spot IF (BioMérieux, Portugal) using an anti-cat fluorescein-conjugate and a cut-off of 1/40.

### Dermatophyte fungi

All samples were cultured on a selective dermatophyte medium (Mycoline, BioMérieux, Portugal), kept at 27°C for a 21-day period and observed daily for detection of fungi colonies. All dermatophyte colonies were observed under 100 $\times$  light microscopy, after acid lactofucsin 0.025% staining.<sup>16</sup> The dermatophyte identification was done according to Saenz in Pemán et al.<sup>17</sup>

### FIV antibodies

FIV antibodies were detected in plasma by immunoblotting using a previously described technique.<sup>18</sup> Positivity was assumed if the plasma samples reacted against the viral core proteins p55, p24, p15 and p10. The absence of reactivity against p24, but positive against p15 and p10 was considered a doubtful result.

### Detection of FeLV antigen

The presence of FeLV antigen in plasma samples was performed by enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (Virachek FeLV, Synbiotics), according to the manufacturer's instructions.

### Detection of FCoV RNA by reverse transcriptase-polymerase chain reaction (RT-PCR)

Viral RNA was extracted from stool with Qiaamp Viral RNA extraction Kit (Qiagen). The genomic region of the untranslated 3'-UTR was amplified by RT-PCR followed by a nested PCR, yielding a product of 177 bp.<sup>19</sup> The RT-PCR reaction was performed with One-step RT-PCR Kit (Superscript, Invitrogen/Gibco BRL) in a 50  $\mu$ l reaction using 10  $\mu$ l of sample and 50  $\mu$ mol of each primer. For the nested PCR 5  $\mu$ l of the previous reaction was added to 45  $\mu$ l of the PCR mixture, containing 10 mM Tris HCl, pH 7.5, 0.01 mM Ethylenediamine-tetraacetic acid (EDTA), 0.5 mM Dithiothreitol (DTT), 1.5 mM MgCl<sub>2</sub>, 2.5 U Taq DNA Polymerase (Pharmacia), 200 mM each dNTP and 50  $\mu$ mol of each primer. The specificity of the 177 bp amplicons was confirmed by *Dra*I hydrolysis of 20  $\mu$ l of the PCR reaction yielding two products of 35 bp and 142 bp.

## Results

### Sample characterisation

Samples were collected from 27 stray cat colonies located in 4/18 counties that settle the Lisbon Metropolitan Area: Lisboa (15 colonies); Barreiro (four colonies); Cascais (two colonies); Sintra (six colonies). 1,178,101 people live within the 533.2 km<sup>2</sup> of these four counties ([www.aml.pt](http://www.aml.pt)).

Of the total cats sampled ( $n = 231$ ), 148 were females (64.1%) and 70 males (30.3%). Gender was not recorded in 13 cases. The cats' ages were estimated: 155 were classified as 1–5 years old (67.1%), 10 as less than 6 months old (4.3%), 29 as between 7 months and 1 year of age (12.6%) and 18 as older than 5 years (7.8%). Age was not recorded in 19/231 cases (8.2%).

### Parasitological survey

*Toxoplasma gondii* IgG antibodies were detected in 47/194 (24.2%) of the sampled cats while only a single

cat was positive for anti-*Leishmania infantum* antibodies ( $n = 180$ ).

Intestinal parasites were identified in 17/74 samples (23.1%), including *Toxocara cati* (10.8%), *Isospora felis* (5.4%), *Uncinaria stenocephala* (2.7%), *Ancylostoma tubaeforme* (1.4%), *Dipylidium caninum* (1.4%) and *Toxascaris leonina* (1.4%). The proportion of positive cats by age class was 11.8% (<1 year); 76.4% (1–5 years) and 11.8% (>5 years). *Otodectes cynotis* was confirmed in 4/182 cats (2.2%).

### Dermatophytes fungi survey

Forty dermatophyte fungi were isolated out of 136 hair samples collected (29.4%). The identified species were *Microsporum canis* (17/136), *Trichophyton mentagrophytes* var *mentagrophytes* (15/136) and *Trichophyton verrucosum* (8/136).

### Virological survey

FIV, FeLV and FCoV distribution by age group and sex are presented in Table 1. No statistical significance was found in relation to sex or confined/unconfined colonies either for FIV, FeLV or FCoV.

FIV seropositivity was detected in 23/226 of the cats (10.2%). On three cats information about gender or age group was unavailable. Therefore, only 20 FIV cases are reported in Table 1. A doubtful result was observed in 13 samples (5.8%). The county sample seropositivity varied from 17.2% in Barreiro ( $n = 29$ ) to 15.1% in Cascais ( $n = 93$ ) and 5.4% in Lisboa ( $n = 74$ ). None positives were found at the county of Sintra ( $n = 23$ ).

For FeLV antigen, 14/198 (7.1%) samples were positive: 4.2% in Lisbon, 13% in Cascais and 11.1% in Sintra. Only 14 FeLV cases are reported in Table 1 due to missing data regarding gender or age group.

FCoV RNA was detected in 9/127 cats (7.1%) at the counties of Lisbon (10.8%) and Cascais (7.8%). Due to lack of information about gender or age group only six FCoV cases are reported in Table 1.

Out of 119 samples tested for FIV, FeLV and FCoV, 89 were negative to the three agents and there was no sample positive to all three agents. Two were positive to FIV and FeLV and one was positive to FIV and FCoV. FIV alone was found in 13 cats; FeLV in eight and FCoV in seven. No statistical association was observed between FIV occurrence and FeLV or FCoV coinfection.

## Discussion

This study aimed to investigate the presence of infectious and parasitic agents in a stray cats population living in supervised colonies. The sampling organisation, data collection and sample transportation to CIISA-FMV proved difficult and resulted in partial gathering of fundamental data like gender or age group.

Although *Toxoplasma gondii* antibodies were detected in 24.2% of the cats, as no oocysts were observed on coprological examination, the potential for environmental contamination and indirect transmission of the disease seems to be very low.

*Toxocara cati* responsible for larva migrans infection was identified in 10.8% of the sampled cats. Therefore, the risk of human transmission through direct contact with these cats is not negligible.

The proportion of intestinal parasites observed (23.1%) was low in comparison with data published by Miró et al<sup>20</sup> in Spain (32.9%) and considering that deworming was not carried out by CATUS volunteers.

Gastrointestinal parasites were detected in all age groups; none showed a higher proportion of infection. This contradicts previous studies by Martínez-Barbosa et al<sup>21</sup> and may reflect the under representation of the age group older than 5 years old in our study (10.6%).

Cats did not show dermal or systemic forms of leishmaniasis. Only one animal (0.6%) was seropositive for *Leishmania infantum*. This cat was also seropositive for FIV. Yet we estimated an FIV and FeLV prevalence of 10.2% and 7.1%, respectively, and leishmaniasis is endemic on dogs living in the investigated counties.<sup>22</sup> This finding reinforces the view that the cat is a rare host of *Leishmania infantum* and does not pose a serious public health threat. Similar results were published by Martín-Sánchez et al<sup>5</sup> in Spain.

The number of otitis caused by *Otodectes cynotis* was rather low (2.2%) in comparison with the range published by Mueller<sup>23</sup> and Payne et al<sup>24</sup>: 3.5–75%. The high frequency of grooming routines among cats of a colony may contribute to this scenario.

Non-zoonotic parasites were also detected like the protozoa *Isospora rivolta* (5/176) that causes diarrhoea in lactating kittens.

The frequency and the dermatophyte species identified in our study confirm that the cat's hair coat adopts the culture image of its surroundings. This case of the unusual isolation of *Trichophyton verrucosum* in eight cats (5.9%) also reported by Moriello et al<sup>25</sup> in the United States. This agent is frequently incriminated with dermatomycosis in ruminants and horses but not in cats or dogs. A possible explanation for a transitory contamination of cat's hair coat could be contacts with fomites, plants or soil contaminated by hair and scale of infected sheep and goat flocks or recreation horses, as these cases were found in colonies located in suburban counties with rural areas. On the other hand, the isolation rate of *Trichophyton mentagrophytes* var *mentagrophytes* (11.9%) may reflect direct or indirect contact with synantropic animal species with potential for tinea transmission, namely rodents such as rats, as the isolation of this dermatophyte from the hair coat of wild animals and synantropic species is well documented.<sup>26</sup> The isolation of *Microsporum canis* (12.5%) was an expected finding because this dermatophyte is the main agent responsible for feline dermatophytosis.<sup>27</sup>

**Table 1.** Distribution of positive results to FIV, FeLV and FCoV according to sex and age group.

	≤6 m	7–11 m	≥1–5 y	≥5 y	Total
Females tested for FIV	6	14	105	9	134
Females tested for FeLV	6	14	101	10	131
Females tested for FCoV	5	4	63	2	74
Females FIV positive	0	0	10	2	12
Females FeLV positive	2	0	6	1	9
Females FCoV positive	0	0	4	0	4
% Females FIV positive	0	0	9.7	22.2	
% Females FeLV positive	33.3	0	5.9	10	
% Females FCoV positive	0	0	6.3	0	
Males tested for FIV	2	14	42	6	64
Males tested for FeLV	3	14	44	6	67
Males tested for FCoV	2	6	27	3	38
Males FIV positive	0	1	4	3	8
Males FeLV positive	0	2	2	1	5
Males FCoV positive	0	1	1	0	2
% Males FIV positive	0	7.1	9.5	50.0	
% Males FeLV positive	0	14.3	4.5	16.7	
% Males FCoV positive	0	16.7	3.7	0	

As stray cats live in close proximity to man, dermatophytoses are becoming a serious public health problem in Europe, especially in the Mediterranean region where the incidence of *Microsporium canis* infection, particularly in children and immunosuppressed people, has been on a steep increase during recent years. Moreover, all attempts made to eliminate the natural source of infection, mainly represented by stray cats, have failed.<sup>6</sup> For its mitigation, integrated medical and veterinary services, better epidemiological surveillance, more strict rules for animal therapy and CNR programmes to control the population size of free-roaming and feral cats are needed.

Recent FIV prevalence studies have reported highly divergent values. In this study we found a proportion of 10.2% in a stray cat population. Differences in population characteristics may explain the observed differences. In the USA, a study including domestic and stray cats in colonies estimated a prevalence of 2.3%.<sup>28</sup> In Canada, a prevalence of 23% was found in feral cats, 5% in feral colony cats and 5% in domestic cats.<sup>29</sup> In Australia, since 1988–2007, values of 6.7–32% were reported within domestic cats population, including sick and healthy animals, and feral cats.<sup>30</sup> In Japan, Maruyama et al<sup>31</sup> found a prevalence of 9.6%. In Portugal, Fevereiro<sup>32</sup> estimated a 23% prevalence in symptomatic cats of the Lisbon area.

FeLV is responsible for an immunosuppressive disease in domestic cats. Transmission occurs via saliva, through the sharing of food and water containers and grooming. The prevalence ranges from 2.3 to 4.3% in the USA.<sup>28,33</sup> In Japan, Maruyama et al<sup>31</sup> estimated 2.9%. In Europe most of the information available regards to wildcats. In our study, we found a prevalence of 7.1% infected cats. The

proportion of positive samples was almost the double in cats ≤1 year (11.1%) comparing with cats >1 year old (6.5%). This probably reflects the efficacy of FeLV vertical transmission and of the grooming route.

FCoV is transmitted by oro-faecal route and the frequency of carrier cats may be as high as 30%.<sup>34</sup> In our sample ( $n = 127$ ) we found nine positive cats for viral RNA (7.1%). Previous reports for FCoV seropositivity in domestic and stray cat populations<sup>34–36</sup> revealed a much higher prevalence (30%, 34%, 18.3%, respectively). However, the assessment of viral frequency through serological status is not a good marker because it indicates past or present exposure to the virus but not viral shedding. To identify carrier cats, detection of viral RNA is more accurate.<sup>12,37</sup> FCoV enteric infections and FIP caused by a genetic viral variant of FCoV, have high prevalence in multi-cat environments with high frequency of virus shedding and stressful conditions.<sup>38</sup> These environmental disease determinants are mitigated on stray cat populations due to their free range condition that reduces the odds of infection.

Increased interest of CNR programmes by municipalities veterinarians and local Public Health physicians has brought stray cats to the forefront of animal and zoonosis control discussion in Portugal.

It is common for old people of the Lisbon Metropolitan Area to care for stray cats without assuming full ownership of them. This scenario allows for daily direct contacts with these cats during feeding as well as indirect contact through fomites, such as clothes and shoes, with their pet cats. Children may also contact with stray cats at schoolyards and public gardens.

The proportion of infectious and parasitic zoonosis estimated by our study was low. Thus, stray cats living in colonies at the Lisbon Metropolitan Area appear to pose low risk to humans or other animal species including pet cats. However, the very young and the very old are high risk groups for infectious and parasitic diseases due to temporary or permanent immunosuppression.<sup>39</sup> Therefore, they may be infected namely by *Microsporium canis*, *Toxocara cati* and *Toxoplasma gondii* as in this study stray cats seem to act as reservoirs of these agents, highlighting the importance of stray cat populations CNR programmes.

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