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Implications of zoonotic and vector-borne parasites to free-roaming cats in central Spain

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ABSTRACT

Cats are definitive hosts and reservoirs for several parasites, some of which are responsible for serious zoonotic diseases. We conducted a case-control study of data from a trap-neuter-return (TNR) programme (years 2014–2017) designed to examine the prevalence of zoonotic parasites in free-roaming cats living in urban areas of central Spain. In the animal population tested (n = 263), we detected a 29.2% prevalence of endoparasites, including high rates of cestodes (12.9%) and *Toxocara cati* (11.7%). While faecal samples showed no *Toxoplasma gondii* oocysts, the seroprevalence of *T. gondii* infection was 24.2%. Antibodies to *Leishmania infantum* were detected in 4.8% of the animals, though all skin and blood samples analyzed were PCR negative for this parasite. Ectoparasites (ticks and fleas) were found in 4.6% of the cat population, and 10.6% of the cats were detected with *Otodectes cynotis*. Finally, 6.3% and 7.9% cats tested positive for feline leukaemia virus and feline immunodeficiency virus, respectively. Our study provides useful information for animal-welfare and public-health, as the parasites detected can affect native wild animals through predation, competition and disease transmission. Our detection of zoonotic parasites such as *L. infantum, T. gondii, T. cati, Giardia duodenalis* and several ectoparasites prompts an urgent need for health control measures in stray cats.

1. Introduction

Cats are definitive hosts to a large number of parasites, some of which cause important zoonoses like the larva *migrans* syndromes (toxocariosis and ancylostomatidosis), toxoplamosis and giardiosis. Besides intestinal parasites, cats are also reservoirs for other vector-borne zoonotic diseases like *Leishmania infantum* infection in endemic regions (Day, 2011; Pennisi et al., 2015) or, as recently shown, *Bartonella* spp. and *Ricketsia* spp. infections (Case et al., 2006; Day, 2011; Ayllón et al., 2012; Diakou et al., 2017).

Stray animals may have significant impacts on public health due to factors such as a lack of preventive measures (e.g. vaccines, deworming), easy access to intermediate hosts (e.g. rats and birds), and unrestricted entry to public areas such as parks and playgrounds. This means that the presence of free-roaming animals is a major risk for the transmission of zoonotic diseases (Otranto et al., 2017a).

Feline colonies are stable groups of free-roaming cats living outdoors in public or private urban areas with access to sources of food. These colonies can be intentionally nourished by people or maintained by human waste (Centonze and Levy, 2002).

A healthy female cat is capable of producing several offspring during her lifespan, which leads to the rapid exponential growth of free-roaming cat populations if there are no control interventions. Overpopulation is detrimental to the animals themselves, poses a risk to public and environmental health, and generates numerous inconveniences in urban areas such as excessive noise and car accidents (Baker and Harris, 2007). Many strategies have been developed and implemented for population control of stray and feral cats including 'trap-neuter-return' (TNR) and 'trap and euthanize' (TE) control programmes (Scott et al., 2002; Schmidt et al., 2009).

Trap-neuter-return interventions consist of trapping the animals, providing them with veterinary care and sterilization, and returning them to the site of capture (Gibson et al., 2002; Levy and Crawford, 2004; Schmidt et al., 2009). The goals of a TNR programme are to reduce or maintain population size, improve the condition and health of cats and lengthen their lifespan. Such initiatives are considered an effective and ethical approach to the population and health control of cat colonies, and are especially effective if other measures are implemented

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in parallel such as the adoption of socialized cats and campaigns to discourage abandonment (Boone, 2015). Controlling cat colonies has a direct impact on public health by diminishing health risks and transmission of zoonotic diseases (Baker and Harris, 2007; Murray et al., 2015). The risks of overpopulation include food and territory conflicts among cats, which promotes the transmission of feline retroviruses through direct contact, scratches or bites (Finkler et al., 2011; Murray et al., 2015).

Trap and euthanize control programmes, besides raising ethical issues, have proven ineffective because of the vacuum effect. This means that if all animals in a given area are wiped out, resource space becomes available, which attracts immigrant or abandoned cats to the same area. Further, the presence of cats is important to ensure an ecological balance, as they are predators of small mammals like birds, rodents and reptiles (Boone, 2015). It is thus essential that veterinarians participate in monitoring feline colonies, and efforts should be made to design and implement appropriate population control methods. Such measures need to consider population biology and economic resources, which is often a limiting factor for TNR programmes (Boone, 2015).

The present study sought to determine the prevalences of endoparasites of zoonotic interest, *L. infantum* and *Toxoplasma gondii* in free-roaming cats living in colonies in the central region of mainland Spain, and to assess the impacts of a TNR programme on these prevalences.

2. Material and methods

2.1. Study population

From 2014–2017, the Animal Protection Society ALBA in Madrid has been undertaking a health control programme for free-roaming cats living in colonies. So far, a large number of cats have been captured, neutered and returned to their site of capture (Griffin et al., 2016). Apart from the TNR programme, animals were tested for feline retroviruses and other pathogens, dewormed and treated, if needed, to ensure that only healthy animals are returned. Treatment after sterilization surgery consists of a topical combination of fipronil, (*S*)-methoprene, eprinomectin and praziquantel (Broadline®, Merial) and a non-steroidal anti-inflammatory drug (meloxicam) plus a broad spectrum antibiotic (amoxicillin/clavulanic acid). All neutered cats are also ear-tipped according to standard recommendations for their subsequent identification (Griffin et al., 2016).

2.2. Sedation and sampling

After sedation (with 80 µg of medetomidine/kg plus 5 mg ketamine/kg), each animal was subject to a thorough physical examination by clinical veterinarians to record age, sex, clinical signs and place of capture, and to collect blood and tissue samples. Blood samples were obtained (for whole blood in EDTA or serum) by jugular venipuncture. Other samples collected were ear swabs, ear tip and skin scrapings, if there were skin lesions, and faeces. These last samples were obtained directly from the rectum using a swab or from the cages where the cats were housed. Samples were kept at 4 °C until processing within 24 h at the laboratory.

2.3. Toxoplasma gondii infection

Antibodies against *T. gondii* were measured in serum using a direct agglutination test (DAT) kit (Toxo-Screen DA; Biomerieux) as described by Desmonts and Remington (1980). We considered an antibody titre of 1:40 to indicate a cat had been exposed to *T. gondii* (Desmonts and Remington, 1980).

2.4. Leishmania infection

2.4.1. Serological diagnosis

For serological tests, specific antibodies to *L. infantum* were detecting using an indirect immunofluorescence antibody test (IFAT) against in-house cultured promastigotes. This test for anti-*Leishmania*-specific immunoglobulin G (IgG) antibodies was performed as described previously using a cut-off ≥ 1:100 to define seropositivity (Ayllon et al., 2008).

2.4.2. Molecular diagnosis

The QIAamp® DNA Micro Kit (50) (QIAGEN®) was used to obtain DNA from blood (100 µl) and ear skin samples according to the manufacturer's instructions. Extracted DNA was eluted in sterilized water (70 µl) and stored at −20 °C until use. A 5 µl aliquot of eluted DNA was used for each polymerase chain reaction (PCR) for *Leishmania* detection and species identification.

Leishmania DNA detection was performed by two PCR methods targeting internal transcribed spacers 1 and 2 (ITS-1 and ITS-2) using the primer pairs LITSR (5′-CTGGATCATTTTCCGATG-3′)/L5.8S (5′-TGATACCACTTATCGCACTT-3′) and L5.8SR (5′-AAGTGCGATAAGTG GTA-3′)/LITSV(5′-ACACTCAGGTCTGTAAAC-3′) as described by Kuhls et al. (2005). The PCR amplification product size was 280–330 bp.

2.5. Enteric parasites

Faeces samples were tested for oocysts, cysts, eggs and larvae of enteric parasites using the modified FLOTAC method plus merthiolate-iodine-formalin staining and Baermann-Wetzel methods followed by examination under a light microscope (Thienpont et al., 1979; Cringoli et al., 2010).

In addition, we used a qualitative immunochromatographic (ICT) commercial strip assay for the rapid simultaneous detection of *Cryptosporidium* and/or *Giardia* (Stick Crypto-Giardia®; Operon, Zaragoza, Spain) on all stool samples. Tests were conducted at room temperature according to the manufacturer's instructions.

2.6. Ectoparasites

Ear swabs were examined under the microscope to determine the presence of ectoparasites such as *Otodectes cynotis* in secretions.

Whole skin was examined to check for skin lesions and ectoparasites. Any parasites found were stored in ethanol 70° until their identification under the microscope using identification keys (Krämer and Mencke, 2001; Bowman, 2002).

2.7. Retrovirus infection

All cats were tested for feline leukaemia virus (FeLV) antigen and antibodies to feline immunodeficiency virus (FIV) using a commercial ELISA kit (PetChek* FIV/FeLV; IDEXX Laboratories) (Tonelli, 1991).

2.8. Ethics

The study was carried out in accordance with Spanish legislation guidelines and with the International Guiding Principles for Biomedical Research Involving Animals issued by the Council for International Medical Science Organizations.

2.9. Statistical analysis

Association between all the variables examined were identified by the Chi-square test (SPSS 17.0). Significance was set at $p < 0.05$.