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Prevalence of Gastrointestinal Protozoa in Feral Cat Population in Qatar

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Introduction

Doha city has a high feral cat population that is estimated to outnumber its human inhabitants by 2–3:1 with a total population of 2–3 million cats according to Qatar Cat Control Unit (QCCU). Doha had a significant rodent problem for decades as in many cities throughout the world. It was difficult to control this huge rodent number. Therefore, cats were introduced in the 1960s, but without any consideration of the possible knock-on effects to human health. Introduced cats have colonized and reproduce rapidly around food and water resources in both urban and rural areas. It is known that cats are natural host for a wide range of helminths and protozoa. Since there were no plans to eliminate cats after they have been introduced to the country, the density of cats increased in an uncontrollable manner. This high cat population has an obvious risk for human and different diseases such as toxoplasmosis would be expected. Hospital records show that human toxoplasmosis is quite widespread in the city, with up to 35% of women of childbearing age being reported to be seropositive, and 41% of the elderly persons of both sexes in the population. These findings highlight the role cats might be playing in the transmission of protozoa in the society. Cats are also hosts to other closely related species of intestinal protozoa. For example, cats can harbour *Isoospora* spp., *Cryptosporidium felis*, *Giardia intestinalis* and *Blastocystis* spp. Given the high density of cats in the city, it is clearly important to assess the prevalence of protozoal infections among these animals as a first step towards achieving a better understanding of their role in the transmission of human infectious disease of feline origin eventually.

Objectives

Since Doha has a high feral cat population, there is a need to understand the role of cats as vectors of human protozoal infections. Our preliminary data indicate that *Blastocystis* spp. and *Toxoplasma gondii* is highly prevalent among the residents of Doha. In this project, it was proposed to estimate the prevalence of gastrointestinal protozoa

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including *Giardia intestinalis*, *Cryptosporidium parvum* and *Blastocystis hominis* in the feral cat population in Doha. A total of 264 fecal samples will be collected from feral cat population from different geographical locations of Doha. Advanced technologies will be used including DNA extraction, RT-PCR and sequencing to provide an accurate assessment of the prevalence.

Methodology Study area and Population

Fresh stool samples were collected from cats in different areas in Qatar. In this study, 37 areas were divided into two geographical regions: outside Doha and inside Doha based on occupation of people. Cats were trapped during the winter (November–April) and the summer (May–October) seasons of 2015. Traps were prepared with fish heads or canned cat food. Cats were retrieved from traps and assessed for sterilization status. Pregnant, lactating female cats and cats estimated to be less than 6 months old were immediately released. Cats older than 6 months were eligible for the study. In order to prevent any repetition and re-sampling of cats already and treated earlier, cat's ear will be tagged with a small metal tag. All project ethical approvals were obtained before the beginning of the project. Samples Collection Fresh stool samples were collected from sterilized cats during the period from February–September 2015 and stored at -20°C by veterinary laboratory of stray cat control unit in Ministry of environment (department of animal resources). A total of 264 samples were processed in order to achieve the aim of this project. Samples were collected in sterile containers labeled with site, where the cat is found, gender and date of collection. The samples were kept and transported on ice and frozen directly after the sample collected. Fecal Examination In order to extract the DNA of the enteric pathogens samples were warmed at 4°C and approximately 200 mg of the stool sample were used for examination. QIAGEN miniamp stool kit was used to extract DNA from the sample following manufacturer's instructions with minor modifications. Lyses buffer ASL was added and mixed with each stool sample. Since cat stool is hard to break, tissue rupture machine was used to ensure the homogenization of the sample and increase DNA recovery. This is followed by vortexing the samples and incubating them at 95°C for 10 minutes to insure complete lysis. After lysis, samples were centrifuged for 10 minutes at 4500 rpm in order to separate and pellet the stool particles. After that, supernatant were placed in new microcentrifuge tubes. Using InhibitEx binding reagent DNA-degrading substances and PCR-inhibitors were separated and removed from the sample. The DNA InhibitEx matrix was centrifugated twice at 14,000 rpm for 3 minutes to pellet the stool and any impurities. 15 μL of proteinase K, 200 μL of the supernatant and 200 μL of the buffer AL were all added to new microcentrifuge and incubated at 70°C for 10 minutes. Proteinase K is used to digest protein and remove contamination and inactivates nucleases which degrade the DNA during the purification process. However, in order to make proteinase K work high temperature is needed to denature proteins. Therefore, samples are incubated at 70°C for 10 minutes. Supernatant part containing DNA was then transferred to a Qiagen Minispin column. Two different washing buffers with optimized pH and salt concentration were added to eliminate the digested proteins and any other impurities. Samples were centrifuged at 14,000 rpm before the addition of each buffer. Finally using AE buffer the DNA was eluted. DNA concentration was measured using Nanodrop (Thermo Fisher Scientific, USA). Primers and Probes Using primer designing software, the primer and probe sets used for detecting parasitic pathogens were designed based on data available in National Center for Biotechnology Information (NCBI) databases. Targeted genes were chosen based on published data and studies describing their sequences, uniqueness, and conservation. Real-time PCR Samples were analyzed by uniplex real-time PCR using Applied Biosystems Cyclor 7500. Protocols were finalized after adjustment of the respective concentrations of primers, probes and the evaluation of several cycling protocols. A proposed protocol based on available literature was a starting point. Two different fluorescence reporter dyes were used in Real-time PCR. SYBR Green was used for *Blastocystis hominis* and TaqMan for other targeted parasites. For both fluorescence reporter dyes amplification reactions were performed in a total 20 μL volume in each well with 17.5 μL master mix and 2.5 μL DNA template. For each plate prepared positive controls consisted of internal controls provided by Hamad Medical Corporation (HMC). Both positive and negative controls were run for each sample. Sample PCR results were compared with both controls and analyzed using 7500 software v2.3.

Results

A total of 264 of stray cat samples were trapped for examination of enteric parasite. The samples were classified according to their gender, area and season. Table (1) summarized the frequency of the cat population examined. Three protozoal parasites (*Giardia intestinalis*, *Cryptosporidium parvum* and *Blastocystis hominis*) were examined using real-time PCR. According to PCR results obtained previously, *Giardia intestinalis* was the only protozoa positively detected. Table (2) shows the prevalence of examined protozoa in cat samples. Figure 1 and figure 2 show the interaction between *Giardia intestinalis* infection and other independent variables (gender, season and area). Table 1. Number of stray cats examined by season, gender and study site from Qatar during 2015 Season Winter Summer Male Female Male Female Site N N N N Outside Doha 44 (16.67%) 39 (14.77%) 25 (9.47%) 23 (8.71%) Inside Doha 34 (12.88%) 20 (7.58%) 37 (14.01%) 42 (15.91%) Total 78 59 62 65 *N, number of samples; the number in brackets indicates the percentage of prevalence. Table 2. Number of subjects in each category and the prevalence (%) of the three species of protozoa by gender, season and area

Number of subjects	<i>Giardia intestinalis</i>			<i>Cryptosporidium parvum</i>			<i>Blastocystis hominis</i>		
	Male	Female	P	Male	Female	P	Male	Female	P
Gender	140	124	0.443 (NS)	5	7.2	0.443 (NS)	0	0	0.443 (NS)
Season	137	127	0.719 (NS)	6.5	5.5	0.719 (NS)	0	0	0.719 (NS)
Area	131	133	0.628 (NS)	5.34	6.77	0.628 (NS)	0	0	0.628 (NS)

*NS: not significant
****The highest prevalence in each category is in bold italics for emphasis.** Benefits to Qatar This study will provide important data for the public healthcare, which they can exploit to determine the role feral cat might be playing in zoonotic diseases in Doha. The training in research methodologies will also foster the interest in research the undergraduate students have and therefore add to the pool of qualified researchers in Qatar.