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Prevalence And Risk Factors Of Toxoplasma-Like And Intestinal Parasites In Cats From Urbanized Area Of Tatarstan, Russia.

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ABSTRACT

This work's objective was to evaluate epidemiologic indicators of the prevalence of Toxoplasma-like endamebas and intestinal parasites in cats in the city of Kazan (Tatarstan, the Russian Federation). Cats' fecal samples (n=148) were examined by the flotation method in the saturated sucrose solution with further microscopy. The general prevalence of the mentioned parasites equaled 17.57%. In the course of estimation of morphometric indicators of the data obtained, the eggs of the following intestinal parasites were found in the examined fecal samples: *Toxocara cati* (4.73%), *Taenia taeniaeformis* (4.05%), *Capillaria aerophila* (3.38%), *Toxoscarisleonina* (3.38%), as well as the oocysts of Toxoplasma-like parasites (3.38%). In three districts of Kazan, an increased risk (OD 5.9-8.1; RR 4.7-6.0)of dissemination of the mentioned parasites in cats ($P<0.05$) was proven to take place. A multidimensional factorial analysis demonstrated the absence of correlation between the risk associated with Toxoplasma-like and intestinal parasites and the sex, age, breed and housing of the cats ($P>0.05$).

Keywords: cat; feces; flotation; oocyst; Toxoplasma; contamination.

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INTRODUCTION

An important role in the dissemination of zoonotic diseases in urbanized areas is played by domestic and synanthropic animals [1]. Soil-inhabiting protozoa may become a reservoir of pathogenic and non-pathogenic microorganisms and a potential source of their dissemination [2, 3]. The sources of soil contamination by the protozoa oocysts and helminth eggs may be represented by various species of domesticated and wild mammals [4]. At the same time, some of them may cause the diseases that are common to both humans and animals, for instance, protist Toxoplasma gondii is an etiological factor of toxoplasmosis.

Cats are considered the main hosts in the transmission of T. gondii because of their direct contact with humans [5]. The primary infecting of a cat is associated with an extraction of millions of oocysts through feces, which leads to a massive contamination of environmental objects and increases the risk of infections among animals and people [6]. According to the data obtained by the researchers, toxoplasmosis screening of domestic animals allowed detecting antibodies to T. gondii in 40.4% of domestic cats and 80.0% of adult stray cats [7]. Some strains of the mentioned parasites circulate in nature, omitting their definitive host. They exist without passing an intestinal phase of development in cats' organisms, proving their facultative heterogeneity.

According to the data provided by Food and Agriculture Organization of the United Nations (FAO), World Organization for Animal Health (OIE), and World Health Organization (WHO), toxoplasmosis is an important problem that exists in humanitarian medicine of all the countries in the world. The prevalence of T. gondii among the population in the Russian Federation reaches 31% [7], and depending on the food culture established in various countries, its prevalence varies from 4 to 95% [8]. The objective of this work was to evaluate the epidemiologic indices of the prevalence levels of Toxoplasma-like parasites in cats in the city of Kazan (Tatarstan, the Russian Federation).

MATERIALS AND METHODS

The object of the research was represented by the cats' fecal samples collected in Kazan city. The subject of the research was represented by the eggs and oocysts of intestinal parasites.

Cat feces sampling

The samples of the feces (n=148) were taken from the domestic (those that were kept at houses and those that had a free access to the outside environment) and stray cats, which belonged to various gender and age categories and lived in various districts of Kazan. In the case of domestic cats, the samples were taken out of litter trays after the defecation, and in the case of stray cats, the samples were taken from the places of their inhabitation. The geographic location of the places of point samples' collection and coordinates were recorded with the help of 2GIS mobile application (Figure 1).

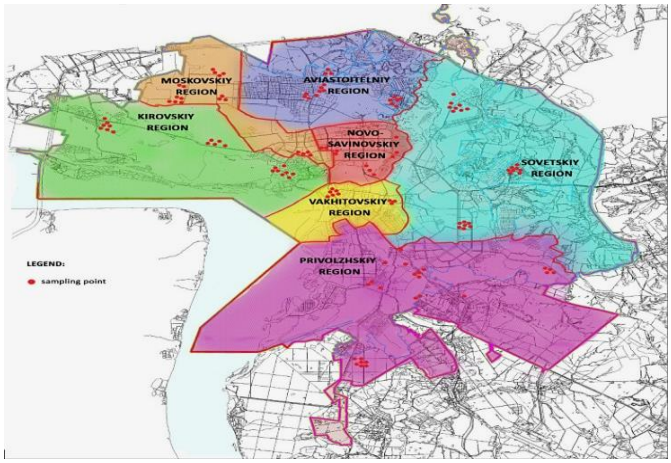

Figure 1: The map of sampling points, where cats' feces were taken in Kazan

Flotation and microscopy

The extraction of oocysts out of the obtained samples was performed by means of flotation in the saturated sucrose solution according to the previously described method [9]. In order to divide the large particles, the obtained feces' samples were sifted through testing sieves that had a diameter of 15 cm and the meshes with the size of not more than 50 mesh (0.3 mm) and not less than 100 mesh (0.15 mm). The particle settling in the working solution was performed by means of centrifuging at 3000 g during 10 minutes; after that, the supernatant was separated, and the residual matter was resuspended in 5 ml of sucrose solution with the relative density of 1.2 N/m^3. The suspension was again centrifuged at 3000 g during 10 minutes, after which supernatant was moved to a separate tube and diluted with distilled water at the ratio 1:10. At the final stage, the samples were centrifuged at the same conditions. The centrifuge 5804R (Eppendorf) with a swinging bucket rotor A-4-44 was used for the work.

Undiluted residual matter, which was obtained after the flotation, was further used for micro-copying: a sample with a volume of 30 mcl was put onto the object plate and examined at the 60-fold increase. For the visualization of oocysts, a digital USB microscope DigiMicro Prof (DigiMicro, China), was used, which has an installed software MicroCapture Pro Version 2.2. For the purposes of microscopic examinations, a microscope Biomed-3 (BioMed Service, Russia) trinocular was used along with the visualization adapter "Digital Camera for Microscope DCM310 (USB2.0)" and "ScopePhoto 3.0" software.

The fact of parasites' belonging to specific species was defined based on morphometric indices, which were compared to reference materials and parasitological atlas [10, 11].

Sporulation of the oocysts

After the final centrifuging stage, each sludge was preliminarily evaluated for the presence of valuable fractions with an increased content of parasites' eggs and oocysts. In the average, 1 to 3 fractions were obtained in one tube. The fractions were sequentially put into separate Petri dishes with the addition of sulfuric acid solution (final concentration of 2%) in the way that the height of the liquid in the dish did not exceed 5 mm. Petri dishes with oocysts were left at the room temperature for 7 days in order to activate the process of oocysts' sporulation. After that, one drop of liquid was taken from each fraction for the aims of further microscopy.

Statistical analysis

The calculation of prevalence with 95% confidence intervals (95% CI) was performed with the help of online VassarStats software using the Chi-square test. Relative risk (RR) and odds ratio (OR) with 95% CI, Z-statistic and associated P-value were calculated using MedCalc statistical software. The analysis was performed with the consideration of the following independent variables: age (under 7 months, 7 months to 2 years, 2-6 years, 7-10 years, above 10 years), sex, breed (pureblooded, not pureblooded), the district of inhabitation (Kirovskiy, Moskovskiy, Sovetskiy, Privolzhskiy, Aviastroitelniy, Novo-Savinovskiy, Vakhitovskiy), and housing conditions (cats that were kept at houses, those that had a free access to the outside environment, and stray cats). The differences in the groups were considered statistically reliable at the values of $P < 0.05$.

RESULTS

In the course of flotation of cats' feces samples with further microscopy, helminth eggs and oocysts were detected, which had the diameter between 10 µm and 90 µm (Figure 2), which is connected with the parasites' belonging to a specific species. Considering morphometric indices of the data obtained, it was proved that the examined samples contained the eggs of Toxocara cati, Taenia taeniaeformis, Capillaria aerophila, Toxoscarisleonina, as well as the oocysts of Toxoplasma-like protozoa.

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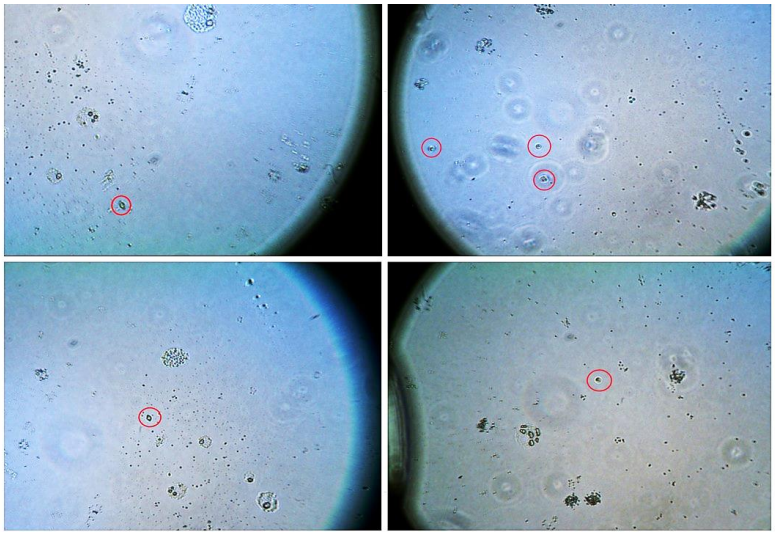

Figure 2: Representational illustrations of helminth eggs and oocysts of Toxoplasma-like parasites, obtained in the course of the microscopy of cats' feces sludge (X40)

The general prevalence of the parasites in cats equaled 17.57% (Table 1). The prevalence of particular species varied from 3.38 to 4.73%, and the most widespread invasion was T. cati. Toxoplasma-like oocysts were found in 3.38% of cases. In two cases, a mixed invasion was observed: in the first case, it involved C. aerophila и T. taeniaeformis, and in the second case – T. catiи T. leonine. In the course of sludge incubation of cats' feces in sulfuric acid solution, a sporulated oocyst Toxoplasma gondii was found.

Table 1: The prevalence of intestinal parasites in cats in Kazan

The type of the parasite	The number of positive samples	Prevalence, %	95% CI
C. aerophila	5	3.38	1.25-8.12
T. cati	7	4.73	2.09-9.87
T. taeniaeformis	6	4.05	1.66-9.00
T.leonina	5	3.38	1.25-8.12
Toxoplasma-like	5	3.38	1.25-8.12
Overall	26	17.57	12.00-24.87

The prevailing independent variable of cats' parasites was the district of inhabitation (Table 2). In the course of the analysis of the infection level among the cats of various Kazan's districts, the allocation of the parasitic diseases was as follows (the number of animals with detected intestinal parasites / general amount of the examined animals): Kirovskiy district 4/24, Moskovskiy district 1/18, Sovetskiy district 1/23, Aviastroitelniy district 5/17, Privolzhskiy district 4/19,Novo-Savinovskiy district 6/26, Vakhitovskiy district 5/21. It was established that the statistically high risk of the infecting can be attributed to Aviastroitelniy(OR = 8.13), 1/18, Sovetskiy district 1/23, Aviastroitelniy district 5/17, Privolzhskiy district 4/19, Novo-Savinovskiy (OR = 5.85) and Vakhitovskiy (OR = 6.09) districts of the city of Kazan ($P < 0.05$).

Table 2: Multivariate analysis of risks factors associated with intestinal parasites in cats from Kazan

Variable	Risk factor	OR	95% CI	Z-statistic	P value
The district of inhabitation	Kirovskiy	3.90	0.66-23.15	1.50	0.134
	Moskovskiy	1.15	0.10-13.52	0.11	0.913
	Sovetskiy	0.89	0.08-10.34	0.10	0.923
	Privolzhskiy	5.20	0.86-31.42	1.80	0.072
	Aviastroitelniy	8.13	1.39-47.36	2.33	0.019*
	Novo-Savinovskiy	5.85	1.08-31.66	2.05	0.040*
	Vakhitovskiy	6.09	1.07-34.72	2.04	0.042*

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Breed	Pureblood	1.00			
	Not pureblood	0.63	0.21-1.88	0.83	0.408
Sex	Female	1.00			
	Male	0.76	0.31-1.85	0.60	0.548
Age	< 6 months	1.08	0.19-6.26	0.08	0.934
	6 months -2 years	0.94	0.17-5.04	0.08	0.939
	2-6 years	0.59	0.10-3.44	0.59	0.553
	> 6 years	1.00			
Housing	Domestic	1.00			
	Free access to the outside	1.71	0.63-4.66	1.05	0.292
	Stray	0.70	0.23-2.14	0.62	0.535

OR – odds ratio; 95% CI – confidence interval; * – significant difference (P < 0.05).

While analyzing the breed factor, invasions were detected in 5/23 of pureblood and 21/141 of stray cats. When the cats were divided according to sex factor, it was stated that the parasites were found in 17/89 of females and 9/59 males. As for the age index, the level of infection dissemination was the following: < 0.5 years (7/33), 0.5 – 2 years (11/58), 2 – 6 years (6/47), > 6 years (2/10). In terms of housing conditions, there was no obvious difference between the various groups, and the values were the following: domestic cats (13/77), domestic cats with a free access to the outside environment (8/31), stray cats (5/40). No statistically significant influence of breed, sex, age and housing on the risk of infecting with intestinal parasites was detected (P > 0.05).

The evaluation of the RR value proved the low level of risk in Moskovskiy and Sovetskiy districts of Kazan (Figure 3). Statistically significant and high rates were detected in Aviastroitelniy district RR = 6.03 (1.29-28.11), Novo-Savinovskiy district RR = 4.73 (1.03-21.70) and Vakhitovskiy districts RR = 4.88 (1.03-23.07), which proves the high risk level of infectious dissemination of intestinal parasites in cats in these districts (P < 0.05).

Figure 3: The allocation of a relative risk (RR) of dissemination of intestinal infections in cats as per the districts of Kazan

DISCUSSION

The general prevalence of intestinal parasites in the examined cats' population in the city of Kazan equaled 17.57%. In the course of analysis of the factors that potentially influence the development, reproduction,and circulation of the parasites, it was established that the prevailing predictor of cats' parasites was the area of habitation, and statistically significant high risk of infectious dissemination is typical for three districts of the city (P<0.05). The breed, sex, age and housing of the examined cats did not demonstrate any statistically significant influence on the risk of infecting (P>0.05).

In spite of the fact that the probability of infecting among the stray cats is relatively high, the OR value among them was lower than the one among the domestic ones. This fact may be connected with the vertical transmission of parasites or infecting of the kittens through their mothers during the first days after the birth, and, as a consequence, after the development of the disease in the early stages of development of the host organism, no eggs or oocysts of the parasites are found in the feces of adult species.

At the same time, the reproduction of the parasites in the host's organism may be slowed down by the host's defense mechanisms or other factors.

According to the data obtained by the researchers of the International Association for Ecology and Health, adult cat species that were held domestically are more likely to be infected with bio-invasions than the younger ones [14]. The quality and the means of nutrition also influence the dissemination of the helminth eggs and oocysts of Protista. The highest level of parasites' dissemination can be observed in those populations that are mainly fed by the prey they hunt. In such groups of animals, the chance of invasions' dissemination is much higher than in the cats that live domestically [15]. The difference in the data obtained by us and by the foreign researches is, in our opinion, represented by the contamination of environment objects with parasites' eggs and oocysts and predomination of a vertical transmission of pathogens.

The technical aspect of the sample preparation causes a negative influence on the coefficient of the extraction of parasites' eggs and oocysts from the samples. For example, the concentration-filtration stage in most cases leads to a significant loss of the number of parasites' oocysts and eggs [16]. Microscopy as a final stage in the majority of the standard methods may also cause fluctuations associated with human subjectivity and experience [17]. The abovementioned factors do not mean that a parasite organism is not able to reproduce and lead to the death of its host and pose a zoonotic risk [18].

CONCLUSION

The general prevalence of the parasites among the cats' population in the city of Kazan equaled 17.57%. In the examined samples, the eggs of T. cati (4.73%), T. taeniaeformis (4.05%), C. aerophila (3.38%), and T. leonina (3.38%) were detected, as well as the oocysts of Toxoplasma-like parasites. In three districts of Kazan a high risk (OD 5.9-8.1; RR 4.7-6.0) of dissemination of the mentioned cats' parasites was determined ($P<0.05$). A multidimensional factor analysis showed the absence of correlation between Toxoplasma-like risk and intestinal parasites and such indices as sex, age, breed and housing of the cats ($P>0.05$).

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