Seroprevalence of Toxoplasma gondii *in household and stray cats of Riyadh, Saudi Arabia*

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Summary
This study was undertaken to determine the seroprevalence of *Toxoplasma gondii* in cats in the area of Riyadh, Saudi Arabia. We examined 200 serum samples collected from stray and household cats for *T. gondii* antibodies by ELISA. The overall seroprevalence was 26%. Seroprevalence was significantly higher ($p < 0.05$) in stray cats (39%) compared to household cats (13%). The prevalence in male and female cats was 31.4% and 20.4%, respectively. The seroprevalence increased with age and was higher in cats over 6 years of age (43%) as opposed to cats less than 4 years old (33%). Seropositivity varied according to the breed. The highest was recorded among cats of American breed (38.5%), followed by Persian (27%), Himalayan (21%), Bengali (11.5%), and Siamese (2%). Antibodies were not reported from the Turkish breed. Overall seroprevalence among cats did not vary significantly with season or with the localities within the Riyadh municipality. We also examined 100 faecal samples from stray and household cats by flotation technique, which revealed an overall prevalence of 12% of *T. gondii* oocycts.

Sieroprevalenza di Toxoplasma gondii *in gatti domestici e randagi, Riyadh, Arabia Saudita*

Parole chiave
Arabia Saudita,
Gatti,
Sieroprevalenza,
Toxoplasmosi.

Riassunto
Lo scopo dello studio è stato quello di determinare la sieroprevalenza di *Toxoplasma gondii* in 200 campioni di siero prelevati da gatti domestici e randagi nella città di Riyadh (Arabia Saudita). La sieroprevalenza complessiva è risultata pari al 26%; la prevalenza di anticorpi contro *T. gondii* è risultata significativamente più alta ($p < 0,05$) nei gatti randagi (39%) rispetto ai gatti domestici (13%). La prevalenza nei gatti maschi è stata del 31,4%, mentre nelle femmine del 20,4% ed è risultata più alta nei gatti di età superiore ai 6 anni (43%) rispetto ai gatti di età inferiore a 4 anni (33%). La sieropositività più alta è stata riscontrata nei gatti di razza americana (38,5%), seguita da quelli di razza persiana (27%), himalayana (21%), bengalese (11,5%) e siamese (2%). Non sono stati riscontrati anticorpi nei gatti di razza turca. La prevalenza non ha mostrato significative variazioni stagionali né territoriali tra le aree all'interno della municipalità di Riyadh. La flottazione dei campioni fecali ha rivelato una prevalenza complessiva di oocisti di *T. gondii* pari al 12%.

Introduction

Toxoplasma gondii is one of the most common protozoan infecting a wide range of animals, and humans (Dubey and Beattie 1988). The infection causes considerable economic losses in the sheep industry. Cats play an important role in the epidemiology of *T. gondii* in humans and animals.

Cats and other felidae, definitive hosts of *T. gondii*, are the only animals that pass oocysts in the faeces; although as intermediate hosts they can harbour infectious tissue cysts. Most feline infections occur post-natally through the ingestion of infected tissue cysts or rarely oocysts, although congenital infection can also occur (Dubey and Jones 2008). Feline infections are typically subclinical; congenitally infected kittens are the most likely to have clinical signs of infection, but previously clinically healthy adult cats may also be affected (Vollaire *et al.* 2005, Dubey and Jones 2008). Common symptoms of *T. gondii* infection in cats include fever, ocular inflammation, anorexia, lethargy, myosyitis, abdominal discomfort, and neurologic abnormalities (Vollaire *et al.* 2005).

Most cats only shed oocysts once in their lives, following infection. However following experimental infection and immunosuppression, repeated shedding occurred in kittens 20-21 days following the immunosuppressive event. In addition, oocysts shedding was induced in a non-immunosuppressed kitten following a second inoculation with infected mouse brain homogenate (Malmasi *et al.* 2009).

Materials and methods

Origin of animals

The main source of household cats in this study was a private pet clinic located in Riyadh, Saudi Arabia. Cats were presented either with clinical signs that warranted veterinary care or for routine general health checks and vaccination. Some cats were admitted for minor veterinary interventions such as spaying. Information detailing age, sex, and area originated from individual cat registration cards. Cats (100) belonged to 6 breeds including Persian (27), Himalayan (22), American (19), Turkish (14), Siamese (12), and Bengali (6).

Stray cats (100) wandering in the streets, parks, and nearby slaughterhouses, restaurants, and residential compounds represented the stray cat sample of this study.

Sampling and testing

Blood samples from household cats were either collected from the animal without sedation or following sedation with a dose of Xylzine hydrochloride (Rompun, Bayer, Leverkusen, Germany) at a dose of 2 mg/kg combined with ketamine hydrochloride (Imalgene, Rhone Merieux) at adose of 5 mg/kg. Stray cats were baited with food containing 10 mg of acepromazine and then were given the same sedative dose in order to enable blood collection. Cats were bled from the jugular vein using 20 g X ½ inch needle into 5 ml syringes without anticoagulant. Blood was transferred into clean tubes and allowed to clot overnight at room temperature, centrifuged for 10 minutes at 1,500 g. Sera were transferred into clean 1.5 ml Eppendorf tubes and stored at - 20°C until they were used.

A volume of 5 grams of rectal stool was collected from each cat enrolled in the study. Faeces were collected into polythene containers and preserved in 2.5% potassium dichromate ($K_2Cr_2O_7$) and used for coprological studies.

Serum samples were subjected to an ELISA method for the detection of *T. gondii* antibodies. This test was performed by using the CHEKIT-Toxotest ELISA Kit (IDEXX Laboratories, Bommeli Diagnostics, AG, and Bern. Switzerland) according to the manufacturer's instructions.

Faecal samples from cats were examined for the presence of oocysts by sugar flotation technique, as described by Dubey and colleagues (Dubey *et al.* 1970).

Statistical analysis

We used the statistical program SPSS in order to analyse data. The data was analysed with Chi-square test and the level was considered significant at $p \leq 0.05$.

Results

Serological examination

Serological examination showed that 52 (26%) out of 200 cats had antibodies to *Toxoplasma gondii* by ELISA (Table I). Serum samples with O.D. values > 40% were considered positive for the presence of *T. gondii* antibodies. Most (< 50%) of the cats that tested positive had O.D. over 100% and only 2 (3.8%) cats recorded an O.D. value of > 200 (high positive), as it is shown in Table I.

In stray cats, antibodies were found in 39 out of 100 (19.5%) tested cats. Antibodies were found in 13 out of 100 tested samples of household cats (6.5%). A significant difference in the seroprevalence between stray and household cats ($p < 0.05$) was

Table I. *ELISA results from stray and household cats.*

Cats	No. negative (%)	No. positive (%)	Class interval for O.D.%				
			41-80 (%)	81-120 (%)	121-160 (%)	161-200 (%)	>200 (%)
Stray	61 (61)	39 (39)	9 (23.1)	10 (25.6)	15 (38.5)	5 (12.8)	0 (0)
Household	87 (87)	13 (13)	0 (0)	3 (23.1)	3 (23.1)	5 (38.4)	2 (15.4)
Total	148 (74)	52 (26)	9 (17.3)	13 (25)	18 (34.6)	10 (19.2)	2 (3.8)

demonstrated. A difference in seroprevalence was also revealed between male (20.4%, 95% CI 0.98-1.37) and female (31.4%) cats screened in this study, however this difference was not statistically significant (Table II). Cats aged 6 years and more showed significantly high prevalence ($p < 0.05$) compared with cats of other age groups.

The highest number of cats (5) showing anti-*T. gondii* antibodies belonged to the American breed, followed by the Persian breed (4), Himalayan breed (2), Bengali and Siamese (1). No antibodies were detected in cats belonging to the Turkish breed.

Examination of faecal samples revealed the presence of oocysts in 17 stray and 7 household cats, giving an overall prevalence of 12%. All shedders cats were also seropositive. There was no significant difference in shedding between stray and household cats ($p > 0.05$).

Discussion

In this study, ELISA was employed to determine the seroprevalence of toxoplasmosis in stray and household cats in the Riyadh area. The results showed that the overall infection rate was 26%.

The prevalence of *T. gondii* antibodies in stray cats (39%) was significantly higher ($p < 0.05$) than that of household cats (13%), likely due to the higher chance of stray cats to get infected. This scenario is expected in light of the dispersion of the infectious stages of *T. gondii* in the environment of stray cats. Moreover, the hunting behaviour of felines further facilitates infection through the consumption of intermediate hosts. This general pattern of infection has been reported for stray versus household cats in various cities of the world, as in Seoul, South Korea (Lee *et al.* 2011); Tahran and Sari, Iran (Haddadzadeh *et al.* 2006, Sharif *et al.* 2009); Ankara, Turkey (Ozkan *et al.* 2008); Lanzhou, China (Wu *et al.* 2011); Colombo, Sri Lanka (Kulasena *et al.* 2011); and La Rioja, Spain (Miro *et al.* 2004). The seroprevalence among household cats further indicates the ubiquity of *T. gondii* and the strong association with its definitive host. The parasite is able to access both cats who live outdoors and those who are kept solely on canned food, if not properly cooked (De Creaye *et al.* 2008, Lopez *et al.* 2008, Lee *et al.* 2010).

Table II. *Comparison between the seroprevalence of* T. gondii *in male and female cats.*

Sex	No. of positive cats (%)	No. of negative cats (%)	Total
Male	20 (20.4)	78 (79.6)	98
Female	32 (31.4)	70 (68.6)	102
Total	52 (26)	148 (74)	200

The prevalence of *T. gondii* antibodies was higher in females. Other investigators have also reported differences in prevalence according to sex (Miro *et al.* 2004, Lee *et al.* 2010, Esteves *et al.* 2014). Moreover, seroprevalence rates also increased with the age of cats, similarly to previous studies.

Variation in prevalence in different breeds was observed in this study. However, further studies are warranted in order to establish a close association between *T. gondii* seroprevalence and cat breed.

The prevalence of infection did not vary significantly according to the different collection sites, or origin, of cats in the city of Riyadh. This could be the result of similar environmental conditions present in Riyadh. There is no information yet on density of stray cats and their spatial distribution within Riyadh city. In this regard, the spatial distribution of areas contaminated by *T. gondii* oocyst in Lyon, France, was described to be highly heterogeneous (Afonso *et al.* 2008). In rural areas of France, local meteorological conditions were also found to influence the spatial distribution of incidence risk in cats (Afonso *et al.* 2008).

The results of the coprological examination revealed an overall prevalence of 12%.

In conclusion, the results of the present study confirm the occurrence of *T. gondii* in cats in Riyadh city. Further studies in additional areas will be necessary to understand the overall epidemiological status of toxoplasmosis in household and stray cats in Saudi Arabia.

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