Prevalence of *Toxoplasma gondii* antibodies in anatolian ground squirrels, *Spermophilus xanthophrymnus* (Rodentia : Sciuridae) from Nigde, Turkey

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SUMMARY

Spermophilus xanthophrymnus (Rodentia: Sciuridae), the Anatolian ground squirrel, a wild rodent found in Central Anatolia of Turkey was studied to investigate the prevalence of antibodies to the protozoon parasite *Toxoplasma gondii*. A total of 105 sera from appearently healthy Anatolian ground squirrels *(Spermophilus xanthophrymnus)* were tested for *T. gondii* antibodies by the Sabin Feldman Dye Test (SFDT). 12 of 105 (11.4%) Anatolian ground squirrels were found to be seropositive for *T. gondii* antibodies at the titer of 1:16 and over. There was no statistically significant difference between seropositivity rates and genders (p>0.05). In conclusion, this study revealed the existence of *T. gondii* in the Anatolian ground squirrels (Spermophilus xanthophrymnus). This is the first report on toxoplasmosis in the Anatolian ground squirrels from Turkey.

KEY-WORDS : *Toxoplasma gondii*, *Spermophilus xanthophrymnus*, Sabin Feldman Dye Test (SFDT).

RÉSUMÉ

Prévalence des anticorps contre *Toxoplasma gondii* chez les écureuils anatoliens, *Spermophilus xanthophrymnus* (Rodentia : Sciuridae) dans la région de Nidge (Turquie). Par M. KARATEPE, C. BABÜR, B. KARATEPE, S. KILIÇ et M. ÇAKIR.

La prévalence des anticorps dirigés contre le protozoaire *Toxoplasma gondii* a été explorée chez les écureuils anatoliens *(Spermophilus xanthophrymnus)*, qui sont des rongeurs sauvages de l'Anatolie Centrale (Turquie). Cent cinq sérums issus d'écureuils apparemment sains ont été testés par le test colorimétrique de Sabin Feldman (SFDT). Douze sérums (11.4%) ont présenté des titres en anticorps supérieurs ou égaux à 1/16 et ont été déclarés séropositifs. Aucune différence significative des titres en anticorps n'a été observée entre les mâles et les femelles (p > 0.05). En conclusion, cette étude, la première réalisée dans cette espèce, montre l'existence d'une infestation possible des écureuils anatoliens *(Spermophilus xanthophrymnus)* par *T. gondii* dans la région de Nigde.

MOTS-CLES : *Toxoplasma gondii*, *Spermophilus xanthophrymnus*, Sabin Feldman Dye Test (SFDT).

Introduction

Toxoplasmosis, a zoonosis of world-wide distribution, is caused by an obligate intracellular parasite *Toxoplasma gondii*. The definitive host of the protozoan is an animal from *Felidae* family (cat, bobcat, puma) and man, domestic or wild animals, as well as birds are intermediate hosts [3, 5, 8, 12, 15, 18]. The disease is transmitted by ingestion of oocysts (shed by infected cats) in contaminated food and water, or bradyzoites (cysts) in the tissues of an infected animal [3, 4, 8]. Many seropositive animals such as lamb, cattle, pork and naturally infected wild animals harbour cysts in their tissues, particularly muscle, indicating that the meat supply is the source for *T. gondii* infection of animals and human beings [3, 5, 6]. The rodents are herbivores and therefore, are likely to be infected by ingesting food or water contaminated with oocysts [3, 10]. The determination of prevalence of *T. gondii* infection in rodents may be of epidemiological importance because rodents can serve as potential sources of tissue cysts for Felidae [3, 6, 8].

The parasite produces a wide range of clinical syndromes in humans, other mammals, and a variety of bird species but uncommonly causes clinically significant disease. Due to variable clinical expression and non-specific haematological and biochemical changes, serological tests such as Sabin Felman Dye Test (SFDT), Indirect Fluoresence Antibody Test (IFAT), Complement Fixation Test (CF), Enzyme Linked Immunosorbent Assay (ELISA), Latex Agglutination Test (LAT) and Indirect Hemagglutination (IHA) tests are preferred for diagnosis of toxoplasmosis. Sabin-Feldman Dye test is still considered as the « gold standart ». [5, 11, 12, 19, 20].

In Turkey, the epidemiology of toxoplasmosis has not been extensively investigated, and few data are available on the distribution and prevalence of the disease. The Anatolian ground squirrel *(Spermophilus xanthophrymnus)* is distributed throughout the Central Anatolia of Turkey [1, 2]. There is no study regarding the occurence of *T. gondii* infections in the Anatolian ground squirrels *(Spermophilus xanthophrymnus)* in Turkey .

The aim of the present study was to determine the prevalence of antibodies to *T. gondii* in the Anatolian ground squirrels .

Materials and Methods

STUDY AREA

This study was performed on Anatolian ground squirrels of the region of Nigde, in the middle of Turkey, where toxoplasmosis was not previously recognized. Blood samples were obtained from Anatolian ground squirrels *(Spermophilus xanthophrymnus)*, between April and August 2003.

ANIMALS AND BLOOD COLLECTION

The Anatolian ground squirrels (65 females and 40 males) were captured and brought alive to the laboratory in a cage with pouring water on their shelter. Records of ages in squirrels were not kept. Ten percent (10%) chloralhydrate solution was inoculated intraperitoneally (1.5-2ml) into the squirrels for anesthesia. Blood samples were collected by cardiac puncture from 105 apparently healthy anaesthetized Anatolian ground squirrels. Sera were obtained by centrifugation at room temperature (25°C), at 1 800g for 10 minutes and were stored at - 20°C until serological analyses were performed.

SEROLOGICAL ASSAY

Serum samples were tested for *T. gondii* antibodies by Sabin Feldman Dye Test (SFDT) in Ankara Refik Saydam National Institute of Hygiene (RSNIH), Department of Communicable Diseases Research. SFDT was performed in accordance with the methods of Sabin-Feldman [14].

As vigorous antigen, 48 hours passage of *T. gondii* Rh strain derived from peritoneous fluid of 3-4 week aged white swiss albino mice were used. As an activator serum, seronegative for *T. gondii* and Mg_2, properdin, C_2, C_3, C_4 rich human serum was used. The sera were first inactivated in 56°C for 30 minutes, then four-fold serum dilutions from 1:4 to 1:1024 were prepared and stored in aliquots of 25 µl in eppendorf tubes. The mixture of vital *T.gondii* tachyzoits and activator serum were added to serum dilutions and incubated in a water-bath at 37°C for 50 minutes. Methylen-blue dye prepared with alkaline soda boraks was added to each tube in equal amounts and kept in a water-bath at 37°C for 10 minutes.

The SFDT result was regarded as positive if more than 50% of tachyzoits did not accept the dye (unstained) at ≥ 1:16 examined under the light microscope (x 400).

STATISTICAL ANALYSIS

The analysis was performed using the « t test ». It was used to evaluate gender related prevalence of *T. gondii*. SPSS for Windows was used for data analysis. Significance was set at $p<0.05$.

Results

Out of 105 sera examined, 12 (11.4%) were positive at the titers ≥ 1:16. It was determined that 11 sera samples were positive at 1:16 dilution and one sample was positive at 1:64 dilution.

The distribution of SFDT titers according to the genders was shown in Table I. Six sera of male squirrels (15%) were considered as seropositive. In females, *T. gondii* prevalence was 9.2% (six sera were seropositive).

No statistically significant difference according to gender was observed between the seropositive and seronegative Anatolian ground squirrels ($p>0.05$).

Gender	Number of tested	Number of positivity (and %)	SFDT Titer	
			1:16	1:64
Female	65	6 (9.2)	6	0
Male	40	6 (15)	5	1
TOTAL	105	12 (11.4)	11	1

TABLE 1. — Distribution of SFDT titers (*T. gondii* antibodies) in Anatolian ground squirrels *(Spermophilus xanthophrymnus)* according to genders (Seropositivity was obtained when antibody titer was superior or equal to 1/16.

Discussion

Toxoplasmosis is one of the most widespread infections in man and animals. The frequency differs from the geographical location [5, 8, 12]. The prevalence of anti-*T. gondii* antibodies in wild rodents has been reported in several countries [6, 7, 9, 10, 13, 16, 17]. The general prevalence of toxoplasmosis in rodents such as *Rattus norvegicus* in various countries varies between 1-30% [6]. FRANTI *et al.* [7] found that 4% of 160 murid rodents (rats and house mice) in Northern California presented antibodies to *T. gondii* by IHA. SMITH and FRENKEL [17] had detected the seropositivity as 3% in mice and rats in USA (Missouri and Kansas) by using the SFDT. In surveys performed among field rodents *(Apodemus agrarius)* in Korea, 15 of 1008 rodents were found seropositive by ELISA [10]. In Panama city, FRENKEL *et al.* [9] found that 53 of 226 *Rattus norvegicus* had antibodies to *T. gondii*.

ROHER *et al.* [13] have been reported the occurence of *T. gondii* cysts and tachyzoites in many tissues of two dead gray squirrels. In the parasitology literature, there is only one investigation that has been reported from USA performed in herbivores including squirrels *(Sciurus spp.)*. Seropositivity rates was found as 18% by the SFDT [17]. In this study, seroprevalence of *T.gondii* was found to be 11.4% in Anatolian ground squirrels *(Spermophilus xanthophrymnus)* from Nigde in Turkey. There was no statistically significant difference in prevalence between genders ($p>0.05$). We are unaware of any previous report of the prevalence of *T. gondii* antibodies in this species. Only one investigation in Turkey was carried out to determine seroprevalence of toxoplasmosis in rodents *(Rattus norvegicus)*. A total of 76 sera was examined and seropositivity rate was found as 2.63% [16].

Seroprevalence result of this study is higher than the result

obtained by the other study performed in Turkey. This higher seroprevalence rates may be related with conducting the study on different animals and different geographical locations.

The Anatolian ground squirrels are herbivores. *T. gondii* cysts have been found in the muscles in these animals, therefore, the Anatolian ground squirrels were confirmed as reservoirs of *T. gondii* infection for other carnivores, and particularly for Felidae. Consequently, squirrels could contribute to the extension of this disease.

In conclusion, this study demonstrated that *T. gondii* is present in the Anatolian ground squirrels *(Spermophilus xanthophrymnus)* in the region of Nigde. We concluded that it is important to make further studies on definitive host and reservoirs (domestic and wild animals) in this area in which the disease was previously not found. However, the epidemiology of toxoplasmosis has not been extensively studied in Central Anatolia. More extended studies are required to determine the seroprevalence rates in human and animals, and its implications for both animal and human health in the region of Central Anatolia.

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