

A Seroprevalence Study of *Toxoplasma gondii* in some bird and animal species of Israel and its Possible Reflection on Environmental Contamination

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ABSTRACT

The prevalence of antibodies to *Toxoplasma gondii* was determined in sera from sheep, chickens and turkeys originating from various geographical regions of Israel. Using a modified agglutination test, antibodies to this parasite were found in 8.9% (41/458) of 4-6 month old sheep from Beer Sheva in the south of the country and 65.6% (84/128) mostly older sheep from a central region, about 20 km south-east of Tel-Aviv. In both areas some animals had titers $\geq 1:400$. Free-range chickens from two areas in the center of Israel were found to be exposed: 12% of 100 chickens in a region approximately 45 km north-east of Tel-Aviv and 10.5% of 57 chickens in Jerusalem found to be seropositive. Some chickens produced titers $\geq 1:144$. Of 45 turkeys tested from a location about 56km south east of Tel-Aviv, 4 (8.8%) had titers $\geq 1:50$. This is the first report of *T. gondii* seroprevalence in turkeys in Israel and the widespread exposure found in these and other domestic animals tested, suggested widespread contamination of the environment with oocysts.

Keywords: *Toxoplasma gondii*; Israel; Chickens; Turkeys; Sheep.

INTRODUCTION

Toxoplasma gondii, an obligatory intracellular parasite, is the cause of toxoplasmosis, which is considered to be a major zoonosis. On the worldwide scale, prevalence rates by serological survey in pregnant women vary from 7% to 51.3%, and in women with abnormal pregnancies and abortions the rates fluctuate from 17.5% to 52.3% (1). Many epidemiological investigations on *T. gondii* infection have been made in both humans and animals in different parts of the world (2, 3, 4). Various Israeli serological studies in humans in different parts of the country have been published (5, 6, 7, 8, 9) with prevalence rates between 20% and 56% depending on ethnicity, religion, and age, as well as socioeconomic status. Seroprevalence studies in domestic animals and birds in Israel are limited in number. In one study, *T. gondii* seroprevalence

in cattle was found to be about 15% of 172 animals tested with titers as high as 1:256 using indirect fluorescent antibody technique (IFA, 10). Antibodies to *T. gondii* were reported in 25% of 372 sheep surveyed by IFA between 1985-1990 (10). In a study by Baneth *et al.* (11), 35.5% of 220 mainly stray dogs from the center of Israel were seropositive using IFA with titers as high as 1:1024. In a cross-sectional study of 1064 cats in the Jerusalem area, 16.8% were seropositive for *T. gondii* using ELISA technique (12). Seropositivity was significantly higher in older cats and in the summer month. In cats from East Jerusalem, seropositivity was significantly higher than in the West Jerusalem areas. In a survey of 96 commercially free-range chickens raised in the north of the country, approximately 45 were seropositive for *T. gondii* using the Modified Agglutination Test (MAT) (13). In a

previous survey of 495 feral pigeons (*Columba livia*) that originated from 3 distinct regions in Israel, 20 (4%) were observed to be seropositive for *T. gondii* (14).

The objective of the present study was to ascertain the seroprevalence of *T. gondii* in domestic ground feeding animals, specifically sheep, chickens and turkeys in different regions of Israel, in order to elucidate possible oocyst dispersion in the environment amongst these ground feeders.

MATERIALS AND METHODS

Animal samples

During the first 4 months of 2004, 5 ml blood was collected from the jugular veins of 4-6 month old sheep (*Ovis aries*) before slaughter at the Jerusalem Municipal Abattoir. The sheep originated from the Beer Sheva region in the south of the country and were raised under extensive conditions in which sheep grazed natural pastures and were supplemented with feed (Group 1.1; Table 1). Also, during the same months, jugular blood samples were extracted from a flock of sheep raised extensively and in the center of the country near Latrun, about 20 km south-east of Tel-Aviv, with animals ranging in age from 5 months to 5 years old (Group 1.2).

Chicken (*Gallus domesticus*) jugular blood samples were obtained from 2.5-year-old free-range egg-layers from the center of the country in a Jewish settlement in Samaria (Group 2.1), in a region approximately 45 km north-east of Tel-Aviv. Specimens were collected in August-December 2003 at the time of slaughter. Blood samples of household free-range chickens from the area of Jerusalem (Group 2.2) were kindly provided by a private veterinarian after aliquot-

ing those from samples purposely taken to screen birds for routine diseases. During the same year, blood samples were collected at the time of slaughter from turkeys (*Melleagris gallopavo*) aged from 5 months and raised in enclosures on the ground on Moshav Komemiyut approximately 52 km South East of Tel Aviv (Group 3). All samples had been centrifuged within 2 hours after removal and their sera stored at -20°C until assayed.

Serologic Examination

Sera from sheep (Groups 1.1 and 1.2) were diluted in serum buffer and measured at titers of 1:25, 1:50, 1:100, and 1:400 using the Modified Agglutination Test (MAT). Sera from chickens in Group 2.1 were diluted two-fold starting at 1:5 dilution using the same MAT assay. Chickens from Jerusalem (Group 2.2) were assayed at titers 1: 6, 1:36, and 1:144. Turkey sera (Group 3) were assayed at titers of 1:25, and 1:50. Serum dilutions for each assay were different due to technical reasons. All testing was performed using the modified agglutination test (MAT) (15). Each plate contained positive and negative controls. All positive test results and 30 randomly taken negative reactions were repeated at least once in order to test the reliability of the test.

RESULTS

Sheep

Of 458 four to six month old sheep (group 1.1), 41 (8.9 %) were seropositive to *T. gondii* at titers \geq 1:25. Three sheep (0.6%) demonstrated positive titers as high as 1:400. Of older sheep tested (group 1.2), *T. gondii* positive seroprevalence was seen in as high as 84/128 (65.6%) of the animals tested.

Table 1: Rate and percentage (in parentheses) of *T. gondii* seropositivity in some ground feeding domestic animals and birds in Israel.

		Titer Number of positives (Percentage of the group)				Total proportion of positives (Percentage)
		1:25	1:50	1:100	1:400	
Animals or birds tested	Beer Sheva sheep (Group 1.1)	26(5.6%)	11(2.4%)	1(0.2%)	3(0.6%)	41/458 (8.9%)
	Latrun sheep (Group 1.2)	28(21.8%)	24(18.7%)	17(13.3%)	15(11.7%)	84/128 (65.6%)
	Samaria free range chickens (Group 2.1)	12(12%)	1(1%)	0(0%)	0(0%)	12/100 (12%)
	East Jerusalem free range chickens (Group 2.2)	4(7.0%)		1(1.7%)	1(1.7%)	6/57 (10.5%)
	Turkeys (Group 3)		1(2.2%)		3(6.7%)	4/45 (9%)

Birds

Free range chickens from both Samaria (group 2.1) and Jerusalem (group 2.2) showed similar *T. gondii* seropositivity rates of 12/100 (12%) and 6/57 (10.5%), respectively. Four out of 45 turkeys (8.8 %, group 3) had *T. gondii* seropositive at titers 1:25.

Reliability of the assay was 100% with positive sera and 30 randomly sampled negative sera displaying the same titers on repeat tests. Dilutions used differed in each survey due to technical reasons or in order to better compare with similar species according to previously published studies.

DISCUSSION

The purpose of the study was to determine *T. gondii* seroprevalence in ground-feeding domestic animals and birds. Presence of antibodies would suggest the level of environmental oocyst contamination. This may reflect on the resultant risk for human infection by ingestion of these animals. Food animals, such as sheep, are assumed to remain infected for life when exposed to *T. gondii* (16). Thus, sheep and poultry were analyzed at the time of slaughter in order to assess the direct risk of *T. gondii* infection of humans from meat consumed from these animals.

Even though the traditional ages that sheep are generally presented for slaughter in this country are young, i.e. 4-6 months of age, cumulative positive *T. gondii* seroprevalence in group 1.2 animals was still close to 9 percent of slaughtered animals. Since sheep have been found to be susceptible to infection after exposure to *T. gondii* and harbor infective tissue cysts for their entire lifespan (16), it is suggested that undercooked lamb is a significant risk for infection in humans. A large proportion of the country's sheep that enter the food market arise from the south of Israel. Hence, this locality was chosen for study purposes. Conditions in which sheep are raised are extensive. Housing conditions allow for free exposure to feral cats, conditions that allow for contamination of cat litter in sheep feed. Group 1.2 was comprised of many older animals (ages were as high as 5 years old). Since a cumulative long lasting IgG antibody derived immune response was measured in this assay, and since continual exposure of the older animals to *Toxoplasma* in contaminated pastures could have taken place, we observed the finding of a higher positive

seroprevalence to *T. gondii* in this group, results similar to those reported by Maruyama *et al.* (17).

Chickens in groups 2.1 and 2.2 were generally older (approximately 2.5 years of age), and were raised as free-range egg layers having free exposure to rodents and feral cats. In fact, in group 2.1, three feral cats were captured in the vicinity of the chicken coup. All were seropositive to *T. gondii* using a MAT assay (results not presented). Thus, it was not expected that titers in Group 2.1 were to be so low. *Toxoplasma* from tissue in seropositive poultry of Group 2.1 sent to the *Toxoplasma* Laboratory of the United States Department of Agriculture (Beltsville, MD, USA) was unable to infect mice (Dubey, J.P, personal communication).

Turkeys in Israel are slaughtered later in life, at 5 months of age and sometimes in conditions that allow for slack biosecurity measures whereby feral cats may readily enter housing premises and contaminate them with infective oocysts. We sampled a small group of this species but still managed to observe infected birds.

The *T. gondii* seroprevalence rate of 4% found in Israeli pigeons in a previous study is in agreement with previous surveys from other countries (14). Although the percentage of seropositive birds is relatively low, a large number of pigeons present around human settlements may account for a large absolute number of positive birds in the population. Hence, the role played by pigeons in the transmission of this disease could be important and their role in the parasite's epidemiology should be further explored.

The study's results strengthen the importance of environmental contamination by infective cat feces to animals that are ground feeders. Prevention of food animals from exposure to feces of feral cats is extremely important in attempts to control animal infection and thus to minimize contamination of human food by *T. gondii* (18).

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