

## **Seroprevalence of *Toxoplasma gondii* in Goats in Two Districts in Northern Palestine**

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### **Abstract**

The objective of this study was to investigate the presence of anti-*T. gondii* in goats raised on farms in the Jenin and Tulkarm districts in the north of Palestine. The investigation was conducted on 14 herds comprising of 280 goats. Blood samples were collected via the jugular veins of 151 goats during the period from January to December 2011. The indirect ELISA test was used for the detection of anti-*T. gondii* antibodies. Results showed that an anti-*T.gondii* IgG antibody was detected in 13.4 % of the samples. The presence of the anti-*T. gondii* antibody was influenced by the location of the goat herds. The highest incidence was in Jenin district (17.44 %) while it was 7.69 % in Tulkarm district. These results indicated the possible contamination of meat and milk of the goats with this parasite, which in turn negatively affects human health.

**Keywords:** *T. gondii*, goats, IgG, Palestine, ELISA

### **Introduction**

Toxoplasmosis is a zoonotic disease that has an impact on human health globally. It is caused by *Toxoplasmosis gondii*, an obligatory protozoan parasite. Wild and domestic cats are the definitive hosts, while other warm blooded animals are considered as intermediate hosts [1-6]. Abortion is seen in goats that are infected with *Toxoplasmosis* during pregnancy [2,7-9].

Cats are the source of oocysts that are excreted with feces which then contaminate pastures. It takes from one to five days before voided sporulate oocysts become infectious [10-13]. The infection of intermediate hosts like goats has 2 sources; sporulated oocysts consumed with contaminated feed or water, and via placenta, where tachyzoites are transmitted to fetuses in the case of infected goats [8,9,14]. It is well documented that *T. gondii* has great negative economic effects worldwide through increases in cases of abortions and the delivery of weak kids [15].

Humans can acquire *T. gondii* by eating raw or inadequately cooked infected goat meat and milk, or uncooked foods that have come in contact with infected meat and milk. Humans can inadvertently ingest oocysts that cats have passed in their stool, and can be transmitted to humans by unwashed fruits or vegetables or in unfiltered water [12].

Goat meat and milk are major commodities for people in Palestine; however, there is no information about the status of this parasite in local herds. The objective of this research is to investigate the prevalence of *Toxoplasmosis* in local goat herds.

### **Materials and methods**

This research was conducted on female goats of 2 - 4 years old of different breeds; Baladi (Black), Shami, Anglo-Nubian and Zaanin. Goats were raised at 2 locations in the north of Palestine, Jenin (n = 86) and Tulkarm (n = 65) districts, under an extensive production system. A total of 14 goat herds (n =

280) were examined for infection of *T. gondii* during the period from January to July 2011. Five ml of venous blood were aseptically collected via the jugular vein of 151 goats and immediately transferred to the Faculty of Veterinary Medicine, An Najah University laboratories. Serum samples were collected following centrifugation of the blood samples at 3000 rpm (1100 g) for 10 min. Sera were stored at  $-20^{\circ}\text{C}$  before being analyzed for *Toxoplasma* antibodies.

#### Assay

The method used in this study was the indirect Enzyme-linked immunosorbent assay for the detection of IgG antibody to *T. gondii*. Commercial ELISA kits were purchased from ATLAS MEDICAL (Cambridge, UK). 100  $\mu\text{l}$  of control (in duplicate) and sample diluents were dispensed into respective wells, 1 well left blank as background control. Five  $\mu\text{l}$  of serum samples were added into wells containing sample diluents, and the microtiter plate was placed into a humidified box and incubated at  $37^{\circ}\text{C}$  for 30 min. After the incubation each well was washed 5 times by filling each well with diluted wash buffer, then inverting the plate vigorously to get all fluid out and blocking the rim of the wells on absorbent paper for a few seconds. After that, 100  $\mu\text{l}$  of Enzyme Conjugate was added to each well and mixed gently by swirling the micro titer plate on a flat bench for 1 min. Next, the micro titer plate was placed into a humidified box and incubated at  $37^{\circ}\text{C}$  for 30 min. After that, a second wash was run. Then, 50  $\mu\text{l}$  of substrate solution A (HRP substrate) was added to each well, followed by adding 50  $\mu\text{l}$  of substrate solution B (TMB) to each well, and mixed gently and incubated at  $37^{\circ}\text{C}$  for 10 min. Finally, 50  $\mu\text{l}$  of stop solution was added to each well to stop color reaction. Optical Density (OD) values of all samples and control were measured at 450 nm against air blank in an ELISA plate reader (DAS Inc., Italy) at 450 nm within 30 min.

#### Interpretation of results

The OD value of the blank wells were used to correct all the OD readings from all wells, and the Cut off value was calculated as  $0.10 +$  of the average OD value of the negative control. A positive result was considered if the OD value was equal to or greater than the Cut off value. OD values less than the Cut off value were considered negative.

#### Statistical analysis

Data was expressed as a percentage of positive samples. Statistical analysis was performed by a simple T test, and the significant level was set at  $P < 0.001$ .

#### Results and discussion

The result showed that of 151 samples, 20 (13.24 %) were positive for the anti *T. gondii* IgG antibody. Jenin district had the highest infections (17.44 %; 15 positive samples out of 86) ( $P < 0.001$ ). However, Tulkarm district had only 5 positive samples out of 65 tested samples (7.69 %), **Table 1**.

The results of the study showed that local goats were infected with the *T. gondii* parasite; however the rate of infection in Palestine (13.24 %) is lower than that reported for other countries [2,10,14,16-20].

The larger infections in Jenin herds (17.44 %) is significantly higher compared to that in Tulkarm district (7.69 %) ( $P < 0.001$ ); this can be explained by the availability of larger areas of pastures used by grazing goats compared to Tulkarm district and the larger number of herds in Jenin [21]. The extensive production system of goats that is adopted in the targeted area can explain the rates of infection. Under this system, besides the low skills of goat farmers, there are few biosecurity measures in place, resulting in factors such as improper feed storage that allow mice, cats and dogs to roam, increasing the chance of the contamination and spread of the disease among goats and sheep contaminate feed. There are also insufficient veterinary services, resulting in the misdiagnosis and treatment of infected animals and the improper disposal of aborted fetuses and placentas which in turn increase the chances of infection [14,17,22].

**Table 1** Infection of *Toxoplasma gondii* in the Jenin and Tulkarm districts.

District	Number of samples	Positive samples	% of positive samples
Jenin	86	15	17.44 <sup>a</sup>
Tulkarm	65	5	7.69 <sup>b</sup>
Total	151	20	13.24

Remark: Different letters in the vertical line are significantly different (P < 0.001).

One-sample test (Test value = 0)						
	t	df	Sig. (2-tailed)	Mean difference	95 % Confidence interval of the difference	
					Lower	Upper
Result	4.785	150	0.000	0.13245	0.0778	0.1871

The infection rates observed by this study were within the international reported rates, which are 11 to 96 % [2]. The reported values from research using similar testing methods were similar to the findings of this study. It was reported that the infection rate in the north west of China was 29.8 % [18], 3.8 % in the north east of China [23], 88 % in Pakistan [17], 52 % in the Punjab of Pakistan [6] and 31.6 % in Afghanistan [16]. In the region of the Middle East, infection rates were 19.3 % in Tabuk in Saudi Arabia [20], 35.1 % in Jordan [10], 27.6 % in Morocco [5] and 29 % in Egypt [15]. Infection rates in other regions include 12 % in Chile [24], 19.5 % and 25.1 % in Brazil [14,7], 37 % in New Zealand [19], 31 % in Uganda [25] and 59.8 % in Bulgaria [2].

The lower infection rates observed in local goat herds can be explained by the type of weather which prevails in the north of Palestine, characterized by hot summer conditions and low precipitation during winter. Higher temperatures and low humidity decrease the numbers of parasites and destroy the oocysts. However, serious measures should be taken at governmental and farm levels to reduce the harmful effects of this parasite.

The results of this study confirm the risk of Toxoplasmosis on human health, because a lot of people consume the milk and meat of infected animals. Mixed grazing in local pastures increases the possibility of transmission of infection from goats to other animals. Goats under extensive production systems, as in Palestine, will spread the disease widely as they keep moving all year round while grazing [14,8,9].

### Recommendations

In order to decrease the chances of infection by the parasite, certain measures should be applied, such as reducing numbers of homeless cats and rodents as much as possible, adoption of biosecurity practices by farmers, and adoption of intensive systems of goat keeping. Application of vaccination programs is not common practice; however, feed animals with anticoccidial supplements (incorporated with monensin or decoquinat) during pregnancy according to veterinary prescription is more efficient [12].

The culling of old animals is an important practice to decrease infection rates, as these are of high risk due to their weak immune system [26,6]. Milk pasteurization and meat cooking are important to decrease the incidence of infection [27].

## Conclusions

The results of this study indicate the possible contamination of meat and milk of goats with this parasite, which in turn negatively affects human health. However, weather conditions have an impact on the spread of the infection.

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