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# Prevalence of Toxoplasmosis in Sheep, Goats and Cattle in Southern Tunisia

**Ibtissem Lahmar**[1], **Arwa Lachkhem**[1], **Darine Slama**[1], **Wahiba Sakly**[1], **Najoua Haouas**[1], **Mohamed Gorcii**[1, 2], **Alexander W Pfaff**[3], **Ermanno Candolfi**[3] **and Hamouda Babba**[1*]

[1]*Laboratoire de Parasitologie-Mycologie Médicale et Moléculaire (code LR12ES08), Département de Biologie Clinique B, Faculté de Pharmacie de Monastir, Université de Monastir, Tunisia*

[2]*Laboratoire de Parasitologie-Mycologie Hôpital Universitaire Fattouma Bourguiba, Monastir, Tunisie*

[3]*Institut de Parasitologie et de Pathologie Tropicale, EA 7292, Fédération de Médecine Translationelle, Université de Strasbourg, France*

[*]**Corresponding author:** Hamouda Babba, Laboratoire de Parasitologie–Mycologie Médicale et Moléculaire (code LR12ES08), Département de Biologie Clinique B, Faculté de Pharmacie de Monastir, Université de Monastir, Tunisie, Tel: +216 97850853; Fax: + 216 73 496 968; E-mail: hamouda.babba@rns.tn

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**Abstract**

The prevalence of *Toxoplasma gondii* infection in ruminants (sheep, goats, and cattle) in Southern Tunisia is largely unknown.

Antibodies and DNA to *T. gondii* were determined in serum and apex of the heart samples of 261 animals (204 sheep, 32 goats and 25 cattle) using the modified agglutination technique test (MAT) and the PCR respectively.

Antibodies to *T. gondii* (MAT, 1:20) were found in 40.2% (95% CI: 33.4%, 47.2%), 34.5% (95% CI: 19.1%, 53.2%) and 12% (95% CI: 03.15%, 32.13%) in sheep, goats and cattle respectively. Seroprevalence significantly ($p<0.05$) varied with species, gender, age, and animals' breed. Older animals (>3 years) and females were significantly ($p<0.05$) more infected than younger and male animals respectively. The seroprevalence was highest in sheep and specifically in the Barbarine breed. The presence of *Toxoplasma* DNA was detected in 11 of 96 samples (11.5%).

The prevalence observed in the present study indicates a widespread exposure to *T. gondii* in South Tunisia. Results indicate, for the first time in Tunisia, that infected sheep and goats represent a potential source of *T. gondii* infection for humans in particular in the South of the country (Gafsa area).

**Keywords:** *Toxoplasma gondii*; Animals; Prevalence; South Tunisia

## Introduction

Toxoplasmosis is one of the most common parasitic zoonosis, caused by the obligate intracellular protozoan *T. gondii*, which can infect almost all warm-blooded animals, including humans and domestic animals [1,2]. Toxoplasmosis is also a public health problem owing to its transmission to humans by ingestion of undercooked meat containing tissue cysts, or by consuming food or drink contaminated with oocysts, or through accidental ingestion of sporulated oocysts from the environment [3]. Meat from *T. gondii*-infected mammals is consumed widely in many countries, including Tunisia, and is known to be source of infection for humans [4,5]. Toxoplasmosis leads to great economic losses in ruminants especially in sheep, cattle and goats by causing embryonic death, fetal death, abortion, stillbirth and reduced flock milk production [6]. Worldwide prevalence of *T. gondii* infection in sheep, goats and cattle are largely investigated. However, in Tunisia very few studies have been conducted in all of Tunisia aiming to detect *T. gondii* infection in horses and sheep [2,7,4,8]. Therefore, the knowledge about the seroprevalence of *T. gondii* in domestic ruminants is of interest in order to implement future strategies on public health programs and to clarify the role of ruminants as a source of infection.

The objective of the present study was to investigate the prevalence of *T. gondii* in sheep, goats and cattle in Gafsa, Southern Tunisia.

## Materials and Methods

### Animals' blood and heart apex

Blood and heart apex samples were collected from 261 ruminants belonging to three species (sheep n=204, goats n=32 and cattle n=25) at the Gafsa abattoir between December 2010 and October 2012. Each sample is accompanied by a collection of epidemiological data.

The animal population was represented by 102 males and 159 females. The different ages of animals were classified into three groups: the first one including young animals [<1 year-old] (n=152), the second including [1 to 3 years-old] animals (n=37) and the third including the older than 3 years-old (n=72), the oldest animal being nine years old. Two sheep breeds were evaluated: the Barbarine breed (n=15) and the Queue Fine de l'Ouest breed (n=189), while only one breed was tested for each of the other species: the cross breed Bastard for goats and the Frison Pie Noir for cattle. Sera were obtained after 3000 rpm centrifugation for 10 minutes and stored at -20°C until testing for anti-*T. gondii* antibodies.

The apex of the heart (10 g) were removed under sterile conditions with a scalpel blade disposable, then kept in sterile 1.5 ml tubes with the same label as the blood sample from the same animal and kept at -20°C.

All animals were handled in strict accordance with good animal practice and the blood samples were drawn via the jugular vein from

each animal at the time of slaughter by exsanguinations as approved by the Tunisian National Ethics Committee.

### Serological examination

Serum samples were analyzed using the Modified agglutination technique test (MAT) (Toxo-Screen®, BioMerieux) to determine the presence of IgG antibodies against *T. gondii*, according to the manufacturer's protocol for the detection of *T. gondii* antibodies in animals. The test was considered positive when a layer of agglutinated parasites formed in wells at dilution of 1:20, while the control antigen and the negative sera showed sediment as a spot or a small ring respectively. Positive and negative control sera were provided in the kit and were included in each test.

### DNA extraction and amplification

DNA was extracted from 25 mg of the homogenized heart apex tissue using the QIAmp DNA tissue Mini Kit isolation kit (Qiagen, France) and stored at -20°C. As positive control, *T. gondii* tachyzoites (RH strain) were obtained from mouse ascites. The presence of *T. gondii* was detected by PCR using the nucleotide sequence of the AF146527 gene as target. Primers were 5'-CTG CAG GGA GGA AGA CGA AAG TTG3' (sense) and 5'-CTG CAG ACA CAG TGC ATC TGG ATT3' (antisense), which amplify a 529 bp fragment of the target gene. PCR reaction was performed in 50 µL reaction mixture containing 10 µL of sample DNA diluted 1:10, 2 µM of each primer, 200 µM dNTP (Invitrogen), 2.5 mM $MgCl_2$ and 1.25 Units of Hot Start Taq DNA Poymerase (Qiagen). Amplification was performed on a Perkin Elmer thermo cycler (GeneAmpPCR systemTM 2400) by 15 min incubation at 94°C, followed by 40 cycles of 30 sec at 95°C, 30 sec at 58.5°C, 30 sec at 72°C and a final 5 min at 72°C. PCR products were analyzed by electrophoresis on 2% agarose gel and visualized on a UV screen using ethidium bromide staining.

### Statistical analysis

Statistical analysis and description of *T. gondii* seroprevalence in different species, gender ages and breeds were performed using the software SPSS.v18, M.S. Excel while the Chi square test was calculated with the EPI Info 6.04 software at 95% confidence interval. The differences were considered statistically significant when $P<0.05$.

## Results

Among the 261 animals tested for IgG anti-*T. gondii*, 96 showed seropositivity, leading to an overall seroprevalence of 36.8% (95% CI: 30.9%, 42.9%). Seroprevalence of *T. gondii* varied significantly ($p<0.01$) among species: 40.2%, 34.5% and 12% in sheep, goats and cattle respectively (Table 1). Moreover, the statistical analysis showed that gender, age and breed affected seroprevalence of *T. gondii* infection. Seroprevalence was higher in females (44%) (95% CI: 36.2%, 52.1%) than in males (25.5%) (95% CI: 17.6%, 35.2%) ($p<0.01$) (Table 1). In addition, percentage of seropositive animals older than 3 years-old (70.8%) was significantly ($p<0.01$) greater than those of middle-aged (29.7%) and young (22.3%) animals (Table 1). Considering the sheep breeds, statistical analysis showed a higher infection rate among the Barbarine breed (Table 1). The analysis of 96 DNA samples that had a positive serology showed the presence of Toxoplasma DNA in 11 samples of cardiac apex (11.5%).

| Factors | Category | Sample size | Number with anti-*T. gondii* antibodies | Seroprevalence (%) | 95% CI | P value |
|---|---|---|---|---|---|---|
| Species | Sheep | 204 | 82 | 40.2 | [33.4-47.2] | p<0.05 |
| | Goats | 32 | 11 | 34.50 | [19.2-53.2] | |
| | Cattle | 25 | 3 | 12 | [03.1-32.3] | |
| Gender | Female | 159 | 70 | 44 | [36.2-52.1] | p<0001 |
| | Male | 102 | 26 | 25.50 | [17.6-35.2] | |
| Age (years) | [<1 year] | 152 | 34 | 22.36 | [16.2-29.9] | p<0.0001 |
| | [1-3years] | 37 | 11 | 29.71 | [16.4-47.1] | |
| | [>3years] | 72 | 51 | 70.84 | [58.8-80.6] | |
| Sheep Breed | Barbarine | 15 | 12 | 80 | [51.3-94.6] | P=0.0002 |
| | Queue Fine de l'Ouest | 188 | 70 | 37.23 | [30.3-44.6] | |

**Table 1:** Seroprevalence of *Toxoplasma gondii* infection in ruminants in the South of Tunisia.

## Discussion

In our study we assessed, for the first time, the prevalence of T. gondii infection in ruminants in the Gafsa region, in Southern Tunisia. The overall seroprevalence was about 37%. In sheep, seroprevalence of anti-*T. gondii* antibodies was 40 % which was higher than that found in the north (1.8%) and the center of the country (19%) as reported by Gharbi et al. [4]. This discrepancy may be related to the difference between the number and the age of population studied and the methods used. Indeed, previous studies were carried out using an ELISA technique which is less sensitive than the agglutination technique used in the present study [9]. The observed seroprevalence in sheep in the current study was higher compared to those reported in other parts of the world, such as Brazil (22.1%) [10], Marrakech (27.6%) [11] and Mexico (29.9%) [12]. However, similar prevalences were reported from the West Indies (44.1%) [13] and Norway (44.3%) [14]. Remarkably, we found a much higher prevalence in Barbarine

sheep (80%, compared to 37%). This result was in agreement with those of Van der Puije et al. [15], who showed a significant difference between breeds. Seropositivity for *T. gondii* in goat (35%) was nearly similar to that reported in Iran (30%) and Brazil (39%) using the indirect immunofluorescence antibody test (IFAT) [16,17]. While seroprevalence is lower in China (14.1%) [18], it is reportedly higher in Ethiopia (74.8%) [19] and in Saudi Arabia (51.7%) [20]. Various risk factors (age, sex, breed, diagnostic test, and climatic variations) may contribute to the difference in seroprevalence in the current study ant other studies of the word.

Regarding cattle, the seroprevalence (12%) of toxoplamosis was higher than that found in West Indies (8.4%) [13], Portugal (7.5%) [21] and Iran (0%) [16], but is comparable to that reported in Poland (12.8%) [22]. Furthermore, this is first report on the seroprevalence of *T. gondii* infection from Tunisian cattle. The difference in prevalence rates between countries is possibly due to different husbandry methods used in these regions. Our findings confirm previous studies stating that seroprevalence of *T. gondii* was highest in sheep, followed by goats and then cattle [15,16,13,21]. Differences in prevalence of toxoplasmosis between animal species might be explained by difference in susceptibility to *T. gondii* infection [23].

Our results showed that older animals (>3 years old) were more likely to be seropositive than younger ones (70% vs 22%, $p<0.01$). Recently, another Tunisian analysis investigating the seroprevalence of the parasite in ovine meat, reported a similar result (38.2% of young sheep and in 73.6% of adult sheep) [7].This is probably due to the increasing exposure to the infective oocysts in the environment [10,15]. We also found higher infection rates in female than in male animals, possibly because of higher susceptibility to protozoan parasites of the female than the male [24].

The significant seroprevalence in sheep and goats could be due to the fact that these animals are raised outdoors as grazing animals and could thus have more contact with oocyst shed by cats in the environment. It is also worth mentioning that the oasis of Gafsa was known for the abundance of fresh water, its biodiversity and its traditional irrigation system. In this area, the highest humidity and the vegetation cover protect the oocysts against desiccation which promotes their survival and sporulation. Furtheremore, we noted that animals were most affected during the culture months in the time of irrigation water abundance and thus growing herbs yet during winter and summer the animals eat dry feeds which limits parasite transmission.

In addition to the serological study, we determined the presence of toxoplasma DNA in the apex of hearts of different animals studied. Indeed, the heart was considered one of the most prone to the formation of toxoplasma cysts [1] and most accessible collection. In the present study, the overall rate of *Toxoplasma* infection in animals for slaughter was 11.5%. This was close (5.7%) to that found by Khayeche et al. [8] who conducted a study on the hearts of sheep in the center of Tunisia on the occasion of the Muslim feast of sacrifice. However, our prevalence was lower than that showed by recent studies in Tunisia [4,7]. Indeed, these authors found Toxoplasma DNA in 50% [7] and 25.5% [4] from the sheep hearts. This discrepancy could be the direct consequence of the difference in the PCR protocols used. In fact, although this technique was highly sensitive, it may give false negative results: (i) the animal may be carrying a low density of parasites in the myocardium, (ii) or the animal hosts the parasite in other organs: the brain , tongue, muscles and retina. However, it could be considered that at least 11.5% of Gafsa slaughtered animals for consumption are able of transmitting toxoplasmosis infection to humans by ingestion of undercooked or grilled meat or by contamination of hands during meat handling.

In conclusion, the present study showed an enhanced prevalence of *T. gondii* infection in sheep, goats and cattle, for the first time, in the South of Tunisia. Suggesting that consumption of meat of those animals in Gafsa area may represent a potential risk factor for human infection.

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