

Original Article

First report on seroprevalence and risk factors of *Toxoplasma gondii* infection in sheep and goats in North Lebanon

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Abstract

Introduction: Toxoplasmosis is of dual importance in both public and veterinary health due to the respective risk of transplacental transmission in primo-infected pregnant women and economic losses caused by abortions in mammals. One of the main routes of *Toxoplasma gondii* transmission to humans is the consumption of raw or undercooked meats containing parasitic cysts. Here, we performed the first epidemiological study to determine the seroprevalence and the risk factors of toxoplasmosis in livestock in Lebanon.

Methodology: Using a modified agglutination test with a cut-off of 1:40, we tested the positivity rate of Immunoglobulin G antibodies in the sera of 100 sheep and 80 goats collected from 18 different livestock farms located in North Lebanon between March and June 2018.

Results: Anti-*Toxoplasma gondii* IgG antibodies were detected in 42% of sheep and 34% of goats. Adults (> 1 year) were significantly more infected by *T. gondii* than the lambs (< 1 year) in both species ($p < 0.05$).

Conclusions: These findings indicated that food animals are highly exposed to *T. gondii* in Lebanon and could be potentially a major risk factor of *T. gondii* infection to humans. Consequently, national prophylactic strategies should be implemented to control and to prevent *T. gondii* transmission between animals and humans.

Key words: *Toxoplasma gondii*; sheep; goats; modified agglutination test; seroprevalence; risk factors.

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Introduction

Toxoplasma gondii (*T. gondii*) is a ubiquitous opportunistic protozoan parasite with a worldwide distribution. It is one of the world's most common parasite with more than 2 billion human infections [1]. Even if the clinical significance of this parasite remains asymptomatic or mild in healthy individuals, *T. gondii* remains a serious cause of morbidity in immunocompromised patients and congenitally infected fetuses and newborns. Cerebral and ocular symptoms are mainly developed in these vulnerable groups [2]. Indeed, the degree of the clinical outcomes depend on several factors including parasitic stage, infection dose, the genotype of *T. gondii* isolate and the host immune status [3]. Moreover, this heteroxenous Apicomplexa has a well-described life cycle involving cats as definitive hosts and a broad range of warm-blooded animals as intermediate hosts. Transmission occurs via ingestion of undercooked meat containing

bradyzoites in tissue cysts and via ingestion of resistant sporulated oocysts by direct contact with cat feces, contaminated soil, food and/or water. In addition, congenital transmission can occur through the transplacental passage of tachyzoites to the fetus [4].

Livestock represents 60% of all mammals on Earth and is used for many purposes, including meat production, milk and dairy products [5], as well as for breeding [6]. In the last decade, consumption of meat and dairy products has increased dramatically. By 2020, the share of developing countries in total world meat consumption will expand from 52% currently to 63% [7]. In Lebanon, small ruminant dairy production is an important part of the agricultural sector, especially in rural areas. More than 40% of the Lebanese population benefits from agriculture. However, Lebanon still imports about 85% of its food needs because of the huge consumption of meat and dairy products [8]. Meat is considered as one of the main sources of *T. gondii* and

can pose a serious problem for vulnerable populations. Notably, the human *T. gondii* infection among Lebanese pregnant women is highly important with seropositivity rate of 82% [9]. No knowledge exists about the seroprevalence and overall risk for toxoplasmosis in livestock in Lebanon. Therefore, the aim of this study was to acquire the first epidemiological data regarding the seroprevalence and associated risk factors for *T. gondii* infection in sheep and goats reared in North Lebanon for human consumption.

Methodology

The study was carried out in Northern Lebanon and Akkar governorates from March to June 2018. The Northern Lebanon governorate covers an area of 2,024 km² with a population of approximately 731,251 people. It is divided into 6 districts including Tripoli, Batroun and Koura. While the Akkar governorate covers an area of 788 km² in north Lebanon with a population of 252,917 people and is characterized by the presence of a relatively large coastal plain, with high mountains to the east. The Mediterranean climate of north Lebanon is characterized by long, hot, dry summers and short, cool, rainy winters. The average annual temperature varies from 15.5°C to 24°C and the average annual humidity varies from 48% to 75%.

The two main animal species, sheep and goat, are considered as one of the major sources of red meat in Lebanon. Blood samples were collected from 100 sheep (*Ovis aries*) and 80 goats (*Capra aegagrus*) upon the individual owner's permission. Animals were originated from 18 different livestock farms located in three districts of Northern Lebanon governorate (Batroun: 7 farms, Koura: 5 farms, Tripoli: 2 farms) and in Akkar governorate: 4 farms. The farms were selected randomly depending on the total number of farms per district. Two to fifteen animals were tested per farm representing 20-30% of the total number of reared animals. The blood was aseptically drawn from jugular veins using 10 ml vacutainer tubes with no anticoagulants. Sera were obtained after blood centrifugation at 2000g/10 minutes and stored at -20°C until serological tests were performed. A standard questionnaire was filled out for each animal that included information about animal gender (male/female), age (lamb: ≤ 1 year/adult: > 1 year), species (sheep/goat), presence of cats in/around the farm and farming system of sampling sites (open/closed system) in order to assess potential risk factors for toxoplasmosis in sheep and goats. As outlined by Dillon in 1992, open or closed system depends mainly

on whether or not the animals interact with their environment [10]. No vaccination for toxoplasmosis and no studies regarding other reproductive pathogens in animals were performed.

Sera samples from sheep and goats were screened with the commercial kit Toxo Screen DA (bioMérieux, Lyon, France), as described previously [11]. This test is a Modified Agglutination Test (MAT) to detect anti-*T. gondii* immunoglobulin G antibodies in serum samples by direct agglutination. Two-fold dilutions of sera from 1:20 to 1:2000 were performed using the dilution buffer. Then, the agglutination was screened after adding to both dilutions 25 µL of formalin-preserved tachyzoites as antigen and 25 µL of 2-mercaptoethanol to remove the IgM antibodies. The reading of plates was performed in 18 hours after incubation at 37°C. Positive and negative controls provided by the kit were included in each plate test. The test was considered positive in case of presence of a button covering about half of the well base at dilution ≥ 1:40 according to the manufacturer's instructions and other previous studies [12].

Data were recorded and analysed using the GraphPad Prism 6.0 (GraphPad Software Inc., San Diego, CA, USA) to explore the relation between the seroprevalence of *T. gondii* antibodies with the different variables such as host species, age and sex. Fisher's exact test was used and the general significance level was set at a p value below 0.05.

Results

In this cross-sectional study, samples were collected from 100 sheep, including 68 females (75%) and 32 males (25%) as well as from 80 goats, including 53 females (66%) and 27 males (34%). The majority of sheep were younger than one year (75%). The distribution regarding age and gender was quite similarly to that observed in goats as shown in Table 1. All samples were positive at both dilution 1:40 and 1:4000. Out of 180 samples, the overall seroprevalence of toxoplasmosis among tested animals was 42.2% (76/180; CI = 0.34-0.49). 49% (49/100; CI = 0.39-0.59) and 33.7% (27/80; CI = 0.23-0.44) seroprevalence was reported in sheep and goats respectively. The presence of anti-*T. gondii* IgG antibodies was higher in sheep than in goats with a statistically significant difference ($p < 0.05$).

The statistical analysis showed no significant association between *T. gondii* infection and the gender of animals in the two species ($p > 0.05$) (Table 1). On the other hand, adults (> 1 year) were significantly more infected by *T. gondii* than the lambs (< 1 year) in both

species. In sheep, 57.3% (43/75) of adults were infected by this parasite compared to 24 % (6/25) in lambs. Similar results were observed in goat flock with 40.6% (26/64) in adults compared to 6.2% (1/16) in young goats (< 1 year) (Table 1). All farms had an open household system and the animals reared in all the farms had contact with cats either inside or outside farm during grazing vegetables.

Discussion

During recent years, toxoplasmosis is a major public health and veterinary problem. It is a leading cause of serious economic losses on a worldwide basis and a common cause of congenital defects or spontaneous abortions in intermediate hosts [4]. According to European Food Safety Authority, Toxoplasmosis is the second greatest cause of death from foodborne disease after salmonellosis [13]. In 2012, the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) declared *T. gondii* to be a foodborne parasite of global interest [14]. This parasite causes tissue cyst formation in different organs of intermediate hosts such as goats and sheep, which can cause risk for human infections if meat is eaten raw or improperly cooked. In Lebanon, in 1973, the *T. gondii* infection was estimated to be 30% and 35% in 200 adults and 50 pregnant women, respectively [15]. These rates were doubled in 2010 as found by Bouhamdan *et al.* [16] where 3426 sera samples of Lebanese women of reproductive age were examined. Recently, in 2017, the overall anti-*T. gondii* IgG and

IgM seroprevalence among 2456 Lebanese pregnant women were 82.6% and 1.8%, respectively [9]. As shown in these previous studies, the prevalence of toxoplasmosis tends to sharply increase among the Lebanese population indicating the possible presence of multiple reservoirs for *T. gondii* infection and consequently a frequent exposure to the parasite. Here, we studied the status of toxoplasmosis in sheep and goats from farms of North Lebanon that were intended later for human consumption.

Out of 180 blood samples collected from different livestock animals in North Lebanon, the seroprevalence of *T. gondii* infection was high (42.2%). Numerous studies performed on livestock showed that the prevalence of toxoplasmosis varies greatly across countries based on various factors such as geographical areas, host species and age, hygiene measures, presence of cats, diagnostic tools and methods used to prevent *T. gondii*. In fact, the high prevalence detected in our animal populations in North Lebanon may be due to the presence of some environmental and anthroponotic factors. For instance, the Lebanese cats are supposed to be highly infected with *T. gondii*. 78.1 % of cats in the Lebanese capital, Beirut, had antibodies to this parasite as reflected by the only published report in Lebanon in 1985 [17]. Furthermore, the seropositivity rate for *T. gondii* infection was variable between farms from 0 to 89%. More than half of the farms (61%; 11/18) had a positivity rate more than 40%. However, all farms included in this study had an open household system that allows direct contact with cats and their

Table 1. Seroprevalence and associated risk factors for *Toxoplasma gondii* infection in sheep and goats from the North of Lebanon.

Animal factors	No. tested (%)	No. Positives	Seroprevalence of <i>T. gondii</i> (%)	p -value	OR	CI 95%
Sheep*	100 (56%)	49	49.00			0.390-0.590
Age group						
< 1 year	25 (25%)	6	24.00	0.005	0.230	0.08-0.65
> 1 year	75(75%)	43	57.33			
Gender						
Male	32 (32%)	12	37.50	0.136	1.989	0.84-4.70
Female	68 (68%)	37	54.41			
Goats*	80 (44%)	27	33.75			0.232-0.443
Age group						
< 1 year	16 (20%)	1	6.25	0.009	0.032	0.00-0.25
> 1 year	64 (80%)	26	40.63			
Gender						
Male	27 (34%)	7	25.92	0.320	1.732	0.62-4.82
Female	53 (66%)	20	37.73			
Total	180 (100%)	76	42.22			0.349-0.495

CI, confidence interval; OR, odds ratio; *Statistically significant association (p -value = 0.0396; OR = 1.886; CI 95% = 1.02-3.46).

excrements. Hence, the oocysts released by infected cats can be spread and contaminate anything that comes in contact with (soil, water, and food) which can be ingested later by sheep, goats and even humans. Due to the mild Mediterranean climate in North Lebanon, temperature and humidity can play an important role in the distribution and transmission of *T. gondii* by affecting the survival and infectivity of the parasite oocysts. [18].

Additionally, the susceptibility of the host species to *T. gondii* infection determines the occurrence and transmission of toxoplasmosis. Our findings revealed that approximately half of sheep population was infected by *T. gondii* parasite (48%) with a significantly higher positivity rate to that found in goat (34%). Sheep is known as the most susceptible host for toxoplasmosis [4]. They are technically grazers; meaning they prefer munching grass, clover, forbs, and other pasture plants low to the ground, putting them in close contact with contaminated environment by the stool of an infected cat. On the other hand, goats are known as browsers meaning they often choose to select leaves, vines, and weeds, often found at the tops of plants, higher off the ground. In the absence of this variety of foods, goats will be forced to graze directly off the ground leading to direct contact with *T. gondii* oocysts.

In our study, the important seroprevalence in Lebanese ovine was higher to those observed as average in Mediterranean countries: 10% in Turkey [19], 26% in Egypt [20], 27.6% in Morocco [21] and 28.3% in Greece [22]. However, higher results were observed in some localities from: Libya 71% [23], France 65.5% [24] and Switzerland 61.6% [25]. Regarding the seroprevalence of *T. gondii* in goats, our results (33.75%) were relatively close to Tunisia 34.5% [26], but lower than data reported in Saudi Arabia 51.7% [27] and Egypt 62% [8].

Considering age, our results outlined that the seroprevalence of *T. gondii* antibodies was significantly higher in adults (50%) than in lambs (17%) suggesting that when animals become older, they are more exposed to oocysts shed by cats through their lifetime (Table 1). Our findings are consistent with other recent studies showing that infection increased with age. For instance, in France, the seroprevalence of *T. gondii* increased from 17.7% for lambs to 89%, for adult sheep [28]. However, contradictory results were obtained by another study performed in Sao Paulo state, Brazil where younger animals were more predisposed to the infection due to low immunity and exploratory habits as suggested by Dubey *et al.* [29].

Among all serologic tests used to detect *T. gondii* antibodies such as Dye Test, Indirect immunofluorescent test, MAT, Latex Agglutination Test and Enzyme-Linked Immunosorbent Assay (ELISA), the MAT had the highest sensitivity and specificity and is used for various hosts including goats, sheep, chicken and pigs [30,31]. By comparison to ELISA which has 90.1% of sensitivity and 85.9% of specificity for the detection of *T. gondii* infection in naturally infected sheep, MAT provided better sensitivity and specificity of 96 % and 88.9%, respectively [32]. According to the kit and by comparison to Dye test that is used as reference test for the serodiagnosis of toxoplasmosis [33], the relative sensitivity and specificity of MAT technique tested for 991 serum samples was 96% and 99%, respectively. The non-specific agglutination is suppressed by using a diluting buffer containing 2-mercaptoethanol, which denatures the IgM antibodies. Although MAT is easy to perform, safe and applicable, some disadvantages are still present. The length of manipulating time and the subjectivity in the interpretation of the results make the test less practical than others. Moreover, the test employs a large amount of formalin-treated whole tachyzoites which agglutinate in the presence of diluted serum containing specific IgG antibodies. It detects only the chronic infections which can underestimate the seroprevalence of toxoplasmosis in the studied population. In addition, the MAT results may be affected by the prozone phenomenon. Consequently, the MAT could be accompanied by other techniques such as ELISA or direct search of parasites in the meat.

There are two major limitations in this study that could be addressed in future research. First, the number of samples in this study was limited and non-representative for all the districts of North Lebanon. Second, the risk of failure reproduction in the studied animals was not assessed due to the possible presence of varieties of pathogens that could cause the abortion. The findings of this study should further be confirmed by well-designed large-scale epidemiological studies.

Conclusions

To our knowledge, this is the first investigation regarding the seroprevalence of antibodies to *T. gondii* in livestock in Lebanon. Our data shows that the seroprevalence of this parasite is relatively frequent among sheep and goats, in particular adults. As the traditional Lebanese food consists of many dishes containing raw meat, our results show that sheep and goats reared in North Lebanon are an important source of infection in the Lebanese human population. Further

epidemiological and population genetic studies are needed to understand the role of livestock in the transmission patterns and as a risk factor for human infections in Lebanon.

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