



## Prevalence of *Toxoplasma gondii* antibodies in raw goat milk at North West Libya

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### Abstract

Drinking of raw goat milk is considered as source of human Toxoplasmosis infections, with high rate of morbidity. This study was aimed to investigate the incidence of *Toxoplasma gondii* in 82 goat raw milk samples in Ab Aissa, Sorman, Sabrath, Aljeelat, and from veterinary clinic at Tripoli. Milk samples were tested to *T. gondii* antibodies by Latex agglutination test. Incidence of *T. gondii* in goat milk was 68% (56/82) with 22/29 positive farm. The results showed that *T. gondii* antibodies were prevalent in all goat ages. Examined milk smears showed toxoplasma cysts in one sample. The results of present study showed a high incidence of toxoplasmosis among goat milk samples in North West Libya, the existence of toxoplasma cysts or tachyzoites in raw milk increases the risk to toxoplasmosis, that the parasite could be transmitted to human through drinking of uncooked milk. Therefore, we suggest that boiling or pasteurization of goat milk before human consumption will reduce the possibility of parasite transmission to the people.

**Key Keywords:** *Toxoplasma gondii*, raw milk, goat, antibodies.



## Introduction:

Toxoplasmosis is an old disease caused by *Toxoplasma gondii*<sup>1</sup>, which is an opportunistic obligatory intracellular protozoan belonging to Apicomplexa phylum, was observed for first time in a North African rodent (*Ctenodactylus gundi*)<sup>2, 3, 4, 5</sup>. It can infects roughly all of warm-blooded animals including domestic animals and humans<sup>2, 6</sup>. The prevalence of *Toxoplasma gondii* seropositivity data indicate that toxoplasmosis is one of the most common humans infections throughout the world, especially in a lower elevated warm areas<sup>7</sup>. It estimate to affect approximately 28% of human population all over the world<sup>8</sup>. Whereas, the seroprevalence In deferent Libyan population is has been estimated to 47%<sup>9</sup>.

Toxoplasmosis resulting from *T. gondii* infection occurs as mild acquired infection in immunocompetent persons, reactivated chronic infection in immunocompromized persons, and congenital or acquired ocular disease<sup>10 11</sup>. The observed human toxoplasmosis also has been linked epidemiologically to consumption of the parasite in food as major sources of infection like unpasteurized goats milk which was well known as source of human infections<sup>12</sup>, and more information with high prevalence rates of *T. gondii* have been reported in goats from around the world up to 75%<sup>10, 14</sup>. Although, limit studies were conducted in North African about antibodies seroprevalence of *T. gondii* in a goat milk, recently, a serological survey carried out in Tunisia, Morocco and Egypt reported that 35%, 9% and 62% in respectively of goats were positive to toxoplasmosis<sup>3, 14, 15</sup>.



Many studies have been approved that the goat milk is more infected than in their meat. Consumption of unpasteurized goat's milk has been associated with human toxoplasmosis, and particularly, human outbreaks by toxoplasmosis were reported mainly by the ingestion of unpasteurized goat milk of affected animals that contains tachyzoites, which facilitates the transmission to the offspring <sup>16</sup>. *T. gondii* transmission is referred to both tachyzoites in milk and suckling trauma, but also tissue cyst excretion due to specific exocytic lipid secretion in mammary cell <sup>17</sup>.

Riemann et al. <sup>18</sup> reported a case of *T. gondii* infection in children who probably contracted the disease by ingesting unpasteurized goat milk. Therefore, it is advisable that milk should be boiled or pasteurized before human consumption as these procedures will inevitably kill any potentially present tachyzoites <sup>19, 5</sup>.

Libya has about 1,265,000 heads of goat <sup>3</sup>, used in meat production, milk is usually left to children and sometimes consumed by goat owners. Although pasteurization will kill *T. gondii* in goat's milk, drinking the raw goat's milk is very usual habit in Libya, especially within the herds' owners. This study aimed to detect *T. gondii* antibodies in raw Libyan goats milk, in Surman and Subrath, Libya.

## **Materials and methods**

### **Ethical consideration**

Ethical approval is not required to pursue this type of study.

### **Collection of goat milk samples**

Eighty two raw milk samples were collected directly from different goats. The samples were from 29 farms, Two at Abi Aissa, Eighteen at Sorman, Six at Sabraths and Three at Aljeelat, and from veterinary clinic at Tripoli



[ figure 1].They included 55 Sorman goat (SG), 11 Sabratha goat (TG), 3 Abi Aissa goat (AG), 3 Aljeelat goat (JG) and 10 Clinic goat (CG). The collection was in a period between 9 November 2018 to 14 April 2019. All samples were collected manually without disinfection of goat teats in dry and sterile plastic 5 ml tubes. Samples were labeled and kept at -4°C until analysis at laboratory of Molecular biology and Biochemistry department, sciences college, Sabratha University, Libya.

### **Latex agglutination test (QUALITATIVE TEST) :**

Milk samples were analyzed using the Latex agglutination technique (TOXO LATIX KIT) to detect specific antibodies in raw goat milk against *T. gondii*, according to the manufacturer's protocol [ Appendix 1]. After bringing of samples and reagents at room temperature, 40  $\mu$ L of undiluted milk sample and two drops of control were added to slide, then transfer 1 drop (20  $\mu$ L) of toxo latex to the samples, mix with stirring sticks, and rotate slowly the slide. After 4-6 minutes we check for agglutination, at the same time compare with the reaction of the control. The presence of agglutination indicates an antibody concentration equal or more than 4 IU/mL.

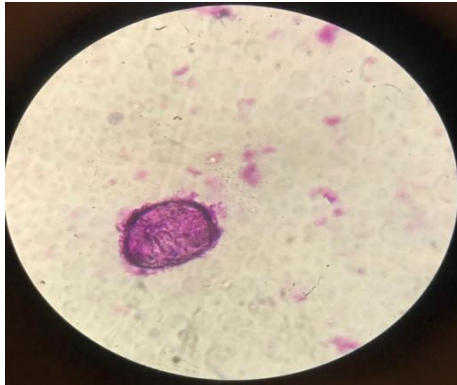
### **Reading and Interpretation**

Examine macroscopically the presence or absence of visible agglutination immediately and and microscopic examination of milk smears stained with Giemsa stain.

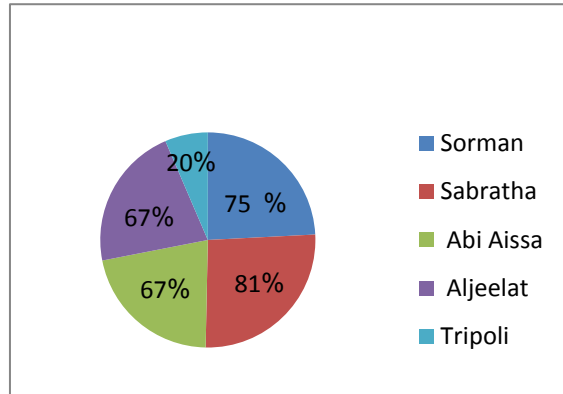
### **Results**

Incidence of *T. gondii* in goat milk was 68% ( 56/82 ) by microscopic examination of Latex agglutination test and the result of microscopic examination of Giemsa stained milk smears showed toxoplasma cyst in one sample (figure 1). Positive *T. gondii* were detected in 41 of 55 SG

(75%), 9 of 11 TG(81%), 2 of 3 JG (67%), 2 of 3AG (67%) and 2 of 10 CG (20%) (Table 1, figure 2).



**Figure 1: showing toxoplasma cyst (arrow) in goat milk smear stained with giemsa stain (Lens, 1000x)**



**Figure 2: Positivity of Toxoplasmosis by region**

**Table 1: Incidence of *T. gondii* antibodies in the examined raw milk samples.**

Goat regions	No. of examined samples	positive latex test n (%)
Sorman	55	41 (75%)
Abi Aissa	3	2 (67%)
Sabraths	11	9 (81%)
Aljeelat	3	2 (67%)
Tripoli	10	2 (20%)
<b>TOTAL</b>	<b>82</b>	<b>56 (68%)</b>

Positive prevalence of *T.gondii* antibodies in goat milk were found in 22 farm of 29. In particular, prevalence rate in single farms were as follows: 8/8 (100%) in farm A, 5/5 (100%) in farm C, E and O , 3/3(100%) in farm G, 2/2 (100%) in farm I, L and M, 4/5 (80%) in farm D, 3/4 (75%) in farm F, 3/8 (38%) in farm B, 1/2 (50%) in farm P, 2/3 (67%) in farm N, 1/1 in farm Q, R, S, T, U, V, W,Y and Z ( Table 2, figure 2). Of the Sorman farms, 14/17 were record a positivity for *T.gondii* antibodies



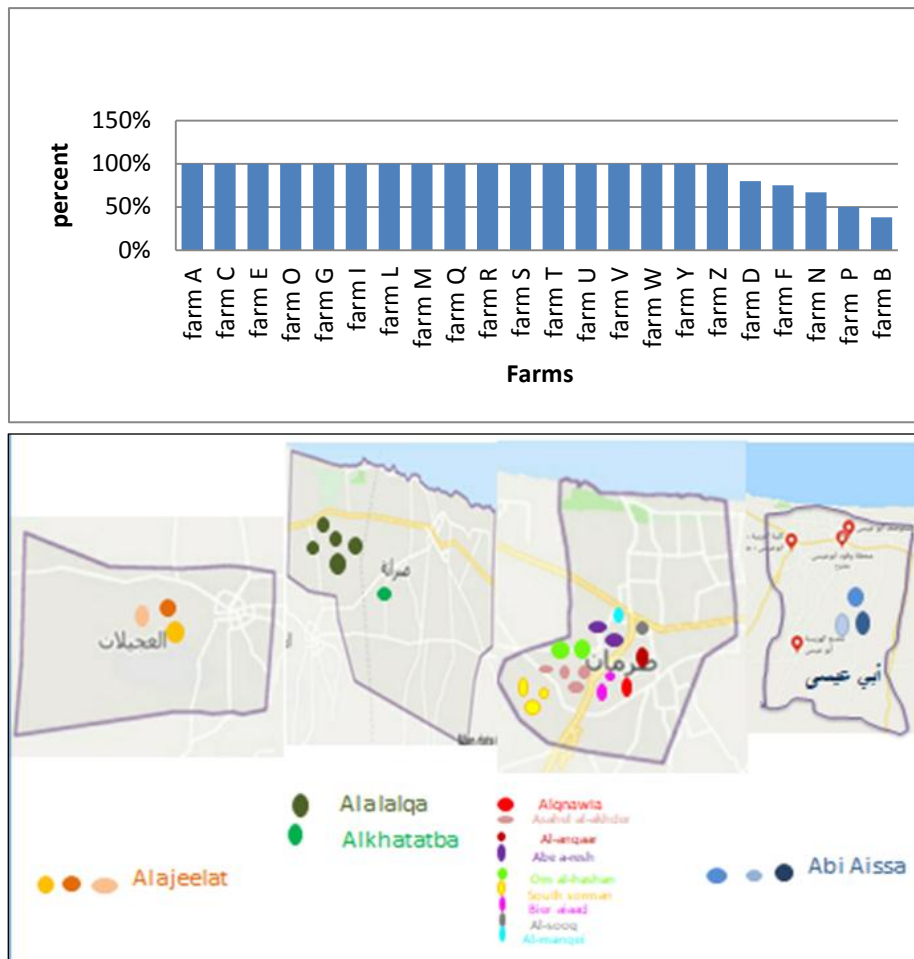
distributed as following: 4 farm at Alsahel Alakhder, 2 farm at Bereash, 2 farm at Om Alhashan, 3 farm at South Sorman, 2 farm at Bear Aiad ,1 farm at Almanqh. While in Sabratha 4/6 farms record positivity , 3 at Alalalqa and One at Alkhatatba. In Abi Aissa the two positive farms were at Suq Alahad (Figur 2,Table 2, Map 1).

**Table 2: Summary of Latix agglutination test results for *T. gondii* in goats milk**

Farm symbol	Number of goats	Region	Results (No. of positive/No. of examined)
H	2	S (ALqnawia)	0:2
I	2	S (ALsahel Alakhder)	2:2
Z1	1	S (Alanqar)	0:1
Z2	1	Abiaissa	0:1
Q	1	S (Abereesh)	1:1
K	2	S (Om Alhashan)	0:2
R	1	T (Alalalqa)	1:1
S	1	T (Alalalqa)	1:1
Z3	1	Alajeelat	0:1
T	1	S (Om Al-hashan)	1:1
Z4	1	T (Al-alalqa)	0:1
Z5	1	T (Al-alalqa)	0:1
L	2	T (Al-alalqa)	2:2
U	1	Alajeelat	1:1
V	1	S (Abereesh)	1:1
B	8	S (South sorman)	3:8
W	1	Abiaissa	1:1
Y	1	Alajeelat	1:1
Z	1	Abiaissa	1:1
D	5	S (ALsahel Alakhder)	4:5
C	5	S (South sorman)	5:5
G	3	S (Beer Aiad)	3:3
P	2	S (Asooq)	1:2
A	8	S(South sorman)	8:8
M	2	S(Asahel Alakhder)	2:2
N	3	S (Beer aiad)	2:3
F	4	S (Almanqai)	3:4
E	5	S (Asahel alakhder)	5:5
O	5	T (Alkhatatbaa)	5:5
<b>Total</b>	<b>82</b>		<b>(56/82) 68%</b>

Regions at North West Libya, S:Sorman, T: Sabratha.

**Figure 3: prevalence rate of *T.gondii* antibodies in each single farms**



**Map 1: incidence distribution at Sorman, Sabratha, Alajeelat and Abiaissa city, (Libya Data map 2019)**

## Discussion

For the first time in Libya we determine a specific *T. gondii* antibodies in goat milk with latex agglutination test. Toxo latex consists of an aqueous suspension of polystyrene particles coated with soluble purified antigens from *T. gondii*. If specific antibodies are present in the sample a clear agglutination will appear. In our study the overall prevalence of anti-*T. gondii* in goat milk was about 68% which was near to that found in north Egypt and higher than that found in South Tunisia and Morocco<sup>3, 14, 15</sup>. The climate and frequency of cats in farms may contribute in different or similarity in results between the current study and other studies of the



word. This study show that exposure of goats to *T. gondii* infection in the regional area examined may be very common, which was ranged in from 100% to 38%<sup>10</sup>.

This study showed that *T. gondii* antibodies were prevalent in all ages (Table 3) which different than that found in Tunisia that likely to be more prevalent in older animals than younger ones<sup>13</sup>. This is may indicate to being there is congenital infection between included goats .

**Table 3: Distribution of *T. gondii* incidense in goat according to the age**

category	Samples size	Number with anti- <i>T. gondii</i> antibodies	prevalence (%)
1-2 year	3	2	67%
1-3 year	10	7	70%
>3 years	63	39	61%

In addition, our results just document that *T. gondii* antibodies can be present in the milk, without conforming that was due to congenitally infected goats or from farm environment. The substantial occurrence of *T. gondii* antibodies in goat milk could be due to the fact that these animals are grazing animals and therefore have more exposure to *T. gondii* oocysts shed in cats stool that contaminate the farms soil, vegetable and irrigation water. In area which this study included that represent North West Libya, the highest humidity and the plants cover and protect the oocysts to promotes sporulation. Furthermore, we collect the samples during winter and spring when growing herbs yet to feeding animal, that may accelerate parasite transmission. Unexpectedly, we observed toxoplasma cyst in milk smear as shown in figure 1, which confirm that the toxoplasma cysts may found in mammary gland of infected animals<sup>17</sup>.





In addition, the presence of tachyzoites in the milk was recorded, in different animals including goats. In specific, the persistence of *T. gondii* tachyzoites in goat milk were experimentally evaluated, and the observed results show that tachyzoites can survive in goat milk from three to seven days at +4°C, and as a result, that the raw goat milk can serve as a source of toxoplasmosis. However, tachyzoites of *T. gondii* are not commonly considered as an rout of oral transmission, because they cannot stand long outside the host and, because they are sensitive to proteolytic enzymes, but, tachyzoites were also shown to survive up to 2 h in pepsin solutions, and can penetrate the oral-pharyngeal mucosa as rout of oral infection<sup>5</sup>. For that reason, and built on our results in this survey and on that previous studies, we confirm that drinking of undercooked milk has a risk of Toxoplasma transmission and has an influence on public health.

## **Conclusion**

The results of present study showed a high incidence of toxoplasmosis among goat milk samples in North West Libya, the existence of toxoplasma cysts or tachyzoites in raw milk increases the risk to toxoplasmosis, that the parasite could be transmitted to human through drinking of uncooked milk. Therefore, we suggest that boiling or pasteurization of goat milk before human consumption will reduce the possibility of parasite transmission to the people. Additionally, we recommended to further molecular study b to confirm the incidence of *T. gondii* in goats milk and for effective control strategies against toxoplasmosis.



## References

1. Wójcik-fatla A, Sroka J, Zajac V, Sawczyn A, Cisak E, Dutkiewicz J. *Toxoplasma gondii* ( Nicolle et Manceaux , 1908 ) detected in *Dermacentor reticulatus* ( Fabricius ) ( Ixodidae ). *Folia Parasitol (Praha)*. 2015;62. doi:10.14411/fp.2015.055
2. Lukman DW, Sudarwanto M. Survival of *Toxoplasma gondii* in Goat Milk after Pasteurization with Low Temperature and Long Time. 2013;(April 2015). doi:10.5829/idosi.gv.2013.11.6.82138
3. Rouatbi M, Amairia S, Amdouni Y, Boussaadoun MA, Ayadi O. *Toxoplasma gondii* infection and toxoplasmosis in North Africa : a review. 2020;6(2019).
4. Sroka J, Kusyk P, Bilska-Zajac E, et al. Seroprevalence of *Toxoplasma gondii* infection in goats from the south-west region of Poland and the detection of *T. gondii* DNA in goat milk. *Folia Parasitol (Praha)*. 2017;64(4 ml). doi:10.14411/fp.2017.023
5. Mancianti F, Nardoni S, D'Ascenzi C, et al. Seroprevalence, detection of DNA in blood and milk, and genotyping of *toxoplasma gondii* in a goat population in Italy. *Biomed Res Int*. 2013;2013. doi:10.1155/2013/905326
6. Ahmed HA, Shafik SM, Alli MEM, Elghamry ST, Ahmed AA. Molecular detection of *Toxoplasma gondii* DNA in milk and risk factors analysis of seroprevalence in pregnant women at Sharkia, Egypt. *Vet World*. 2014;7(8):594-600. doi:10.14202/vetworld.2014.594-600
7. Bowerman, R.J. Seroprevalence of *Toxoplasma gondii* in rural India: a preliminary study. *Trans. Soc. Trop. Med. Hyg*;1991 85: 622
8. Saad NM, Hussein AAA, Ewida RM. Occurrence of *Toxoplasma gondii* in raw goat, sheep, and camel milk in Upper Egypt. *Vet World*. 2018;11(9):1262-1265. doi:10.14202/vetworld.2018.1262-1265
9. Gashout A, Amro A, Erhuma M, et al. Molecular diagnosis of *Toxoplasma gondii* infection in Libya. *BMC Infect Dis*. 2016;16(1). doi:10.1186/s12879-016-1491-5



10. Webster JP, Dubey, J.P. Toxoplasmosis of Animals and Humans. *Parasit Vectors*. 2010;3(1):2-3. doi:10.1186/1756-3305-3-112
11. SMITH JL. Foodborne Toxoplasmosis. *J Food Saf*. 1991;12(1):17-57. doi:10.1111/j.1745-4565.1991.tb00063.x
12. Sadek O, Abdel-Hamed Z, M. Kuraa HM. Molecular detection of toxoplasma gondii dna in raw goat and sheep milk with discussion of its public health importance in assiut. *Assiut Vet Med J*. 2015;61(145):166-177.
13. Arwa Lachkhem IL, Wahiba Sakly DS. Prevalence of Toxoplasmosis in Sheep, Goats and Cattle in Southern Tunisia. *J Bacteriol Parasitol*. 2015;06(05):10-13. doi:10.4172/2155-9597.1000245
14. Benkirane A, Essamkaoui S, El Idrissi A, Lucchese L, Natale A. Indagine sierologica sulle più comuni cause di aborto infettivo nei piccoli ruminanti in Marocco. *Vet Ital*. 2015;51(1):25-30. doi:10.12834/VetIt.389.1814.1
15. Al-Kappany YM, Abbas IE, Devleeschauwer B, Dorny P, Jennes M, Cox E. Seroprevalence of anti-Toxoplasma gondii antibodies in Egyptian sheep and goats. *BMC Vet Res*. 2018;14(1):1-5. doi:10.1186/s12917-018-1440-1
16. Tenter AM. Toxoplasma gondii in animals used for human consumption. *Mem Inst Oswaldo Cruz*. 2009;104(2):364-369. doi:10.1590/S0074-02762009000200033
17. Diagnosis of Infection with Toxoplasma Gondii in Pregnant Women , Neonates and Immunocompromised Patients Eskild Petersen.; 2005.
18. De Paula RC. September 14, 2009. *J Venom Toxins Incl Trop Dis*. 2010;16(2):311-323.
19. Tenter AM, Heckeroth AR, Weiss LM. Erratum to “Toxoplasma gondii: from animals to humans” [Int. J. Parasitol. 30 (2000) 1217–1258]. *Int J Parasitol*. 2001;31(2):217-220. doi:10.1016/s0020-7519(01)00125-4.