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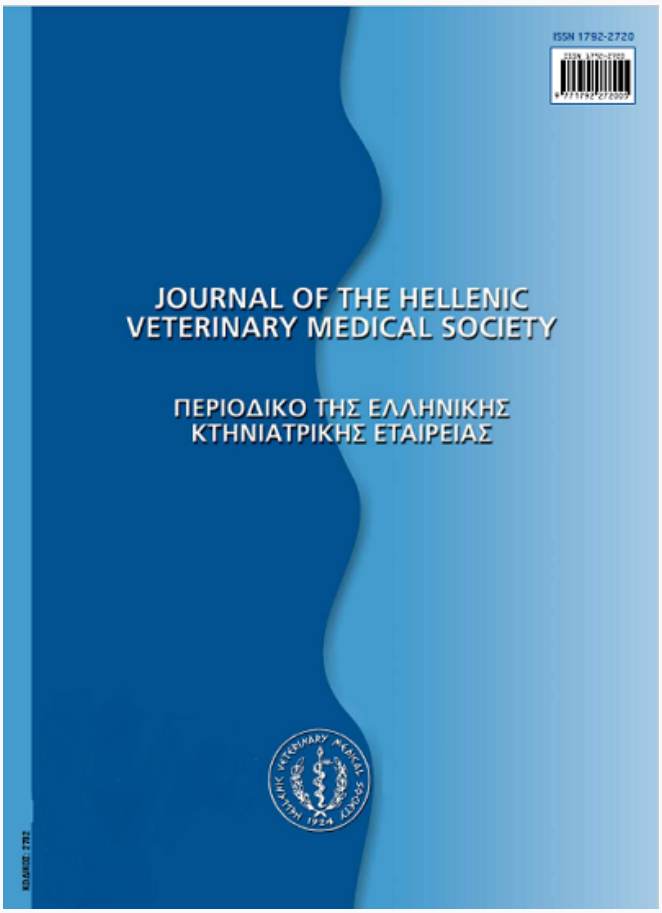

The zoonotic protozoan of sheep carcasses in the north of Algeria: A case of ovine toxoplasmosis

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Research article
Ερευνητικό άρθρο

The zoonotic protozoan of sheep carcasses in the north of Algeria: A case of ovine toxoplasmosis

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ABSTRACT. *Toxoplasma gondii* is a zoonotic protozoan parasite of great importance in veterinary and public health. The aim of the present study was to determine the seroprevalence for *T. gondii* in 580 sheep sera slaughtered for human consumption in the slaughterhouses of El Harrach by a commercial kit ELISA (enzyme-linked immunosorbent assay), and to evaluate the presence of *T.gondii* in 335 sheep from 580 (335 oesophagi and 335 diaphragms) by.the histopathological analysis. Antibodies to *T. gondii* were found in 8.28% (48 /580) of sheep. All positive sheep were male. Seropositivity for *T. gondii* increased with age, but the difference was not statistically significant.. While the seroprevalence was significantly higher in summer and in the North /Center of Algeria. Thus, season and origine of animals were considered as risk factors associated with *T. gondii* infection. Histopathological analysis showed that only 2 sheep presented dubious cysts of *T. gondii*. However, tissues cysts compatible with *Sarcocystis spp.* were visible in the histological sections of 94.03% (315/335) of sheep. These results suggest that infection with *T. gondii* in sheep is present in the north of Algeria and as sheep with antibodies usually carry tissue cysts, this indicates that undercooked lamb and mutton may indeed be a sources of human toxoplasmosis.

Keywords: Sheep, Toxoplasmosis, *T.gondii*, ELISA, Tissue cyst.

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1. INTRODUCTION

Sheep are commonly infected with a cosmopolitan zoonotic infection, Toxoplasmosis, caused by the coccidian protozoan parasite, *T. gondii* (Dubey, 2009), which naturally infects human beings, wild and domestic animals, as well as birds. It is a geographically wide spread infection (Germani Fialho and al., 2009). Also, it has substantial medical and veterinary significance (Tenter and al., 2000). Infection with *T.gondii* during pregnancy in sheep has a major economic impact and represents a serious risk for congenital disease including embryonic or fetal death and mummification, abortion, stillbirth, and neonatal death (Dubey, 2009). In humans, infection results from the ingestion of oocysts released into the environment with the faeces of cats, from the consumption of raw or lightly cooked meat containing tissue cysts, or through congenital transmission (Dixon, 1992). The ingestion of undercooked infected lamb is considered as an important source of infection for humans (Dubey, 2009). Approximately one-third of the world population is likely to be exposed to this parasite (Tenter and al., 2000). While toxoplasmosis is often mild or asymptomatic, it can be a devastating illness in immunocompromized patients and in congenitally infected infants (Dixon, 1992). Seroprevalence of *T. gondii* in sheep has been reported extensively in different countries and the positive rates ranged from 3% to 95% (Dubey, 2009). In Algeria, the incidence of toxoplasmosis in man or its prevalence in sheep is not well known. As a result, its impact on sheep production still remains unknown and abortions are attributed to other diseases such as brucellosis, Also, the possible sources of infection to humans through lamb consumption are not well established. Considering the importance of toxoplasmosis and the lack of epidemiological information of ovine toxoplasmosis in Algeria, the presented study aimed to obtain data on the prevalence of *T. gondii* infection in 580 sheep in the slaughterhouses in the north of Algeria, particulary those of El Harrach, and the probable role of ovine meat consumption in human toxoplasmosis. To this end, ELISA test and histological technique were established for the examination of sheep sera and the tissue fragments.

2. MATERIALS AND METHODS

2.1. Sample collection and storage

Samples from randomly selected sheep were collected. The animals came from different regions of Algeria and were intended for human consumption. Most of them males and few females were sampled because females are normally kept for breeding purpose, age was estimated and animals were divided into three age groups: ≤ 1 year, 1.5-3 years, and 3-5 years. A total of 580 blood samples were collected directly from the jugular vein during bleeding in sterile assay tubes. Each animal was then tagged on the right anterior limb for oesophagus and diaphragm collection. The samples were tagged, refrigerated, and transported immediately to the laboratory of parasitology and mycology of the Superior National Veterinary School - Algiers -. In the laboratory, sera were separated after centrifugation at 3000 rpm for 10 minutes and stored at - 20 °C in microtubes until assayed by ELISA technique and the tissue fragments (oesophagi and diaphragms) were stored in 10% buffered formalin and were submitted to the laboratory of anatomy pathological ENSV- Algiers - for histopathological evaluation and identification of the parasite.

2.2. Serological analysis

Serological analysis of 580 sera of sheep was realised at the laboratory of parasitology and mycology of the Universitary Hospital of Mustapha Bacha - Algeirs -. Toxoplasma-specific antibodies were measured by a commercial kit ELISA (ID Screen® Toxoplasmosis Indirect Multi-species, ID vet, Grabels - France). According to the manufacturer's instructions, 90 microliter (ul) of the dilution buffer 2 were distributed into the 96 polystyrene wells of the ELISA microplate coated with Toxoplasmosis P30 Antigen. Ten ul of the positive and negative control were included in A1 and B1 cupules for the negative control, and C1 and D1 cupules for the positive one, the sera were deposited at 10 µl/well (into the other cupules) and incubated for 45 min ± 4 min at 21 °C (± 5 °C). Next, the plates were washed 3 times with washing buffer, 100 ul of Anti-multi-species IgG-HRP peroxidase-labeled conjugate previously diluted 1:10 in dilution buffer 3 was added and incubated for 30 min ± 3 min at 21°C (± 5 °C). Then, the wells were washed, 100 ul of substrate solution was distributed and incubated for 15 min ± 2 min at 21°C (± 5°C) in the darkness, the reaction was stopped by adding 100 ul of stop solution. The optical density (OD) of each well was measured at 450 nm using a absorbance microplate reader (BIO-RAD PR

4100). The test results were interpreted according to the manufacturer's instructions. The test is validated if the average optical density value of the positive controls ODpc > 0.350 and the ratio: OD of the positive controls / OD of the negative controls > 3.5. For each sample, the percentage (S/P%) was calculated according to the schema provided by the manufacturer: S/P% = Optical density of sample × 100/ Optical density of positive control. Samples with S/P% ≤ 40% were considered to be negative, samples with S/P% between 40% and 50% were considered to be dubious, samples with S/P% between 50% and 200% were considered positive, whereas values ≥ 200% indicated acute toxoplasmosis.

2.3. Histological examination

670 samples were studied from 335 sheep (335 oesophagi and 335 diaphragms). The fixed samples were cut into 0.5 cm-thick sections, dehydrated through serial dilutions of ethanol and xylene, before being embedded in paraffin wax using routine procedures. From each block, four to six sections 4 to 5 micron thick were cut, deparaffinised, rehydrated and stained with haematoxylin and eosin (H&E). All the slide was analyzed by a light microscopy (Leica DMLS®, objective (40×, 100×) for presence of tissue cysts of *T. gondii* or histopathological lesions.

2.4. Statistical analysis

Statistical analyses of *T. gondii* prevalence from different regions, age groups, gender and season were performed by Chi Square test using the software program Microsoft Excel 2010. The differences were considered statistically significant if P < 0.05.

3. RESULTS

3.1. Research of antibodies against *T. gondii* by ELISA

3.1.1. The overall seroprevalence of *T. gondii*

The overall *T. gondii* seroprevalence was 8.28% (48 /580) with S/P% ranging from 79% to 336%. While 2/580 (0.34%) samples gave a dubious results and 530/580 (91.38%) were negative. The chi-square test was very significant between the positive and negative results. From 48 seropositive animals 13 sera had S/P% ≥ 200, while 35 sera had S/P% between 50% and 200% (Figure 1). The chi-square test was very significant between the two levels of seropositive cases (S/P% between 50% and 200%, S/P% ≥ 200).

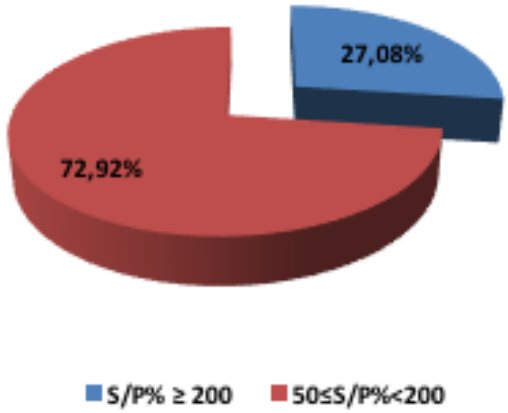

Figure 1: Repartition of the 48 seropositive sheep according to S/P% value.

3.1.2. Seroprevalence of *T.gondii* according to the risk factors

Results of the seroprevalence of *T. gondii*. according to the risk factors are shown in the following Table.

3.2. Research of *T. gondii* tissue cysts by histological technique

It was difficult to identify cysts of *T. gondii* in histological sections stained with HE by a light microscopy, beacause the parasite can be confused with other protozoan parasites (*Sarcocystis*,

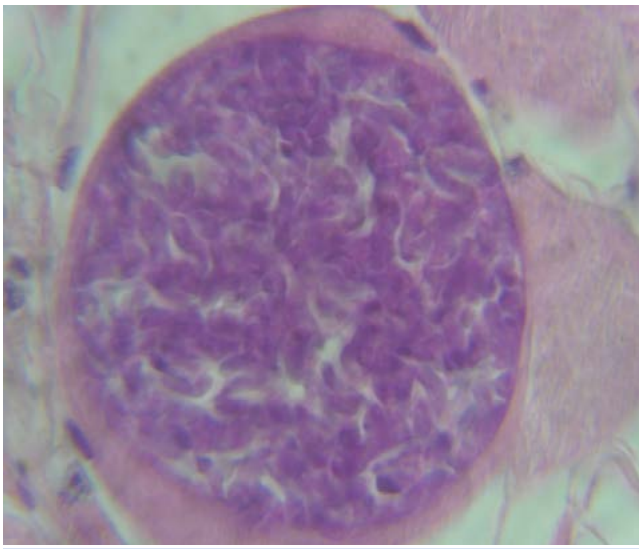

Figure 2: Dubious tissue cyst of *T.gondii* in diaphragm (H&E, 1000×)

Table: Seroprevalence of *T.gondii* in sheep according to the risk factors

Factors	Category	Sheep tested		Sheep with *T.gondii* antibodies		95%CI	Degree of significance and P value
		N	%	N	%		
Gender	Male	574	98.96	48	8.36	6 -10.5	
	Female	6	1.03	0	0		
Age Year (s)	≤ 1	87	15	5	5.75	0.9- 10.6	Not significant p = 0.375
	[1.5-3]	288	49.65	22	7.64	4.6-10.7	
	[3-5]	205	35.34	21	10.24	6.1-14.4	
Season	Winter	144	24.82	0	0		Very significant (p < 0.0001)
	Spring	144	24.82	7	4.86	1.3-8.4	
	Summer	144	24.82	32	22.22	15.4-29	
	Autumn	148	25.51	9	6.08	2.2-9.9	
Region (in Algeria)	Center	335	57.75	37	11.04	7.7-14.4	Very significant (p < 0.01) Without south
	Western	169	29.13	5	2. 96	0.4-5.5	
	Eastern	65	11.20	6	9.23	2.2-16.3	
	South	11	1.89	0	0		

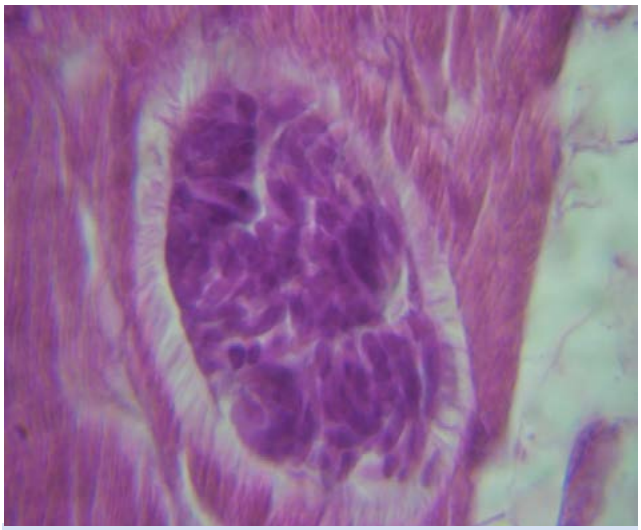

Figure 3: *Sarcocystis* spp cyst in oesophagus, thin wall with hair like projections (H&E, 1000×).

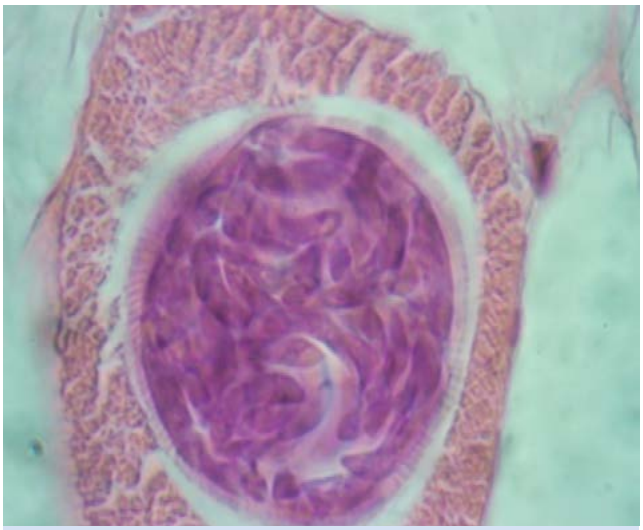

Figure 4: *Sarcocystis* spp cyst in oesophagus, thick wall with radial striations (H&E, 1000×).

Hammondia, Neospora). Dubious tissue cysts of *T.gondii* were found in 2 sheep corresponding to a prevalence of 0.59% from 335 sheep examined and 4.16% from 48 seropositive sheep. One cyst was found in the diaphragm and the other in the oesophagi

Observed tissue cysts were spherical, had diameters of 40 to 50 um and surrouded by a thin wall cyst (Figure 2). However, tissue cysts compatible with *Sarcocystis spp.* were visible from both muscles tissues of 94.03% (315/335) of the sheep. These cysts

variable in size and in shape were widely dispersed throughout the tissues and according to the morphology of their walls cyst, two types of microcysts were differentiated. *Sarcocystis* with thin wall cyst with hair like projections (Figure 3) and *Sarcocystis* with thick wall cyst with radial striations (Figure 4). No significant histopathological changes were found in the evaluated organs.

4. DISCUSSION

4.1. Overall seroprevalence

The choice of the ELISA technique for this study is justified by the fact that it is considered to be one of the most sensitive immunologic techniques. A number of studies have been conducted on the seroprevalence of *T. gondii* infection in sheep from various geographical regions in the world. The present results agree almost with those obtained in Algeria by Dechicha and al. (2015) who studied sheep from different herds in the north of Algeria and found 11.59% positivity for 276 serum samples using the indirect fluorescent antibody test (IFAT). Also, similar results were observed in Chile where 12% from 408 sheep were positive to *T. gondii* antibodies by the indirect haemagglutination test (IHAT) (Gormana and al., 1999). However, our results were higher than the 7.7% obtained by Da Silva and Langoni (2001) in Brazil through IFAT and the 3.0% reported by Wang and al. (2011) in the northeastern China by IHAT. In addition, low prevalence was observed in the present study compared to those obtained in some localities from : China (20.3%) by using modified agglutination test (MAT) (Yin and al., 2015), Morocco (27.6%) by ELISA (Sawadogo and al., 2005), Ghana (33.2%) by ELISA (Van Der Puije and al., 2000), Portugal (33.6%) by means of the modified agglutination (Lopes and al., 2013), Iran (35%) by IFAT (Sharif and al., 2007), Mexico (37.9%) by IFAT (Cruz-Vazquez and al., 1992), Italy (49.9%) by ELISA (Vesco and al., 2007), Caribbean islands (65.25%) using an in house ELISA (Hamilton and al., 2014), Brazil (75%) of samples with ELISA and (80%) by using IFAT (Rossi and al., 2011). Serbia (85%) by the modified agglutination test (Klun and al., 2006).

The differences observed could be due to the sampling techniques, husbandry methods used in the different regions, frequency of cats on the farms and the climatic variations from one region to another, which are essential elements in epidemiological studies. In addition, hygiene also presented a greater risk of *T. gondii* infections (Liu and al., 2015). Another probable explanation may be related to strain variation, since *T. gondii* strains from South America present significant genetic differences from Eurasia, Africa and North America populations (Lehmann et al., 2006). A positive association was observed between seroprevalence of *T. gondii* and the presence of cats in the herds, indicating that the presence of, and intimate contact with, feline species is important in the epidemiology of toxoplasmosis (Lopes and al., 2010). Cats are, however, likely to be found in almost all areas where sheep are kept, and the probability that a young cat may shed oocysts on a farm will always be present and any fecal material from infected cats will represent a hazard (Skjerve and al., 1998).

In Algeria, sheep raised in an extensive system and fed on fresh bulk feed or pasture presented a greater risk of toxoplasmic infection. These results support the scenario of the presence of sporulated *T.gondii* oocysts in the local environment.

Lopes and al. (2010) noted that, the lack of mineral supplementation, also had an influence in toxoplasmosis infection, this could be related to a decrease in immune defenses. Sheep that received supplementation were shown to be more immunocompetent than those that did not receive mineral supplements.

In the present study, 13 sera had a high titre of antibodies (≥ 200). This may be an indication of frequent exposure to the parasite on farms (Lopes and al., 2013). The seroprevalence demonstrated in this study indicates levels of environmental contamination with oocysts, which could potentially offer an alternative route of transmission for humans through contaminated fruit or vegetables or water (Hamilton and al., 2014).

4.2. Seroprevalence according to the risk factors

This study used samples from abattoir where the majority of the slaughtered animals were male. The difference in the occurrence of *T. gondii*-specific antibodies between genders was not considered. However gender-related tendency of prevalence had been reported previously and some data had suggested that the sex was a significant factor in determining

previous exposure to *T. gondii* infection in sheep (Clementino and al., 2007). Thus, some reports indicated that female animals were more susceptible than males to infection with *T.gondii* (Wang and al., 2011,Van Der Puije and al., 2000, Clementino and al., 2007) which was probably due to the lower levels in immune response or antibody persistence of females in some periods of their lives (Yin and al., 2015). Furthermore, according to some authors, the prevalence in males was higher than females (Alvarado-Esquivela and al., 2013, Holec Gąsior and al., 2015). However, according to the last, these results may differ because the male and female population consisted of a different number of animals, thus, the group of males represented only 5.6% of the tested population of animals (Holec Gąsior and al., 2015).

Results from our study showed an increase in *T. gondii* seroprevalence with age confirming that a major source of infection for sheep is likely to be through the consumption of sporulated oocysts from the environment, and suggesting that most sheep acquired the infection post-natally (Dubey, 2009).These results are similar to those of some previous investigations (Wang and al., 20011, Van Der Puije and al., 2000, Rossi and al., 2011, Clementino and al., 2007, Holec Gąsior and al., 2015, Gebremedhin and al., 2014, Katser and al., 2011) indicating that age was an important factor for being seropositive as a measure of the cumulated life-time risk (Katser and al., 2001). In contrast, Rahman and al. (2014) noted that seroprevalence for young and adult sheep was similar. The infection may have occurred because of poor hygiene conditions at the farm and ingestion of food or water contaminated with oocysts (Lopes and al., 2013).

On the contrary, other researchers found that there was no correlation between the seroprevalence and age and that age was not a crucial risk factor for *T. gondii* infection (Yin and al., 2015, Alvarado-Esquivela and al., 2013). Also, a study conducted in Italy, noted that the seroprevalence was already 39.6% in young animals less than 1 month of age, and suggested that these animals received *Toxoplasma*-specific IgG-antibodies from their mothers through the colostrums and milk or congenitally during the later part of gestation (Vesco and al., 2007).

Yin and al. (2015) showed in their study that the season was considered as a risk factor associated with *T. gondii* infection and found that the seroprevalence was higher in summer and in spring, compared to winter (Yin and al., 2015). According to them, in spring and summer, the climate is warm and damp, conditions which are favorable for the survival of *T. gondii* oocysts. In addition, cats are more active at warm temperature and expand their range which lead to oocysts widely distribution (Yin and al., 2015). Also, in Ethiopia, a study revealed that the risk of *T. gondii* infection was significantly higher in sheep sampled during the dry season where the climate was more suitable for survival of the oocysts than those sampled during wet season (Gebremedhin and al., 2014). In Algeria, the climate in summer, spring and autumn is more suitable for survival of the oocysts compared to winter where we note a high mean rainfall and low mean temperature. This might be a reflection of fluctuations or differences in rate of transmission between seasons. In Mexico, the observations of a study performed on ovine toxoplasmosis showed that sheep in the driest (600 mm of mean annual rainfall) municipality had the highest seroprevalence of *T. gondii* infection and *T. gondii* oocysts remained longer in an environment with little rainfall because there is little wash or removal by the rain (Alvarado-Esquivela and al., 2013). These results suggested that environment climatic variables including mean annual temperature and mean annual rainfall are important factors correlating with the seroprevalence of *T. gondii* infection in sheep and have epidemiological significance and point toward a limitation in reporting an overall seroprevalence of *T.gondii* infection in sheep (Alvarado-Esquivela and al., 2013).

Differences in *T. gondii* prevalence across geographic locations were also reported in Scotland (Katser and al., 2011), Ghana (Van Der Puije and al., 2000), China (Yin and al., 2015), Mexico (Alvarado-Esquivela and al., 2013), Ethiopia (Gebremedhin and al., 2014) and Morocco (Sawadogo and al., 2005). According to (Katser and al., 2011) this distribution disequilibrium may be due to the spread and survival of oocysts on

pasture and lambing areas (Katser and al., 2011). While Gebremedhin and al. (2014) recorded that this might be due to climatic differences between the area which influence the tenacity and infectivity of oocysts (Gebremedhin and al., 2014). In our study, the high prevalence of toxoplasmosis in Northern Algeria (Center, Eastern and Ouestern) may be due to the high relative humidity which is favourable to the viability of *T. gondii* oocysts compared to the south with dry environments. While, significant differences in prevalence were found among the three regions in the north of Algeria, these differences may be attribuated to the variable levels of contamination with *T. gondii* oocysts in different regions where sheep were exposed.

4.3. Detection of tissue cysts of T. gondii by histological technique

From Brazil, Da Silva and Langoni (2001) found that the examination of both brains and diaphragms by histopathological technique was negative in all examined sheep, while, forty of the sheep (7.7%) were IFAT positive. Also, Cremers et al. (1991) did not find *Toxoplasma* in smears of swine and ovine tissues. Esteban Redondon and al. (1999) reported the difficulty in detecting the parasite in tissue sections from large animals due to the low density of microorganisms and the limitation of sample size, as the parasite may be present in the unexamined tissues (Esteban Redondon and al., 1999). Also, a negative result from any sample does not necessarily mean that the whole tissue is free of the parasite (Barreto Tenório Nunes and al., 2015). The location and number of tissue cysts in animals difered with hosts and the strain of *T .gondii* (Dubey, 1998). In higher mammals (cattle, cats, sheep, goats) more tissue cysts were present in muscular tissues than in the brain (Dubey, 1998). However, the results from (Esteban Redondon and al., 1999) showed that *T. gondii* was more frequently and consistently detected within brain and heart tissues of sheep given the higher dose of *T.gondii* suggesting that the brain and heart are the favoured site for detection of *T. gondii* in experimentally infected adult sheep (Esteban Redondon and al., 1999). No significant histopathological changes were found in the evaluated organs. According to Barreto Tenório Nunes and al. (2015), the histological alterations may differ between studies, especially with regard to the intensity of the mononuclear infiltrate observed in tissues targeted by the parasite. According to the last, the predominant finding histopathologically was the presence of mononuclear cell infiltrate in the heart and a perivascular cuff in the cerebrum and the cerebellum from sheep tissue. Dubey (1998) noted that intact tissue cysts probably do not cause any damage to the tissue and can persist without causing any inflammatory response by the host and only in cases where cysts have ruptured releasing parasites does a severe inflammation occur along with local necrosis (Dubey, 1998). As observed in the present study, this technique may have low sensitivity, because most of the samples that were positive in serological evaluation were negative by histology. We therefore consider that the results were insufficient for this technique to be adopted in routine diagnostic laboratories and we recognize that cysts are difficult to identify in histological sections, and that it is therefore more appropriate to use an association with another techniques in order to increase the chances of identifying the parasite.

5. CONCLUSION

T. gondii infection in sheep used for human consumption is prevalent in the present study, suggesting a dispersion of oocysts and parasite reservoir hosts in the environment. Season, region are risk factors of seropositivity in sheep. Considering the presence of *T. gondii* in slaughtered sheep from the study area in the north of Algeria, meat should be considered as a source of infection in the human population when consumed raw or undercooked. In Algeria, this risk is further reduced due to the customary prolonged cooking of meat. Also, the infection by oocysts eliminated by cats is another source of contamination which needs to be considered. Prevention of the spread of the disease is essential .The present serological survey does not represent a national population prevalence, other studies must be carried out to determine the true prevalence of ~~the~~ sheep toxoplasmosis in different regions in Algeria.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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