BF-1849
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RESEARCH ARTICLE Indian Journal of Animal Research

Seroprevalence of Sheep Toxoplasmosis in North-eastern of Algeria and Determination of Potential Risk Factors

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10.18805/IJAR.BF-1849

ABSTRACT

Background: Toxoplasma gondii is an obligatory intracellular protozoan capable of infecting humans and several animal species. The aim of the study was to assess the seroprevalence in sheep in five regions located in north-eastern of Algeria and determine potential risk factors for the disease. The estimation of seroprevalence and the determination of risk factors make it possible to apply strategies to reduce the rate of infestation on sheep farms.

Methods: The study was conducted between November 2021 and March 2023. The indirect enzyme-linked immunosorbent assay (ELISA) method was used to detect the presence of anti- *T. gondii* IgG antibodies in blood plasma from 368 animals, both sexes. Moreover, to identify the potential risk factors for *T. gondii* infection, a survey through breeders' questionnaires was conducted. Breeders' questionnaires were also used in a survey to find possible risk factors for T. gondii infection. For statistical analysis, the Khi2 and ANOVA tests were used with multiple range tests (multiple range).

Result: The true individual seroprevalence was 37.7%. The seroprevalence at the herd level was 94.44% (95% CI). Gender (femelle) and age (> 5 years) were identified as risk factors, also the presence of cats, rats and birds (p= 0.0001). Other risk factors were significant statistically: Herd size (less than 20 p= 0.001), type of food (pasture and concentrate p= 0.01), source of water (River p= 0.01), season (winter p= 0.01).

Key words: Elisa, North-eastern of Algéria, Risks factors, Sheep toxoplasmosis, Seroprevalence.

INTRODUCTION

The obligate intracellular protozoan *Toxoplasma gondii* causes a widespread infection in humans and several warm-blooded animal species (mammals and birds). It is detrimental to the health of individuals as well as animals. Only domestic cats are true hosts; other animals just act as intermediary hosts. These hosts are exposed to the parasite through infected meat, milk and water. Farm animals can become affected by oocyst-infected products, which are a common source of contamination (Tenter, 2009; Dubey, 2019).

The zoonotic characteristic of Toxoplasmosis constitutes a threat to both human and animal health as well as to the economy, due to its economical losses and serious diseases in animals (Celik *et al*., 2018).

Both domestic and wild animals have been shown to have toxoplasma infections. Sheep and goats are more commonly infected than cattle or chickens among raised animals for food (Tenter *et al*., 2000; Hill and Dubey, 2013). Goats and sheep are reported to be a major source of infection in a number of countries (Tenter, 2009; Hill and Dubey, 2013).

Stillbirths and abortions in ruminants are significantly increased by T. gondii infection (Cenci-Goga *et al*., 2011) and cause large financial losses for the world's sheep, goat and cattle industries (Kortbeek *et al*., 2004; Radostits *et al*., 2006). Numerous non-specific (such as fever and dyspnea) and specific symptoms (such as lymphadenopathy, chorioretinitis, fever, lethargy, depression, vomiting and diarrhea.) are produced by it (Rouatbi *et al*., 2019).

Anti-*T.gondii* antibody seroprevalence in sheep ranged widely over the world, from 0% to 100% (Tenter *et al*., 2000). This disparity in incidence is dependent on a variety of risk variables, which may be related to the animals' surroundings (such as theexistence of cats, the local climate, altitude and methods used to control animals) or to the animals themselves (such as age, sex and species) (Tenter *et al*., 2000).

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How to cite this article: Ilyes, B., Amal, T., Touhami, N.A.K., Abdeslam, M., Nassim, O. and Ahmed, B. (2025). Seroprevalence of Sheep Toxoplasmosis in North-eastern of Algeria and Determination of Potential Risk Factors. Indian Journal of Animal Research. 1-6. doi: 10.18805/IJAR.BF-1849.

Submitted: 02-08-2024 **Accepted:** 19-12-2024 **Online:** 20-01-2025

Thus, it is imperative to prevent T. gondii infection in sheep for the benefit of public health as well as effective domestic animal breeding.

Despite being widespread in Algeria, there is limited research on the prevalence of toxoplasmosis in ruminants and other animals. As a result, the *T. gondii* infection's state is still unknown and poorly understood. The analysis of Ouchetati *et al*. (2021) revealed a pattern of rising infection since 2015 (R2 = 0.129, p > 0.05).

We performed a cross-sectional study on sheep in northeastern Algeria to evaluate the seroprevalence of T. gondii infection in domestic ruminants and to determine the main risk variables linked to the infection.

MATERIALS AND METHODS

Study area and samples

A cross-sectional study was carried out between November 2021 and March 2023 in north-estern of Algeria. The survey was conducted in 36 farms issue from 5 provinces of northeastern Algeria (Constantine, Annaba, Souk ahras, Om bouaghi, Skikda) (Fig 1) and additional data were collected from each farm sampled: Age, sex, herd size, type of breeding, type of food, quality of water, presence of cats and rats to determine potential risk factors.

According to (Thrusfield, 2005), the sample size was calculated using a 40% predicted prevalence, a 5% desirable precision and a 95% level of confidence. A total number of 368 Blood samples were collected from the jugular in 10 mL EDTA tubes and centrifuged at 3000 rpm for 15 min. The plasma was conserved at -20°C.

A flock of animals is considered positive if one animal of the same herd possesses anti-T.gondii antibodies.

Serologic examination

Using a Toxoplasmosis Indirect ELISA Multi-species kit (ID Screen, ID.VET. Innovative Diagnostics, Montpellier, France), the plasma was examined for the presence of anti-*T. gondii* antibodies in accordance with the manufacturer's instructions. This ELISA test's sensitivity is 100% and its specificity is 96%, according to the manufacturer's data.

Data analysis

The statistical program used was R i386 3.0.2 for Windows Front-end GUI. The Khi2 and ANOVA tests were used with multiple range tests (multiple range). The threshold value of different tests was p<0.05.

RESULTS AND DISCUSSION

The true individual seroprevalence in five province studies was 37.7%. (95% CI) and the number of cases was positive (139/368). The seroprevalence at the herd level was 94.44% (95% CI) and the number of herds considered positive was 34/36.

According to our study (Table 1), animals aged over 5 years old (49.27%) were the most infested compared to animals aged 2 to 5 years old (41.39%) and under 2 years old (24.77%) (p<0.001). The prevalence among females (40.39%) was significantly higher than among males (25.75%) (p<0.001). Farms whose numbers were between 20 and 50 (31.88%) were the least infested compared to farms whose numbers were less than 20 (52.94%) or greater than 50 (39.06%) (p<0.001). No significant differences were recorded in relation to type of breeding, intensive breeding (38.09%) and semi-extensive breeding

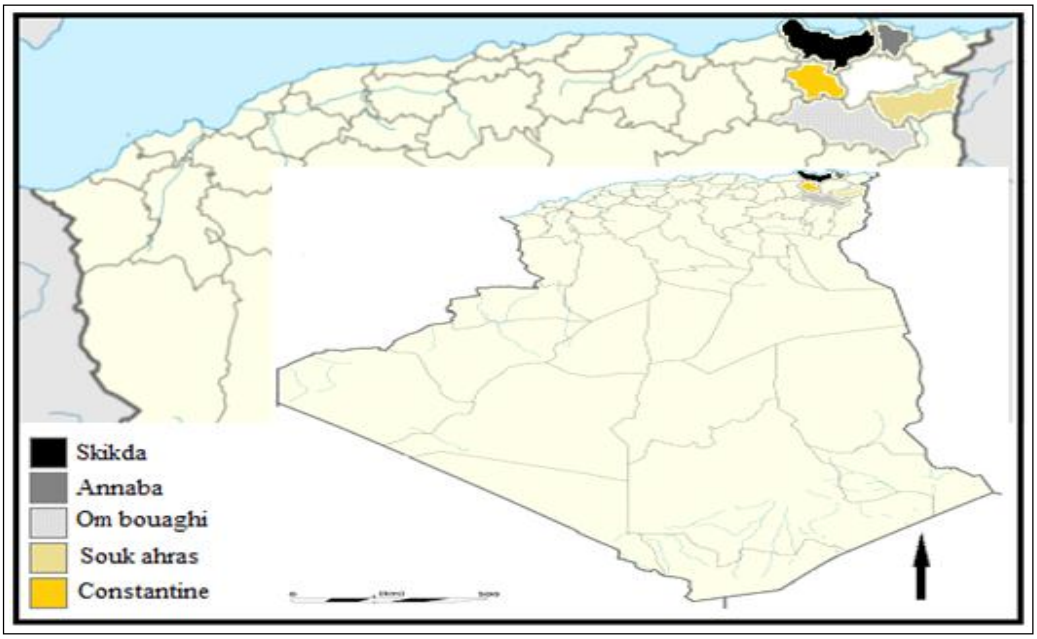

Fig 1: Presentation of the study area.

(37.70%). Seroprevalence is higher in animals whose hygiene mode of box storage food is poor (41.66%) compared to those whose hygiene is good (34.90%) ($p<0.05$) and is higher in animals in pasture and supplemented with concentrate (44.01%) compared to animals in pasture only (34.54%) and animals in pasture and fed with concentrate and hay (26.92%) ($p<0.01$).

Seroprevalence is significantly associated with the season. The results showed that the seroprevalence is higher in winter (47.73%) compared to the spring season (26.03%) ($p<0.001$) and is significantly associated with the quality of water; herds that consume river water are more infested (51.89) than herds that consume tap water (33.91) ($p<0.01$). The Constantine region showed the lowest seroprevalence (22.72) and the highest was recorded in Skikda (46.98%) ($p<0.05$). Finaly, the presence of cats, rats and birds ($p<0.0001$) was significantly associated with seroprevalence in this study.

The seroprevalence recorded in our study (37.7%) was less than that recorded in France. 65.6% (Dumètre *et al*, 2006), 46.5% in spain (Jiménez-Martín *et al*., 2020), 53.8% in Italia (Condoleo *et al*., 2023), 50.6 % in Romania (Hotea *et al*., 2021), 52.8% in Ethiopia (Jilo el al., 2021), 41.7% in Estonia (Maarja-Tagel *et al*., 2019), 56.6% in Lebanon (Khalife *et al*., 2022), 42.85% in India (Pandit *et al*., 2021), 60.7 % in lybia (Hailazakis *et al*., 2021), 60.1% in Senegal (Dahourou *et al*., 2019), 64.4 % in south of Africa (Tagwireyi *et al*., 2019), 39.1 % in Egypt (Abdelbaset *et al*., 2020) and it was higher than recorded in bénin 1.4% (Tonouhewa *et al*., 2019), 34.3% in Brazil (De Moura *et al*., 2021), 31.29% in china (Jia *et al*., 2023), 35.9% in mexico (Suazo-Cortez *et al*., 2020), 4.5% in turkey (Aktaş and Aydın, 2020), 30% in morocco (Benkirane *et al*., 2015), 19.7% in Tunisia (Guesmi *et al*., 2023), 25.6% in Algeria (Abdallah *et al*., 2019), 35.9% in algeria also (Ouchene *et al*., 2023).

The variation in global prevalence seen in this study and others previously noted may be explained by the degree to which the small ruminants had access to contaminated feed and water, climate variables and the diagnostic procedures used (Dubey, 2004; Innes *et al*., 2009).

Animals aged over 5 years old (49.27%) were the most infested in our study, these results are similar to those founded by Abdallah *et al*. (2019) and Niaz *et al*. (2016) Jittapalapong *et al*. (2005). This might be the result of the

Table 1: Risk factors associated to infection.

Variables		N	No. of positives	Pourcentage (%)	P-value
	<2	113	28	24.77	0.001
(Age years)	2 to 5	186	77	41.39	
	>5	69	34	49.27	
Gender	Male	66	17	25.75	0.001
	Femelle	302	122	40.39	
Herd size	<20	102	45	52.94	0.001
	20 To 50	138	44	31.88	
	>50	128	50	39.06	
Type of	Intensive	63	24	38.09	
Farming	Semi-extensive	305	115	37.70	
Food	Pasture	55	19	34.54	0.01
	Pasture and concentrate	209	92	44.01	
	Pasture and concentrate and hay	104	28	26.92	
Water	River	79	41	51.89	0.01
Quality	Tap water	289	98	33.91	
Season	Winter	199	95	47.73	0.001
	spring	169	44	26.03	
Storage food	Good	212	74	34.90	0.05
box cleanless	Bad	156	65	41.66	
Regions	Souk-ahras	51	23	45.09	
	Constantine	88	20	22.72	0.05
	Oum bouaghi	72	27	37.5	
	Annaba	74	30	40.54	
	Skikda	83	39	46.98	
Presence of cats	Yes	305	104	34.09	
	No	63	35	55.55	
Presence of birds	Yes	368	139	37.77	0.0001
	No	0	0	0	
Presence of rats	Yes	361	135	37.39	
	No	7	4	57.14	

adults' prolonged exposure to the *T. gondii* infection (Dubey, 2019).

According to Dubey *et al*. (1998), females may have weaker immunological resistance at particular stages of their lives, which can explain the higher sensitivity. In Algeria, small herds are typically managed by traditional methods. Firstly, cats have easy access to the livestock's food. Second, the animals graze frequently and every day, a transition from extensive to intensive grazing and back again occurred. Thirdly, there are no animal hygiene procedures, including organizing feeding or cleaning (Abdallah *et al*. (2019).

In this study seroprevalence is higher in animals whose hygiene mode of box storage food is poor (41.66%). These results are comparable to those of Cosendey-KezenLeite *et al*. (2014) because farm hygiene, cleaning and disinfection practices may be crucial in the transmission of T. gondii infections to livestock. This is because cleaning decreases the risk of oocyst contamination of the facilities and can also minimize exposure to infected intermediate hosts, such as rodents.

According to various research studies (Dubey *et al*., 1996; Ruiz and Frenkel, 1980), the high oocyst contamination of agricultural soil may be the cause of this high prevalence. Grazing systems, where several herds graze each day, are responsible for these agreements, as a result, herds of animals were in significant danger of coming into contact with contaminated feed and grass throughout the grazing season (Abdallah *et al*., 2019). The higher seroprevalence in winter season in our study could be explained because sheep in winter are typically confined throughout the night and on days of severe weather and they are supplied supplemental feed consisting of hay, grass silage and concentrates and cats have easy access to the livestock's food.

In our study constantine region where dry climate showed the lowest seroprevalence (22.72) and the highest was recorded in coastal area in skikda (46.98%) ($p<0.05$). The parasite's ability to survive and spread epidemiologically is negatively impacted by a dry climate (Dubey, 2019; Tegegne *et al*., 2016). On the contrary, in the coastal cities of Algeria, the high levels of wetness increase the likelihood that oocysts will survive in the environment and that they will come into contact with contaminating sources, resulting in increased seroprevalence (Dubey, 2019; Tilahun *et al*., 2019; Jones *et al*., 2001).

Seroprevalence is significantly associated with the presence of cats, rats and birds ($p<0.0001$) in this study, the prevalence at farms whose cats are present and have access to box food storage was 34.09%. This founding are different than showed by ramzan *et al* (2009). The observation of a higher seroprevalence in domestic ruminants, which supports the high incidence of toxoplasmosis in women seen in Algeria, affirms the role of cats as a source of *T. gondii* infection (Messerer *et al*., 2014; Berredjem *et al*., 2017). Even on farms without the presence of cats, the seroprevalence was higher (55.55%). That could be explained by the possibility that animals became infected for the first time outside of these farms or in contaminated pasture by other stray cats. More research is needed to determine how long the infection remains in the animals.

The rate of infection in local populations of birds and rodents determines the rate of infection in cats because cats contract the infection by eating these animals. Cat infection rates would rise as a result of prey animals becoming more prone to contracting the disease when there are more oocysts in the environment (Hill *et al*., 2005). Wide natural infection of the environment exists because a cat could eliminate millions of oocysts after consuming as little as one bradyzoite or one tissue cyst and one infected mouse may contain numerous tissue cysts (Frenkel *et al*., 1970; Dubey, 2001).

CONCLUSION

This study showed widespread *T. gondii* infection in sheep that produce food in northeastern Algeria and enhanced knowledge of Toxoplasma infection. All of the provinces involved in the study's animal farms had anti-Toxoplasma antibodies, suggesting that the parasite is widely present. It is the first published estimate of seroprevalence in the areas under investigation. In addition to educating the public on *T. gondii* prevention, treatment and method of transmission, further research should be done to examine the disease's economic impact.

ACKNOWLEDGEMENT

The present study was supported by ministery of researchs fonds.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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