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Original Scientific Paper

First report on ovine toxoplasmosis in Bosnia and Herzegovina a case study from Srednja-Bosna Canton

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ABSTRACT

Toxoplasma gondii is a major foodborne zoonotic parasite, with sheep meat identified as a high-risk transmission vehicle to humans. This preliminary study aimed to determine the seroprevalence of *T. gondii* in sheep intended for human consumption in Bosnia and Herzegovina. Liver samples from 250 sheep were collected at slaughterhouse located in Canton Sarajevo and tested for anti-*T. gondii* IgG antibodies using an indirect ELISA on meat juice. An exceptionally high seroprevalence of 98.8% was detected, one of the highest rates reported globally. The near-universal exposure suggests widespread environmental contamination and intense infection pressure in the studied region. Given the strong epidemiological link between undercooked mutton and human toxoplasmosis, these findings indicate a significant public health risk. Effective mitigation should focus on consumer education about safe meat preparation and the adoption of integrated One Health strategies to reduce transmission at the animal-human interface.

1. Introduction

Toxoplasma gondii is a globally distributed zoonotic protozoan parasite (*Buxton*, 1990) whose transmission pathways, life cycle, and persistence in the external environment render it highly relevant in the context of food safety (*Belluco et al.*, 2016). Toxoplasmosis is a significant public health concern and is classified among the most important foodborne diseases, with *T. gondii* ranked as the fourth most important foodborne parasite worldwide and the second in Europe. Human infection is primarily acquired through oral ingestion of infective stages: oocysts from contaminated sources, such as water, soil, or unwashed vegetables, or tissue cysts present in raw or undercooked meat from infected animals (*Bouwknegt et al.*, 2018; *FAO/WHO*, 2014). In Europe, it is estimated that 30–63% of human toxoplasmosis cases are attributable to the consumption of undercooked meat (*Thomas et al.*, 2022). Among different meat types, sheep (lamb) meat has been identified as posing a particularly high risk of transmission (*Marin-Garcia et al.,* 2022).

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Sheep are highly susceptible to *T. gondii* infection and frequently develop lifelong latent infections with tissue cyst formation, particularly within muscle (*Condoleo et al*., 2023; *Innes et al.*, 2009). Global serological surveys have revealed a high prevalence of infection in sheep, with a meta-analysis reporting a mean seroprevalence of 33.9% (*Ahaduzzaman & Hasan*, 2022). Considerable variability exists between regions, influenced by climatic and husbandry factors; under favourable conditions (e.g., warm, humid climates with high environmental oocyst contamination), flock-level prevalence can exceed 90% (*Cenci-Goga et al*., 2013). In the Balkans, particularly high values have been documented—for example, a seroprevalence of 84.5% in Serbian sheep, among the highest recorded globally (*Klun et al*., 2006). Multiple epidemiological studies have implicated lamb/mutton consumption as a major risk factor for human toxoplasmosis. Case-control studies in Norway and France demonstrated a significant association between the consumption of undercooked lamb and the disease; therefore, mutton is considered the highest-risk meat type—exceeding pork, poultry, and beef—due to its high tissue cyst burden (*Thomas et al*., 2022). Similarly, a U.S. study found that consumption of "rare" lamb markedly increased infection risk (*Thomas et al*., 2022). Consequently, toxoplasmosis is recognised as a major foodborne zoonosis, with mutton serving as an important transmission vehicle to humans.

In Bosnia and Herzegovina, no published data are available regarding *T. gondii* occurrence in sheep meat. However, international literature consistently demonstrates that sheep are frequently infected throughout their lifespan, and their meat can harbour infectious cysts that pose a public health hazard. In this context, a preliminary investigation was undertaken to detect anti-*Toxoplasma gondii* antibodies in sheep livers collected at the slaughter line from animals intended for human consumption.

2. Materials and methods

2.1 Sampling

Sampling was carried out in slaughterhouse in Canton Sarajevo, with all animals originating from five farms located in Srednja-Bosna Canton. From each farm, 50 liver samples were taken from sheep older than 1 year of age during slaughter in June 2025, while the meat of slaughtered animals, after veterinary inspection, was placed on the market for human consumption. A total of 250 livers from sheep were sampled and immediately delivered to the laboratory and frozen at -20°C. Before the start of the analysis, the livers were thawed in the refrigerator, after which the meat juice was extracted and used for further analysis.

2.2 Laboratory analysis

A total of 250 meat juice samples underwent analysis using a commercial indirect ELISA kit for the detection of anti-*Toxoplasma gondii* IgG antibodies (ID Screen® Toxoplasmosis Indirect Multi-species, Innovative Diagnostics, Grabels, France), following manufacturer-provided guidelines. All meat juice samples were diluted at a 1:2 ratio as recommended by the manufacturer. Optical density (OD) readings were obtained using an ELISA plate reader (Epoch,BioTek, USA) set to 450 nm wavelength with a reference filter at 620 nm. As per the manufacturer's specifications, the sensitivity and specificity of this ELISA are 98% and 95%, respectively.

3. Results and discussion

The present study revealed an extremely high seroprevalence of *Toxoplasma gondii* in sheep from central Bosnia and Herzegovina, with 247 out of 250 animals (98.8%) testing positive for IgG antibodies. This seropositivity is among the highest ever reported for ovine toxoplasmosis. By comparison, a recent global meta-analysis estimated an average seroprevalence of only about 33.9% in sheep worldwide (*Ahaduzzaman & Hasan*, 2022). Even within the Balkans, a region already known for elevated *T. gondii* rates, our findings stand out. For example, a large survey in neighbouring Serbia found 84.5% of sheep seropositive, one of the highest national rates documented to date (*Klun et al*., 2006). Our nearly universal seropositivity exceeds even that benchmark, suggesting that exposure to *T. gondii* in the sampled population was essentially ubiquitous.

Several factors could explain this remarkable prevalence. All sampled animals originated from Srednja-Bosna Canton, an area whose environmental and husbandry conditions likely facilitate intense transmission. Sheep in this region are typically raised in free-range or extensive systems, allowing frequent contact with soil, water, and feed that can be contaminated by oocysts shed from cats (the definitive hosts). In general, flocks managed in open

environments with high cat presence tend to show higher infection rates (*Condoleo et al*., 2023). Climatic conditions also play a role: *T. gondii* oocysts survive best in mild, moist environments, leading to especially high sheep infection levels in certain climates (*Cenci-Goga et al*., 2013). The Bosnian farms in this study could offer a similarly conducive setting, given the nearly 99% exposure rate observed. The fact that only one liver was definitively negative (with two inconclusive) suggests that almost every sheep brought to slaughter had been infected with *T. gondii* at some point in its life. This is consistent with the notion that sheep are highly susceptible to *T. gondii* and often acquire lifelong infections on pasture (*Condoleo et al*., 2023). It also aligns with prior reports that sheep are frequent carriers of *T. gondii* cysts compared to other livestock, like cattle (*Thomas et al*., 2022). The only available study from Bosnia and Herzegovina to date was recently conducted in pigs (*Muftić et al*., 2025), reporting an animal-level seroprevalence of 13.3% and farm-level seropositivity of 44.8%. These findings confirm the occurrence of *T. gondii* infection among domestic livestock in the country.

It is important to acknowledge that our detection method – an ELISA on meat juice – indicates exposure (presence of antibodies) rather than direct confirmation of viable parasites in tissues. However, abundant serological exposure could imply a high likelihood that infective tissue cysts are present in the meat. Previous studies have shown a good correlation between seropositivity and the finding of cysts or DNA in sheep tissues (*Marín-García et al*., 2022). For instance, one survey in France recovered live *T. gondii* from about one-third of seropositive lambs under 1 year old (*Thomas et al*., 2022). Our results provide the first substantive data on ovine toxoplasmosis in Bosnia and Herzegovina, and they suggest that infection is widespread to an extraordinary degree in at least the studied region. This finding closes an information gap for the country and aligns it with observations from other high-prevalence areas. It underscores that Bosnia, like many parts of Europe, harbours a substantial reservoir of *T. gondii* in the sheep population, which has important implications for food safety.

These results carry a clear public health message: consumers in Bosnia and Herzegovina (and by extension, the wider Balkans) should avoid eating undercooked sheep meat. If a large proportion of regional mutton is infected (as our data indicate), it could be a major contributor to the overall burden of disease. Fortunately, the risk can be greatly mitigated by proper food handling. Cooking meat to safe internal temperatures (≥67 °C for whole cuts of lamb, as recommended by health agencies) will reliably inactivate *T. gondii* cysts (*EFSA*, 2007). Freezing meat at sub-zero temperatures for an adequate duration is also known to kill the parasite (*Dubey et al.,* 1990; *Dubey*, 2004). Compared with areas where rare ovine meat is favoured, in countries or communities where mutton is traditionally eaten well-done, like in Bosnia and Herzegovina, the incidence of toxoplasmosis from meat is much lower (*Cenci-Goga et al.*, 2013). In contrast, cultural practices of consuming raw or lightly cooked meats can greatly increase infection risk. Notably, a case-control study in the United States identified rare lamb consumption as one of the most significant risk factors for acute toxoplasmosis (*Thomas et al*., 2022). Similarly, outbreaks and epidemiological investigations in Europe have repeatedly linked undercooked lamb to toxoplasmosis cases in humans (*Thomas et al.*, 2022). In our context, any local customs of tasting raw meat during preparation or dishes involving undercooked lamb (such as certain traditional sausages or liver dishes) would pose a serious hazard and should be discouraged. Vulnerable populations, pregnant women in particular, must be made aware of these risks. Even a single exposure to raw or undercooked infected mutton during pregnancy can lead to severe consequences (congenital toxoplasmosis) (*Rostami et al*., 2020). Prior research has shown that pregnant women who consume raw/undercooked sheep products have a significantly higher likelihood of seroconversion (*Chung & Tirabassi*, 2021). Therefore, our findings should prompt the strengthening of educational campaigns on safe meat preparation and perhaps targeted advice during prenatal care in Bosnia and Herzegovina. Another implication is the need for vigilance in the meat production and inspection chain. Unlike many bacterial or viral pathogens, *T. gondii* cannot be detected by routine meat inspection – the cysts are microscopic and cause no overt changes in the meat's appearance. All the sheep in this study were slaughtered under veterinary supervision and passed routine *ante-mortem* and *post-mortem* checks (since they were destined for human consumption). The fact that virtually all were seropositive underscores that *T. gondii* contamination is invisible to standard meat hygiene protocols. This suggests that additional measures might be warranted if one aims to reduce toxoplasmosis risk. For example, farms could implement control strategies, such as limiting cat access

to sheep feed and water sources, controlling rodent populations (to reduce intermediate hosts that cats prey on), and possibly using vaccines where available. According to official statistics, a total of 64,718 sheep were slaughtered in Bosnia and Herzegovina during 2024, of which 54,511 were lambs and 9,207 were older animals (*BHAS*, 2025). Our livers were collected exclusively from the latter category, which is the primary source of mutton available for human consumption. Given that this group is directly relevant to consumer exposure, the exceptionally high seroprevalence observed in our study underscores the potential public health importance of mutton consumption in the country.

This preliminary investigation has several limitations that should be considered when interpreting the results. First, our sampling was geographically limited to one region and one point in time. While the results strongly indicate a high prevalence in that area, they may not capture the situation in other parts of Bosnia and Herzegovina. It is possible that regions with different climates, farming practices, or degrees of cat exposure could have lower (or higher) *T. gondii* prevalence in small ruminants. Second, as noted, we relied on an indirect ELISA test on meat juice to infer infection status. This method detects antibodies that diffuse from the tissue into fluid during freezing/thawing; it has the advantage of using slaughterhouse samples without requiring blood collection. The single negative result could represent a truly uninfected animal, probably from an atypical management system, or seroconversion that has not occurred yet. It could also theoretically be a false negative (given the kit specificity ~95%, a few false results are expected). Without confirmatory tests (such as PCR or bioassay for parasite presence), we must assume the serology reflects true exposure, but we did not directly prove that each seropositive meat contained viable parasites. In future studies, it would be valuable to complement serology with molecular detection (e.g. PCR on tissue samples) to confirm the presence of *T. gondii* DNA in the meat. Such data could quantify how heavily infected the tissues are (parasite load), not just whether exposure occurred. Additionally, genotyping any detected parasites could reveal which strains circulate in Bosnian sheep – information relevant to understanding virulence and potential human health outcomes.

Despite these limitations, our study provides a crucial first look at *T. gondii* in the meat production chain in Bosnia and Herzegovina. The findings raise important questions and directions for further research. One priority is to investigate risk factors at the farm level: for example, does infection correlate with flock size, presence of cats, feeding practices, or geographical features (altitude, rainfall, etc.)? Identifying key risk factors could help in designing interventions to reduce transmission on farms. Another worthwhile avenue is to assess the public health impact more directly – for instance, by measuring *T. gondii* seroprevalence in the human population of the region and examining if people who frequently consume home-raised mutton have higher infection rates. This could solidify the causal link between the high infection pressure in sheep and actual human toxoplasmosis cases locally.

4. Conclusion

This preliminary study revealed an exceptionally high seroprevalence (98.8%) of *Toxoplasma gondii* IgG antibodies in sheep livers collected (in Srednja Bosna Canton) at slaughter in Bosnia and Herzegovina. This is among the highest prevalence levels reported globally and suggests that environmental contamination and animal exposure to *T. gondii* in the region are extensive. While this suggests extensive exposure of sheep to *T. gondii* in the region, serology alone does not confirm the presence of viable tissue cysts. Future studies using molecular and biological methods are, therefore, essential to determine the occurrence of viable parasites in edible tissues and to evaluate the accuracy of serology as a surveillance tool. Given the well-documented risk of human infection from consuming undercooked mutton, these results indicate a potentially significant public health concern. Additionally, broader epidemiological surveillance across multiple regions and seasons, coupled with human seroprevalence data, is needed to fully understand the transmission dynamics and zoonotic risk. Until such data are available, these findings should be interpreted with caution, while still highlighting the importance of safe meat preparation and the value of a One Health approach to toxoplasmosis prevention.

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