Impact of *Toxoplasma gondii* on Serum Protein Levels and Mineral Content in Sheep and Goats at a Small Dairy Farm in White Nile State, Sudan

Abstract

Aim:This study aims to investigate the effects of *Toxoplasma gondii* infection on the serum proteins and macro elements (Sodium, Potassium, Calcium, Magnesium and Phosphate) for sheep and goat. ***Introduction***: In sheep and goats, Toxoplasma infection is a major cause of abortion and stillbirth. Subclinical infections are also quite common in adult animals of affected flocks and herds. Serum proteins and macro elements plays an essential role in animal metabolism.***Material and Methods:***Serological survey of *T. gondii* was conducted using Latex Agglutination Test (LAT) for 20 sera collected from sheep and goats (8 and12 respectively) inwhite Nile State, Sudan. ***Results*:**Out of twelve goats' serum and eight sheep serum samples, seven samples were found to be positive for Toxoplasma (58.33%) in goats and four samples were found to be positive for Toxoplasma (50%) in sheep, the remaining samples were negative. It was observed that serum Total protein (Tp)level was increased significantly ($p<0.05$) in both species, and albumin (Alb) level decreased significantly($p<0.05$) in goats, while Globulin (Glob) wassignificantly ($p<0.05$) low in sheep compared with control animals. The results showed thatsome minerals (Na and K) concentrations in the serum were higher in bothsheep and goats compared to the controls. ***Conclusion***: In conclusion infestation of goats with *Toxoplama gondii* increasesthe total protein, globulins and significantly decreases the albumin concentrations. For sheep theinfestation increases the total protein, albumin and significantly decreases the globulin concentrations. Sheep and goats infected with *Toxoplasmagondii*showed increased serum sodium, potassium and magnesiumlevels affecting most of the enzyme systems

Key Word: Macro elements, Total proteins, Toxoplasma, sheep and goat.

Introduction

The global consumption of livestock, particularly sheep and goats, is particularly prevalent in developing nations (Mazinani and Rude, 2020). Among the numerous pathogens affecting these animals, *Toxoplasma gondii* stands out as an obligate intracellular protozoan belonging to the phylum Apicomplexa, subclass Coccidia (Bandyopadhyay*et al*., 2022.) Toxoplasmosis, caused

by *T. gondii*, is a common parasitic zoonosis affecting a wide range of warm-blooded animals, including humans and domestic livestock (Stelzer*et al.*, 2019; Stanić, and Fureš, 2020)

Sheep and goats, in particular, exhibit high rates of chronic *T. gondii* infection, often resulting in abortion and stillbirth (Nayeri*et al.*, 2021). Subclinical infections are also prevalent in adult animals within affected flocks and herds. The severity of toxoplasmosis in these animals correlates with the gestational stage; infections during early gestation can lead to fetal death, resorption, and abortion, while infections later in gestation may have minimal clinical effects, resulting in the birth of normal but infected and immune lambs (Dubey and Beattie, 1988; Buxton *et al.*, 2007).

Beyond reproductive and economic losses, *T. gondii* infection in animals poses significant public health concerns. Consumption of infected meat or milk can facilitate zoonotic transmission, with the parasite being transmitted through various routes such as direct contact with animals, contaminated faeces, soil, herbage, or contaminated food and water (Jittapalapong *et al.*, 2005). The transmission cycle of *T. gondii* involves both definitive and intermediate hosts, as well as inter-host transmission (Calero-Bernal *et al*., 2022; Lymbery, and Thompson, 2012).

Minerals such as phosphorus (P), calcium (Ca), magnesium (Mg), iodine (I), manganese (Mn), copper (Cu), selenium (Se), and zinc (Zn) play crucial roles in governing successful reproductive processes(Fadlalla, 2022). These minerals, originating mainly from external sources, constitute approximately 4% of an animal's body weight and serve structural, physiological, catalytic, and regulatory functions (Bednarek and Bik, 1994; Suttle, 2000). Imbalances in mineral levels, whether due to excess or deficiency, can lead to alterations in serum levels and contribute to various pathological conditions, including infectious diseases (Asín*et al.*, 2021)

Therefore, the aim of this study is to investigate the effects of Toxoplasma gondii infection on serum proteins and macro elements in sheep and goats. Specifically, we aim to examine the impact of *T. gondii* infection on the content of selected minerals and total proteins in these animals.

Material and methods

This study was conducted on a farm located in White Nile State,, Sudan. The farm comprised a mix of eight male sheep and twelve female goats. The aim was to investigate the impact of Toxoplasmosis on serum total proteins, and mineral levels. The study was conducted on the local breeds. The age of more than three years, with the history of weakness, apportion, ticks infestation, loss of appetite, low milk production and reduced fertility were selected.

Sampling: Blood samples were collected from the jugular vein of both sheep and goats. The samples were transported to the Parasitology Laboratory at the College of Veterinary Medicine, University of Bahri. Upon arrival, serum was separated from the clotted blood and stored at -20°C until further analysis. Initially, the sera were qualitatively examined for anti-*T. gondii* antibodies using the Latex Agglutination Test (LAT) according to the manufacturer's instructions (Spinreact, S.A./S.A.U, Spain). Sera showing positive or doubtful reactions were subsequently diluted in twofold serial increments (1:2 up to 1:128) for further testing.

All sera were subjected to appropriate analytical procedure to determinate different biochemical parameters. Serum calcium was determined by the Photometric Test CPC method according to the procedure described by (Gitelman, 1967). The concentration of the phosphate PO_4 in serum sample was determined by (Shervedani and Bagherzadeh 2008). Serum sodium and potassium was determined colormetrically according to the method of Trender (Trender, 1951). Serum magnesium was determined by Howard method (Howard, 1964).

Serum total protein levels were assessed in each serum sample using the methodology outlined by Lowry *et al*., (1951). Serum albumin was quantified colorimetrically employing the dye binding technique with Bromocresol Green (BCG), following the protocol outlined by Young (1975). Serum globulin concentrations were determined using Enzyme-Linked Immunosorbent Assay (ELISA), as per the procedure outlined by Piomelli *et al*., (1998).

Statistical analysis: Statistical analysis was performed using SPSS-version 20 software **(**IBM, Armonk, NY, USA.**).**An independent sample T-test was used to analyze the effects of toxoplasmosis. Parametric data were expressed in tables as means ± SDs.

Results

As depicted in Table 1, examination of eight blood samples collected from sheep showed that four samples (50%) tested positive, whereas the remaining four samples tested negative.

Similarly, analysis of twelve blood samples obtained from goats revealed that seven samples (58.33%) tested positive for the presence of the target analyte, while the remaining five samples (41.66%) yielded negative results.

Table (1): percentage of *Toxoplasmagondii* in the farm

Parasite	**species**	**Tested samples**	**Positive**	**Negative**	**Chi-square value**	**P-value**
Toxoplasma	Sheep	8	4 (50%)	4 (50 %)	0.13	0.71
	Goats	12	7 (58.33%)	5 (41.66 %)		

Form serum protein concentrations results were illustrated in table (2).In sheep, toxoplasmosis infection lead to increase in total protein and albumin and significant ($p<0.05$) decreased in globulins concentrations in positive sheep compared with negative sheep. For goats, toxoplasmosis infection lead to increasein total protein and globulins and significant ($p<0.05$) decreased in albumin concentrations in positive goats compared with negative goats.

Table (2): Serum protein concentrations inG\L ingoats and sheep with Toxoplasmosis

Parameter	Sheep		Goats	
	Control (negative)	Case (Positive)	Control(negative)	Case Positive
TP G\L	65.36±5.73	77.57±2.94	70.16±13.03	92.75±5.33
Alb G\L	48.74±3.69	64.77±3.99	58.82±11.19	58.31±4.72*
Glob G\L	16.61±2.04	12.79±6.12*	11.34±2.79	34.43±4.98

TP= Total proteins, Alb= Albumin, Glob = Globulin,*Significantly different from control at ($P<0.05$). *Significantly different from control at ($P<0.01$).

For minerals the results were shown in table (3): In sheep, toxoplasmosis infection lead to increase in Na, K, Ca, Mg and PO4 in positive sheep compared to the negatives. While in goats, toxoplasmosis infection lead to significant ($p<0.05$) increased in Na^+ and Mg concentration and significant ($p<0.001$) increased in K. Also, increased in both Ca and PO_4 in the positive goats compared with negatives.

Table (3): Serum minerals concentrations in m.mol/L in sheep and goats with toxoplasmosis

Parameters	Sheep		Goats	
	Control(negative)	Case (Positive)	Control(negative	Case (Positive)
Na m.mol/L	125.47±4.13	126.61±4.02	137.18±1.83*	139.62±1.91
K m.mol/L	3.55±0.26	3.79±0.30	4.44±0.09***	4.44±0.06
Cam.mol/L	2.26±0.04	2.30±0.07	2.44±0.03	2.36±0.02
Mg m.mol/L	0.68±0.06	0.92±0.22	0.95±0.01*	0.96±0.01
PO_4m.mol/L	1.16±0.03	1.18±0.05	1.24±0.05	1.29±0.01

*Significantly different from control at ($P<0.05$).
*Significantly different from control at ($P<0.01$).

Discussion

Small ruminants, such as sheep and goats, are particularly vulnerable to *T. gondii*, resulting in various problems in these animals (Holec-Gąsior, and Sołowińska, 2023).

In the present study Latex agglutination test was used to detect the sero-prevalence of *Toxoplasma gondii* in sheep and goats in a small farm in western Omdurman Sudan.

The current investigation revealed that over 50% of the goats (seven out of twelve) and sheep (four out of eight) tested positive for Toxoplasma infection, signifying a significant prevalence

among the livestock population at the farm. These findings are consistent with previous research (Dubey, 2016b), which underscores the high susceptibility of sheep and goats to *T. gondii* infections, making this protozoan parasite a leading cause of reproductive losses in small ruminants globally. While much of the research has focused on sheep, toxoplasmosis holds similar, if not greater, significance as a cause of abortion in goats (Dubey, 2010b). A recent meta-analysis conducted in Africa, spanning data from 1969 to 2016, estimated an overall prevalence of 26.1% (with a range of 17.0–37.0%) for sheep and 22.9% (with a range of 12.3–36.0%) for goats (Tonouhewa *et al*., 2017).

For sheep with toxoplasmosis there was an increase in total protein and albumin concentrations and decreased a significant ($p<0.05$) in globulin concentrations.On the other handgoats with toxoplasmosis infection there was an increase in total protein and globulins concentrations and significant ($p<0.05$) decreased in albumin compared with negative ones. These results are in agreement with that recorded by Roland*et al.*,(2022) who reported that, a significant increase in albumin and ALT ($p=0.04$) was observed between small ruminants infested with toxoplasmosis compared to the control group. These results are also similar to those obtained by Nora *et al*.,(2018), who indicated that ALT and albumin activities were increased in animals infected with *T. gondii*. These results could be explained by the fact that during toxoplasmic infestation there is a visceral hypertrophy, especially in the liver (El-Sayed *et al*., 2016).Also Kaneko *et al*., (2008) and Roussel, *et al*., (1997)reported that some of the parameters were significantly altered due to protozoan infections. The reduction of serum total protein and albumin concentrations were observed in Toxoplasma, Eimeria, and Cryptosporidium infections. It has been reported that protein requirement increase in the presence of any lesions on the body and chronic organ disease (El-Manyawe*et al.*, 2010).A significant decrease of total serum proteins in goats with internal parasitic infections was recorded by Moudgil *et al*., (2017). This was attributed to protein-losing gastroenteropathy and malabsorptionof proteins from damaged intestinal mucosa(Jesse *et al*., 2019). The increased concentrations of total serum protein in goats with internal parasites werealso observed by Hassan *et al*., (2012).

Minerals play a vital role in maintaining homeostasis by being involved in all living processes, either in the capacity of structural elements or as regulators of almost all metabolic processes (Bauman and Currie, 1980).A significant increase ($P>0.05$) of sodium, potassium and

Phosphatein small ruminantsreported inthis study table (3) was in contradiction to that obtained by Soheir *et al*., (2010) who reported low levels of sodium andpotassium in theserum of infected goats compared to controls. This low level may be attributed to diarrhea and reduced absorption in the infected tissues of intestine (Cirak*et al.*,2004).For calcium concentrations, our results showed thatthere is no significant change in theserum calcium of goat and significant increase ($P<0.01$) in magnesium concentration regarding the negative controls. This was in disagreement with Geng *et al.*,(2001) who reported thatin Toxoplasma infected goats, serum showed a reduction in magnesium, calcium and potassium concentration. But in our study themean positiveserum Mg concentration of sheep isnot significantly increased compared to the negative controls, no significant differences wereobserved in comparison to that reported by Seyrek*et al*., (2004) who reported that the mean concentration of Mg in theserum was higher but not significant ($P>0.05$) in seropositive sheep than in their controls.Magnesium (Mg) is most abundant divalent cation in intracellular fluid. It is needed in many enzymes such as alkaline phosphatase, ATPase(Maji *et al.,* 2023). Sheep and goats are highly susceptible to the infections with *T. gondii*and this protozoan parasite is considered as a major cause of reproductive losses in small ruminant'sworldwide (Ali *et al*., 2019).

Conclusion

Infestation with Toxoplasma in sheep elicits a pronounced elevation in total protein and albumin levels, concomitant with a significant reduction in globulin concentrations. Conversely, Toxoplasma gondii infestation in goats demonstrates an augmentation in total protein and globulin levels, coupled with a noteworthy decline in albumin levels, potentially impacting various enzymatic pathways. Furthermore, sheep and goats afflicted with toxoplasmosis exhibit heightened serum concentrations of sodium, potassium, and magnesium. These findings underscore the imperative for stringent protozoan disease management strategies in small-scale farming operations to mitigate substantial economic repercussions on animal productivity.

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Competing Interests

Authors declare no conflict of interest.

Availability of Data and Material

Data are available within the submitted article.

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