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J.K and F.G designed, structured and coordinated the study. R.N.C coordinated the fieldwork but also collected data with G.J.T.T and R.N.C and K.K.A.P extracted and analyzed the data. R.N.C prepared the first draft of the manuscript. J.K, and F.G, critically reviewed the manuscript. All authors read and approved the final version of the manuscript.

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Metabolic Profiles Associated with *Toxoplasma gondii* Infestation in Goats and Sheep in Cameroon

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Abstract:

Toxoplasmosis has important implications for animal productivity and health, as well as for human health and welfare. The present study was to identify the metabolic factors associated with *Toxoplasma gondii* infestation in sheep and goats in Cameroon. A cross-sectional study was conducted in 200 small ruminant farms during a period from April to October 2021. A total of 1061 small ruminants were sampled and the serums obtained were analyzed first with the indirect multi-species ELISA for toxoplasmosis and then once the groups were formed, some metabolic parameters were analyzed in both the control and the *T. gondii* infested animals groups in order to highlight the parameters associated with toxoplasmic infestation. 329 animals tested positive for *T. gondii* with an individual prevalence of 31.01% (95% CI: 28.23 - 33.79). A positive and significant association was obtained between the prevalence of toxoplasmosis and variations in albumin (p=0.015), ALT (p=0.001) and progesterone (p=0.03). Furthermore, a significant correlation was observed between the prevalence of toxoplasmosis and region (p=0.0001), species (p=0.0001), sex (p=0.0002), age (p=0.0002) and breed (p=0.01), production targets (p=0.04) and hygiene level (p=0.04). Several physiological factors were associated with significant (p<0.05) variation in albumin, ALT and progesterone in *Toxoplasma gondii* infested small ruminants, including age and gestation. Infestation of sheep and goats with *T. gondii* promotes severe increase in albumin and alanine aminotransferase, and significant hypoprogesteronemia that can lead to abortion. Understanding the factors associated with this infestation is essential for the implementation of effective control programs to reduce its impact on small ruminant farms.

INTRODUCTION

The livestock sector is currently establishing itself as a safe and huge value of the Cameroonian economy (Zakari, 2021). It contributes nearly 165 billion FCFA to the formation of the Gross Domestic Product (GDP) and provides income to about 30% of the rural population (MINEPIA, 2019). The production of small ruminants has an important place in the pastoral economy since it contributes to 20% of the protein needs of Cameroonians (FAO, 2018). In spite of considerable governmental efforts, this production remains insufficient because it is faced with food constraints, poorly performing genetic material and the pathological factor (MINEPIA, 2018). This last one is at the origin of 30% of losses in breeding (Grace *et al*., 2015). Among these multiple pathologies, the abortive pathologies are a source of important numerical and economic losses in the farms (Diallo *et al*., 2020; Elandalousi *et al*., 2015). Among the causes of abortions in small ruminants, toxoplasmosis appears to be the most important cause of abortions of infectious origin (Cremoux *et al*., 2017).

Some studies on the seroprevalence of zoonotic abortifacient diseases have been reported in small ruminants (Alemayehu *et al*., 2021; Charbal & Meriem, 2021; Deconinck *et al*., 2021; Hotea *et al*., 2021; Li *et al*., 2021; Li *et al*., 2020; Sidibe *et al*., 2019). These studies point out the multiplicity and complexity of this abortive infestation either at the individual or herd level. During abortions, the clinical manifestations and epidemiological characteristics are not specific. At best, these elements can point to a suspicion, hence the need for laboratory analysis. Analytical diagnosis uses direct methods with search for the causal agent or indirect methods (search for antibodies). These costly diagnostic methods are not within the reach of breeders (Degbe *et al*., 2018).

In order to improve the tools necessary for a rapid and less costly diagnosis of infestation, several previous studies have reported the unfavorable consequences of *Toxoplasma* on the clinical health status of the host (Alekish *et al*., 2017; Nora *et al*., 2018; Tothova *et al*., 2016). However, the implication of biochemical parameters in the abortion process of infectious origin remains unclear. In view of the prevalence of toxoplasmosis and its economic implication on animal production and in humans, it should be considered with more vigilance. A study of the variation of metabolic profiles during *Toxoplama gondii* infestation in goats and sheep in Cameroon is crucial to develop new diagnostic studies of this pathology in livestock.

MATERIALS AND METHODS

Study area

The study was carried out in the northern regions of Cameroon, namely the Adamawa (6°49’59’’ LN and 13°15’0’’ LE), North (8° 30′ LN**,** 14° 00′ LE) and Far North (11°30′43’’ LN**,** 14°33′03″ LE) regions (Fig. 1). These regions are well known for their involvement in ruminant production in Cameroon as more than 75% of the national small ruminant population is located in these regions. The production system of small ruminants in these areas was mainly extensive and they were mainly raised for meat production. This part of Cameroon represents a prime area for improving small ruminant production in Cameroon.

Ethics approval and consent to participate

The ethics and scientific research committee of the Higher Institute of Health of Bangangté-Cameroon (N° UdM-BUR-CPR-2021/006) gave its ethical approval to this research. The regional delegations in charge of animal health allowed the survey in the three northern regions of Cameroon, Adamawa (N°0020/21/RA/DREPIA/ SRSV), North (N°0025 /21/RN/ DREPIA/SRSV) and Far North (N°0017/21/MINEPIA/ SG/DREN). Small ruminant farmers were informed of the purpose of the study and the approximate duration of the interview, and their informed consent was sought prior to their participation in the survey.

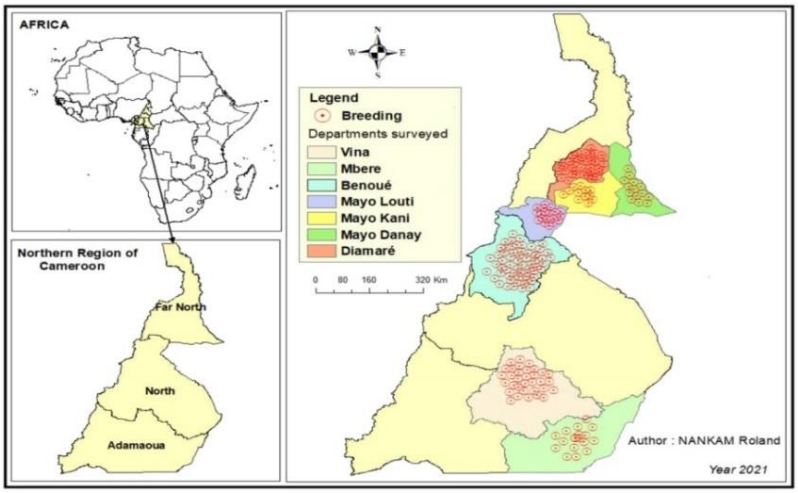

Fig. 1. Map showing the study regions (Adamawa, North, Far North) in Cameroon.

Animal selection

A cross-sectional study was conducted in 200 small ruminant farms during a period from April to October 2021. A total of 1061 small ruminants were obtained according to the formula proposed by Musallam et al. (2015) with a seroprevalence of 4.47 obtained by Sidibe *et al*. (2019) in Mali. An absolute precision of 5% was applied with a 95% confidence interval (CI).

☐ Minimum sample size of flocks

$$N = \frac{Z^2}{d} X \frac{Hse\ X\ P + 1 - Hsp\ X\ (1-P)\ X\ 1 - Hse\ X\ (P-1) - Hsp\ X\ (1-P)}{Hse + Hsp - 1^2}$$

- N : Sample size (number of farms to be selected) ;
- Z : Surface where we find (1- α) of the normal curve (Z) (Z=1,65) ;
- P: Known or attributed prevalence.
- HSe : Sensitivity of the ELISA used in the herd (HSe =1) ;
- HSp : Specificity of the ELISA used in the herd (HSp = 1) ;
- d: Absolute accuracy of 10%, with a confidence interval of 95%.

☐ Minimum sample size of animals in selected herds

$$K = \left[1 - \left(1 - P^{\frac{1}{d}}\right)\right] X \left[N - \frac{d}{z}\right] + 1$$

- K : Minimum number of animals to be sampled on each farm;
- P : Probability of detecting at least one positive animal (prevalence);
- d : Expected number of infected animals on a farm;
- N : Number of farms;
- The minimum number of animals is 5 depending on the number of animals provided by the breeder in the selected farms.

Data and blood collection

The sheep and goat flocks were not selected strictly at random, but a certain representativeness of the national herd was sought by diversifying the types of flock (sedentary or transhumant) and the geographical areas. On this occasion, a questionnaire was filled in with the aim of bringing out information on the herd (the number of adult females, the recent introduction of animals (including partial returns from transhumance), the number of abortions and stillbirths during the year and the pathology of the young from birth to one month). This questionnaire also allowed us to trace the reproductive career of the female in chronology, with emphasis on any abortive episodes and their clinical consequences. The blood collected in dry tubes during the visit of flock, was left to

rest for 30 min. The fresh serum obtained was collected with a micropipette and divided into aliquots in identified cryotubes which were then transported to the laboratory in a cooler containing carbohydrate ice and stored at -20°C until analysis.

Serological diagnosis of *Toxoplama gondii*

Serum samples were analyzed with the indirect multi-species ELISA for toxoplasmosis (ID.vet, Grabels, France). As a prelude, serum samples and controls were diluted 1:10 and tested. The microplate ELISA reader measured the optical densities (OD) at 450 nm. The ODs obtained were used to calculate the Substrate/Product percentage (S/P %) by the following equations: S/P (%) = (OD sample /DOpc) ×100. Samples with an S/P% less than or equal to 40% were considered negative; if the S/P% was between 40% and 50%, the result was considered doubtful and considered positive if the S/P% was greater than or equal to 50%.

Biochemical analysis

After identification of sheep and goats serologically infested by *Toxoplasma gondii* using the indirect ELISA method, the animals were grouped into two groups. A "control" group, with animals recognized as negative by the test, and a "case" group, of sheep and goats identified as positive by the test. Once the groups were formed, some metabolic parameters were analyzed (Table 1) in both the control and the *T. gondii* infested animals groups in order to highlight the parameters associated with toxoplasmic infestation.

Table 1. Analysis Method of metabolic parameters

Parameter		Analysis Method	Kit used
Organic elements	**Albumin**	Colorimetric	DUTCH® Kit
	Total Cholesterol	Enzymatic, Colorimetric	DUTCH® Kit
	Glucose	Enzymatic, Colorimetric	DUTCH® Kit
	Creatinine	kinetic colorimetric of Jaffé	DUTCH® Kit
Enzyme	**ALT**	Kinetics.IFCC, UV-37 ° C	DUTCH® Kit
	AST	Kinetics.IFCC, UV-37 ° C	DUTCH® Kit
Hormone	**Progesterone**	Indirect ELISA	" Erbalisak®" ref : IME00017ER2

UV= Ultraviolet, IFCC= International Federation of Clinical Chemistry and Laboratory Medicine, ALT= Alanine Aminotransferase, AST= Aspartate Aminotransferase, ELISA= Enzyme-Linked Immunosorbent Assay

Statistical analysis

Statistical analysis was performed using R i386 4.1.2 and Sphinx 5.1 for Windows. The effects of the different variables were tested by analysis of variance; the effects of time on each variable were evaluated by Dunnett's test, and comparisons between groups were made by Student's or Fischer's test. The conditions required for their applications were the independence of the data between the groups; the normality of the distribution of the data in each group, and the homogeneity of the variances. All data were represented as mean ± SD (Standard Deviation) at the 5% threshold.

RESULTS

Seroprevalence of *Toxoplasma gondii* at flock and individual (animal) level.

The results of toxoplasmosis seroprevalence at the herd and individual (animal) level are presented in Table 2. A total of 74 herds was tested positive for *T. gondii* with a herd level prevalence of 37% (95% CI: 30.31 - 43.69). At the individual level, 1061 small ruminants over one month of age were tested. 329 animals tested positive for *T. gondii* with an individual prevalence of 31.01% (95% CI: 28.23 - 33.79).

Table 2. Seroprevalence of toxoplasmosis on small ruminant farms in the northern regions of Cameroon (Adamawa, North and Far North).

Variables	N	*Toxoplasma gondii*		
		Prevalence n (%)	95 % CI	P value
Flock prevalence	200	74 (37)	30.31 – 43.69	0.0001*
Individual prevalence	1061	329 (31.01)	28.23 – 33.79	0.0001*

Values in a column with "*" differ significantly at P < 0.05; CI= Confidence interval, N= total number of animals and the number of flock, n= number of positive animals.

Univariate analysis of risk factors associated with *T. gondii* infestation

Risk factors associated with toxoplasmosis seropositivity in small ruminants in a univariate analysis at P<0.05 are presented in Table 3. Significant correlation was observed between individual-level prevalence of toxoplasmosis and region (p=0.0001), species (p=0.0001), sex (p=0.0002), age (p=0.0002) and breed (p=0.01). The prevalence of *T. gondii* was also significantly correlated with production targets (p= 0.04) and hygiene level (p=0.04).

Table 3. Univariate analysis of the association between risk factors and toxoplasmosis seropositivity in the northern regions of Cameroon (Adamawa, North and Far North)

Variables		N	Toxoplasmosis		
			Prevalence n (%)	95 % CI	P value
Region	Adamawa	202	93 (46.04)	39.16 - 52.91	0.0001*
	North	476	146 (30.01)	26.53 - 34.81	
	Far North	202	90 (23.50)	19.25 - 27.74	
Species	Sheep	540	194 (35.93)	31.87 - 39.97	0.0001*
	Goats	521	135 (25.91)	22.14 - 29.67	
Gender	Female	775	263 (33.94)	30.60 - 37.26	0.0002*
	Male	286	66 (23.08)	18.19 - 27.96	
Age	< 5 months	46	14 (30.43)	17.13 - 43.73	0.0002*
	[6-12 months[	229	53 (23.14)	17.68 - 28.60	
	[1-3 years [	502	143 (28.49)	24.69 - 32.62	
	[3-5 years]	284	119 (41.90)	36.16 - 47.64	
Physiological status	Pregnant	291	111 (38.14)	32.56 - 43.72	0.13
	Non-Pregnant	484	152 (31.40)	27.27 - 35.54	
Breed	Sahelian goat	3	0 (0.00)	-	0.01*
	Djallonke goat	409	103 (25.18)	20.97 - 29.39	
	Djallonké Sheep	322	114 (35.40)	30.18 - 40.62	
	Kirdi	104	32 (30.77)	21.89 - 39.64	
	Fat tail sheep	11	1 (9.09)	0.00 - 26.08	
	Oudah	206	79 (38.35)	31.71 - 44.98	
	Sokoto goat	6	0 (0.00)	-	
Breeding objectives	Financial	139	72 (51.80)	43.49 - 60.10	0.04*
	Consomption	51	16 (31.37)	18.63 - 44.10	
	Pleasure	10	1 (10)	0.00 - 28.59	
Hygiene level	Own	48	12 (25)	12.75 - 37.25	0.04*
	Very clean	8	2 (25)	0.00 - 55	
	Dirty	134	54 (40.30)	31.99 - 48.60	
	Very dirty	10	6 (60)	29.63 - 90.36	

Values in a column with "*" differ significantly at P < 0.05; CI= Confidence interval,. N= total number of animals, n= number of positive animals.

Metabolic parameters associated with *T. gondii* infestation

The metabolic parameters associated with toxoplasma infestation in small ruminants in this study are presented in Table 4. A positive and significant association was obtained between the prevalence of toxoplasmosis and changes in albumin (p=0.015), ALT (p=0.001) and progesterone (p=0.03). This association results in an increase in albumin and ALT in toxoplasmosis-infected animals, and a decrease in progesterone levels. However, significant interaction between species and serological status was observed on some metabolic parameters (Table 5). In particular albumin (p=0.04), glucose (p=0.01) and progesterone (p=0.04). Furthermore, similar significant variations according to age groups were observed (Table 6). Depending on the physiological status of the females (pregnant and non-pregnant), significant variations in glucose (p=0.03), creatinine (p=0.04), AST (p=0.04), ALT (p=0.03) and progesterone (p=0.0001) were observed (Table 7).

Table 4. Variations in metabolic parameters according to toxoplasma infestation in small ruminants in the northern regions of Cameroon.

Parameters	Metabolic variations (Mean ± SD)			t-test	P value
	Total (200)	Negative (100)	Positive (100)		
Cholesterol (mmol/L)	1.96±0.92	2±0.95	1.93±0.90	0.31	0.75
Albumin (g/dl)	13.78±9.31	12.02±6.30	14.66±10.45	2.36	0.015*
Glucose (mmol/L)	1.48±1.24	1.74±0.99	1.35±1.33	1.32	0.18
Creatinine (mg/dl)	10.50±19.26	12.66±19.61	9.42±19.21	0.65	0.52
AST (IU/L)	43.35±22.08	46.30±22.32	41.73±21.93	1.02	0.31
ALT (IU/L)	14.02±11.35	13.43±13.43	14.96±6.84	3.98	0.001*
Progesterone (ng/ml)	5±1.71	5.67±0.89	4.69±1.91	2.20	0.03*

*= p-value significative, SD= standard deviation, ALT= Alanine Aminotransferase, AST= Aspartate Aminotransferase.

Table 5. Variation of metabolic parameters according to the species of small ruminants infested by *T. gondii*

Metabolic Parameters	Metabolic variations (Mean ± SD)				F-test	P value
	Negative (n= 100)		Positive (n=100)			
	Sheep	Goats	Sheep	Goats		
Cholesterol (mmol/L)	1.8 ± 0.83	2.2 ± 1,01	1.7 ± 1	2.1 ± 0.79	0.06	0.79
Albumin (g/dl)	13.8 ± 8.07	10.2 ± 3.34	13.4 ± 6.4	15.7 ± 13	2.18	0.04*
Glucose (mmol/L)	40.5 ± 19	22.2 ± 11.7	18,7 ± 16.5	29.1 ± 28.4	5.96	0,01*
Creatinine (mg/dl)	10 ± 10.9	14.4 ± 25.9	5 ± 2.9	13.1 ± 25.5	0.08	0.76
AST (IU/L)	46.4 ± 15.1	46.2 ± 27.5	43.8 ± 16.8	40 ± 25.9	0.07	0.78
ALT (IU/L)	14,3 ± 7.9	15,7 ± 5.8	13,7 ± 19	13,2 ± 6.3	0.09	0.60
Progesterone (ng/ml)	5.5 ± 1.2	6 ± 0.4	5 ± 2	5 ± 2	3.49	0,04*

*= p-value significative, SD= standard deviation, ALT= Alanine Aminotransferase, AST= Aspartate Aminotransferase.

Table 6. Variation of metabolic parameters according to age groups of small ruminants infested with *T. gondii*

Metabolic Parameters	Metabolic variations (Mean ± SD)				F-test	P value
	Negative (n= 100)		Positive (n=100)			
	Young	Adults	Young	Adults		
Cholesterol (mmol/L)	1.8 ± 0.7	2.2 ± 1.1	2 ± 1.3	1.9 ± 0.8	1.25	0.26
Albumin (g/dl)	14.9 ± 7.5	9.7 ± 4	12.3 ± 4.8	15.8 ± 12.1	2.95	0.04*
Glucose (mmol/L)	35.5 ± 18	27.9 ±18	19.2 ± 12.7	26.8 ± 27.6	2.30	0.04*
Creatinine (mg/dl)	9.5 ± 12.1	15.3 ± 24.4	11.9 ± 25.2	8.3 ± 16.1	0.78	0.38
AST (IU/L)	41.6 ± 11.5	50.3 ± 28.3	39.1 ± 11.6	42.4 ± 24	1.17	0.28
ALT (IU/L)	14.4 ± 7.7	15.3 ± 6.5	12.5 ± 4.3	13.8 ± 16.1	0.21	0.88
Progesterone (ng/ml)	6 ± 1.5	5.5 ± 1.1	3 ± 1.7	4.9 ± 1.9	2.49	0.04*

*= p-value significative, SD= standard deviation, ALT= Alanine Aminotransferase, AST= Aspartate Aminotransferase.

Table 7. Variation of metabolic parameters according to physiological status of small ruminants infested with *T. gondii*

Metabolic Parameters	Metabolic variations (Mean ± SD)				F-test	P value
	Negative (n= 100)		Positive (n=100)			
	Pregnant	Non-Pregnant	Pregnant	Non-Pregnant		
Cholesterol (mmol/L)	2.3 ± 1.2	1.8 ± 0.5	2.1 ± 1.2	1.9 ± 0.7	0.41	0.52
Albumin (g/dl)	14.5 ± 6.4	12.8 ± 8	14 ± 3.4	12 ± 6.3	1.23	0.26
Glucose (mmol/L)	24.2 ± 21	36 ± 18.2	37.6 ± 31.8	16 ± 13.1	4.72	0.03*
Creatinine (mg/dl)	11.2 ± 14	19.8 ± 29.5	7.2 ± 5.2	9.6 ± 23	2.18	0.04*
AST (IU/L)	55.5 ± 14	44.6 ± 31.5	38.5 ± 15.2	44.8 ± 29.8	2.33	0.04*
ALT (IU/L)	11.8 ± 5	12.4 ± 8	17.4 ± 8.1	13.1 ± 4.5	4.39	0.03*
Progesterone (ng/ml)	5.67 ± 0.9	5,35 ± 0,15	3.69 ± 1.9	4,35 ± 0,5	6.32	0,00*

*= p-value significative, SD= standard deviation, ALT= Alanine Aminotransferase, AST= Aspartate Aminotransferase.

DISCUSSION

Toxoplasmosis is a neglected endemic disease of livestock in Cameroon. It is considered an obstacle to livestock production, more specifically to small ruminant production, because of the significant economic losses it causes (Sidibe *et al*., 2019). But beyond that, it constitutes a real public health problem for certain groups of the Cameroonian population (Guemgne *et al*., 2019). This study examined the dynamics of the metabolic profile associated with toxoplasma infestation in order to facilitate their diagnosis in small domestic ruminants in Cameroon.

The individual seroprevalence of *Toxoplasma gondii* obtained in the present study (31.01% (329/1061) with 35.93% in sheep and 25.91% in goats) was lower than those obtained in China (61.65%) (Li *et al*., 2021), in Romania (50.64% in sheep and 75% in goats) (Hotea *et al*., 2021) and Egypt in sheep (62%) (Al-Kappany *et al*., 2018). However, lower prevalence have been found in small ruminants in Mexico (14.8%) (María de la Luz *et al*., 2022), in Mali (4.47 %) (Sidibe *et al*., 2019) and in Bangladesh (12.2%)

(Sah *et al*., 2018). The different sero-detection techniques used could explain the differences observed. Moreover, the studies carried out in hot zones sometimes reaching temperatures of 45ºC, did not favour the survival of oocysts in the environment for more than an hour thus limiting the indirect mode of transmission (Sidibe *et al*., 2019).

Univariate analysis in this study showed the Adamawa region (46.04% (95% CI: 39.16-52.9) had significantly (p=0.0001) high *T. gondii* seroprevalence. This region has a savannah climate with a dry winter according to the Köppen-Geiger classification, unlike the other regions in the study (North and Far North) (Données-Mondiale, 2022). This type of climate favors the development and expansion of this pest (Fayez *et al*., 2021; Qin *et al.*, 2015). The sheep species was significantly more infected (35.93% (95% CI: 31.87 - 39.97)) (p=0.0001) with *T. gondii* than the goats in these areas. This result was similar to those obtained in Tunisia (Lahmar *et al*., 2015). Sheep are generally associated with large cattle for grazing unlike goats, which favors greater contact with this infectious agent. Females (33.94% (95% CI: 30.60 - 37.26)) tested significantly more infested with *T. gondii* than males as observed in the Lahmar study in Tunisia (Lahmar *et al*., 2015). Small ruminants aged 3-5 years old were significantly more infested with *T. gondii* than young animals (41.90% (95% CI: 36.16 - 47.64)). This observation was also highlighted by Qin *et al*. (2015) and was associated with continuous and increasing exposure to infectious oocytes in the environment (Qin *et al*., 2015).

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 similar with those reported by Alekish *et al*. (2017), Nora *et al*. (2018) and Tothova *et al*. (2016), who found 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), as well as acute inflammation and dehydration which could lead to an increase in ALT and albumin (Tothova *et al*., 2016). Furthermore, the significant decrease (p=0.04) of progesterone observed in infested females is related to the abortion caused by the disease. This result is in agreement with those obtained by María de la Luz (María de la Luz *et al*., 2014) in his study on the role of hormones on *T. gondii* infection. Indeed, progesterone plays an important role in the maintenance of gestation. Its decrease could therefore lead to abortion.

Several physiological factors were associated with significant variation (p<0.05) in albumin, ALT and progesterone in *T. gondii* infested small ruminants, including age and gestation. In the recent study, these factors may not interfere in the results because the animals were divided into equal groups according to age and physiological status. Associated with this the samples were collected in the same period avoiding variations in temperature and humidity.

CONCLUSION

Infestation of sheep and goats with *Toxoplama gondii* promotes severe increase of albuminemia, alanine aminotransferase, and significant hypoprogesteronemia that can lead to abortion. Further studies will be needed to define the mechanisms of these consequences.

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None declared

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All data generated or analyzed during this study are included in this published article.

Consent for publication:

Not applicable.

Competing interests

The authors declare that they have no financial or personal interests or relationships that could have influenced the work presented in this article.

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