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Seroprevalence of *Neospora caninum*, *Toxoplasma gondii* and *Brucella* Species in Sheep

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ABSTRACT

Numerous infections are accountable for abortion in small ruminants, resulting in significant economic repercussions, as well as possessing the potential risk for humans. The current study was undertaken to assess the identification of different risk factors as well as the seroprevalence associated with sheep abortion in *Neospora caninum, Toxoplasma gondii*, and *Brucella* species infections. Ninety serum samples from sheep were examined using ELISA to detect antibodies against *Toxoplasma* and *Neospora*. The results revealed that 54(60%) and 7(7.7%) were seropositive for *T. gondii* and *N. caninum*, respectively. For these samples the Rose Bengal test was used to detect antibodies against *Brucella* species. The results showed that 26(28.8%) were seropositive serologically to *Brucella* species. There were 17(18.8%) samples showed mixed infection *of T. gondii* and *B. melitensis*, 4(4.4%) were mixed showed infection with *T. gondii* and *N. caninum,* and 1(1.1%) was mixed infection between *N. caninum* and *B. melitensis*. Only one (1.1%) sample showed mixed infection for the three infections together. The prevalence of miscarriage in sheep was observed. The seropositivity of toxoplasmosis, Neosporosis, and Brucellosis in sheep needs further study to elude the transmission to human especially those of zoonotic importance, so attention should be considered to more investigations concerning these diseases in animals and humans in the studied area.

Keywords: *Brucella*, Egypt, ELISA, *Neospora*, Small ruminant, *Toxoplasma*.

INTRODUCTION

Worldwide, around 1.5 billion small ruminants produce meat and milk each year, contributing as significant food sources and sources of income (Watkins et al., 2021). All focus groups well-thought-out sheep the most main livestock species, followed by cattle (Wodajo et al., 2020). Additionally, Greece is one of the top producing nations, producing 840.140 tons of milk/year, and the milk production from small ruminants is significant for developed Mediterranean countries, contributing significantly to rural income, and bolstering the national economy (Moutos et al., 2022).

Abortion constitutes a significant health encounter that influences sheep and goats productive and reproductive efficacy (Haif et al., 2021). For that, most recent studies dealt with diseases causing abortion in small ruminants (Tesfaye et al. 2020).

Abortion in sheep and goats occur as a result of both infectious and non-infectious causes. This led to a significant economic consequence, such as the loss of the fetus and a decrease in milk production. Furthermore, the agent responsible for causing abortion may also have transmitted a risk to humans (Stone, 2012; Mammeri et al., 2013 and Van Engelen et al., 2014).

Abortion in both sheep and goats is a substantial problem in numerous countries globally including Jordan (Hailat et al., 2018), South Dakota State (USA) (Holler, 2012), Borana zone (Ethiopia) (Tesfaye et al., 2020).

Two essential intracellular parasites, *T. gondii* and *N. caninum*, are significant contributors to newborn death and abortion in ruminants raised for food around the world. It is conceivable to see a correlation between the epidemiology of these parasites in both sheep and cattle since they share a common source of pasture and water (Malekifard et al., 2022).

N. caninum is widely recognized as a significant etiological factor for bovine abortion. However, new research has also highlighted its significance as an etiology in sheep and goats. More understanding of the pathophysiology of ovine neosporosis, was cleared by Arranz-Solís et al. (2015). Fereig et al. (2016) recorded that *N. Caninum* is responsible for storms of abortion and increase the animal culling proportion. Moreover, *N. caninum* induces reproductive imperfect performance and has the affinity to cause persistent infections (Chernick et al., 2018).

T. gondii, *N. caninum*, and *B. melitensis* are prevalent pathogens that induce severe clinical conditions in a majority of livestock animals. Concerning to *B. melitensis* and *T. gondii*, zoonotic agents that are prevalent in Egypt and other countries, *T. gondii*, *N. caninum*, and *B. melitensis* are recognized as infectious pathogens causing abortion in various animals. The incorrect identification of these abortion cases can result in significant financial losses due to the implementation of inappropriate control measures (Fereig et al., 2022).

Serological tests, like the Enzyme-Linked Immunosorbent Assay (ELISA) for the dam, and specialized direct tests, like the Polymerase Chain Reaction (PCR) for the fetus, are carried out in order to accurately determine the diagnosis of the infectious agent responsible for abortion (Asadpour et al., 2012). Currently, a wide range of commercially available diagnostic approaches for the identification of ovine abortion are applied, encompassing both traditional and emerging methodologies. Histopathological examination of fetal tissues has emerged as the primary approach for identifying the etiology of ovine abortion resulting from protozoal infections (Shaapan, 2016).

In Egypt few and scattered studies focused on *N. caninum, T. gondii*, and *B. melitensis* as etiological agents of abortion in sheep. The study interested mainly with the detection of *N. caninum, T. gondii*, and *B. melitensis* as causes of abortion in sheep, using serological analysis.

MATERIALS AND METHODS

1. Animals and samples collection

From 90 adult sheep of the Baladi breed, aged (2 years), blood samples were taken from them in two different locations in the Menoufia governorate; 55 blood samples were taken from a flock of sheep raised in Kafr Tanbidi and 35 blood samples were taken from a flock of sheep raised in Shebein El Kom. Males, females who had had previous abortions history, pregnant and non-pregnant females all had their samples taken.

Table (1): Blood samples collected from sheep:

According to		Samples
Locality	KafrTanbidi	55
	ShebinElkom	35
	Total	90
Season	Winter	34
	Summer	56
	Total	90
Age	> 2 years	90
	Total	90
Sex	Male	20
	Female	70
	Total	90
Breed	Baladi breed	90
	Total	90
Pregnancy	Previous abortion	30
	Pregnant	31
	Non-pregnant	9
	Males	20
	Total	90

2. Samples:

2.1. Serum samples (Blood without anticoagulant).

Blood samples (5 ml) were collected using sterile disposable syringes from the jugular vein of sheep, then placed in a sterile glass tube and allowed to settle for about 30 minutes. Subsequently, it was subjected to centrifugation at 3000 for 10 minutes in order to obtain a clear serum that was free from hemolysis. The serum that had been separated was carefully stored in aliquots that were appropriately labelled, and then stored at -20 °C until they were ready to be tested.

2.2. Rose Bengal Plate test (RBPT).

RBPT was performed as described by Morgan et al. (1969). Its *B. abortus* strain stained with Rose Bengal in lactate buffer (PH 3.65±0.05). It was obtained from Veterinary Serum and Vaccines Research Institute, Abbasia, Cairo, Egypt.

3. Detection of Toxoplasma IgG antibodies in sheep:

This was carried as the method described by Lind et al. (1997) and Byomi et al. (2018). A commercial kits (Pishtaz Teb Diagnostics-MA *Toxoplasma* IgG _96 _02, Catalogue No. PT- *Toxoplasma* – IgG -96. Iran) were used for antibody detection against *Toxoplasma gondii* in sheep samples. The test procedures were followed according to manufacture. ELISA reader with 630 nm (reference) filters (model ELx808 Absorbance Reader, BioTek Instruments, Inc., USA) was used to read the developed color of the microtiter plate at specified wave length.

4. Materials used for ELISA examination for Neospora:

The test was carried out according to González-Warleta et al. (2011). *N. caninum* ELISA Kit was obtained from Bio K 218/2 - Bio K 218/5 For serum - Sero Blocking –Monowell. The test procedures were followed according to manufacture. ELISA reader with 630 nm (reference) filters (model ELx808 Absorbance Reader, Bio Tek Instruments, Inc., USA) was used to read the developed color of the microtiter plate at specified wavelength.

5. Statistical analysis

It was carried according to Nazanin Alavi et al. (2015) and Byomi et al. (2019a and b) where the data of samples including all the current variables including (locality, season, age, sex, breed, pregnancy, and abortion) were collected. The association between positive samples and these animal attributes was tested using a univariate logistic regression analysis model in IBM SPSS Statistics for Windows version 21.0 (IBM SPSS Inc., Armonk, NY).

The association between animal attributes (locality, season, age, and sex) and seropositivity results of *N. caninum*, *T. gondii*, and *B. melitensis* were determined using a multivariate logistic regression model. At first, a univariate logistic regression model was applied to identify the association between each animal element with *N. caninum*, *T. gondii*, and total infection status. The significance of this collinear association was detected by using chi-square at $P< 0.05$, with the variable judged as most biologically plausible kept in the multivariate analysis. All variables that passed the previous 2 steps were incorporated into a binary logistic regression model. A manual backward stepwise selection approach was used to select variables in that model to keep only variables with $P< 0.05$ in the final model. All two-way interactions between variables retained in the model were assessed. Testing for confounder was did out by controlling the change of logic of factors by deleting a suspected factor from the model.

RESULTS

1. Seroprevalence of T. gondii, N. caninum, and B. species in tested sheep serum samples:

ELISA was applied to determine *T. gondii* and *N. caninum* antibodies, and the results revealed 54(60%) and 7(7.7%) were positive for *T. gondii* and *N. caninum*, respectively. However, the results of the Rose Bengal test revealed 26(28.8%) were seropositive for *Brucella* species as illustrated in (table 2).

Table (2): Seroprevalence of *T. gondii* by ELISA, *N. caninum* by ELISA, and *B. species* by Rose Bengal test in tested samples:

Total samples	***T. gondii* by ELISA**		***N. caninum* by ELISA**		***Brucella Species* by Rose Bengal**	
No.	**No.**	**%**	**No.**	**%**	**No.**	**%**
90	54	60	7	7.7	26	28.8

2. Seroprevalence of mixed infection of (T. gondii, N. caninum, and Brucella spp.)

Table (3) showed 17(18.8%) sheep serum samples had serologically mixed infection of *Brucella* spp., and *T. gondii*, as well as mixed infection of *Toxoplasma* and *N. caninum* was 4(4.4%) sheep serum samples and one (1.1%) sheep serum sample showed mixed infection of *N. caninum* and *B. species.* Only one (1.1%) sheep serum sample showed mixed infection of the three infective agents together.

Table (3): The mixed infection of *T. gondii*, *N. caninum,* and *B. species*:

Total samples	*Toxoplasma* & *Neospora*		*Toxoplasma* & *Brucella*		*Neospora* & *Brucella*		*Toxoplasma*, *Neospora* & *Brucella*	
No.	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
90	**4**	**4.4**	**17**	**18.8**	**1**	**1.1**	**1**	**1.1**

3. Risk factors associated with T. gondii, N. caninum, and B. species among tested sheep serum samples:

3.1. Regarding sex,

By ELISA 12/20 (60%) of rams samples were positive for *T. gondii*, 42/70 (60%) of ewes were positive for *T. gondii* and for *N. caninum* no rams tested positive but there were 7/70(10 %) ewes were positive. Rose Bengal test reported 8/20 (40 %) rams were positive, while 21/70 (30%) ewes were positive (Table 4).

Table (4): Effect of sex seroprevalence of *T. gondii*, *N. caninum* and *B. melitensis*:

Sex	**Total**	***Toxoplasma* by ELISA**		***Neospora* by ELISA**		***Brucella* by Rose Bengal test**	
		Positive	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**
Male	20 22.2%	12 (60%)	8 (40%)	00 (0%)	20 (100%)	8 (40%)	12 (60%)
Female	70 77.8%	42 (60%)	28 (40%)	7 (10%)	63 (90%)	21 (30%)	49 (70%)

3.2. Effect of season,

By ELISA 35/56 (62.5%) and 4/56 (7.14%) were positive for *T. gondii* and *N. caninum* respectively in summer, while in winter19/34 (56%) and 3/34 (8.8 %) were seropositive for *T. gondii* and *N. caninum* respectively. In summer rose Bengal test showed that20/56 (35.7%) were positive for *Brucella species*, while 6/34(17.6 %) were positive in winter (Table 5).

Table (5): Effect of season on Seroprevalence of *T. gondii*, *N. caninum* and *Brucella spp.* according to season:

Season conditions	**Total**	***Toxoplasma* by ELISA**		***Neospora* by ELISA**		***Brucella* by Rose Bengal test**	
		Positive	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**
Warm (spring and summer)	56 62.2%	35 (62.5%)	21 (37.5%)	4 (7.2 %)	52 (92.8%)	20 (35.7%)	36 (64.2%)
Cold (autumn and winter)	34 37.8%	19 (55.8%)	15 (44.2%)	3 (8.8%)	31 (91.2%)	6 (17.6%)	28 (82.4%)

3.3. For the locality,

31/55 (56.3 %) and23/35 (65.7%) were positive for *T. gondii*, respectively, in Kafr Tanbidi and Shebin Elkom. While, 5/55 (9.1%) and 2/35 (5.7%) were positive for *N. caninum*, respectively, in Kafr Tanbidi and Shebin Elkom. Rose Bengal test reveled 9/55 (16.4%) and 17/35 (48.6%) were positive for *Brucella species*, in Kafr Tanbidi and Shebin Elkom respectively (Table 6).

Table (6): Effect of locality on seroprevalence of *T. gondii*, *N. caninum* and *Brucella species:*

Locality	Total	*Toxoplasma* by ELISA		*Neospora* by ELISA		*Brucella* by Rose Bengal test	
		Positive	Negative	Positive	Negative	Positive	Negative
Kafr Tanbidi	55 61.1%	31 56.4%	24 43.6%	5 9.1%	50 90.9%	9 16.4%	46 83.6%
Shebin Elkom	35 38.8%	23 65.7%	12 34.3%	2 5.7%	33 94.3%	17 48.6%	18 51.4%

4. Statistical analysis of some risk factors associated with T. gondii, N. caninum, and Brucella species among tested sheep samples.

4.1. Effect of Locality on the prevalence of N. caninum, T. gondii and Brucella Spp.

The statistical analysis showed a non-significant effect of locality in *N. caninum* infection, although the number of infections with *N. caninum* in sheep in Kafr Tanbidi was more than in Shebin Elkom 1.6 times, no significant effect related to locality for *T. gondii* infection**,** No significant effect of locality was found in infection with *Brucella spp.* However, infection in Shebin Elkom was 5 times more than infection in Kafr Tanbidi (Table7)**.**

Table (7): Effect of locality on the prevalence of *N. caninum, T. gondii* and *Brucella* spp:

N. caninum		B	S.E.	Wald	Df	Sig.	Exp(B)	95% C.I. for EXP(B)	
								Lower	Upper
Step 1[a]	Locality (1)	.501	.866	.334	1	.563	1.650	.302	9.011
	Constant	-2.803	.728	14.820	1	.000	.061		
T. gondii		B	S.E.	Wald	Df	Sig.	Exp(B)	95% C.I. for EXP(B) Lower	Uppr
Step 1[a]	Locality(1)	.000	.518	.000	1	1.000	1.000	-363	2.758
	Constant	.405	.456	.789	1	.374	1.500		
*Brucella*Spp.		B	S.E.	Wald	Df	Sig.	Exp(B)	95% C.I.for EXP(B) Lower	Upper
Step 1[a]	Locality (1)	-1.574	.497	10.024	1	.002	.207	.078	.549
	Constant	-.057	.338	.029	1	.866	.944		

4.2. Effect of sex on the seroprevalence of N. caninum, T. gondii and Brucella spp.:

The statistical analysis showed that there is no significant effect of sex on the prevalence of *N. caninum and T. gondii*, and *Brucella spp.*, although the infection in females was 1.2 times more than in males (Table 8).

Table (8): Effect of sex on the seroprevalence of *N. caninum, T. gondii and Brucella spp:*

N. caninum		B	S.E.	Wald	Df	Sig.	Exp(B)	95% C.I. for EXP(B)	
								Lower	Upper
Step1[a]	Sex	19.006	8987.421	.000	1	.998	179497204.405	.000	.
	Constant	-21.203	8987.421	.000	1	.998	.000		

T. gondii		B	S.E.	Wald	Df	Sig.	Exp(B)	95% C.I. for EXP(B)	
								lower	Upper
Step1[a]	Sex	.000	.518	.000	1	1.000	1.000	.414	3.996
	Constant	.405	.456	.789	1	.374	1.500		
Brucella Spp.		B	S.E.	Wald	Df	Sig.	Exp(B)	95% C.I. for EXP(B)	
								Lower	Upper
Step1[a]	Sex	.251	.579	-189	1	.664	1.286	.414	3.996
	Constant	-1.099	.516	4.526	1	.033	.333		

4.3. Effect of abortion on the prevalence of N. caninum, T. gondii and Brucella spp.:

No significant effect between previously aborted, pregnant, and non-pregnant animals on the prevalence of *N. caninum*. On the other hand a high significant effect of abortion in infection with *Toxoplasma gondii* was reported, the infection in previously aborted sheep was more significant.

Totally significant effect of abortion in infection with *Brucella* was reported. Previously aborted and pregnant sheep were more susceptible to infection than male and non-pregnant sheep (Table 9).

Table (9): Effect of previous abortion on the prevalence of *N. caninum, T. gondii* and *Brucella* spp.:

N. caninum		**B**	**S.E.**	**Wald**	**Df**	**Sig.**	**Exp(B)**	**95% C.I. for EXP(B**	
								Lower	**Upper**
Step 1[a]	Abortion			1.464	3	.691			
	abortion(1)	18.529	8987.418	.000	1	.998	111412034.313	.000	.
	abortion(2)	.000	16132.913	.000	1	1.000	1.000	.000	.
	abortion(3)	19.593	8987.418	.000	1	.998	323094899.509	.000	.
	Constant	-21.203	8987.418	.000	1	.998	.000		
T. gondii		B	S.E.	Wald	Df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	Abortion			16.984	3	.001			
	abortion(1)	-1.147	.597	3.699	1	.054	.317	.099	1.022
	abortion(2)	-.182	.811	.050	1	.822	.833	.170	4.088
	abortion(3)	1.792	.761	5.548	1	.019	6.000	1.351	26.649
	Constant	.405	.456	.789	1	.374	1.500		
*Brucella*Spp		B	S.E.	Wald	Df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	Abortion			9.862	3	.020			
	abortion(1)	-1.135	.797	2.026	1	.155	.321	.067	1.534
	abortion(2)	.875	.847	1.069	1	.301	2.400	.457	12.613
	abortion(3)	.965	.633	2.325	1	.127	2.625	.759	9.076
	Constant	-1.099	.516	4.526	1	.033	.333		

4.4. Effect of season on the prevalence of N. caninum, T. gondii and Brucella spp.:

No significant effect of season on prevalence of infection with *N. caninum*, although the number of animals infected in worm conditions more than in cold, Also no significant effect was observed for Season on prevalence of infection with *T. gondii*, although the infection in worm season more than in cold. Almost significant effect of season in infection with *Brucella*, found that infection in worm season was 2.5 times more than infection in cold weather (Table 10).

Table (10): Effect of season on the prevalence of *N. caninum*, *T.gondii* and *Brucella* spp.:

N. caninum	B	S.E.	Wald	Df	Sig.	Exp(B)	95% C.I.for EXP(B) Lower	Upper
Step 1[a] Season (1)	.230	.797	.083	1	.773	.795	.167	3.789
Constant	-2.335	.605	14.918	1	.000	.097		
T. gondii	B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B) Lower	Upper
Step 1[a] Season (1)	.274	.442	.385	1	.535	1.316	.553	3.130
Constant	.236	.345	.468	1	.494	1.267		
Brucella. spp	B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B) Lower	Upper
Step 1[a] season(1)	.953	.529	3.239	1	.072	2.593	.919	7.316
Constant	-1.540	.450	11.725	1	.001	.214		

DISCUSSION

Brucella species, *T. gondii, and N. caninum* are significant pathogens known for their ability to induce abortion in various animal species across diverse geographical regions. Little research on the seroprevalence of these pathogens and their possible related risk factors in sheep and goats has not been undertaken in Egypt. Therefore, this study was conducted on small-scale flocks of Baladi sheep, situated in two distinct areas within the Menoufia governorate of Egypt. The animals exhibited a documented history of reproductive abnormalities, such as pyometra, abortions, stillbirths, and the birth of poor lambs. Additionally, there was a significant presence of cats in close proximity to the sheep flocks.

In this study, *T. gondii* higher prevalence rate was recorded and can be compared to the seroprevalence reported by Villagra-Blanco et al. (2019) using indirect ELISA (41.1%). Similarly, in western Mexico Caballero-Ortega et al. (2008) applied Immunofluorescence assay and reported prevalence rate (29.1%) as well as Gondim et al. (1999) in Brazil reported seroprevalence rate (18.75%) using latex agglutination test (LAT). However, lower seroprevalence rate in our study was disagree with that reported by Hamilton et al. (2014) in sheep from Dominica (67%) and Montserrat (89%) using an in-house ELISA. Notwithstanding these disparities, the authors reached a consensus that sheep possess the potential to serve as sentinels for identifying environmental pollution in soil, water, and crops caused by infective protozoa oocysts, including *T. gondii*. This is mostly due to their distinctive feeding habits. Sheep are herbivorous animals that

have a high tend susceptibility to consuming diseases found in close proximity to the ground, such as apicomplexan oocysts. These pathogens can then be transmitted to other organisms that serve as definitive or intermediate hosts (Gazzonis et al., 2016).

The cause of infection may be related to the abundant cat population in contact with sheep flocks, which had substantial effect in the *Toxoplasma* transmission, also the agro-climatic condition of Menoufia governorate with the contamination of feed and water by sheep feces, the adult age and breed of the examined animals. In the study conducted by Al-Kappany et al. (2010), it was observed that the grazing pattern, in sheep flocks were grazed on a daily basis, resulted in a significant prevalence of *Toxoplasma* in sheep. This finding suggests that there is a high likelihood of environmental contamination with infective oocysts in pastures and food during the grazing process. In Egypt that there is a significant population of stray cats, which are highly prevalent and widely distributed and around 97.4% of these stray cats in Egypt are infected with *T. gondii,* indicating a substantial risk of environmental contamination. This contamination arises from the presence of sporulated oocysts that can remain infectious in soil and water for extended periods (Byomi et al., 2018).

This study reported that the seroprevalence of *N.caninum* was 7/90 (7.7%) in sheep by using ELISA, which was lower than the results in Iran 10% (4 seropositive of 70 dams) of their sheep samples (Asadpour et al., 2012). In difference, studies in Brazil demonstrated that the seroprevalence of *N. caninum* was 1.81 % and 3.2 % by Soares et al. (2009) and Vogel et al. (2006), respectively. Meanwhile, in Italy, Gaffari et al. (2006), in an extensive study on sheep, goats, and aborted feti, reported a serologically of about 17 % of aborted feti and 3% of abortive sheep were positive *N. caninum* and by molecular technique reported 15% of aborted feti were positive *N. caninum* (Nayri et al., 2022)

Also, in Argentina was 3% (Hecker et al., 2013) by IFAT, in Costa Rica 10.9% obtained by Villagra-Blanco et al. (2019); in Switzerland (10.3 %) by Hassig et al. (2003), close to the percentages detected in Northwest Spain (10.1%) by Panadero et al. (2010) and in Grenada (Caribbean West Indies) (13%) (Sharma et al., 2015) using ELISA, however, (Patarroyo et al., 2013) recorded (78.6% by dot-ELISA) in Colombia.

The observed variety in these findings could potentially be attributed to several factors, including the prevalent practice of co-grazing sheep with beef cattle, the existence of dogs within animal farms, disparities in managing practices, variations in environmental circumstances, and discrepancies in the serological techniques employed. It is possible that the presence of neosporosis in animals is a contributing factor. Therefore, in order to effectively control infections caused by these apicomplexan parasites in ovine farms, it will be imperative to enhance management practices, provide educational resources to sheep owners, and offer additional veterinary support.

This study revealed non-significant effect of season on the prevalence of *Toxoplasma* and *Neospora*. Other researchers documented difference in prevalence in worm and cold season that may be attributed to the fact that sporulated oocysts, which are responsible for infection, had the ability to stay viable in warm conditions and humid environment found in agricultural regions like Menoufia Governorate (Byomi et al., 2018). In contrast, these oocysts are not able to stay in dried and cold environment. This finding is consistent with the research conducted by Robert-Gangneux and Dardé (2012) as well as Figliuolo et al. (2004), who reported that the only flock that tested negative for *N. caninum* had implemented a firm practice for monitoring bovine neosporosis. These

studies suggest that differences in altitude and temperature within regions may account for variations in seroprevalence, with warmer zones promoting higher rates of infection and oocyst sporulation compared to colder regions.

According to the locality, the difference between the groups was insignificant, revealed that no relationship was found between seroprevalence in sheep and areas of collection in the present study. This might be referred to the agro-climatic nature with humid rainy weather, sheep grazing behavior, drinking from stream water, lifestyle in areas of sheep breeding in Menoufia Governorate as a whole, and presence of cats in contact with sheep are effective factors for obtaining *parasites* oocysts by animals (Lopes et al., 2010).

The present investigation revealed a notable seroprevalence of *Brucella* spp., as determined by the Rose Bengal test, with a rate of 28.8% (26 out of 90). According to (FAO), Brucellosis is classified as a transboundary contagious animal disease (TAD). FAO defines TADs as illnesses that hold substantial commercial, trade, and food concern implications for numerous nations. Trans-Allelic Divergence (TADs) possess a notable propensity for transnational dissemination, potentially escalating to epidemic levels, hence necessitating collaborative efforts among multiple nations for effective control, management, or containment. The North African nations are susceptible to various transboundary animal diseases (TADs) due to their geographical positioning, proximity to the Sahel region, and specific limitations on the financial resources allocated to national veterinary services and the livelihoods of livestock owners in the area (Kardjadj, 2018). Hence, it is imperative to conduct thorough investigations into the epidemiology of these diseases, as well as the limitations associated with their eradication and the strategies implemented for their control. According to McDermott and Arimi (2002), brucellosis is a prevalent and overlooked disease that imposes significant burdens on both animals and humans in low-income countries. Furthermore, the lack of effective control measures exacerbates the problem.

The current survey aimed to detect Brucellosis seroprevalence through using the Rose Bengal test. The results showed that 28.8% (26 out of 90) were positive for *Brucella* antibodies. The observed seropositivity percentages in this study were found to be higher compared to the findings reported by Diab et al. (2018), who documented a seropositivity rate of 11%. Additionally, Diab et al. (2018) concluded that *Brucella* infection is prevalent in the northern and western regions of Egypt, warranting further investigation about *Brucella* isolation and associated risk factors in the studied area .Previous studies conducted in various regions of Egypt, including Kafr El-Sheikh (Hegazy et al., 2011b), Alexandria (Hosein et al., 2016), and several other governorates, have reported different prevalence rates of a certain phenomenon. Specifically, Hosein (2015) found a prevalence rate of 6.92%, Lamyaa (2005) reported 8.52%, Lobna (2006) reported 8.2%, and Samaha et al. (2008) reported 4.8%.

In their study, Horton et al. (2014) observed a relatively reduced seroprevalence of sheep brucellosis in countries other than Egypt, with a prevalence rate of 3.08%. In a similar vein, Patel et al. (2017) documented a prevalence rate of 4%, Rahman et al. (2011) reported findings indicating a rate of 7%, and Tsehay et al. (2014) found an incidence rate of 8.70%.

The variations in the prevalence of brucellosis can be ascribed to factors such as the temporal and spatial aspects of sampling, as well as individual's tendencies in reporting cases. The prevalence of our results closely aligned with Kaoud et al. (2010), found the prevalence rate of 21.20% throughout several governorates in Egypt. Furthermore, our findings exhibited a strong resemblance to the prevalence rates

18.09% and 16.4% reported by Mahboub et al. (2013) and Nagati & Hassan (2016) respectively. Additionally, Al-Majali et al. (2007) was closely aligned with the prevalence of sheep brucellosis at 33.1% and Ahmed et al. (2010) at 24%.

In this study a nearly equivalent effect of sex on the prevalence of brucellosis was observed among males and females. These findings align with previous studies that have suggested a comparable susceptibility to brucellosis between male and female animals (Radwan et al., 1992; Yibeltal, 2005; Al-Busultan et al., 2007 and Ashenafi et al., 2007).

Numerous surveillances have reported a higher prevalence in males compared to females (Ahmed et al., 2016; Alrodhan, 2017 and Abdelbaset et al., 2018). The increased incidence of infection in males could be attributed to their regular movement, such as during grazing, trading activities, or mating. These activities may render males more liable to contracting the infection. Previous researches have consistently reported a higher prevalence of the condition in females compared to males (Omer et al., 2010; Haggag et al., 2016 and Hosein et al., 2016).

The dissimilar prevalence rate seen in both males and females in our study could be explained by the analogous treatment protocols employed for males and females, as noted by Al-Rawahi (2015) who revealed that that brucellosis seroprevalence was not influenced by the gender.

Current study found that there were (18.8%) of sheep samples mixed between *T. gondii* and *Brucella spp.*, higher than that of Fereig et al. (2022) who found (4.4%) mixed infections with *T. gondii* and *Brucella*. Also current study found that (4.4%) of sheep samples were mixed between *T. gondii* and *N. caninum*, nearly close to Fereig et al. (2022), who reported (4.2%) *T. gondii* and *N. caninum* mixed infection; also (1.4%) mixed infection of *N. caninum* and *Brucella spp*., and three pathogens mixed infections (0.6%), while in the current study there was (1.1%) sheep sample mixed between *N. caninum* and *Brucella* spp. and (1.1%) sample was mixed infection for three agents.

CONCLUSION

This study aimed to determine the seroprevalence of three infectious agents, namely *T. gondii*, *Brucella* spp., and *N. caninum*. The results revealed that *T. gondii* had the greatest seroprevalence rate at, followed by *Brucella* spp. then *N. caninum* with a lower seroprevalence rate. In order to effectively control infectious agents causing abortion in small ruminants in Egypt, it will be imperative to enhance management methods in ovine farms, provide educational resources to sheep owners, and offer additional veterinary care.

REFERENCES

Abdelbaset, A.E.; Abushahba, M.F.; Hamed, M.I.; and Rawy, M.S. (2018). Sero-diagnosis of brucellosis in sheep and humans in Assiut and El-Minya governorates, Egypt. *International journal of veterinary science and medicine, 6*, S63-S67.

Ahmed, M.; Elmeshri, S.; Abuzweda, A.; Blauo, M.; Abouzeed, Y.; Ibrahim, A.; Salem, H.; Alzwam, F.; Abid, S., and Elfahem, A. (2010). Seroprevalence of brucellosis in animals and human populations in the western mountains region in Libya, December 2006–January 2008. *Eurosurveillance, 15*(30), 19-25.

Ahmed, W.A.; Majeed, S.A.; Ameer, A.H.A.; Mahmmod, N.D.; Saeed, N.I., and Hanaa, L.Y. (2016). Sensitivity and specificity of various serological tests for detection of brucella spp. infection in male goats and sheep. *Advances in Microbiology, 6*(02), 98.

AL-Busultan, A.; AL-Bassam, L.; AL-Shididi, A. and AL-Hashemi, B.(2007). Cross sectional study on the seroprevalence of brucellosis in sheep, goat and man in Diyala governorate. Mirror of Research in Veterinary Sciences and Animals, 7 (1), 1-19.

Al-Kappany, Y.M.; Rajendran, C.; Ferreira, L.R.; Kwok, O.C.; Abu-Elwafa, S.A.;Hilali, M. and Dubey, J. P. (2010).High prevalence of toxoplasmosis in cats from Egypt: isolation of viable *Toxoplasma gondii*, tissue distribution, and isolate designation. *J. Parasitol.*, 96(6): 1115-8.

Al-Kappanyy.M.; Abbas I.E.;DevleesschauwerB.;Dorny P.; Jennes M. and Cox E.(2018). Seroprevalence of anti-Toxoplasma gondii antibodies in Egyptian sheep and goats, BMC Veterinary Research, 14:120.

Al-Majali, A.M.; Majok, A.A.; Amarin, N.M. and Al-Rawashdeh, O. F. (2007). Prevalence of, and risk factors for, brucellosis in Awassi sheep in Southern Jordan. Small Ruminant Research, 73(1-3), 300-303.

Al-Rawahi, A. (2015). The epidemiology of brucellosis in the Sultanate of Oman. (PhD), Murdoch University.

Al-Rawahi, A. (2015). The epidemiology of brucellosis in the Sultanate of Oman. (PhD), Murdoch University.

Alrodhan, M. (2017). Serological Investigation of Caprine Brucellosis at Saniyah District. Kufa Journal for Veterinary Medical Sciences, 8(1), 94-99.

ArranzSolís D.; Benavides J.; Regidor-Cerrillo J.; Fuertes M.; Ferre I.; Ferreras M.; Collantes-FernándezE.; Hemphill A.; Valentín Pérez V. and Ortega-Mora L.M. (2015) Influence of the gestational stage on the clinical course, lesion development and parasite distribution in experimental ovine neosporosis, Veterinary Research 46:19.

Asadpour,R.; Jafari-Joozani, R. and Salehi, N. (2013) Detection of Neospora caninum in ovine abortion in Iran J Parasitic Diseases 37(1):105–109

Ashenafi, F.;Teshale, S.;Ejeta, G.; Fikru, R. and Laikemariam, Y. (2007). Distribution of brucellosis among small ruminants in the pastoral region of Afar, eastern Ethiopia. Revue scientifique et technique, 26(3), 731-739.

Byomi, A.; Zidan, S.; Salama, A.;Elsify, A.; Hadad, G. and Eissa, N. (2018). Public health implications of toxoplasmosis in animals and women in selected localities of Menoufia governorate, Egypt. Assiut Veterinary Medical Journal, 64(157), 120-130.

Byomi, A., Zidan, S., Elkamshishi, M., Sakr, M., Elsify, A., Eissa, N., & Hadad, G. (2019a). Some associated risk factors with *Coxiella burnetii* in sheep, humans and ticks in Menoufiya governorate, Egypt. *Bioscience Research, 16*(S1-2): 121-138.

Byomi, A., Zidan, S., Sakr, M., Elsify, A., Hussien, H., Eissa, N., & Hadad, G. (2019b). *Coxiella burnetii* Infection in Milk of Cattle and The Risk of Human Infection in Menoufia Governorate. *Journal of Current Veterinary Research*, *1*(2), 140-148.

Caballero-Ortega, H.; Palma, J.M.; García-Márquez, L.J.; Gildo-Cárdenas, A. and Correa, D. (2008). Frequency and risk factors for toxoplasmosis in ovine of various regions of the State of Colima, Mexico. Parasitology 135, 1385–1389.

Chernick, A.; Ambagala, A.; Orsel, K.; Wasmuth, J.D.; Marle, G. and Meer, F. (2018). Bovine viral diarrhea virus genomic variation

within persistently infected cattle. Infect. Genet. vol., 58: 218-223.

Diab, M.S.; Elnaker, Y.F.; Ibrahim, N.A.; Sedeek, E.K. and Zidan, S.A.A. (2018). Seroprevalence and Associated Risk Factors of Brucellosis in Sheep and Human in Four Regions in Matrouh Governorate, Egypt. *World*, *8*(4), 65-72.

Fereig, R.M.; Aboulaila, M.R.; Mohamed, S.G.A.; Mahmoud H.Y.A.H.; Ali, A.O.; Ali, A.F.; Hilali, M.; Zaid, A.; Mohamed, A.E.A. and Nishikawa, Y. (2016). Serological detection and epidemiology of *Neosporacaninum*and Cryptosporidium parvum antibodies in cattle in southern Egypt. Acta Trop. 162: 206-211.

Fereig, R.M.; Wareth, G.; Abdelbaky H.H.; Mazeed A.M.; El-Diasty M.; Abdelkhalek, A.; Mahmoud H.Y.A.H.; Ali, A.O.; El-tayeb A.;Alsayeqh, A.F. and Frey C.F.(2022). Seroprevalence of Specific Antibodies to Toxoplasma gondii, Neospora caninum, and Brucella spp. in Sheep and Goats in Egypt, Animals, 12, 3327.

Figliuolo, L.P.C.; Kasai, N.;Ragozo, A.M.A.; de Paula, V.S.O.; Dias, R.A.; Souza, S.L.P. and Gennari, S.M.(2004). Prevalence of anti-Toxoplasma gondii and anti-Neospora caninum antibodies in ovine from Sao Paulo State, Brazil. Vet. Parasitol. 123 (3–4), 161–166.

Gaffari, A.; Giacometti, M.; Tranquillo, V.M.;Magnito, S.;Cordioli, P. and Lanfranchi P. (2006). Serosurvey of roe deer, chamois and domestic sheep in the Central Italian Alps. J Wild Dis 42: 685–690.

Gazzonis, A.L.; Álvarez-García, G.;Zanzani, S.A.; Ortega Mora, L.M.;Invernizzi, A. and Manfredi, M.T. (2016).Neospora caninum infection in sheep and goats from northeastern Italy and associated risk factors. Small Rumin. Res. 140, 7–12.

Gondim, L.F.P.; Barbosa, H.V.; Ribeiro, C.H.A. and Saeki, H.(1999).Serological survey of antibodies to Toxoplasma gondii in goats, sheep, cattle, and water buffaloes in Bahia State, Brazil. Vet. Parasitol. 82, 273–276.

González-Warleta, M.; Castro-Hermida, J.A.; Carro-Corral,C. and Mezo M. (2011).Prev Vet Med. 1;101(1-2):58-64. Epub 2011 Jun 6.

Haggag, Y.N.; Samaha, H.A.; Nossair, M.A. and Mohammad, H.S. (2016). Monitoring of Ruminant Sera for the Presence of Brucella Antibodies in Alexandria Province. *Alexandria Journal for Veterinary Sciences, 51*(2).

Haif, A.; Khelifi-Ouchene, N.A.; Khelifi, M.;Ouchetati, I.; Zeroual, F. and Ouchene, N. (2021). Abortive diseases and their various associated risk factors in small ruminants in Algeria: a systematic review. Tropical Animal Health and Production, 53, 1-14.

Hailat, N.;Khlouf, S.; Ababneh, M. and Brown C. (2018).Pathological, Immunohistochemical and Molecular Diagnosis of Abortions in Small Ruminants in Jordan with Reference to Chlamydia abortus and Brucella melitensis Vet J, 38(1): 109-112.

Hamilton, C.M.; Katzer, F.; Innes, E.A. and Kelly, P.J.(2014). Seroprevalence of Toxoplasma gondii in small ruminants from four Caribbean islands. Parasit. Vectors 7, 449.

Hassig, M.; Sager, H.;Reitt, K.; Ziegler, D.; Strabel, D. and Gottstein, B. (2003).Neospora caninum in sheep: a herd case report. Vet Parasitol 117(3):213–220.

Hecker, Y.P.; Moore, D.P.;Manazza, J.A.;Unzaga, J.M.; Spath, E.J.; Pardini, L.L.; Venturini, M.C.; Roberi, J.L. and Campero,

C.M.(2013). First report of seroprevalence of Toxoplasma gondii and Neospora caninum in dairy sheep from Humid Pampa, Argentina. Trop. Anim. Health Prod. 45, 1645–1647.

Hegazy, Y.M.; Molina-Flores, B.; Shafik, H.; Ridler, A.L. and Guitian, F.J. (2011b). Ruminant brucellosis in Upper Egypt (2005–2008). *Preventive Veterinary Medicine,* 101(3), 173-181.

Holler, LD. (2012). Ruminant abortion diagnostics. The veterinary clinics of North America. Food Anim Pract; 28:407–18.

Horton, K.C.; Wasfy, M.; Samaha, H.; Abdel-Rahman, B.; Safwat, S.; Abdel Fadeel, M.; Mohareb, E. and Dueger, E. (2014). Serosurvey for zoonotic viral and bacterial pathogens among slaughtered livestock in Egypt. *Vector-Borne and Zoonotic Diseases,* 14(9), 633-639.

Hosein, H. (2015). Molecular Epidemiological Investigation on Brucella Infection in ruminants. (PhD), Beni Suef University.

Hosein, H.; Rouby, S.; Menshawy, A. and Ghazy, N. (2016). Seroprevalence of camel brucellosis and molecular characterization of Brucella melitensis recovered from dromedary camels in Egypt. *Res. J. Vet. Pract,* 4(1), 17-24.

Kaoud, H.; Zaki, M.M.; El-Dahshan, A. and Nasr, S. A. (2010). Epidemiology of brucellosis among farm animals. *Nature and Science,* 8(5), 190-197.

Kardjadj, M. (2018). Epidemiological situation of transboundary animal diseases in North African countries-proposition of a regional control strategy. *Trop Anim Health Prod, 50*(3), 459-467.

Lamyaa, M. (2005). Public health importance of brucellosis in Menofia Governorate. Ph. D. Thesis, Zoonoses, Fac. Vet. Med., Benha University.

Liu, Z-K.; Li, J-Y. and Pan, H.(2015). Seroprevalence and risk factors of Toxoplasma gondii and Neospora caninum infections in small ruminants in China. Preventive Veterinary Medicine 118, 488–492.

Lind, P.; Haugegaard, J.;Wingstrand, A. and Henriksen, S.A.(1997). The time course of the specific antibody response by various ELISAs in pigs experimentally infected with Toxoplasma gondii. *Veterinary Parasitology*, 71(1), 1-15.

Lobna, M. (2006). Epidemiological studies on Brucellosis in animal and man with special reference to prevention and control. Ph. D. Thesis, Zoonosis, Fac. Vet. Med., Benha University.

Lopes, W.D.; Santos, T.R.; da Silva, R.S.; Rossanese, W.M.; de Souza, F. A.;Rodrigues, J.; de Mendonça, R.P.; Soares, V.E. and da Costa, A.J.(2010).Seroprevalence of and risk factors for *Toxoplasma gondii* in sheep raised in the Jaboticabalmicroregion, São Paulo State, Brazil. *Res. Vet. Sci.,* 88(1): 104-6.

Mahboub, H.D.; Helal, M.A.; AbdEldaim, M.A.; Abd El-Razek, E.M. andElsify, A.M. (2013). Seroprevalence of Abortion Causing Agents in Egyptian Sheep and Goat Breeds and Their Effects on the Animal's Performance. *Journal of Agricultural Science,* 5(9): 92-101.

Malekifard, F.;Batavani, R. and Khodadadi, A.(2022). investigation of toxoplasma Gondii and Neospora caninum as cause of ovine abortion in affected flocks of Urmia, Northwestof Iran. Bulgarian Journal of Veterinary Medicine, 2022, 25, No 2, 308–317

Mammeri, A.;Alloui,M.N.;Keyoueche,F.Z. and Benmakhlouf,A.(2013).

Epidemiological survey on abortions in domestic ruminants in the governorate of Biskra, Eastern arid region of Algeria. J. Anim. Sci. Adv., 3: 406-418.

McDermott, J.J. and Arimi, S. (2002). Brucellosis in sub-Saharan Africa: epidemiology, control and impact. *Veterinary microbiology*, 90(1-4), 111-134.

Morgan, W.J.; MacKinnon, D.J.; Lawson, J.R. and Cullen, G.A. (1969). The rose bengal plate agglutination test in the diagnosis of brucellosis. *Vet Rec*, *85*(23), 636-641.

Moutos, A.;Doxani, C.; Stefanidis, I.;Zintzaras, E. and Rachiotis G. (2022). Knowledge, Attitude and Practices (KAP) of Ruminant Livestock Farmers Related to Zoonotic Diseases in Elassona Municipality, Greece. *European Journal of Investigation in Health, Psychology and Education*. 12(3):269-280. https://doi.org/10.3390/ejihpe12030019

Nagati, S.F. and Hassan, S.K. (2016). Diagnosis of Brucella Infection in Sheep and Goat and Evaluation of the associated Practices in Animal Contacts; American Journal of Infectious Diseases and Microbiology, Vol. 4, No. 5, 95-101.

Nayri, T.; Sarvi, S.;Moosazadeh, M. and Daryani A.(2022).The Global Prevalence of *Neospora caninum* Infection in Sheep and Goats That Had an Abortion and Aborted Fetuses: A Systematic Review and Meta-Analysis;Front Vet Sci. 2022; 9: 870904.

Nazanin Alavi, M.D.; Nasreen Roberts, M.B.B.S.;Mrcpsych, U.K.;Frcpc, M.S.C.; Chloe Sutton,B.S.C. Nicholas Axas, M.S.W. and Leanne Repetti R.N.(2015). Bullying Victimization (Being Bullied) Among Adolescents Referred for Urgent Psychiatric Consultation: Prevalence and Association with Suicidality, Canadian Journal Psychiatry, 60(10):427–431.

Omer, M.; Musa, M.; Bakhiet, M. and Perrett, L. (2010). Brucellosis in camels, cattle and humans: associations and evaluation of serological tests used for diagnosis of the disease in certain nomadic localities in Sudan. *Rev Sci Tech*, 29(3), 663-669.

Panadero, R.;Painceira, A.; Lopez, C.; Vazquez, L.; Paz, A.; Diaz, P.; Dacal, V.; Cienfuegos, S.; Fernandez, G.; Lago, N.;Díez-Baños, P. andMorrondo, P.(2010). Seroprevalence of Toxoplasma gondii and Neospora caninum in wild and domestic ruminants sharing pastures in Galicia (Northwest Spain). Res. Vet. Sci. 88, 111–115.

Patarroyo, J.H.; Vargas, M.I.; Cardona, J.A.; Blanco, R.D. and Gómez, V.E.(2013).Frecuenciaserológica de infecciónporNeosporacaninumenovinoseneldepartamento de Córdoba, Colombia. Rev. MVZ Córdoba 18 (3), 3886–3890.

Patel, K.B.; Patel, S.; Chauhan, H.; Thakor, A.; Pandor, B.; Chaudhari, S.; Chauhan, P. and Chandel, B. (2017). Comparative Efficacy of Serological Tests for Detection of Brucella Antibodies in Sheep and Goats. *Journal of Animal Research*, 7(6), 1083-1087.

Radwan, A.; Bekairi, S. and Prasad, P. (1992). Serological and bacteriological study of brucellosis in camels in central Saudi Arabia. Revue Scientifique et Technique-Office International des Epizooties, 11, 837-837.

Rahman, M.; Faruk, M.; Her, M.; Kim, J.; Kang, S. and Jung, S. (2011). Prevalence of brucellosis in ruminants in Bangladesh.

Veterinarni Medicina, 56(8), 379-385.

Robert-Gangneux, F. and Dardé, M. (2012): Epidemiology of and Diagnostic Strategies for Toxoplasmosis. *Clin. Microbiol. Rev.,* 25(2): 264-296.

Samaha, H.; Al-Rowaily, M.; Khoudair, R.M.; and Ashour, H.M. (2008). Multicenter study of brucellosis in Egypt. Emerging infectious diseases, 14(12), 1916.

Shaapan, R.M. (2016): The common zoonotic protozoal diseases causing abortion. J. Parasit. Dis., 40(4): 1116-1129.

Sharma, R.N.; Bush, J.; Tiwari, K.;Chikweto, A. andBhaiyat, M.I.(2015). Seroprevalence of Neospora caninum in sheep and goats from Grenada, West Indies. Open J. Vet. Med. 5, 219–223

Soares, H.S.; Ahid, S.M.M.; Bezerra, A.C.D.S.; Pena, H.F.J.; Dias,R.A. and Gennari, S.M. (2009). Prevalence of anti-Toxoplasma gondii and antiNeosporacaninum antibodies in sheep from Mossoro´, Rio Grande do Norte. Brazil. Vet Parasitol., 160(3–4):211–214

Stone, D.M.;Kumthekar,S.;Chikweto,A.; Thomas,D.; Tiwari,K. and SharmaR.N. (2012). Exposure to zoonotic abortifacients among sheep and goats in Grenada. Int. J. Anim. Vet. Advan., 4: 113-118.

Tesfaye, A.; Sahele, M.; Sori, T.;Guyassa, C. and Garoma A.(2020).Seroprevalence and associated risk factors for chlamydiosis, coxiellosis and brucellosis in sheep and goats in Borana pastoral area, southern Ethiopia BMC Veterinary Research 16:145 1-8.

Tsehay, H.; Getachew, G.;Morka, A.; Tadesse, B. and Eyob,H. (2014). Seroprevalence of brucellosis in small ruminants in pastoral areas of Oromia and Somali regional states, Ethiopia. *Journal of Veterinary Medicine and Animal Health*, 6(11), 289-294.

Van Engelen, E.;Luttikholt,S.;PeperkampK.;Velle ma,P.and Van den Brom,R.(2014). Small ruminant abortions in the Netherlands during lambing season 2012-2013. Vet. Rec., Vol. 174. 10.1136/vr.102244.

Villagra-Blanco, R.; Barrantes-Granados, O.; Montero-Caballero,D. , Romero-Zúñiga J. and Dolz G. (2019) Seroprevalence of Toxoplasma gondii and Neospora caninum infections and associated factors in sheep from Costa RicaParasite Epidemiology and Control 3.

Vogel, F.S.F.;Arenhart, S. andBauermann, F.U. (2006). AnticorposantiNeosporacaninume mbovinos. ovinos e ubalinos no Estado do Rio GrandedoSulCienc Rural 36:1948–1951.

Watkins, P.J.;Jaborek, J.R.; Teng, F.; Day, L.;Castada, H.Z.; Baringer, S. and Wick, M. (2021). Branched chain fatty acids in the flavour of sheep and goat milk and meat: A review. Small Ruminant Research, 200, 106398.

Wodajo, H.D.; Gemeda, B.A.;Kinati, W.; Mulem, A.A., van Eerdewijk, A. and Wieland, B. (2020). Contribution of small ruminants to food security for Ethiopian smallholder farmers. Small Ruminant Research, 184, 106064.

Yibeltal M. (2005). A seroprevalence study of small ruminant brucellosis in selected sites of the Afar and Somali regions, Ethiopia. (DVM thesis), Addis Ababa University, Debre Zeit, Ethiopia, Faculty of Veterinary Medicine.