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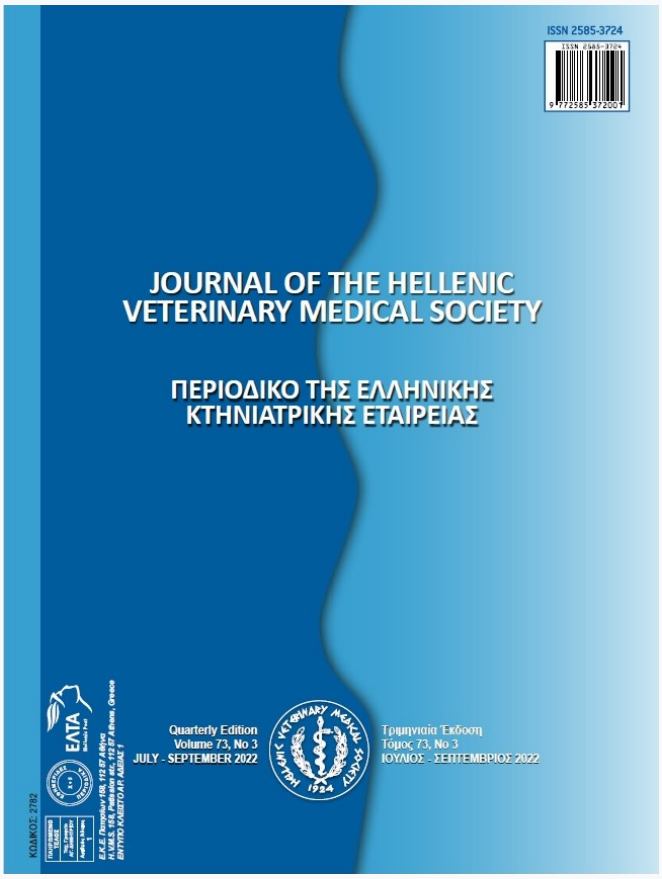

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Research article
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Oxidant/Antioxidant Status and Certain Trace Elements Relationship in Hair Goats Naturally Infected by *Neospora caninum*

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ABSTRACT: This study was performed to determine the prevalence of *Neospora caninum* in female hair goats raised in different locations of the Siirt province of Turkey, and to investigate the total antioxidant status (TAS), total oxidant status (TOS), Zinc (Zn), and Copper (Cu) levels of the infected goats. The animal material of the study consists of a total of 184 female hair goats that are between 1 to 6 years old with the random sampling method. Investigation of the *N.caninum* antibodies was then performed using a commercial ELISA kit. The results of the ELISA tests reveal that the prevalence of *N. caninum* in the province of Siirt was 10.33% (19/184) positive, 7.61% (14/184) suspected, and 82.07% (151/184) negative. According to the ELISA test results, 10 seropositive and 10 seronegative sera samples were used in the TAS, TOS, Zn, and Cu analysis. The TAS ($p<0.01$) and Zn ($p<0.001$) levels of goats positive with *N. caninum* were found to be lower compared to those of the seronegative goats, while their TOS and Cu levels were higher ($p<0.05$). This study was the first to determine the *N.caninum* prevalence in the goats raised in the province of Siirt. It was concluded that it would be beneficial to take precautionary measures, as well as further studies with larger scopes to be performed which should also involve dogs as the definitive host. Furthermore, the oxidative stress parameters (TAS, TOS) and certain trace elements (Zn, Cu) were found to be affected by Neosporosis, which should be useful in the diagnosis and treatment of the disease.

Keywords: Goat, *Neospora caninum*, Oxidative stress, Trace element, Siirt

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INTRODUCTION

Neospora caninum is an intracellular parasite first detected in Norway in puppies with congenital encephalomyelitis (Dubey et al., 2007; Uzêda et al., 2007)**.** Despite not being considered a zoonotic disease, humans with *N. caninum* antibodies were also reported (Liu et al., 2015). The definitive host of *N. caninum* are the domestic and wild canids. The intermediate hosts are the herbivores (cattle, sheep, goats, horses, bison, and deer), and are infected by consuming in the oocytes excreted along with the feces of the definitive host (Sharma et al., 2015; Gharekhani et al., 2016). The disease causes neuromuscular defects, paralysis, and death in dogs, while it causes abortion and newborn deaths in cattle, sheep, and goats and significant economic losses (Dubey, 2003; Figliuolo et al., 2004; Uzêda et al., 2007; Sharma et al., 2015). The disease is considered amongst the primary causes of abortion in cattle (Da Silva Andrade et al., 2013). Experimental studies have shown that the disease can also cause congenital infection in sheep and goats (Gharekhani et al., 2016).

Significant alterations in blood parameters and biochemistry of the host animals have been reported (Sahin and Akgül, 2006; Ayaz et al., 2007). In a study where the presence of *N. caninum* antibodies was investigated serologically in sheep that have aborted, certain hematological values were found to be altered significantly more in disease suspicious animals, compared to the serologically negative animals (Har and Başbuğan,2019).

Oxidative stress plays a part in the pathogenesis of numerous diseases (Miller et al., 1993). The control of the balance between pro-oxidants and antioxidants is essential to sustain biochemical and vital functions. Oxidative damage can occur in cases where this balance is lost in favor of the pro-oxidants (oxidative stress) (Irak et al., 2018).

The metabolism of the trace elements is also important for organisms to sustain a healthy life. Animals suffering from parasitic diseases become more susceptible to vitamin and trace element deficiencies (Değer et al., 2005; Akış and Dede, 2009). Zinc and copper are trace elements that partake in immune system, cellular respiration, redox reactions, and protein synthesis. Both copper and zinc are also the co-factors of the superoxide dismutase (SOD) enzyme, which plays an important role in the antioxidant system, and they help reduce the peroxidation speed of the free radicals (Costanzo et al., 1995; Akış and Dede, 2009).

Neosporosis is one of the important causes of reproduction problems in animal husbandry, and the studies which investigated its presence in goats are limited in numbers. These studies also lack investigations regarding the infection rates of *N.caninum* and the severity of the impact of the disease (Uzêda et al., 2007). This study was performed to determine the seroprevalence of *N. caninum* in the hair goats raised in different locations of the province of Siirt, and to determine the relationship between the infection and the total antioxidant status (TAS), total oxidant status (TOS), Zinc (Zn), and Copper (Cu) levels of the infected animals.

MATERIALS AND METHODS

The Study Area

The present study was performed in the Kurtalan, Baykan, Aydınlar, and Central districts of the Siirt province in Turkey (Fig.1).

Animal Material and Sample Collection

The animal material of the study consists of a total of 184 female hair goats that are between 1 to 6 years old with the random sampling method. Blood samples were collected from the jugular veins of the animals into sterile non-anticoagulant vacuum tubes. The samples were centrifuged at 3000 rpm for 10 minutes, and the sera were transferred into 1.5 ml microtubes. The samples were kept at -20°C till the serological and biochemical analyses.

Serological examination

The ELISA analyses of the study were performed in the Science and Technology Application and Research Center of the Siirt University. A commercial ELISA kit (*N. caninum* Ab ELISA Kit, IDEXX, USA) was used to determine the *N. caninum* antibodies. The plates were scanned in 450 nm wavelength using the ELISA microplate scanner (Thermo Scientific Multiskan Go, Thermo WellWash). The obtained values were then put into calculations using the formula specified in the kit process.

$$Value\% = \frac{OD\ (Sample) - OD\ (Negative\ Control)}{OD\ (Positive\ Control) - OD\ (Negative\ Control)} \times 100$$

Accordingly, if the value is equal to or greater than 40, then it is interpreted as positive. If the value is equal to or greater than 30 and less than 40, then it is interpreted as suspect. Finally, if the value is less than 30 then it is interpreted as negative.

Biochemical Analysis

According to the ELISA test results, 10 seropositive and 10 seronegative sera samples were used in the TAS, TOS, Zn, and Cu analyses.

Measurement of Total Antioxidant Status (TAS)

TAS levels were measured using commercially available kits (Relassay, Turkey). The novel automated method is based on the bleaching of the characteristic color of a more stable ABTS (2,2 ′ - Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical cation by antioxidants. The assay has excellent precision values, which are lower than 3%. The results were expressed as mmol Trolox equivalent/L (Erel, 2004).

Measurement of Total Oxidant Status (TOS)

TOS levels were measured using commercially available kits (Relassay, Turkey). In the new method, oxidants present in the sample oxidized the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction was enhanced by glycerol molecules abundantly present in the reaction medium. The ferric ion produced a colored complex with xylenol orange in an acidic medium. The color intensity, which could be measured spectrophotometrically, was related to the total amount of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide and the results were expressed in terms of micromolar hydrogen peroxide equivalent per liter (μol H2O2 equivalent/L) (Erel, 2005).

Measurement of Zinc and Copper

Commercial measurement kits (Zinc Assay Kit, Copper Assay Kit, Rel Assay Diagnostics, Turkey) were used in zinc and copper analysis. Any zinc present in the sample changes the normally red color of 5-Br-PAPS into light pink under the base environment. The absorbance change taking place at 548 nm wavelength is linearly proportional to the amount of zinc in the sample. The copper present in the samples, on the other hand, changes the normally red-orange color of the DiBr-PAESA substance into purple. The absorbance change in 572 nm wavelength is proportional to the amount of copper in the sample.

Statistical analysis

The data obtained in the study were analyzed using the SPSS V16.0 program. The relationship between grouped variables was determined by chi-square analysis and the relationship between grouped variables in the biochemical analysis was determined by independent sample t-test.

Ethical approval

Ethical approval for this study was obtained from the Siirt University Local Ethics Committee for Animal Experiments. (Decision date and number: 14/03/2018-2018/02/05).

RESULTS

The results of the ELISA tests show that *N. caninum* seroprevalence in the province of Siirt is 10.33% (19/184) positive, 7.61% (14/184) suspected, and 82.07% (151/184) negative. Seropositivity was most commonly encountered in the 6-year-old group (28.60%), while it was the least encountered in the 2 years old group (1.60%) (Table 1). Between the locations where the animals were obtained from, the highest seropositivity was found in the Aydınlar district (11.40%), while the lowest was in the central district (8.90%). While no statistically significant difference was determined between the locations in terms of seropositivity ($p>0.05$), a meaningful difference was present between the age groups ($p<0.01$) (Table 2).

Table 1. Distribution of *N.caninum* seropositivity based on age

Age	No. of animals	Positive	
		(n)	(%)
1	11	1	9.10
2	61	1	1.60
3	39	3	7.70
4	38	5	13.20
5	21	5	23.80
6	14	4	28.60
Total	184	19	10.33

The TAS, TOS, Zn, and Cu values for the *N.caninum* seropositive and seronegative goats are given in Table 3. The difference between the two groups in terms of TAS ($p<0.01$), Zn ($p<0.001$), and Cu ($p<0.05$) levels were determined to be statistically significant. The TOS level of the infected group was also higher compared to the TOS level of the control group, but the difference was statistically insignificant.

DISCUSSION

Neosporosis is considered a significant problem in particular for cattle breeding (Utuk and Eski, 2017), but it also causes abortions and infected births in sheep and goats, resulting in severe economic losses (Cayvaz and Karatepe, 2011). Since clinical findings alone are not enough in the diagnosis of the disease

Table 2. *N.caninum* seropositivity based on age and location

Variables		No. of animals	Positive		Negative		Suspicious		p
			(n)	(%)	(n)	(%)	(n)	(%)	
Age									
	1-3	111	5	4.50	101	91.00	5	4.50	**
	4-6	73	14	19.20	50	68.50	9	12.30	
Location									
	Center	45	4	8.90	39	86.70	2	4.40	
	Kurtalan	74	8	10.80	56	75.70	10	13.50	NS
	Baykan	30	3	10.00	27	90.00	0	0.00	
	Aydınlar	35	4	11.40	29	82.90	2	5.70	

NS: Non-significant, **: p<0.01

Table 3. Serum TAS, TOS, Zn, and Cu levels of the animals infected with *N. caninum* and that of the control group

Parameters	Group	Mean ± SEM	P Values
TAS	Control	0.65 ± 0.04	0.007
	Infected	0.46 ± 0.05	
TOS	Control	9.81 ± 0.30	0.164
	Infected	10.60 ± 0.45	
Zn	Control	102.97 ± 5.77	0.00
	Infected	46.95 ± 1.87	
Cu	Control	96.86 ± 2.48	0.041
	Infected	105.26 ± 2.92	

in goats, serological tests like enzyme-linked immune-sorbent assay (ELISA), immunofluorescent antibody test (IFAT), and direct agglutination test (DAT) are being used in its diagnosis (Cayvaz and Karatepe, 2011; Gharekhani et al., 2016). Amongst these, ELISA is reportedly more sensitive and specific (Dubey, 2003; Gharekhani et al., 2016). The ELISA test was therefore used in the present study to determine the seroprevalence of the disease in goats.

Various studies have been performed all over the world to determine the neosporosis prevalence in goats. In studies in Brazil performed using the IFAT method, Figliuolo et al. (2004) reported 6.4% seropositivity and showed that the highest seropositivity was determined in goats above 4 years of age. Other studies performed in the same country include the studies of Uzêda et al. (2007) with 15%, Moraes et al. (2011) with 17.39%, and Topazio et al. (2014) with 4.59% seropositivity. In a study performed in Argentina using the IFAT method, a prevalence of 5.5% was reported with a statistically significant difference between the locations included in the study (Gos et al., 2017).

For the studies using the ELISA method, Al-Majali et al. (2008) performed a study in Jordan that reported a 5.7% seropositivity. While that study reported no statistical difference between the included locations, a statistically significant difference was reported between the age groups (older than 4 years old). Iovu et al. (2012) reported a 2.34% seropositivity for Romania and claimed statistically significant differences between both locations and age groups (higher in adults). Czopowicz et al. (2011) report seropositivity of 9% in Poland as a result of their ELISA study, while Nasir et al. (2012) reported 8.6% in Pakistan¸Bartova and Sedlak (2012) reported 6% in the Czech republic, Diakou et al. (2013) reported 6.9% in Greece, Gazzonis et al. (2016) reported 5.7% in Italy, and Sun et al. (2020) reported %8.55 in China. Meanwhile, Gharekhani et al. (2016) and Díaz et al. (2016) performed studies in Iran and Spain, with seropositivity results of 6.2% and 6%, respectively. Both studies have also reported that, in terms of age groups, adult goats had higher seropositivity.

According to the results of the meta-analysis to determine the seroprevalence of *N. caninum* in goats worldwide; It has been reported that the presence of dogs in farms increases the possibility of seropositivity, there is a higher rate of seropositive animals in the Americas, and the infection is widely distributed worldwide (Rodrigues et al., 2020). In a study con-

ducted to determine the risk factors for N. caninum infections in goats in Taiwan, it was concluded that reduced entry of farmworkers to other farms may help reduce N. caninum infections in dairy goats (Chiang et al., 2020).

Similar studies performed in Turkey to determine the presence of *N. caninum* report the seropositivity between 0% and 25.9%. Amongst the studies performed using the ELISA method, Sevgili et al. (2003) report seropositivity of 5% in Şanlıurfa, while Utuk et al. (2011) report 11.4%, 23.5%, and 2.43% seropositivity for Elazığ, Erzurum, and Kırşehir, respectively, Cayvaz and Karatepe (2011) report %25.9 seropositivities for Niğde, Zhou et al. (2016) report 4.2% and 0% for Konya and Karaman, respectively, Utuk and Eski (2017) report 15.21% for Kilis, and Utuk and Eski (2019) report 8.9% seropositivity for Adana.

The seroprevalence determined in the present study is higher compared to the findings of the studies performed in Adana (Utuk and Eski, 2019), Konya, Karaman (Zhou et al., 2016), Kırşehir (Utuk et al., 2011) and Şanlıurfa (Sevgili et al., 2003), while it is similar to the findings of study performed in Elâzığ (Utuk et al., 2011), and lower than the findings of the studies performed in Erzurum (Utuk et al., 2011), Niğde (Cayvaz and Karatepe, 2011) and Kilis (Utuk and Eski, 2017).

The differences between the results of this study and other studies may be due to differences in diagnostic methods, a pattern of studies, number, and breeds of goats, presence of transplacental transmission, presence and number of the definitive host, and farm management differences.

While no statistically significant difference was determined between the female hair goats from different locations as part of the present study, a significant difference between the age groups was determined, similar to the studies of others previously mentioned (Figliuolo et al., 2004; Al-Majali et al., 2008; Cayvaz and Karatepe, 2011; Iovu et al., 2012; Díaz et al., 2016; Gharekhani et al., 2016), which was particularly true for the older animals. The reason for this age discrepancy might be the longer exposure of older animals to the contaminated forage areas, increasing the likelihood they would consume sporulated oocytes and horizontal contamination risk.

Various studies report that the hematological (Har and Başbuğan) and biochemical (Bottari et al., 2014) parameters of the animals infected with *N. caninum* are altered. Bottari et al. (2014) have performed a study where they showed that experimental models infected with *N. caninum* and *T.gondii* had lowered serum total protein and albumin levels. The same study reported increased Thiobarbituric acid reactive substances (TBARS) and Advanced oxidation protein products (AOPP) levels in infected animals which characterize the formation of lipid peroxidation related to protein oxidation -a bio-indicator of both cellular lesions and tissue damage-, along with nitric oxide (NO) levels that are associated with an immune reaction. In a study performed to evaluate the NO and AOPP levels of the naturally infected goats, animals with *N. caninum* seropositivity were reported to have higher NO ($P<0.001$) and AOPP levels (Tonin et al., 2015).

In the study conducted by Glombowsky et al., (2017); dairy cows were tested for N. caninum by immunofluorescent antibody testing (IFA) and divided into three groups (seronegative, n = 30; seropositive and asymptomatic, n = 30; seropositive and symptomatic, n = 30). It was determined that thiobarbituric reactive acid substances (TBARS) levels increased and butyrylcholinesterase (BChE) activity decreased ($P < 0.05$) in seropositive asymptomatic or symptomatic animals. Reactive oxygen species (ROS) levels and adenosine deaminase (ADA) activity were increased and glutathione S-transferase (GST) activity decreased ($P < 0.05$) in only seropositive symptomatic dairy cows compared to seronegative dairy cows. Based on these results, it has been observed that seropositive animals show cell damage associated with oxidative stress and inflammation, mainly in those with symptomatic infections.

In the present study, goats that were seropositive with *N. caninum* had a lower TAS level compared to seronegative goats ($p<0.01$), while they had relatively higher TOS levels. These findings support the idea that oxidative stress parameters of animals infected with *N.caninum* are affected by the infection (Bottari et al., 2014; Tonin et al., 2015; Glombowsky et al., 2017).

Trace element deficiencies that usually surface during parasitic inflectional diseases are the results of a complex mechanism (Chaturvedi et al., 2004; Akış and Dede, 2009). Studies performed in this regard show that, due to various reasons associated with parasitic diseases, serum zinc levels drop significantly during their course (Seyrek et al., 2004; Van Wey-

enbergh et al., 2004; Taşkapan et al., 2007; Akış and Dede, 2009). In the present study, the serum Zn levels of the goats infected with *N.caninum* were found to be lower than that of the control groups', and the difference was statistically significant ($p<0.001$). A decrease in zinc levels can be explained as the acute phase reaction against the parasitic infection.

Studies by various researchers have shown that blood copper levels are also affected by parasitic infections (Hucker and Young, 1986; Akış and Dede, 2009). Seyrek et al. (2004) performed a study where they showed that the copper levels of sheep infected with toxoplasmosis have increased and that this situation is a response to the infection caused by the acute phase protein called "ceruloplasmin". In the present study, the Cu levels of goats infected with *N.caninum* were found to be statistically higher ($p<0.05$) than those of the control group.

CONCLUSION

This study is the first to reveal the *N.caninum* seroprevalence in goats raised in the province of Siirt. Based on the findings of the study, which are also in line with existing results reported by various researchers, *N.caninum* causes a decrease of TAS and Zn levels in infected goats, while it causes an increase in TOS and Cu levels. These findings will be useful in the diagnosis and treatment of the disease. Dogs play an important role in the spreading of the infection as the definitive host. Considering the severe economic losses associated with *N.caninum*, in addition to preventive measures like the periodic controls of dogs, limiting the access of dogs to feed and water sources of other animals, destruction of the waste placenta and aborted fetuses to prevent horizontal contamination, it would also be beneficial to perform further studies with larger scopes which also include dogs as part of the study.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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