

## Seroprevalence of *Neospora Caninum* and *Toxoplasma Gondii* in Honamlı Goats in Burdur Province

Mustafa ESER<sup>1\*</sup>, Kemal VAROL<sup>2</sup>

<sup>1</sup>Anadolu University Open Education Faculty Health Programs, Yunusemre Campus, TR-26470 Eskisehir, Turkey

<sup>2</sup>Burdur Mehmet Akif Ersoy University, Food, Agriculture and Livestock Vocational College of Burdur, Department of Veterinary, 15030, Burdur, Turkey

### ABSTRACT

*Neospora caninum* and *Toxoplasma gondii* are intracellular protozoan parasites that cause economic losses in goats. Both parasites are similar in features and cause abortions in goats. For this reason, in this study, it was aimed to determine the seroprevalence of *N. caninum* and *T. gondii* in Honamlı goats bred in Burdur province. The study material consisted of 273 Honamlı goats aged between 2-7 years in 11 different goat herds. While 69 of these goats were aborted in the 5th month of pregnancy, the remaining 189 were normal goats that gave birth to healthy kid. In the findings; *N. caninum* specific antibodies were determined in eight (2.9 %) blood serum taken from the goats included in the study. In the blood serum of three goats (1.1 %), *N. caninum* suspectable antibody positivity was detected. *Toxoplasma gondii* specific antibodies were determined in seven (2.6 %) blood serum taken from the goats included in the study. In the blood serum of three goats (1.1 %), *T. gondii* suspectable antibody positivity was detected. As a result, it was observed that *N. caninum* and *T. gondii* had low seroprevalence levels in Honamlı goats bred in Burdur province.

**Keywords:** ELISA, Goat, Honamlı Goat, *Neospora caninum*, *Toxoplasma gondii*, Seroprevalence.

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### Burdur İlinde Honamlı Keçilerinde *Neospora Caninum* ve *Toxoplasma Gondii*'nin Seroprevalansı

#### ÖZ

*Neospora caninum* ve *Toxoplasma gondii* keçilerde ekonomik kayıplara neden olan hücre içi yerleşim gösteren protozoer parazitlerdir. Her iki parazitte özellik olarak benzerlik göstermekte ve keçilerde abortlara yol açmaktadır. Bu nedenle bu çalışmada, Burdur ilinde yetiştirilen Honamlı ırkı keçilerde *N. caninum* ve *T. gondii*'nin seroprevalansının belirlenmesi amaçlanmıştır. Çalışma materyalini 11 farklı keçi sürüsünde, yaşları 2-7 yaş arasında değişen toplam 273 adet Honamlı ırkı keçi oluşturmuştur. Bu keçilerin 69 tanesini gebeliğin beş aylık döneminde abort yapan keçiler oluştururken, kalan 189 tanesini normal sağlıklı yavru doğuran keçiler oluşturmaktadır. Bulgularda; çalışmaya dahil edilen keçilerden alınan kan serumlarının sadece (% 2.9) 8' inde *N. caninum* spesifik antikor varlığı saptanmıştır. Üç keçinin kan serumunda (% 1.1) ise, *N. caninum* şüpheli antikor pozitiflik belirlenmiştir. Çalışmaya dahil edilen keçilerden alınan kan serumlarının, sadece (% 2.6) 7' inde *T. gondii* spesifik antikor varlığı saptanmıştır, Üç keçinin kan serumunda (% 1.1) ise, *T. gondii* şüpheli antikor pozitiflik belirlenmiştir. Sonuç olarak, Burdur ilinde yetiştirilen Honamlı ırkı keçilerde *N. caninum* ve *T. gondii*'nin düşük seroprevalans düzeyine sahip olduğu görülmüştür.

**Anahtar kelimeler:** ELISA, Keçi, Honamlı Keçisi, *Neospora caninum*, *Toxoplasma gondii*, Seroprevalans.

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ORCID ID; ME: 0000-0003-1542-2989, KV: 0000-0002-3057-2865.

\*Corresponding author e-mail: meser961@anadolu.edu.tr

## INTRODUCTION

Neosporosis is a protozoan disease caused by *Neospora caninum*. It causes cyst formation in host tissues (Dubey 1999). This disease causes abortions in domestic livestock and deer. It also causes infections in the nervous system in horses and dogs. Dogs are the last hosts of *N. caninum*. It is also known that wolves (gray and coyote worms) are the last host of the disease. Although neosporosis does not show any specific clinical signs in adult cattle, it causes abortions in sheep, goats and young pregnant cattle. In addition, calves born from cattle infected with *N. caninum* are weak and in poor condition. Neuromuscular anomalies can be observed in calves born this way in a few days after birth (Aktaş et al. 2005). Dogs play an important role in the spread of the disease. Cattle and goats should not be grazed on pastures where dogs are overpopulated (Dubey and Lindsay 1996). Today, vaccine development studies are carried out to prevent the disease. Vaccines prepared from tachyzoites of the agent against neosporosis are used in regions where the disease is common (Andrianarivo et al. 1999).

Toxoplasmosis is one of the most common parasitic zoonotic diseases worldwide caused by *Toxoplasma gondii* (Tenter et al. 2000). *T. gondii* was first detected in the tissues of a hamster-like wild rodent named *Ctenodactylus gundi* by Nicolle and Manceaux (1908) in the laboratories of the Pasteur Institute in Tunisia in 1908 (Dubey 2008, Dubey 2009). It is an obligate intracellular protozoan belonging to the class Coccidea of the Apicomplexa subphylum. They cause toxoplasmosis infection by localizing in all cells except erythrocytes of humans and many animals. Cats are the last host of the parasite. Its intermediate hosts are poultry, mammals and humans (Kolören and Dubey 2020). Toxoplasmosis causes abortions in cattle, sheep and goats. The disease is transmitted to humans by consumption of *Toxoplasma* infected meats and food and beverages contaminated with sporulated oocysts from the final host, and causes an important public health problem (Dubey and Beattie 1988, Tenter et al. 2000, Aköz et al. 2009).

*N. caninum* and *T. gondii* are intracellular protozoan parasites that cause economic losses in goats. Both parasites are similar in features and cause abortions in goats (Dubey and Beattie 1988, Dubey and Lindsay 1996, Tenter et al. 2000, Aköz et al. 2009). *Toxoplasma gondii* has zoonotic properties. It causes serious health problems that threaten public health. Developing an effective treatment method against diseases and performing preventive medicine in a healthy way will be possible by determining the epidemiological spread of these diseases. The aim of this study was to evaluate the seroprevalence of *N. caninum* and *T. gondii* in Honamlı goats bred in Burdur province, Turkey.

## MATERIAL and METHODS

### Study Area

The research was evaluated at the meeting of Anadolu University Animal Experiments Local Ethics Committee, dated 04.06.2018, with registration number 18-37. According to the decision numbered 825172047.09100/2018-4, there was no ethical objection. In addition, this research was supported by Anadolu University Scientific Research Projects Commission as project number 1805S215.

### Animals and Serum

This study was carried out on Honamlı goats, which are one of our domestic goat breeds, bred in Burdur province, Turkey. The study material consisted of 273 Honamlı goats aged between 2-7 years in 11 different goat herds. While 69 of these goats were aborted in the 5th month of pregnancy, the remaining 189 were goats that gave birth to normal healthy kid.

Eight milliliter of blood was taken from the vena jugularis of goats into vacuum tubes with coagulation factor. Collected blood was brought to the laboratory and centrifuged at 3500 rpm for 10 minutes. Extracted serums were placed in capped plastic tubes and stored at -20 °C until testing.

### Detection of *N. caninum* and *T. Gondii* antibodies

In order to investigate *N. caninum* and *T. gondii* antigens from the serums, noncompetitive Enzyme Linked Immunosorbent Assay (ELISA) kits (Sinogeneclon ELISA Kit) were used in accordance with the procedure (Sandwic ELISA Method-Noncompetitive). As a result of the test, the plates were read in an ELISA Microplate reader (Biotek Synergy/H1/USA) at a wavelength of 450 nm. As a result of the test, the % inhibition value was obtained. It was calculated according to the specific procedure of the kit with the specified formula. In the tests applied for both parasites, positivity and negativity were determined according to the following criteria; Test validity: Positive control mean  $\geq 1.00$ ; Negative control mean  $\leq 0.10$ .

Critical Calculation (CUT OFF): Critical = Average of Negative control well + 0.15.

Negative verdict: Example OD < Critical Calculation (CUT OFF) TG and NC are Negative.

Positive decision: Example OD  $\geq$  Critical Calculation (CUT OFF) TG and NC are Positive.

### Statistical analysis

The findings were evaluated using the IBM SPSS 22.0 for Windows package program. Cross-tabs were used to determine the compositional distribution of the variables. Spearman Correlation analysis was used to determine the relationship between variables.

## RESULTS

In the findings; *N. caninum* specific antibodies were determined in only eight (2.9 %) blood serum taken from the goats included in the study. In the blood serum of three goats (1.1 %), *N. caninum* susceptible antibody positivity was detected. *T. gondii* specific antibodies were determined in only seven (2.6 %) blood serum taken from the goats included in the study. In the blood serum of three goats (1.1 %), *T. gondii* susceptible antibody positivity was detected (Table 1 and Table 2).

When the aborted goats are examined in terms of *N. caninum*; two goats were determined as *N. caninum* seropositive and one goats were determined as *N. caninum* susceptible. The distribution of *N. caninum* according to herds; one goat in Ovacık 1, and one goat in Ovacık 2 were determined as seropositive. One goat in Kuzköy 1 were determined as susceptible (Table 1).

When the non-aborted goats are examined in terms of *N. caninum*; six goats were determined as *N. caninum* seropositive and two goats were determined

as *N. caninum* susceptible. The distribution of *N. caninum* according to herds; one goat in Bağısaray 1, two goat in Bağısaray 3, one goat in Bağısaray 4, one goat in Kuzköy 2, and one goat in Ovacık 2 were determined as seropositive. One goat in Bağısaray 1, and one goats in Bağısaray 2 were determined as susceptible (Table 1).

When the aborted goats are examined in terms of *T. gondii*; one goat was determined as *T. gondii* seropositive. The distribution of *T. gondii* according to herds; positivity was determined in Ovacık 2 (Table 2).

When the non-aborted goats are examined in terms of *T. gondii*; six goats were determined as *T. gondii* seropositive and two goats were determined as *T. gondii* susceptible. The distribution of *T. gondii* according to herds; one goat in Bağısaray 1, one goat in Bağısaray 2, one goat in Bağısaray 3, one goat in Bağısaray 4, one goat in Çeltikçi 1, and one goat in Ovacık 2 were determined as seropositive. One goat in Bağısaray 1, and two goats in Bağısaray 3 were determined as susceptible (Table 2).

**Table 1.** Distribution of *N. caninum* in aborted and non-aborted goats by herds.

<i>N. caninum</i>		Aborted goats			Total	Non-aborted goats			Total
Village		Negative	Positive	Susceptable		Negative	Positive	Susceptable	
Bağısaray 1	Count	6	0	0	6	28	0	1	29
	% of Total	8.3%	0.0%	0.0%	8.3%	13.9%	0.0%	0.5%	14.4%
Bağısaray 2	Count	8	0	0	8	27	1	1	29
	% of Total	11.1%	0.0%	0.0%	11.1%	13.4%	0.5%	0.5%	14.4%
Bağısaray 3	Count	7	0	0	7	25	2	0	27
	% of Total	9.7%	0.0%	0.0%	9.7%	12.4%	1.0%	0.0%	13.4%
Bağısaray 4	Count	9	0	0	9	30	1	0	31
	% of Total	12.5%	0.0%	0.0%	12.5%	14.9%	0.5%	0.0%	15.4%
Bağısaray 5	Count	3	0	0	3	7	0	0	7
	% of Total	4.2%	0.0%	0.0%	4.2%	3.5%	0.0%	0.0%	3.5%
Çeltikçi 1	Count	7	0	0	7	21	0	0	21
	% of Total	9.7%	0.0%	0.0%	9.7%	10.4%	0.0%	0.0%	10.4%
Çeltikçi 2	Count	1	0	0	1	9	0	0	9
	% of Total	1.4%	0.0%	0.0%	1.4%	4.5%	0.0%	0.0%	4.5%
Kuzköy 1	Count	6	0	0	6	13	0	0	13
	% of Total	8.3%	0.0%	0.0%	8.3%	6.5%	0.0%	0.0%	6.5%
Kuzköy 2	Count	10	0	1	11	8	1	0	9
	% of Total	13.9%	0.0%	1.4%	15.3%	4.0%	0.5%	0.0%	4.5%
Ovacık 1	Count	7	1	0	8	13	0	0	13
	% of Total	9.7%	1.4%	0.0%	11.1%	6.5%	0.0%	0.0%	6.5%
Ovacık 2	Count	5	1	0	6	12	1	0	13
	% of Total	6.9%	1.4%	0.0%	8.3%	6.0%	0.5%	0.0%	6.5%
Total	Count	69	2	1	72	193	6	2	201
	% of Total	95.8%	2.8%	1.4%	100.0%	96.0%	3.0%	1.0%	100.0%

**Table 2.** Distribution of *T. gondii* in aborted and non-aborted goats by herds.

<i>T. gondii</i>		Aborted goats			Total	Non-aborted goats			Total
Village		Negative	Positive	Suspectable		Negative	Positive	Suspectable	
Bağsaray 1	Count	6	0		6	27	1	1	29
	% of Total	8.3%	0.0%		8.3%	13.4%	0.5%	0.5%	14.4%
Bağsaray 2	Count	8	0		8	28	1	0	29
	% of Total	11.1%	0.0%		11.1%	13.9%	0.5%	0.0%	14.4%
Bağsaray 3	Count	7	0		7	24	1	2	27
	% of Total	9.7%	0.0%		9.7%	11.9%	0.5%	1.0%	13.4%
Bağsaray 4	Count	9	0		9	30	1	0	31
	% of Total	12.5%	0.0%		12.5%	14.9%	0.5%	0.0%	15.4%
Bağsaray 5	Count	3	0		3	7	0	0	7
	% of Total	4.2%	0.0%		4.2%	3.5%	0.0%	0.0%	3.5%
Çeltikçi 1	Count	7	0		7	20	1	0	21
	% of Total	9.7%	0.0%		9.7%	10.0%	0.5%	0.0%	10.4%
Çeltikçi 2	Count	1	0		1	9	0	0	9
	% of Total	1.4%	0.0%		1.4%	4.5%	0.0%	0.0%	4.5%
Kuzköy 1	Count	6	0		6	13	0	0	13
	% of Total	8.3%	0.0%		8.3%	6.5%	0.0%	0.0%	6.5%
Kuzköy 2	Count	11	0		11	9	0	0	9
	% of Total	15.3%	0.0%		15.3%	4.5%	0.0%	0.0%	4.5%
Ovacık 1	Count	8	0		8	13	0	0	13
	% of Total	11.1%	0.0%		11.1%	6.5%	0.0%	0.0%	6.5%
Ovacık 2	Count	5	1		6	12	1	0	13
	% of Total	6.9%	1.4%		8.3%	6.0%	0.5%	0.0%	6.5%
Total	Count	71	1		72	192	6	3	201
	% of Total	98.6%	1.4%		100.0%	95.5%	3.0%	1.5%	100.0%

In correlation findings; while there was a low and statistically insignificant positive correlation between abortion and *T. gondii* ( $r=0.073$ ;  $p=0.230$ ); a low and statistically insignificant negative correlation was

found between abortion and *N. caninum* ( $r= - 0.004$ ;  $p=0.941$ ). A moderate and statistically significant positive correlation was found between *T. gondii* and *N. caninum* ( $r=0.615$ ;  $p<0.001$ ) (Table 3).

**Table 3.** Correlation findings between *T. gondii*, *N. caninum* and abortion.

Spearman's rho		Abortion	<i>T. gondii</i>	<i>N. caninum</i>
Abortion	Correlation Coefficient	1.000	.073	-.004
	Sig. (2-tailed)	.	.230	.941
	N	273	273	273
<i>T. gondii</i>	Correlation Coefficient	.073	1.000	.549**
	Sig. (2-tailed)	.230	.	.000
	N	273	273	273
<i>N. caninum</i>	Correlation Coefficient	-.004	.549**	1.000
	Sig. (2-tailed)	.941	.000	.
	N	273	273	273

\*\*Correlation is significant at the 0.01 level (2-tailed).

## DISCUSSION

*Neospora caninum*, protozoan parasite of the phylum Apicomplexa that lives as obligate intracellular tissue cysts. It causes a disease called neosporosis, which affects many systems (Donahoe et al. 2015). In order to determine the seropositivity of *N. caninum* in goats, ELISA test, Indirect Fluorescent Antibody Test (IFAT) methods are used. In studies conducted according to these methods, the lowest rate of seropositivity was found to be 0.7 % and the highest 26.6 % in the world (Naguleswaran et al. 2004, Tembue et al. 2011). There are limited studies on the seroprevalence of *N. caninum* on goats in Turkey. For the first time in Turkey, Sevgili et al. (2003) determined the *N. caninum* antibody level as 5 % in goats in Şanlıurfa by ELISA test. In addition, Sevgili et al. (2003) reported that this rate did not show a significant difference on race and age. Afterwards, Ütük et al. (2011) determined 13.8 % positivity in Saanen goats and 2.4 % positivity in Hair goats with ELISA test in Elazığ, Erzurum and Kırşehir provinces. Cayvaz et al. (2011) obtained a positivity rate of 25.9 % with ELISA test in goats in the Niğde region. Özdamar et al. (2021) determined seropositivity in 8.69% of goats with ELISA test in 5 different study centers in Ordu's Mesudiye district. According to these studies, the lowest rate of seropositivity in goats in Turkey was 2.4 % and the highest was 25.9 %. In parallel with this information, in our study; *N. caninum* specific antibodies were determined in only 8 (2.9 %) blood serum taken from the 273 goats included in the study. In the blood serum of three goats (1.1 %), *N. caninum* suspectable antibody positivity was detected (Table 1). When the findings are compared with the results of other studies; it showed similarity to the values determined in hair goats in Elazığ, Erzurum and Kirsehir provinces. It showed a lower course than the values detected in other studies.

*Toxoplasma gondii*, which has zoonotic importance, causes important reproductive system diseases in both humans and animals (Ataseven et al. 2006). Its distribution in the world varies. Age, education, lifestyle, nutrition and cleanliness play an important role in this difference (Dubey and Battie, 1988). In order to determine *T. gondii* seropositivity in goats, ELISA test, IFAT test, Sabin-Feldman Dye Test (SFDT), Indirect Hemagglutination (IHA) Complement Fixation Test (CFT), Modified Agglutination Test (MAT), Direct Agglutination Test (DAT) and Latex Agglutination Test (LAT) methods have been used (Karaca et al. 2007, Czopowicz et al. 2011, Almería et al. 2018, Bozukluhan et al. 2018). Many studies have been carried out using these methods in the world. According to these studies, the rate of *T. gondii* seropositivity in goats varies between 5.6 % and 100 %. *Toxoplasma gondii* seroprevalence in goats in Turkey was determined for the first time by

Weiland and Dalchow (1970). According to this study, the seropositivity of *T. gondii* in goats in Turkey was reported as 51.6 % (Ataseven et al. 2006). Babür et al. (1997) determined the seroprevalence of *T. gondii* as 63.15 % by using SFDT test in goats in Çankırı province. Muz et al. (2013) determined 35.9 % positivity by using ELISA test in Hatay province. Ataseven et al. (2006) determined the seroprevalence of *T. gondii* as 72.7 % using SFDT test in local goat breeds in Eastern and Southeastern Anatolia Regions. Karaca et al. (2007) determined the seroprevalence of *T. gondii* as 80.61 % by using SFDT test in goats in Van province. In another study conducted in the same province, was reported that the seroprevalence of *T. gondii* was 33.3 % using the IHA test (Tütüncü et al. 2003). Ural et al. (2009) determined the seroprevalence of *T. gondii* as 81.08 % in SaneenxKilis goats and 82.53 % in Angora goats using the SFDT test in Ankara. Yağcı et al. (1997) reported that *T. gondii* seroprevalence was determined as 54 % using the SFDT test in goats in Ankara province. It has been reported that *T. gondii* seroprevalence was determined as 43.87 % using the SFDT test in Angora goats in Eskişehir (Babür et al. 1999). It has been reported that the seroprevalence of *T. gondii* was determined as 95.24 % using the SFDT test in Aleppo goats in Kilis province (Beyhan et al., 2013). According to studies conducted with different methods in goats in Turkey, *T. gondii* seropositivity rates vary between 12.1 % and 95.24 %. In parallel with this information, in our study; *T. gondii* specific antibodies were determined in only seven (2.6 %) blood serum taken from the goats included in the study. *T. gondii* suspectable antibody positivity was detected 1.1 % in goats (Table 2). When the findings are compared with the results of other studies; it was determined that *T. gondii* seroprevalence in Honamlı breed goats in Burdur province was lower than other studies. This result suggests that the goats are constantly fed on the rocky areas in the region with vegetation. In addition, it is known that the breeders in the region take serious control measures in their herds in order to ensure breeding and prevent diseases. Among these measures; it is thought that the selection of female progenies who have better condition and complete their development stages without any disease as breeding and the inclusion of breeding goats that did not abort in the previous pregnancy periods in the production contribute to this result.

Neosporosis progresses with non-specific symptoms such as abortion, congenital anomaly and stillbirth in domestic animals (Björkman and Uggla 1999). Cayvaz et al. (2011) stated that there was no statistically significant relationship between aborted goats and *N. caninum* seropositivity. Similarly, Özdamar et al. (2021) reported that there was no statistical relationship between aborted goats and *N. caninum* seropositivity. In this study, a low and statistically insignificant

negative correlation was found between abortion and *N. caninum* ( $r = -0.004$ ;  $p = 0.941$ ) (Table 3). Similarly, *T. gondii* is known to cause abortion in goats (Unzaga et al., 2014). It was determined that the seropositivity of *T. gondii* in aborted goats in Adana and Hatay provinces was 15.1 % by ELISA and 12.1 % by IHA. Öz et al. (1995) reported that there was no statistically significant difference ( $p > 0.05$ ) between aborted goats and non-aborted goats. In this study, a low and statistically insignificant positive correlation between abortion and *T. gondii* ( $r = 0.073$ ;  $p = 0.230$ ) (Table 3).

*N. caninum* is similar to *T. gondii* in many features (Björkman and Uggla 1999). Therefore, *N. caninum* was identified as *T. gondii* and misdiagnosed (Dubey 1999, Reichel 2000). It is stated that especially *T. gondii* and *N. caninum* give a cross-reaction in the diagnosis (Gondim et al. 2017). For this reason, research on *N. caninum* should also be investigated in terms of toxoplasmosis for a correct diagnosis. Bartova and Sedlak (2012) stated in their study conducted in the Czech Republic that all samples found positive for *N. caninum* were also positive for *T. gondii* antibodies.

In our study; seropositivity was determined for both *T. gondii* and *N. caninum*. And also there was a moderate and statistically significant positive correlation was found between *T. gondii* and *N. caninum* ( $r = 0.615$ ;  $p < 0.001$ ) (Table 3).

## CONCLUSION

In this study, two important parasitic abortion factors, *N. caninum* and *T. gondii* were studied together in Honamlı goats reared in Burdur, Turkey. A new literature has been added to the very few literature, especially on the seroprevalence of *N. caninum* in Turkey. As a result of the study, the presence of both parasites, which cause abortion in goats in the region, was reported. It has been demonstrated once again that both diseases are extremely important for public health in the region with the consumption of raw or undercooked goat meat and goat milk.

**Conflict of interest:** The authors declared that there is no conflict of interest.

**Ethical Approval:** This study has received permission with, Anadolu University HADYEK number 825172047.09100/2018-4 and 04.06.2018 date. In addition, the authors declared that they comply with the Research and Publication Ethics.

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