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Short Paper

Seroprevalence and associated risk factors of *Toxoplasma gondii* in sheep in Erzurum province, Eastern Anatolia region, Turkey

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Abstract

Background: Toxoplasmosis causes economic losses due to abortion, neonatal death and reproductive diseases in infected sheep. Erzurum is one of the most important cities in Turkey where sheep farming is done. **Aims:** The aim of this study was to evaluate seroprevalence of *Toxoplasma gondii* and related risk factors in sheep brought to the slaughterhouse in Erzurum province of Eastern Anatolia region, Turkey. **Methods:** Nine-hundred and sixty sheep brought to slaughterhouse from Erzurum center and districts were used in this study. The data on age, breed, abortion history of sheep and whether or not they had contact with cats were recorded. The presence of *T. gondii* antibodies was determined by enzyme-linked immunosorbent assay (ELISA) kit in blood samples taken from sheep just before slaughter. **Results:** According to the study results, 44 (4.58%) of 960 sheep were found to be seropositive. Seroprevalence was found to be highest in the $\geq 2 < 3$ age group with 5.29% ($P > 0.05$), and it was more common in Akkaraman breed compared to Morkaraman breed ($P < 0.01$). It was determined that 43 (97.73%) of the 44 seropositive sheep had contact with cats ($P < 0.01$) and 12 of them (27.27%) had abort history. **Conclusion:** The study results identify the presence of *T. gondii* in the sheep from Erzurum province of Eastern Anatolia region, Turkey.

Key words: Risk factors, Seroprevalence, Sheep, *Toxoplasma gondii*

Introduction

Toxoplasma gondii is an apicomplexan, obligate intracellular protozoan parasite and a pathogen capable of infecting almost all mammals and birds (Dubey, 2002). *Toxoplasma gondii* in sheep was first diagnosed in 1954 (Jones and Dubey, 2012), and it is well known that sheep are an important intermediate host of *T. gondii* (Tzanidakis *et al.*, 2012). *Toxoplasma gondii* causes economic losses due to abortion, neonatal death and reproductive diseases in infected sheep (Thaller *et al.*, 2011; Hammond-Aryee *et al.*, 2015) and is also of zoonotic importance as it can be transmitted to humans through contaminated meat and milk (De Santana Rocha *et al.*, 2015; Amairia *et al.*, 2016).

Various risk factors associated with *T. gondii* have been reported in sheep including age, presence of cat in the farm, the size of the farm, climate conditions and geographical location (Abu Samra *et al.*, 2007; Andrade *et al.*, 2013). In addition, some sheep breeds are reported to be more susceptible to *T. gondii* infection than others (Dubey and Welcome, 1998; Williams *et al.*, 2005). In adult sheep, infection may be subclinical, but often the only symptom is abortion (Hassing *et al.*, 2003).

In seroprevalence studies conducted on sheep in different areas/cities in Turkey for *T. gondii*,

seropositivity was determined between 13% and 99.19% (Aköz *et al.*, 2009; Cicek *et al.*, 2011). There are not any studies on the seroprevalence of *T. gondii* in sheep in Erzurum province, Eastern Anatolia region, Turkey.

The total number of small ruminant in the province of Erzurum is 717.843 and its share in the Eastern Anatolian region is approximately 19.3% (Kopuzlu *et al.*, 2016). Therefore, the region has an important place in sheep farming. The aim of this study was to determine the seroprevalence of *T. gondii* and to evaluate risk factors such as age, breed, contact with cats and presence of abortion in sheep brought to the slaughterhouse in Erzurum province in the Eastern Anatolia region of Turkey.

Materials and Methods

Study area and animal

This study was conducted between May-November 2015 in Erzurum (39° 57' 23" N 41° 10' 12" E) which is located in the Eastern Anatolia region of Turkey. In this study, 960 sheep were used from city center of Erzurum and from its districts brought to the slaughterhouse located in Erzurum. Sheep breed, age, from which district, contact with cats and abort history were recorded according to the information received from the animal

owner (Ethical Committee Decision Number: 2014/6).

Blood samples were taken from the vena jugularis of the sheep into sterile tubes without anticoagulant tubes (BD Vacutainer System, Plymouth, UK) just before the slaughtering and were centrifuged at 1.000 g for 15 min at 4°C to separate the serums. The obtained serums were kept at -80°C until analyses. All samples were analyzed at the same time.

Serological investigations

A commercial enzyme-linked immunosorbent assay (ELISA) kit was used for the detection of *T. gondii* in sheep serum samples (CHEKIT TOXOTEST, IDEXX Laboratory, USA). The kit was used in accordance with the manufacturer's instructions.

Statistical analysis

Chi-square independence test was used to determine statistical significance between age groups and breeds in *T. gondii* seropositive sheep. Chi-square test was used to determine statistical significance between those who have aborted or not, and those who have contact with cats or not. Significant level was determined as $P < 0.05$. SPSS 20.0 package program was used in the analysis.

Results

In this study, 44 (4.58%) of 960 sheep were positive for antibodies against *T. gondii*. Overall seroprevalence of *T. gondii* antibodies in sheep in Erzurum and districts were given in Table 1.

Table 1: Overall seroprevalence of *T. gondii* antibodies in sheep in Erzurum and districts

District	No. tested	No. positive	Seroprevalence (%)
Aşkale	10	4	40
Aziyiye (central district)	52	0	0
Çat	58	8	13.80
Horasan	82	0	0
Karayazı	38	2	5.30
Palandöken (central district)	145	8	5.51
Pasinler	170	5	2.94
Tekman	60	8	13.33
Tortum	30	0	0
Şenkaya	155	5	3.22
Yakutiye (central district)	160	4	2.50
Total	960	44	4.58

Seroprevalence of *T. gondii* antibodies in sheep according to age groups were given in Table 2. When the distribution of the age factor was evaluated statistically, it was determined that there was no significant difference between the age groups ($P > 0.05$).

Table 2: Seroprevalence of *T. gondii* antibodies in sheep according to age groups

Age groups (year)	No. tested	No. positive	Seroprevalence (%)
≥1<2	32	0	0
≥2<3	472	25	5.29
≥3<4	413	19	4.60
≥4<6	43	0	0

Seroprevalence of *T. gondii* antibodies in sheep according to breed groups were given in Table 3. It was determined that the difference between breed was significant statistically ($P < 0.01$).

Table 3: Seroprevalence of *T. gondii* antibodies in sheep according to breed groups

Sheep breed	No. tested	No. positive	Seroprevalence (%)
Akkaraman	105	12	11.43 ^a
Morkaraman	855	32	3.74 ^b

Different letters (^{a,b}) indicate significant differences at $P < 0.01$

It was determined that 27.27% of the sheep were positive against *T. gondii* antibodies have abortion history ($P < 0.01$).

In the study, 97.73% of the seropositive sheep had contact with cats ($P < 0.01$), while 2.27% of those without contact were identified.

Discussion

Different seroprevalence studies have been performed in sheep at different provinces in Turkey from 1976 to 2014. The lowest seropositivity rate was found in Konya province with 13%, and the highest rate was found in Afyon province with 99.19% (Akoz *et al.*, 2009; Cicek *et al.*, 2011). Although there is no study performed in Erzurum on *T. gondii* seroprevalence in sheep, Altintas (1975) reported that in 1975 sheep brought from Erzurum to Ankara for slaughter had a seropositivity rate of 31%. In this study, 4.58% seropositivity was determined in sheep brought for slaughter from Erzurum and its districts, and this rate is the lowest in seroprevalence studies that have been made on sheep until now in Turkey. It is known that arid, sub arid, high altitude and cold climate areas are not suitable for the survival of *T. gondii* oocysts, whereas humid, rainy, hot and low altitude areas are more suitable for them (Gebremedhin *et al.*, 2014; Ahmed *et al.*, 2016). Summers are short and arid, winters are long, snowy and very cold in Erzurum. Furthermore, Erzurum, with an altitude of 1860 meters above sea level is the highest province in Turkey. The reason for the *T. gondii* seroprevalence being lower in Erzurum compared to other regions in Turkey is probably in connection with its climate and geographical structure.

When *T. gondii* seropositivity was evaluated according to age, different results and different interpretations depending on these results emerged. It is indicated that the seroprevalence increases with age in sheep (Dubey, 2009; Ahmad, 2015; Hammond-Aryee *et al.*, 2015). Puije *et al.* (2000) suggest that the reason of the increase in seroprevalence with age is that older animals are exposed to risk factors for a longer period of time. Lashari and Tasavar (2010), reported that seropositivity was most commonly seen at 16-28 months old and lowest at 68-80 months. It is indicated that this situation may be due to the poor resistance of *T. gondii* to animals aged 16-28 months (Jabar and Jori, 2013). In the presented study, although there was no statistical

difference between ages, the highest positive rate was found in the $\geq 2 < 3$ age group with 5.29% and then in the $\geq 3 < 4$ age group with 4.60%. Similar to this study, Hammond-Aryee *et al.* (2015) determined that highest seropositivity was between 28-40 months.

Infected sheep may not always show clinical signs of toxoplasmosis, but often the most important symptom is abortion (Dubey, 2009). It is indicated that the *T. gondii* seropositivity in sheep with abortion is reported to 35.18% in Kayseri (Inci *et al.*, 1999), 46.8% in Elazığ (Aktas *et al.*, 2000), 97.4% in Kars (Mor and Arslan, 2007), 96% in Silopi during the studies performed in Turkey (Leblebici and Yildiz, 2014). In this study, it has been determined that 12 of 44 sheep (27.27%) had an abortion history according to the anamnesis data from animal owners.

The cats are both final and intermediate hosts of the *T. gondii* and they have a vital role in the spread of oocysts and in transmission of the infection to other species. Therefore, the cat presence is an important factor that increases risk of possible infection in sheep (Ahmad *et al.*, 2015). Studies on *T. gondii* seroprevalence and risk factors in sheep have also shown that cats have intense contact with sheep (Leblebici and Yildiz, 2014; Ahmad *et al.*, 2015). In this study, 43 (97.73%) of 44 seropositive sheep had contact with cats, supporting previous studies.

Some researchers reported that some sheep breeds were more susceptible to *T. gondii* than other breeds (Williams *et al.*, 2005). However, Sevgili *et al.* (2005), in a study conducted in Akkaraman, Ivesi and Morkaraman sheep in the Sanliurfa region, determined that there was no statistical difference between the breeds in terms of seropositivity. In this study, Akkaraman and Morkaraman sheep were used, and it was found that Akkaraman sheep were more sensitive than Morkaraman sheep and this difference was statistically significant. Ahmad *et al.* (2015) found that salt range sheep were more sensitive than another breed.

In conclusion, the study results identify the presence of *T. gondii* in the sheep from Erzurum province of Eastern Anatolia region, Turkey. The data obtained from this first study to determine the seroprevalence of *T. gondii* in the sheep in Erzurum province shows that cats are an important factor in the spread of the infection. For this reason, contamination of the special feed and water of the sheep with cat feces should be prevented. Although the determined seropositivity rate in this study is low compared to other studies in different regions of Turkey, it should be considered because the disease threatens both animal and human health. In addition, it is necessary to carry out extensive studies to determine prevalence, control and prevent of *T. gondii* in sheep in Erzurum province.

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Conflict of interest

The authors declare that they have no conflict of interest.

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