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Research Article

Detection of Anti-*Neospora caninum* Antibodies in a Goat Flock in Kilis Province of Turkey

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ABSTRACT

Neospora caninum is a significant agent causing abortion in cattle. There are many studies about cattle neosporosis due to its economic impact; however, the number of studies on neosporosis in goats is limited. In this pilot study, we aimed to detect anti-*Neospora caninum* antibodies in Kilis and Shami goats from the same flock in Kilis province of Turkey. For this aim, 92 sera samples were obtained from dairy goats between the ages of 1and 10 years, and tested with commercially available competitive enzyme-linked immunosorbent assay (c-ELISA) kit. At the end of the study, it was detected that 23.91% (11/46) of Shami and 6.52% (3/46) of Kilis goats were positive against anti-*N.caninum* antibodies and overall prevalence was 15.21% (14/92). According to our statistical examination, significant difference was observed between seropositivity rates of Kilis and Shami goats using the Chi Square test ($P<0.05$). In this study, we presented the rates of exposure to *N.caninum* in two different breeds of goats in Kilis province of Turkey.

Key words: *Neospora caninum*, goat, c-ELISA, Kilis, Turkey

INTRODUCTION

Neospora caninum is a tissue cyst forming; obligate intracellular coccidian parasite. In domestic animals, dogs are both intermediate and definitive hosts, while cattle, sheep, goat and horses are intermediate hosts. Hosts are infected either by horizontal postnatal or by transplacental ways (Dubey, 2003). Both exogenous and endogenous forms of transplacental transmission occur, which have different effects on the epidemiology of the disease. While exogenous form causes epidemic abortions, endogenous form is the source of persistently infected calves and has an important role in the spread of disease in herds (Goodswen *et al.*, 2013). Neosporosis causes big economic losses in cattle industry due to abortions, fetal deaths, resorption, mummification, autolysis, stillbirths, premature culling, diminished milk production and repeat breeder problems (Simsek *et al.*, 2008; Pişkin and Utuk, 2009; Utuk *et al.*, 2016).

As in the case of cattle, abortion, fetal death and stillbirths are the signs of caprine neosporosis (Utuk *et al.*, 2011). Serological, molecular and histopathologic techniques are used for the detection of antibodies, DNA and different developmental forms of the parasite in blood and tissues. Enzyme-linked immunosorbent assay (ELISA), indirect fluorescence antibody test (IFAT) and the enzyme immunoassay (EIA) tests are used to determine the rates of exposure to *N.caninum* in goats in Turkey and different parts of the world (Donahoe *et al.*, 2015).

In this pilot study, with c-ELISA test, we aimed to determine the rates of exposure to *N.caninum* in two different breeds of goats in the same flock from Kilis province of Turkey.

MATERIALS AND METHODS

Blood samples were collected with disposable needles into vacutainer tubes from the *Vena jugularis* of 92 dairy goats (46 Kilis and 46 Shami breeds) between the ages 1 and 10 years in Kilis province of Turkey. All animals were female. The goats were in the same flock and collected from different parts of the city for slaughtering. Breeds were determined according to their morphological properties and owner's anamnesis. Age could not be determined precisely due to the owner's impatient manner.

The clotted bloods were centrifuged at 4500 rpm for 5 min. The sera obtained were collected into 1.5 mL centrifuge tubes, and stored at -20ºC until tested. A commercially available competitive enzyme-linked immunosorbent assay (c-ELISA) kit (VMRD, USA) was

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used to determine anti-*N.caninum* antibodies. The test was done according to the instructions of the manufacturer. The mean optical density (OD) was determined at 630 nm for each well by using a microplate reader (ELx 800 UV, Universal Microplate Reader, Bio-Tec Instrumens, Inc). The percent inhibition for each test sample was calculated, and the samples with values of ≥30% inhibition were considered as positive, and those with the values <30% inhibition were regarded as negative (Utuk *et al.,* 2011; Utuk *et al.,* 2016).

The chi-square test (x^2) was used to determine the correlation of seropositivity rates between Kilis and Shami goats. The differences were considered as statistically significant, when probability P values were <0.05. SPSS (ver.20). Packaged software was used in the statistical calculations.

This study was approved by Adana Veterinary Control Institute's experimental animal ethic committee (approved protocol no.25471393.13/1368).

RESULTS

At the end of the study, it was detected that 23.91% (11/46) of Shami and 6.52% (3/46) of Kilis goats were positive against anti-*N.caninum* antibodies, and overall prevalence was 15.21% (14/92) (Table 1). Based on our statistical examination by using the Chi Square test (P<0.05), we observed significant difference between seropositivity rates of Kilis and Shami goats.

Table 1: Percentage of Anti-*N.caninum* antibodies in respect of goat breeds.

Breed	Total number	Negative	Negative (%)	Positive	Positive (%)
Shami	46	35	79.08	11	23.91
Kilis	46	43	93.47	3	6.52
Total	92	78	84.78	14	15.21

DISCUSSION

Due to its economic impact, neosporosis is an important problem in cattle breeding. In recent years, the number of studies on neosporosis in different kinds of domestic and wild animals has increased to provide a clear understanding of the epidemiology of the disease (Utuk *et al.,* 2016). The studies on the goat neosporosis started in 1990s with case reports from stillborn and aborted goat fetuses. These reports provide information on the macroscopic and microscopic lesions in aborted samples, and create awareness on the goat neosporosis (Dubey, 2003; Dubey, 2011; Utuk *et al.,* 2016). In 2000s, the common objective of the studies was to determine the risk factors, defense measures and the rates of exposure to the *N.caninum* in the goat flocks from different countries (Dubey, 2011).

Serologic tests have widely been used for determining the rates of exposure to *N.caninum.* In Brazil, the seroprevalence rates were ranging from 3.3% to 40% at individual-level and from 16.4% to 75% at herd-level (Faria *et al.,* 2007; Uzeda *et al.,* 2007; Moraaes *et al.,* 2011; Andrade *et al.,* 2013; Santos *et al.,* 2013). In other studies, the seroprevalence rates were 6.6% (106/1594) in Argentina (Moore *et al.,* 2007), 0.5% (5/1060) in Poland (Czopowicz *et al.,* 2011), 15.5% (18/116) in Slovakia (Cobadiova *et al.,* 2013), 6% (15/251) in Czech Republic (Bartova and Sedla, 2012), 6.9 % (26/375) in Greece (Anastasia *et al.,* 2013), 8.6% (13/142) in Pakistan (Nasir *et al.,* 2012), 13% (18/138) in West Indies (Sharma *et al.,* 2015), 7.0% (3/486) in Sri Lanka (Naguleswaran *et al.,* 2004), 7.23% (47/650) in China (Liu *et al.,* 2015), 0.9% (4/464) in Korea (Jung *et al.,* 2014), 0% (0/24) in Taiwan (Ooi *et al.,* 2000), 2% (2/302) in Jordan (Abo-Shehada and Abu-Halaweh, 2010), and 6.2% (28/450) in Iran (Gharekhani *et al.,* 2016). In these studies, animals were chosen from various climatic regions, breeds, sexes and ages. Additionally, different diagnostic serological tests (ELISA, IFAT, EIA) and different cut-off values were used.

To the best of our knowledge, there are four studies on goat neosporosis in Turkey, and the seroprevalences of *N.caninum* were determined as 25.9% (47/181) in Nigde (Cayvaz and Karatepe, 2011), 4.2% (8/189) in Konya, 0% (0/60) in Karaman (Zhou *et al.,* 2016), 2.43% (1/41) in Kırsehir, 23.52% (4/17) in Erzurum, 11.42% (8/70) in Elazig (Utuk *et al.,* 2011) and 5% (9/180) in Sanliurfa (Sevgili *et al.,* 2003) provinces. Same c-ELISA kit and same procedures were used in three of these studies, while Zhou *et al.* (2016) used home-made ELISA with recombinant antigen, NcSAG1. In this study, with c-ELISA, we determined the overall seroprevalence as 15.21% (14/92) in Kilis and Shami goats in the same flock from Kilis province of Turkey. The prevalence in Shami and Kilis goats was 23.91% and 6.52%, respectively. In their studies, Utuk *et al.* (2011) and Sevgili *et al.* (2003) report on the breed, sex and ages of animals. Cayvaz and Karatepe (2011) also report on the age and sex, while Zhou *et al.* (2016) do not provide detailed information, except the total number of goats.

In goat neosporosis, while some researchers found statistically significant differences between different breeds and ages, others could not determine any difference between these variants. For example, Uzêda *et al.* (2007), Jung *et al.* (2014), Sevgili *et al.* (2003), Cayvaz and Karatepe (2011) did not find any association between age groups, while Al-Majali *et al.* (2008) found a high seroprevalence older than 4 years of age. Likewise, Sevgili *et al.* (2003) and Utuk *et al.* (2011) did not find any association between breeds, whereas according to Al-Majali *et al.* (2008) and Dubey (2003) breed is a risk factor for *N.caninum*. The common opinion of researchers on sex is that there is no significant difference in the seroprevalence between males and females (Faria *et al.,* 2007; Nasir *et al.,* 2012; Gharekhani *et al.,* 2016). In this study, all animals were female. We could not statistically evaluate the age groups; however, we detected difference between Kilis and Shami breeds. Although animals were in the same flock, they were gathered from different parts of Kilis for slaughtering, and the owner was a buyer rather than a breeder.

In general, when we investigated the serological research on goat neosporosis, we observed a high fluctuation ranging from 0% to 40%. This may be associated with different sample sizes, management systems, nutrition features, owners education, activity of veterinary services, different geographic and climatic conditions, population size of domestic and wild definitive and intermediate hosts, specifity and sensitivity

of diagnostic tests and differences in their cut-off values (Faria *et al.*, 2007; Al-Majali *et al.*, 2008; Utuk *et al.*, 2011; Santos *et al.*, 2013; Utuk *et al.*, 2016).

Conclusion

In conclusion, the use of an international standard test method and same cut-off value and optimized sampling may be effective for reaching sound serologic results. At present, there is no effective treatment for neosporosis. For prevention against caprine neosporosis, veterinarians, veterinary technicians and farmers should be educated. Serological monitoring and culling positive dams is important in preventing vertical transmission. Reducing populations of farmers' dogs and wild canids and limiting their access to food and water sources, placental membranes and carcasses of aborted foetuses may be effective in preventing horizontal transmission (Faria *et al.*, 2007; Al-Majali *et al.*, 2008; Utuk *et al.*, 2011; Utuk *et al.*, 2016). This pilot study has enabled us to collect data on the exposure rate in a goat flock gathered from Kilis province of Turkey. In the light of this study, we will widen our research area in Kilis and to other provinces of Turkey.

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