### **Original papers**

# Prevalence of anti-Neospora caninum antibodies in Iranian goats

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**ABSTRACT.** Many species of animals play the role of intermediate host for *Neospora caninum*. Although the neosporosis has a global distribution, however there is no information on goats' infection in Iran. The main goal of present study was to determine the seroprevalence of *N. caninum* in goats of western Iran. A total of 450 sera were taken and analyzed for antibodies to *N. caninum* using ELISA technique. Of those, 28 (6.2%) examined goats were found to be seropositive (95%, CI=6.2±2.2%). In which male goats (11.1%) had the highest IgG against *N. caninum* with more than 2 years-old (54.4%). There was significant difference between prevalence and age. Positive association were in animals with history of abortion (18/30, 60%) and IgG against *N. caninum*. No correlations were found between male and female. This was the first report on *N. caninum* infection in Iranian goats and may be responsible for abortion and economic losses in goat husbandry in the region.

Key words: goat, Neospora caninum, ELISA, Iran

#### Introduction

Neospora (N.) caninum is an Apicomplexan obligate intracellular parasite that was first isolated in puppies with congenital encephalomyelitis from Norway in 1984 [1]. The parasite has since been recognized as a major cause of abortion in cattle and neuromuscular disorders in dogs [2,3]. Domestic and wild canids are definitive hosts, and a widerange of animals like small ruminants play role of intermediate hosts [4]. Excretion of N. caninum oocysts could act as a risk factor when emerged in feces and infected definitive hosts habitat. These could cause stillbirth and miscarriage to cattle and other intermediate hosts [5]. Congenital infection has been experimentally induced in sheep and goats [2].

N. caninum is a ubiquitous infection worldwide [1]. In Iran, N. caninum infection were also reported 15.8–46% in cattle [3,5–8], 10.6–33% in dogs [7,9,10], 37% in water buffalo [11], 3.2–5.8% in

camel [12,13], 28–40.8% in horses [14], 52% in donkeys [14], and 1.1–5.7% in sheep [15,16]. Epidemiology, economy and clinical importances of *N. caninum* infection in goats have been remained uncertain [4]. It seems neosporosis can be causes of abortion, neonatal mortality and perhaps clinical signs in goats similar to those reported from toxoplasmosis [17].

The earlier investigations indicated that a wide range of animals have been exposed to *N. caninum* infection [1,5]. However, there is no published information of *N. caninum* infection in Iranian goats. Thus, the present study was aimed to evaluate seroprevalence of *N. caninum* infection in domestic goats (*Capra hircus*) in western Iran.

#### **Materials and Methods**

**Field of study.** The location of sampling was Hamedan province in western Iran (34°49'N and 48°40'E, 1595 m above sea level). It is located in a

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Animals -		Age (year, %)		Sex (%)		Abortion (%)		Flock size	
		<2	>2	Male	Female	+	_	30–99	>100
Goats	Examined ats	205 (45.6)	245 (54.4)	54 (12)	396 (88)	30 (7.6)	366 (92.4)	52 (57.8)	38 (42.2)
(450)	Seropositive	7 (3.4)	21 <sup>S</sup> ,a (8.6)	6 (11.1)	22NS,b (5.6)	18 (60)	10S,c (2.7)	3 (5.8)	9S,d (23.7)

Table 1. The percentage of *N. caninum* infection in different age groups, sex and flock size of examined goats in Hamedan province, western Iran

NS, non-significant; S, significant

a: P=0.024 (df=1,  $\chi^2=5.086$ ); b: P=0.112 (df=1,  $\chi^2=2.513$ ); c: P<0.0001 (df=1,  $\chi^2=138.39$ );

mountainous region and has continental type of climate with low humidity of average annual rainfall of 317.7 mm and temperature 11.3°C. The farm animal husbandry has an important role in national economy, especially, in this region besides of agricultural activities. According to Iranian Veterinary Organization (IVO, 2012), an average population of 200,000 goats are reared in this region.

Animals. During the period of this cross-sectional study from September 2013 to September 2014, 90 flocks of goats under traditional rearing were examined. All flocks had a population size between 30–300 goats of which divided into two groups (30–99 goats and 100–300 goats, Table 1). At the beginning of the investigation, all animals were recorded and their sex, age, sheepdogs contact and abortion registered. The animals were divided into two groups, less than 2 years-old and over 2 years-old goats (Table 1). A total of 450 goats were randomly selected and 5ml of blood samples were taken from jugular vein [18]. The sera were removed after centrifugation at 1000×g for 10min and stored at -20°C until laboratory evaluation [5].

**ELISA examination.** Anti-*Neospora* antibodies (IgG) were detected using a commercially available *N. caninum* ELISA kit (ID Screen®Neosporosis indirect multi-species; ID-Vet company, France). The presence of antibody was determined by calculating of S/P% (Sample to positive  $\geq$ 50% =positive) according to the manual instruction.

**Statistic evaluation.** Statistical analysis was performed using non-parametric Chi-square test ( $\chi^2$ ) with confidence interval (CI) of 95% (SPSS 16.0, SPSS Inc., Chicago, IL, USA). *P*-value of <0.05 was considered as significant.

#### Results

A total of 28 (6.2%) of 450 sera were positive for IgG against *N. caninum* (95% CI=6.2±2.2%) (Table 1). Seropositivity in flock level were 13.3% (12/90); the prevalence rate was recorded 5.8% and 23.7% in flocks with 30–99 and 100–300 population size, respectively ( $\chi^2$ =6.098, P=0.013, OR=5). The highest prevalence was significantly found in goats (54.4%) with 2 years-old ( $\chi^2$ =5.086, P=0.024, OR=2.6). There were no significant differences in the prevalence between male (11.1%) and female (5.6%) goats in the all age groups ( $\chi^2$ =2.513,  $\chi^2$ =0.112). During the course of the study, abortion was significantly recorded in 18 out of 30 (60%) seropositive goats ( $\chi^2$ =138.39,  $\chi^2$ =0.0001, OR=53.4).

#### **Discussion**

Several serological techniques, i.e., enzyme linked immune-sorbent assay (ELISA), immuno-fluorescent antibody test (IFAT), and direct agglutination test (DAT) used to detect *N. caninum* infection [7,8]. According to Dubey and Lindsay [2] sensitivity and specificity of ELISA technique is higher than others.

Information on the prevalence of goats' neosporosis is important to implement effective control programs. The prevalence was low in line with reports from Iraq (5.6%), Czech Republic (6%), Costa Rica (6.1%), Brazil (6.4%) and Argentina (6.6%) [20–23] and lower than that reported in Thailand (23.6%) and Turkey (25.9%) [2,24].

In this study, the seroprevalence was much higher in flocks with 100-300 goats (P=0.013, OR=5). In earlier works, the risk of seropositive

d: P=0.013 (df=1,  $\chi^2=6.098$ )

case increased with the flock size due to increasing number of dogs [1,4,7]. Thus the flock size played a role as a risk factor for *Neospora* infection in this work.

The prevalence of infection in age group over 2 years-old was close to that reported by Ghattof and Faraj [23] in Iraq (10% in <1yr and 13.8% in >1yr) and Cayvaz and Karatepe [24] in Turkey (13.8% in <3yr and 42.3% in >3yr) [23,24]. In contrast, it was much higher in goats (78%) with less than one year-old in Brazil [25]. It may be due to horizontal transmission of *N. caninum* and exposure of older animals for a long period in the region [5].

The prevalence of *N. caninum* infection showed no significant differences between male and female same as reported data by other researchers [23,25,26]. According to Gharekhani and Heidari [5], the hormonal differences play important role in host susceptibility to *N. caninum* infection. Since estrogen excretion enhances antibody production and androgen suppress T and B cells immune responses. In female animal, the immunity to the infection can be broken down as a result of various factors (e.g. nutrition, age, pregnancy, and environment).

In small ruminants, detection of antibody against N. caninum in fetal fluids and/or serum is useful to identify the role of the parasite in abortion [16,19]. Most of the seropositive goats had significantly abortion in their histories. This was taken together with earlier investigations support the notion that seropositive goats had association with abortion [19,25,27]. Discrepancies in the rates might be attributed to difference in diagnostic methods, study design, experimental strategies, climatic variations, frequency of canids inside and suburb of the farms and farm management [5]. The Iranian goats reared in extensive management system which allowed roaming free on pasture during the day and having a direct contact with dogs. This may be increased the chance of infection through environment, food and water [16].

This is the first report of *N. caninum* infection in Iranian goats. The results indicated that neosporosis may partially be responsible for abortion and economic losses in goats' husbandry in this region. Thus further molecular investigations and bioassays on the goats' neosporosis will reveal more information about economic effects of this parasite which it will be useful for establishing control programs.

#### Acknowledgements

We greatly appreciate of Hamedan Veterinary Office staff members for sampling.

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Received 8 February 2016 Accepted 16 April 2016