

## Neosporosis in Sheep and Different Breeds of Goats from Southern Jordan: Prevalence and Risk Factors Analysis

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**Abstract:** A cross sectional study was performed to investigate the epidemiology of *Neospora caninum* infection in Southern Jordan. A total of 320 sheep and 300 goats from 38 and 24, sheep and goat flocks, respectively, were randomly sampled and assayed for presence of antibodies against *N. caninum*. A structured pre-tested questionnaire was administered to collect information on flocks' health and management. A multivariable logistic regression model was constructed to investigate risk factors associated with seropositivity to *N. caninum*. The individual true seroprevalence of *N. caninum* in sheep and goats was 4.3 and 5.7%, respectively. The sheep and goat flock level true seroprevalence for *N. caninum* was 45.8 and 48.7%, respectively. The logistic regression model revealed small herd size, having more than one dog and grazing in communal pastures as risk factors for *N. caninum* seropositivity in both sheep and goats. Damascus breed goats were more likely to be seropositive than goats of other breeds.

**Key words:** *Neospora caninum*; seroprevalence; sheep; goats; risk factors

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### INTRODUCTION

*Neospora caninum* is now well recognized as a cause of abortion in cattle and neuromuscular disorders in companion animals<sup>[1]</sup>. This disease has rarely been described in other domestic animal species<sup>[2]</sup>. The principal mode of transmission is believed to be transplacental<sup>[3,4]</sup>, although transmission through the ingestion of contaminated colostrums in newborns has been reported previously<sup>[5,6]</sup>. Carnivores, the definitive hosts, acquire infection by ingestion of infected tissues (horizontal transmission) or via the placenta. They can shed oocysts in their feces for 3 weeks. Oocysts became infectious for other animals after going through sporulation in the external environment<sup>[7]</sup>.

Buxton et al.<sup>[8]</sup> demonstrated that experimental infection of sheep and goats with tachyzoites of *N. caninum* produce a syndrome similar to that in cattle. Natural infection of ovine with *N. caninum* has been reported twice<sup>[9,10]</sup>.

Serological surveys suggested that neosporosis is widespread throughout the world<sup>[7]</sup>. Studies that address the epidemiology of *N. caninum* in small ruminants are

very scarce. The only available reports in the Middle East, investigated the prevalence of *N. caninum* in horses, cattle, buffalos and wild canines<sup>[7,11]</sup>.

The objective of this study was to investigate some epidemiological aspects on neosporosis in sheep and goats in Southern Jordan.

### MATERIALS AND METHODS

**Study design:** This cross sectional study was performed in two major governorates in the southern part of Jordan, namely; Tafelah and Ma'an. More attention was paid to sample flocks from El-Esse city in Tafelah and Shobak city in Ma'an since most flock owners migrate during the summer season to these two cities for grazing. Vaccination against brucellosis, pox, anthrax and FMD is practiced and supervised by the government. Vaccination against these diseases is usually performed during September and October (directly before the beginning of the winter season). Vaccination against *Neospora* is not practiced in Jordan.

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A cross sectional study with two stage sampling method was performed during the period between May and August 2007. The two sampling stages were selection of flocks and selection of animals within each flock. Selection of flocks was performed randomly based on the density of sheep and goats flocks in each governorate. Thirty four flocks were sampled from Tafelah and 28 flocks were sampled from Ma'an. Sample number was determined for sheep and goat separately since those are two different animal species. For sheep and goats, sample size was collected based on a proposed prevalence of 10% and a confidence interval of 95%. Sample size was then adjusted to the actual population of sheep and goats in the study area. Sample size was 320 head for sheep and 300 head for goats. Ten percent of the animals were sampled from flocks of 150 or less flock size and only 5% of the animals were sampled from flocks of more than 150 flock size. Animals were randomly selected from each flock using a table of random digits. These animals were sampled from 38 and 24 sheep and goats flocks, respectively. In all the studied flocks, the abortion rate was less than 5%. Ram exchange is not practiced in all studied flocks.

**Serological analysis:** The serum samples were tested for antibodies against *N. caninum* using a commercial enzyme immuno-assay kit (Bommeli Diagnostic, Bern, Switzerland). Serum samples (diluted 1:10) were added in duplicate to the *Neospora* antigen-coated microplates, which were incubated at room temperature for 90 min. Then, the conjugate provided in the kit was added to each well and incubated for 90 min. The reaction was revealed adding the supplied chromogen, and after 12 min, the reaction was stopped. Plates were read at 405 nm, and the test results were expressed as optical density (OD) values; which were analyzed using the equations provided by the manufacturer to determine which serum samples were negative or positive to *N. caninum*-specific antibodies. The test sensitivity and specificity are 97.5% and 95.1%, respectively.

**Statistical analysis:** The true *Neospora* seroprevalence was determined by adjusting to the test sensitivity and specificity using the following equation<sup>[12]</sup>:

$$TP = (AP+Sp-1)/(Se+Sp-1)$$

Where TP is true prevalence, AP is apparent prevalence, Sp is test specificity and Se is test sensitivity.

Univariable analysis was performed using chi-square test. The impact of the different studied variables on seropositivity in individual sheep and goats was assessed using a multivariable logistic regression model. Variables with  $P \leq 0.05$  (two-sided) in the univariable analysis were offered to the model. To adjust for clustering effect, a random effect approach was used in constructing the logistic models. Statistical analyses were performed using SPSS software version 12 (SPSS Inc., Chicago, IL, USA).

## RESULTS

**Prevalence of neosporosis in sheep and goats:** The apparent individual prevalence of *N. caninum* in sheep and goats was 8.9 and 10.2%, respectively. The adjusted true individual prevalence was 4.3 and 5.7% in sheep and goats, respectively. On the flock level, the apparent prevalence of *N. caninum* was 47.3% (18 out of 38 flocks) and 50% (12 out of 24 flocks) for sheep and goats, respectively. The adjusted true flock level prevalence of *N. caninum* was 45.8 and 48.7% for sheep and goat flocks, respectively. At both levels (individual and flock), No statistical differences were detected in the prevalence of *N. caninum* between the two animal species. No differences were detected in the prevalence of *N. caninum* in the different studied governorates. According to age, the seroprevalence of *N. caninum* was significantly higher ( $\chi^2 = 14.7$ ,  $P < 0.05$ ) in sheep and goats older than 4 years of age than in younger animals.

**Risk factors analysis:** Table 1 represents the distribution of the investigated variables among *N. caninum*-positive and negative sheep and goat flocks from the southern part of Jordan. The chi-square univariable analysis revealed 5 variables for sheep and 6 variables for goats with  $P < 0.05$ . Significant factors in the univariable analysis were further analyzed using multivariable logistic regression models. The models revealed small flock size, having more than one dog in the farm and grazing in communal areas as factors for *N. caninum* seropositivity in sheep and goats (Tables 2 and 3). Using well water was identified as a risk factor for *N. caninum* seropositivity in sheep only (Table 2). Breed (Damascus breed) was identified as a risk factor for *N. caninum* in goats.

Table 1: Distribution of *N. caninum* seropositive sheep and goat flocks according to the different investigated risk factors.

Variable	Category	Sheep		Goats	
		N	No. +ve (%)	N	No. +ve (%)
Flock size	Small	12	8 (67)*	8	5 (63)*
	Medium	14	5 (36)	6	2 (33)
	Large	12	6 (42)	10	5 (50)
Breed	Damascus	-	-	8	6 (75)*
	Local	-	-	9	3 (33)
	Mixed breed	-	-	7	3 (43)
Management	Free browsing	28	13 (46)	15	8 (53)
	Tethering	10	5 (50)	9	4 (44)
Mixed raising (goats with sheep)	Yes	17	8 (47)	16	9 (56)*
	No	21	10 (48)	8	3 (38)
Addition of new animals	Yes	20	9 (45)	19	10 (53)
	No	18	9 (50)	5	2 (40)
No of dogs	1	16	4 (25)	10	4 (40)
	≥ 2	22	14 (64)*	14	8 (57)*
Usage of disinfectants	Yes	19	10 (53)*	13	6 (46)
	No	19	8 (42)	11	6 (54)
Veterinary service	Yes	13	6 (46)	9	5 (56)
	No	25	12 (48)	15	7 (47)
Source of water	Well	27	15 (56)*	14	7 (50)
	Tap water	11	3(27)	10	5 (50)
Graze at communal pasture	Yes	21	12 (57)*	11	8 (73)*
	No	17	6 (35)	13	4 (31)
Visiting the live animal market	Often	15	7 (47)	12	7 (58)*
	Not often	23	11 (48)	12	5 (42)

\**P*-value ≤ 0.05 by chi-square analysis and offered to the logistic regression model.

Table 2: Logistic regression analysis of variables associated with sheep flock seropositivity to *Neospora caninum* in Jordan.

Variable	B	S.E.	OR	95% CI <sub>OR</sub>	P
Constant	-1.3	0.12	-	-	0.001
Small flock size	1.8	0.11	1.9	1.1, 2.9	0.03
Having more than one dog	0.8	0.15	2.4	2.1, 6.1	0.021
Grazing in communal areas	2.1	0.02	1.2	1.0, 3.1	0.02

Table 3: Logistic regression analysis of variables associated with goat flock seropositivity to *Neospora caninum* in Jordan.

Variable	B	S.E.	OR	95% CI <sub>OR</sub>	P
Constant	-1.3	0.12	-	-	0.001
Small flock size	1.8	0.11	1.9	1.1, 2.9	0.03
Having more than one dog	0.8	0.15	2.4	2.1, 6.1	0.021
Grazing in communal areas	2.1	0.02	1.2	1.0, 3.1	0.02

### DISCUSSION

This cross sectional study is the first to investigate the epidemiology of neosporosis in sheep and goats in the Middle East region. Worldwide, *N. caninum* seroprevalence studies in small ruminants are very scarce, and none attempted to investigate associated risk factors.

In this study, the seroprevalence of *N. caninum* in sheep and goats were 4.3 and 5.7%, which is significantly lower than that detected in Brazil<sup>[13,14]</sup> and much higher than that reported in England<sup>[15]</sup>. For goat, the reported prevalence was closely similar to that reported by Faria, et al.<sup>[16]</sup>. On the other hand, the flock level seroprevalence of *N. caninum* in this

study, in sheep and goats, were significantly higher than that reported by Romanelli et al.<sup>[13]</sup>, and lower than that reported by Figluolo et al.<sup>[14]</sup>. The differences in farming management and climate may in part explain these significant differences in the prevalence. Climatic factors affect the abundance of viable parasitic stages in the environment for definitive and intermediate hosts and influence the overall prevalence. It is known that moist conditions are favorable for development of *N. caninum* oocysts, potentially making transmission to intermediate hosts more efficient<sup>[2,4]</sup>. In this regard, the hot dry weather in Jordan affects development of *N. caninum* oocysts.

Our results suggest an association between age and seropositivity to *N. caninum*, where older sheep and goats are more likely to be positive than younger animals. These findings are in disagreement with previous observations<sup>[13,14]</sup>. In cattle, age association with seropositivity was reported<sup>[17]</sup>. A positive association between age and seropositivity may indicate that horizontal transmission, in addition to the previously reported vertical transmission<sup>[14]</sup>, is possible.

Small flock size was detected by the logistic model as a risk factor for *N. caninum* seropositivity. Similar observations were reported in cattle<sup>[18]</sup>. The lower prevalence of *N. caninum* in particular and parasitic infections, in general, on larger farms can probably be explained by better hygiene-sanitary conditions and handling practices. In goats, Damascus breed was more likely to be seropositive to *N. caninum* than other breeds. This finding is unexpected since Damascus goat farms are usually more organized and have higher biosecurity levels and expected to have lower seropositive goats for *N. caninum*. More research is needed to investigate genetic resistance of this breed to neosporosis.

In this study, grazing in communal pastures and having more than one dog were detected as risk factors for *N. caninum* infection. These finding may further suggest the idea of horizontal transmission. Figluolo et al.<sup>[14]</sup> reported no association between seropositivity of *N. caninum* in sheep and presence of dogs. In cattle, the role of dogs in transmission of *N. caninum* has been documented<sup>[18,19]</sup>.

This study should emphasize more the role of this protozoan (*N. caninum*) as an abortion agent and the need for routine protozoan search in case of ovine or caprine abortion. We believe that sanitary education measures with the aim to inform breeders about disease and the presence of *N. caninum* in tissues and

fetal fluids will be important for a better validation of the protozoan involvement in abortions.

Finally, it is worth mentioning that cross sectional survey as a tool for risk factor analysis have few limitations. Cross sectional studies evaluate both presence of a disease and risk factors associated with the disease at the same time. Therefore, cross sectional studies take a snapshot of the situation at a specific moment<sup>[20]</sup>.

### CONCLUSION

This study documents for the first time the epidemiology of *N. caninum* infection in small ruminants in Jordan. The prevalence of *N. caninum* in sheep and goats was 4.3 and 5.7%, respectively. Small flock size, presence of dogs in the farm and grazing in communal pastures were identified as risk factors for seropositivity to *N. caninum* in southern Jordan. Further studies are needed elucidate the epidemiology in the whole country.

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