Investigation of anti-Neospora caninum antibodies and disease-related risk factors in goats

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Summary
Neospora caninum is a parasitic protozoan that causes abortion, stillbirth, and premature culling in goats. The aims of this study were (i) to determine the prevalence of anti-N. caninum antibodies in goats in the Adana province of Turkey (ii), to identify risk factors for the disease, and (iii) to provide collective data on goat neosporosis. For this purpose, 383 sera were collected from goats of different breeds, ages, and sexes from 15 counties of Adana. A commercially available c-ELISA test kit was used to detect anti-N. caninum antibodies. To identify risk factors that influence the prevalence of neosporosis, an oral survey was conducted, and the data collected were evaluated by the logistic regression analysis. The prevalence was determined as 8.9% (34/383) at the individual level and 66.6% (10/15) at the flock level. Statistical analysis indicated that the co-presence of sheep, the animals’ being of pure breed and the purchasing of animals from different flocks are the factors that increase the prevalence of neosporosis, while feeder disinfection decreases it.

Keywords: goat, neosporosis, risk factors, c-ELISA

Goat breeding is an important livestock industry in areas without suitable pastures, in macquis groves, and in mountainous regions of Turkey. One of essential components of goat breeding is to rear healthy offspring every year and to maintain the sustainability of the flock. Abortions that cannot be avoided are the biggest problem of goat breeding (30, 31). In goats, abortion is the loss of foetus at any time of gestation, and it usually occurs in the last 2 months of pregnancy. Various stress factors, nutritional disorders, poisonings, hormonal disorders, genetic factors, and infectious agents are major causes of abortion. Infectious agents are bacteria, viruses, fungi, and protozoa (31). According to the classical literature, the most important abortifacient protozoan in small ruminants is Toxoplasma gondii. Recent research, however, indicates that N. caninum may also be significant (30, 39).

Neospora caninum is a tissue-dwelling parasitic protozoan in the phylum Apicomplexa. Canidae are both the final and intermediate hosts of the parasite, while ruminants are intermediate hosts. In the domestic cycle of N. caninum, the most important final and intermediate hosts are dogs and cows, respectively. Disease can be transmitted both horizontally and vertically. In the horizontal transmission, dogs are infected by eating the bradyzoite-contaminated meat of intermediate hosts, whereas the intermediate hosts are infected by oral uptake of water or food contaminated with sporulated oocysts. In pregnancy, vertical transmission occurs in two ways: as endogenous or exogenous. Endogenous transplacental transmission results from the reactivation of an existing persistent infection within a cow and leads to the birth of a persistently infected calf. Exogenous transplacental transmission, on the other hand, is the infection of a cow with oocysts, which leads to abortion (17, 39, 44). Epidemic, endemic and sporadic abortions occur in infected cattle (32). Furthermore, early foetal deaths, stillbirths, and neonatal mortalities occur due to foetopathic effects of the parasite. Foetal deaths result in economic losses due to increased calving intervals and delayed lactation. In addition, increased culling of valuable stock and decreased value of herds with high prevalence rates are considered as important problems for animal breeding (38).

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Recent studies have shown that *N. caninum* causes abortions, foetal deaths, and stillbirths in goats, just as it does in cattle. Histopathologic and molecular techniques are used in the diagnosis of neosporosis, while the prevalence of the diseases is determined by serologic methods (c-ELISA, i-ELISA, NAT, IFAT) (39).

The aims of this study were (i) to determine the prevalence of anti-*N. caninum* antibodies in goats at individual and flock levels in the Adana province of Turkey, (ii) to identify risk factors for the disease, and (iii) to provide collective data on goat neosporosis.

**Material and methods**

Sera samples were obtained from 383 goats of different breeds, ages, and sexes from 15 counties of Adana, and stored at –20°C until used (Fig. 1). While the counties were grouped according to their altitudes (< 100 m > 100 m), the goats were grouped according to breeds (hair goat and others), ages (≤ 3 and ≥ 4 years), and sexes (male and female).

To determine risk factors for neosporosis, the goat owners were asked questions about rearing systems, mix-breeding, pasture type, dominant breed, abortion and reproductive problems, disinfection, animal purchasing, and the presence of dogs. The data collected were evaluated by logistic regression analysis. The results of the statistical analysis are shown as an estimated relative risk (odds ratio-OR) and a 95% confidence interval (CI). The statistical significance level was determined as P < 0.05 (Tab. 1).

A commercial c-ELISA test kit (VMRD, USA) was used to detect anti-*N. caninum* antibodies. Samples with percent inhibition values ≥ 30 were confirmed as positive, and those with percent inhibition values < 30 as negative.

This study was approved by the Ethics Committee of Adana Veterinary Control Institute (05.05.2016/1369).
Results and discussion

At the end of the study, the prevalence was determined as 8.9% (34/383) at the individual level and 66.6% (10/15) at the flock level. Percent inhibition values ranged from 30.34056 to 95.41463 in positive samples and from −48.8132 to 29.72136 in negative samples. Statistical analysis showed that the co-presence of sheep, the animals’ being of pure breed and the purchasing of animals from different flocks are factors that increase the prevalence of neosporosis, while feeder disinfection is a factor that decreases it (P < 0.05) (Tab. 1).

According to the classical literature, *T. gondii* is the most important abortifacient protozoon in small ruminants (30, 31). In the 1990s, *N. caninum* was detected in stillborn and aborted goat fetuses, which created the awareness of goat neosporosis. After the 2000s, studies focused on risk factors, prevention, and prevalence rates of *N. caninum* in goat flocks from different countries (39). Although there have been many studies on cattle neosporosis in recent decades, neosporosis has not been sufficiently investigated in other livestock and wild animals. Economic losses caused by *N. caninum* and the epidemiological characteristics of the parasite still remain unknown (3, 17, 32).

According to different serological studies, the prevalence of the disease amounted to 6% in Africa, 0.7-7.23% in Asia, 0.47-15.5% in Europe, 3.8-5.8% in North America, 1.05-17.7% in South America (Tab. 2) and 0-25.9% in Turkey (Tab. 3). According to our global assessment, the average seroprevalence of goat neosporosis was 4.54% (118/2598) in Asia, 3.88% (147/3781) in Europe, 4.68% (15/320) in North America, 7.29% (684/9374) in South America, 5.99%

| Variable | Category            | No. tested | No. positive | % positive | Odds ratio | 95% CL      | X^2     | P Value |
|----------|---------------------|------------|--------------|------------|------------|-------------|---------|---------|
| Altitude | < 100 m             | 179        | 17           | 9.5        | 1.14       | 0.60-2.16   | 0.160   | 0.689   |
|          | > 100 m             | 204        | 17           | 8.3        |            |             |         |         |
| Breed    | Hair goat           | 230        | 17           | 7.4        | 1.56       | 0.77-3.17   | 1.572   | 0.210   |
|          | Others              | 153        | 17           | 11.4       |            |             |         |         |
| Sex      | Female              | 341        | 31           | 9.1        | 1.27       | 0.40-3.98   | 0.175   | 0.675   |
|          | Male                | 42         | 3            | 7.1        |            |             |         |         |
| Age      | ≤ 3 years           | 192        | 16           | 8.3        | 1.14       | 0.56-2.31   | 0.141   | 0.707   |
|          | > 4 years           | 191        | 18           | 9.4        |            |             |         |         |
| Rearing system | Semi-extensive | 358        | 31           | 8.7        | 1.43       | 0.41-5.07   | 0.322   | 0.570   |
|          | Intensive           | 25         | 3            | 12.0       |            |             |         |         |
| Co-presence of sheep | Yes    | 173        | 22           | 12.7       | 2.22       | 1.13-4.36   | 5.750   | 0.016*  |
|          | No                  | 210        | 12           | 5.7        |            |             |         |         |
| Pasture type | Common          | 329        | 32           | 9.4        | 1.70       | 0.54-5.35   | 0.857   | 0.354   |
|          | Own                 | 74         | 2            | 5.6        |            |             |         |         |
| Dominant breed | Pure             | 309        | 32           | 10.4       | 3.83       | 0.93-15.63  | 4.323   | 0.038*  |
|          | Crossbreed          | 74         | 2            | 2.7        |            |             |         |         |
| History of abortion | Yes          | 256        | 26           | 10.2       | 1.61       | 0.75-3.45   | 1.561   | 0.211   |
|          | No                  | 127        | 8            | 6.3        |            |             |         |         |
| History of postpartum problems | Yes    | 172        | 16           | 9.3        | 1.09       | 0.57-2.07   | 0.070   | 0.792   |
|          | No                  | 211        | 18           | 8.5        |            |             |         |         |
| History of infertility | Yes         | 169        | 19           | 11.2       | 1.60       | 0.84-3.06   | 2.092   | 0.148   |
|          | No                  | 214        | 15           | 7.0        |            |             |         |         |
| Shelter disinfection | No            | 148        | 18           | 12.2       | 1.79       | 0.93-3.85   | 3.209   | 0.070   |
|          | Yes                 | 235        | 36           | 6.8        |            |             |         |         |
| Feeder disinfection | No            | 334        | 34           | 10.2       | 1.11       | 1.07-1.15   | 5.474   | 0.019*  |
|          | Yes                 | 49         | 0            | 0          |            |             |         |         |
| Animal purchasing | No             | 304        | 22           | 7.2        | 2.29       | 1.08-4.87   | 4.903   | 0.027*  |
|          | Yes                 | 79         | 12           | 15.2       |            |             |         |         |
| Dogs around the feeders | Yes        | 292        | 26           | 8.9        | 1.01       | 0.47-2.15   | 0.001   | 0.970   |
|          | No                  | 91         | 8            | 8.8        |            |             |         |         |

Explanation: * – P < 0.05
| Continent | Country | Region | Test | Kit | Cut-off | SE | SP | No. tested | No. positive | % positive | Reference |
|-----------|---------|--------|------|-----|---------|----|----|------------|-------------|------------|-----------|
| Africa    | Sudan   | Khartoum state | c-ELISA | VMRD | ≥ 30 | – | – | 100 | 6 | 6 | (20) |
| China     | Qinghai province | i-ELISA, IFAT* | IDEXX | ? | 98.6, 98.3 | 650 | 47 | 7.23 | (24) |
| Iraq      | Wasit province | i-ELISA | IDvet | ≥ 50 | – | – | 106 | 6 | 5.6 | (16) |
| Iran      | Hamedan province | i-ELISA | IDvet | ≥ 50 | – | – | 450 | 28 | 6.2 | (15) |
| Jordan    | Northern Jordan | i-ELISA | BIO-X | – | 95 | 96 | 302 | – | 2 (CTP) | (1) |
| Jordan    | Southern Jordan | i-ELISA | IDEXX Chekit | ? | 97.5, 95.1 | 300 | 17 | 5.7 (CTP) | (2) |
| Korea     | Northern, central and southern regions | i-ELISA | IDEXX | ? | 97.6, 98.5 | 464 | 4 | 0.9 | (22) |
| Pakistan  | Punjab | c-ELISA | VMRD | ≥ 30 | 96 | 99 | 142 | 13 | 8.6 | (29) |
| Sri Lanka | Various parts of the country | ih-ELISA, IFAT*, WB* | – | ? | – | – | 486 | 3 | 0.7 | (28) |
| Germany   | Hesse | i-ELISA | IDvet | ≥ 50 | – | – | 415 | 2 | 0.48 | (42) |
| Czech Republic | Eight different regions | c-ELISA, IFAT* | VMRD | ≥ 30 | – | – | 251 | 15 | 6 | (7) |
| Greece    | Various regions | i-ELISA | – | ? | – | – | 375 | 26 | 6.9 | (4) |
| Italy     | Milan, Bergamo, Varese | ih-ELISA, WB* | – | ? | – | – | 414 | 24 | 5.7 | (14) |
| Poland    | Entire country | i-ELISA, IFAT* | IDEXX Chekit | ? | 98.6, 98.3 | 1060 | 5 | 0.47 | (10) |
| Romania   | Four different regions | i-ELISA | IDEXX Chekit | ≥ 50 | 98.6, 98.3 | 512 | 12 | 2.3 | (21) |
| Slovakia  | Eastern Slovakia | c-ELISA | VMRD | ≥ 30 | – | – | 116 | 18 | 15.5 | (9) |
| Spain     | Galicia | c-ELISA | VMRD | ≥ 30 | – | – | 638 | 45 | 6 | (11) |
| North America | Eastern Caribbean | Grenada | i-ELISA | IDvet | ? | 100 | 100 | 138 | 8 | 5.8 | (35) |
| Mexico    | Veracruz | iELISA | IDEXX | ? | 100, 98.9 | 182 | 7 | 3.8 | (19) |
| Argentina | Córdoba, Buenos Aires | IFAT | – | 1 : 50 | – | – | 1594 | 106 | 6.6 | (26) |
| Argentina | La Rioja Province | IFAT | – | 1 : 100 | – | – | 2922 | 162 | 5.5 | (18) |
| Brazil    | Paraíba State | IFAT | – | 1 : 50 | – | – | 306 | 10 | 3.3 | (12) |
| Brazil    | Bahia State | IFAT | – | 1 : 100 | – | – | 384 | 58 | 15 | (41) |
| Brazil    | Minas Gerais State | IFAT | – | 1 : 50 | 98 | 99 | 667 | – | 10.7 (CTP) | (5) |
| Brazil    | Maranhão State | IFAT | – | 1 : 25 | – | – | 46 | 8 | 17.39 | (27) |
| Brazil    | Paraíba State | IFAT | – | 1 : 50 | – | – | 975 | 26 | 2.7 | (33) |
| Brazil    | Santa Catarina State | IFAT | – | 1 : 50 | – | – | 654 | 30 | 4.58 | (37) |
| Brazil    | São Paulo State | NAT | – | 1 : 25 | – | – | 923 | 161 | 17.7 | (25) |
| Brazil    | Pernambuco State | IFAT | – | 1 : 50 | – | – | 174 | 5 | 2.9 | (6) |
| Brazil    | Piauí State | IFAT | – | 1 : 50 | – | – | 202 | 4 | 2 | (6) |
| Brazil    | São Paulo State | IFAT | – | 1 : 50 | – | – | 394 | 25 | 6.4 | (13) |
| Brazil    | Pernambuco State | IFAT | – | 1 : 50 | – | – | 319 | 85 | 26.6 | (36) |
| Brazil    | Rio Grande do Norte State | IFAT | – | 1 : 50 | – | – | 381 | 4 | 1.05 | (23) |

Explanation: * – confirmation tests; c-ELISA – Competitive Enzyme-Linked Immunosorbent Assay; i-ELISA – Indirect ELISA; ih-ELISA – In house ELISA; WB – Western Blotting; IFAT – Indirect Fluorescent Antibody Test; NAT – Neospora Agglutination Test; SE – Sensitivity; SP – Specificity; CTP – Corrected true seroprevalence
(970/16173) globally (Tab. 4), and 14.44% (91/630) in Turkey (Tab. 3). In this study, the individual prevalence was determined as 8.9% (34/383), which is below the average for Turkey (14.44%), but above the world’s average (5.99%).

Flock-level prevalence was reported as 12-50% in Jordan (1, 2), 13.3% in Iran (15), 32.1% in Italy (14), 0.9% in Poland (9), 38% in Spain (11), 53.2% in Argentina (26) and 16.4-75.2% in Brazil (5, 33). In this study, we determined the flock-level prevalence as 66.6% (10/15), which is higher than the above-mentioned rates, except for Brazil (5). Both individual and flock-level prevalence determined in the present study are above the world’s average, and our results suggest that goat neosporosis is endemic in Adana and Turkey.

In previous studies, the relationship between the disease and various risk factors was examined, and conflicting results were obtained. In some studies, the age, breed, presence of dogs in flocks, and season are presented as risk factors important for the prevalence of neosporosis, which is not confirmed by some other studies (1, 2, 6, 8, 13, 14, 16, 22, 26, 34, 37, 39, 41). As a general consensus, the sex of the animals is thought to have no effect on the prevalence of the disease (6, 12, 14-16). In this study, statistical analysis indicates that the co-presence of sheep, the animals’ being of pure breed and the purchasing of animals from different flocks are factors that increase the prevalence of neosporosis, while feeder disinfection decreases it (P < 0.05) (Tab. 1).

Dramatic differences in the results of serological studies are due to different serological tests, cut-off values, specificity, and sensitivity rates, as well as wrong sampling (39, 40). Low cut-off values result in high sensitivity and low specificity, while high values have opposite effects (43). With regard to the ELISA kits and IFA tests used in different serological studies, it can be observed that cut-off values range from 30 to 50, dilutions from 1/25 to 100, sensitivities of ELISAs from 95% to 100%, and specificities from 95.1% to 100% (Tab. 2). It should be kept in mind that non-optimized serological tests may result in false positivity or negativity, and wrong sampling may also have adverse effects on test results.

In conclusion, to understand the epidemiology of neosporosis and to develop appropriate protection and control strategies, it is important to ensure harmonization among laboratories, to use the same test methods, cut-off values, and dilution ratios, as well as tests with the same specificity and sensitivity rates, and to keep proper records of the determinants of the disease related to the species, host, and environment in large scale studies at national and regional levels.

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