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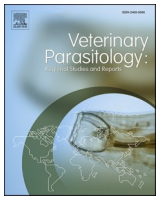

Short Communication

# Seroprevalence of *Neospora caninum* in dairy goats from northern South Africa: A preliminary study

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ABSTRACT

*Neospora caninum* is an intracellular protozoan parasite with a global distribution, known to cause abortions in livestock. This study aimed to determine *N. caninum* seroprevalence in dairy goats with a history of reproductive failure in South Africa. Blood samples were collected from 131 dairy goats across five farms in three provinces and tested for *N. caninum* antibodies using the commercial indirect ELISA (IDvet Screen® *Neospora caninum*), followed with confirmatory testing with western blot (WB). The ELISA detected antibodies in one goat (1/131) [95 % CI: 0–2.8 %], while no positives were detected by WB. While *N. caninum* infection and associated abortions do not currently pose a major concern, farmers are encouraged to perform surveillance for potential infections.

## 1. Introduction

*Neospora caninum* is an apicomplexan, cyst-forming parasite that causes significant disease in cattle and dog, and sporadically affects sheep and goats (Dubey et al., 2017). Neosporosis in goats is widely distributed and prevalent in many parts of the world. Abortions, fetal death, and still births have been reported in *N. caninum* infected goats (Salehi et al., 2021; Schnydrig et al., 2017; Nunes et al., 2017). While few cases of *N. caninum*-related abortion have been reported in goats, the seroprevalence and relevance of infection in these species has been insufficiency studied. In South Africa, the most common infectious causes of abortion in goats include *Chamydophila abortus*, Rift Valley fever, Wesselsbron disease and *Coxiella burnetti* (Department of Agriculture, 2016; Hobson, 2021). Abortions in animals lead to economic losses for farmers, with infectious diseases being the primary cause. However, very low seroprevalences of *N. caninum* infection in goats have been reported in Africa (Rodrigues et al., 2020; Semango and Buza, 2024). Limited studies in Africa have reported seroprevalences ranging from 0 % to 66.7 % (Fereig et al., 2022; Julie et al., 2019). To prevent horizontal transmission, it is crucial to avoid exposure to pastures contaminated with oocysts. Goats are browsers and feed predominately on leaves of scrubs or trees and because of this feeding behavior they have limited exposure to infective parasites that are transmitted through feces in the environment (Hoste et al., 2010). Goats are also easily adaptable to resource-poor areas and are commonly raised for meat, milk, and in many cases kept for traditional or religious purposes. Although dairy goat farming remains a niche industry in South Africa, there has been increased demand for goat milk and dairy products in recent years. The main dairy goat breeds in the country include the British Alpine, Toggenburg and Saanen (Bosman et al., 2015). Little is known about the distribution and significance of *N. caninum* infection in dairy goats in South Africa. The study aimed to determine the seroprevalence of *N. caninum* in dairy goat flocks with a history of abortions in the northern parts of South Africa. (See Table 1.)

## 2. Materials and methods

### 2.1. Animals and blood samples

Blood samples were collected from 131 dairy goats across three

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**Table 1**
Frequency of anti-*N. caninum* specific IgGs in dairy goat flocks in South Africa.

| Farm | Samples | ELISA (% positive) | Breed | Province | Mixed stock farming |
|---|---|---|---|---|---|
| 1 | 25 | 0 | British Alpine Toggenburg Saanen | North West | Cattle |
| 2 | 23 | 1 (4.3 %) | Saanen | Gauteng | Horses |
| 3 | 40 | 0 | Saanen | Gauteng | Sheep |
| 4 | 18 | 0 | Toggenburg Saanen | Gauteng | Cattle Sheep |
| 5 | 25 | 0 | British Alpine | Limpopo | Cattle |
| **Total** | 131 | 1 | | | |

provinces of South Africa (Gauteng, North West and Limpopo) (Fig. 1). A multistage sampling approach was used, with farms having a history of abortion sampled as the primary unit and stratified across provinces. Within each farm, dairy goats were randomly or purposively sampled, prioritizing older breeding goats. A formula by Thrusfield (2018) was used to calculate the required sample size with 95 % confidence:

$$n = \left(1.96^2 \times P_{exp}(1 - P_{exp})\right) \Big/ d^2$$

where $n$ = required sample size, $P_{exp}$ = expected prevalence and $d$ = desired absolute precision. The sample size was adjusted by multiplying it with the design effect ($D$) for multi-stage sampling (Bennett et al., 1991):

$$D = 1 + \rho(m - 1)$$

where $\rho$ is the intra-cluster correlation coefficient (ICC) and $m$ is the average cluster size.

The absence of *N. caninum* seroprevalence data in milk-producing goats in South Africa led to an assumed expected prevalence of 2 %, based on previous studies in Africa. With a desired precision of 5 % and using $\rho = 0.1$ and $m = 30$, $D$ was calculated to be 3.9. Therefore, at least 121 goats from at least four flocks were required. The following data was collected, farm location, goat breed and the presence of other animals, particularly dogs.

### 2.2. Serology

Following the manufacturer's instructions, a commercial indirect ELISA (IDvet Screen®) that uses an anti-ruminant conjugate was used to detect antibodies against *N. caninum*. The test has a sensitivity (Se) of 99.6 % [CI 95 %: 98.9–100 %] and a specificity (Sp) of 98.9 % [CI 95 %: 97.4–100 %] for cattle sera (Alvarez-García et al., 2013). Samples with a sample to positive control ratio (S/P) ≥50 % were considered positive, 40 % to 50 % was doubtful and < 40 % was negative. Samples that tested positive on ELISA were further tested using a confirmatory WB. For the WB, antigen preparation ($2 \times 10^7$ tachyzoites per nitrocellulose membrane), electrophoresis under reducing conditions, electrotransfer of proteins and immunoblotting were carried out following the procedure described by Lopez-Ureña et al., 2023. Sera were diluted at 1/20 in blocking solutions (5 % powdered skim milk 0.05 % tris-buffered saline-Tween 20 (TBS-T) and the secondary antibody was used diluted at 1/1000 in TBS-T (monoclonal anti-goat/sheep IgG antibody conjugated with peroxidase, Sigma, Ref. A9452). The recognition of a clear 17–19 kDa antigenic fraction on WB was considered positive (Alvarez-García et al., 2003).

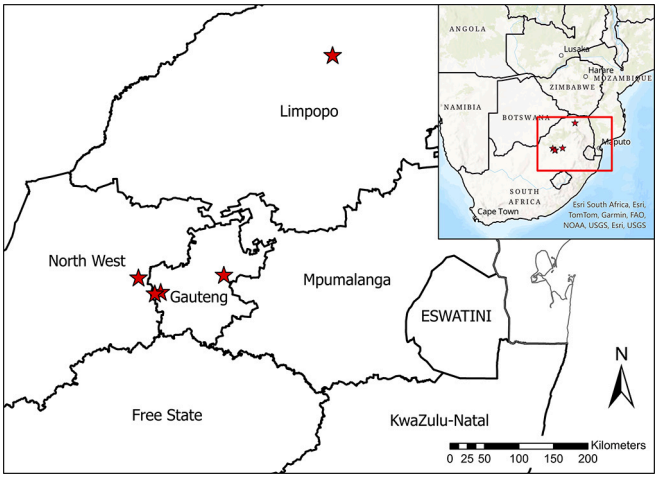


**Fig. 1.** Map showing sampled farms with dairy goats tested for *Neospora caninum* infection.

### 2.3. Statistical analysis

Seroprevalence was calculated with exact binomial 95 % confidence interval. Seropositivity to *N. caninum* was assessed for association with breed, location, type of production, contact with dogs and mixed stock farming using the chi-squared ($\chi^2$) test. Risk factors with a $P$-value <0.05 were considered statistically significant.

## 3. Results

*Neospora caninum* antibodies were detected in 1/131 (0.76 %) goats using the ELISA, with the upper one-sided 97.5 % confidence limit for *N. caninum* seroprevalence at 2.8 %. However, none of the samples tested positive on western blot, indicating that the ELISA-positive sample was likely a false positive. Due to the absence of positive results, no risk factor analysis was possible. All farms had dogs, practised mixed stock farming, and pastured and supplemented their goats.

## 4. Discussion

The low prevalence of *N. caninum* observed in goats using ELISA aligns with the low prevalence found in cattle, suggesting that the infection is not as widespread in the country compared to other regions of the world (Tagwireyi et al., 2024). None of the 131 goats in the study tested positive for antibodies against *N. caninum* with the confirmatory western blot test, indicating a zero seroprevalence in dairy goats in the northern regions of South Africa. This is the first such study in the country and the findings are consistent with the very low seroprevalence reported in other African countries, such as Egypt (0 %), Tanzania (1 %) and Mozambique (3.8 %) (Alsacia et al., 2017; Fereig et al., 2022; Thomas et al., 2022). The single positive ELISA result, confirmed as a false positive by western blot, may have been due to cross-reactivity. Although abortions had been reported in all sampled flocks, *N. caninum* could not be implicated as the cause, and other abortifacient agents should be considered. However, further data on abortion rates and serosurveys in goats that have aborted are needed to explore potential association between abortion and *N. caninum* infection.

The presence of *N. caninum* infection in dairy cattle in two of the study provinces (Gauteng, 5.2 % and North West, 2.3 %) was confirmed in a concurrent study (Tagwireyi et al., 2024). The failure to detect the parasite in goats in the same provinces where cattle tested positive may be attributed to the feeding habits of goats. As browsers, goats primarily feed on the leaves of shrubs and trees, which limits their exposure to infective parasites transmitted through feces on pasture (Hoste et al., 2010). In contrast, cattle are grazers and have more direct contact with infective parasites found in pastures contaminated with feces. Another reason for not reporting of *N. caninum* infection in goats could be due to non-existent or inadequate surveillance platforms for abortifacients particularly in low and medium income countries (LMICs) (Semango and Buza, 2024). This could lead to underreporting of infection. This is supported by Rodrigues et al. (2020), who reported that despite Africa

having a significant proportion of the world's goat population, there are very few published studies on the seroprevalence and risk factors of *N. caninum* in goats. The lack of available information does not imply that the pathogen is not present in animals in African countries. This is especially relevant for South Africa, where *N. caninum* has been reported in other species, but not yet in goats. (Jardine and Dubey, 1992; Tagwireyi et al., 2024).

## 5. Conclusion

No evidence of *N. caninum* infection was found in the dairy goat herds examined in this study. However, this finding highlights the need for further, more comprehensive research across South Africa to accurately determine the disease status. Future studies should involve larger, more representative samples and focus on identifying the risk factors associated with the transmission of infection. Additionally, these studies should explore biosecurity, biocontainment, and risk factor management strategies for herds, as no effective vaccine currently exists.

## Ethical statements

This study was conducted under terms approved by the University of Pretoria, Faculty of Veterinary Science and Faculty of Humanities Research Ethics Committees (REC160–21) and the Department of Agriculture, Land Reform and Rural Development (12/11/1/1/6/2441/(HP).

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## CRediT authorship contribution statement

**Whatmore Munetsi Tagwireyi:** Writing – review & editing, Writing – original draft, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation. **Gema Alvarez Garcia:** Writing – review & editing, Validation, Supervision, Conceptualization. **Darshana Morar-Leather:** Writing – review & editing, Supervision. **Luis Neves:** Writing – review & editing, Supervision, Conceptualization. **Peter N. Thompson:** Writing – review & editing, Supervision, Methodology, Formal analysis.

## Declaration of competing interest

The authors declare no competing interests.

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## References

Alsacia, A.N., Alfeu, C., Aida, C.C., Rosângela, S.U., Bárbara, P.S., Luis, F.P.G., Müller, R.-A., Maria, Â.O.-A., Carlos, A.D.M., Nelson, M., 2017. Serological survey of *Neospora caninum* and *Besnoitia besnoiti* in cattle and goats from smallholder farms in Angónia, Tete Province, Mozambique. African Journal of Rural Development (AFJRD) 2 (2), 303–311. https://doi.org/10.22004/ag.econ.262853.

Alvarez-García, G., Collantes-Fernández, E., Costas, E., Rebordosa, X., Ortega-Mora, L., 2003. Influence of age and purpose for testing on the cut-off selection of serological methods in bovine neosporosis. Vet. Res. 34 (3), 341–352. https://doi.org/10.1051/vetres:2003009.

Alvarez-García, G., García-Culebras, A., Gutiérrez-Expósito, D., Navarro-Lozano, V., Pastor-Fernández, I., Ortega-Mora, L.M., 2013. Serological diagnosis of bovine neosporosis: a comparative study of commercially available ELISA tests. Vet. Parasitol. 198 (1–2), 85–95. https://doi.org/10.1016/j.vetpar.2013.07.033.

Bennett, S., Woods, T., Liyanage, W.M., Smith, D.L., 1991. A simplified general method for cluster-sample surveys of health in developing countries. World Health Statist. Quart. 44 (3), 98–106.

Bosman, L., van Marle-Köster, E., Visser, C., 2015. Genetic diversity of South African dairy goats for genetic management and improvement. Small Rumin. Res. 123 (2-3), 224–231. https://doi.org/10.1016/j.smallrumres.2014.12.003.

Department of Agriculture F. a. F, (2016). Goat Training Manual. Retrieved on 30 November 2024 https://www.dalrrd.gov.za/images/Branches/AgricProducHealthFoodSafety/genetic-resources/goat-training-manual-book_a4-layout_.pdf.

Dubey, J., Hemphill, A., Calero-Bernal, R., Schares, G., 2017. Neosporosis in Animals. CRC Press. https://doi.org/10.1201/9781315152561.

Fereig, R.M., Wareth, G., Abdelbaky, H.H., Mazeed, A.M., El-Diasty, M., Abdelkhalek, A., Mahmoud, H.Y., Ali, A.O., El-Tayeb, A., Alsayeqh, A.F., 2022. Seroprevalence of specific antibodies to *Toxoplasma gondii, Neospora caninum*, and Brucella spp. in sheep and goats in Egypt. Animals 12 (23), 3327. https://doi.org/10.3390/ani12233327.

Hobson, M., 2021. Angora goat health guide: Keeping biological integrity intact. Stockfarm 11 (6), 60–61 https://hdl.handle.net/10520/ejc-vp_stock_v11_n6_a25.

Hoste, H., Sotiraki, S., Landau, S.Y., Jackson, F., Beveridge, I., 2010. Goat–nematode interactions: think differently. Trends Parasitol. 26 (8), 376–381. https://doi.org/10.1016/j.pt.2010.04.007.

Jardine, J., Dubey, J., 1992. Canine neosporosis in South Africa. Vet. Parasitol. 44 (3-4), 291–294. https://doi.org/10.1016/0304-4017(92)90124-r.

Julie, B., Paul, A.J., Antoine, M.M.R., 2019. Seroprevalence of two abortive parasites: *Toxoplasma gondii* and *Neospora caninum* in domestic animals in Franceville, Gabon. Afr. J. Parasitol. 60, 1–5.

Nunes, A.C., Yamasaki, E.M., Kim, P.C., Melo, R.P., Ribeiro-Andrade, M., Porto, W.J., Mota, R.A., 2017. Transplacental transmission of *Neospora caninum* in naturally infected small ruminants from northeastern Brazil. Pesqui. Vet. Bras. 37 (09), 921–925. https://doi.org/10.1590/S0100-736X2017000900004.

Rodrigues, A.A., Reis, S.S., de Sousa, M.L., da Silva Moraes, E., Garcia, J.L., Nascimento, T.V.C., da Cunha, I.A.L., 2020. A systematic literature review and meta-analysis of risk factors for *Neospora caninum* seroprevalence in goats. Prevent. Vet. Med. 185, 105176. https://doi.org/10.1016/j.prevetmed.2020.105176.

Salehi, B., Amouei, A., Dodangeh, S., Daryani, A., Sarvi, S., Safari-Kharyeki, M.R., Salehi, S., Hosseini, S.A., Hosseininejad, Z., 2021. Molecular identification of *Neospora caninum* infection in aborted fetuses of sheep, cattle, and goats in Mazandaran Province, Northern Iran. Iran. J. Parasitol. 16 (3), 483. https://doi.org/10.18502/ijpa.v16i3.7102.

Schnydrig, P., Vidal, S., Brodard, I., Frey, C.F., Posthaus, H., Perreten, V., Rodriguez-Campos, S., 2017. Bacterial, fungal, parasitological and pathological analyses of abortions in small ruminants from 2012-2016. Schweiz. Arch. Tierheilkd. 159, 647–656. https://doi.org/10.17236/sat00136.

Semango, G.P., Buza, J., 2024. Review of the current status on ruminant abortigenic pathogen surveillance in Africa and Asia. Vet. Sci. 11 (9), 425. https://doi.org/10.3390/vetsci11090425.

Tagwireyi, W.M., Thompson, P.N., Garcia, G.A., Morar-Leather, D., Neves, L., 2024. Seroprevalence and associated risk factors for *Neospora caninum* infection in dairy cattle in South Africa. Parasitol. Res. 123 (8), 298. https://doi.org/10.1007/s00436-024-08309-8.

Thomas, K.M., Kibona, T., Claxton, J.R., de Glanville, W.A., Lankester, F., Amani, N., Buza, J.J., Carter, R.W., Chapman, G.E., Crump, J.A., 2022. Prospective cohort study reveals unexpected aetiologies of livestock abortion in northern Tanzania. Sci. Rep. 12 (1), 11669. https://doi.org/10.1038/s41598-022-15517-8.

Thrusfield, M., 2018. Veterinary Epidemiology. John Wiley & Sons. https://doi.org/10.1002/9781118280249.