Seroprevalence and associated risk factors of Toxoplasma gondii in goats and sheep in the Khomas region of Namibia

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Abstract

This study aimed to determine the seroprevalence levels of *T. gondii* in small ruminants (goats and sheep) and the associated risk factors in the Khomas region of Namibia. A total of 299 and 345 sheep and goat sera, respectively, were collected from 22 farming establishments. An IDEXX Toxotest Ab®, a commercial ELISA kit, was used to test for IgG antibodies to *T. gondii*. Overall, 3.68% (11/299) of the sheep sera were positive, and 61.54% (8/13) of the sheep flocks tested had at least one positive animal. Only 0.29% (1/345) of the goat sera were positive, and only one of the 19 goat flocks had at least one positive animal giving a herd-level prevalence of 5.26%. Sheep flocks had significantly greater animal-level and flock-level prevalences than goats (p < 0.05) and were 13.14 times more likely to be seropositive (OR = 13.14; CI 95%: 1.686-102.382) than goat flocks. A questionnaire was also administered to identify any putative risk factors associated with seropositive cases. Eight risk factors were evaluated, including the total number of goats, total number of sheep, farm size, average rainfall, level of Feliformia, history of abortions and the presence of domesticated cats and strays. Seropositivity to *T. gondii* was positively associated with the total number of sheep at the farming establishment, history of abortions and farm size (p < 0.05), but not goats. The study concluded that sheep were probably more susceptible to toxoplasmosis than goats and that the *T. gondii* seroprevalence level in the Khomas region was very low compared to other countries.

Introduction

*Toxoplasma gondii* is an intracellular protozoan pathogen associated with livestock reproduction losses through abortions, stillbirths, mummifications, infertility, early embryonic death and foetal resorption (Tagwireyi et al., 2019; Al-Malki, 2021; Omonijo et al., 2022). In addition, toxoplasmosis, caused by *T. gondii*, is an important zoonosis, and it is estimated that this parasite infects one-third of the world's human population (Innes, 2010; Zhang et al., 2013; Tegegne et al., 2016; Abbas et al., 2020; Kolören and Dubey, 2020).

*T. gondii* is presumed to occur worldwide (Al-Malki, 2021), although not all countries have conducted studies to confirm its presence in small ruminant livestock. In Namibia, for example, the only studies regarding *T. gondii* involved wildlife and seroprevalence studies in humans (Joubert and Evans, 1997; Colf et al., 2020; Seltmann et al., 2020) but not in livestock. In addition, *T. gondii* seroprevalence rates ranging from 11–67.25% in small ruminants (sheep and goats) were determined in South Africa and Zimbabwe (Tagwireyi et al., 2019; Omonijo et al., 2022) but not in Namibia.

Concerning toxoplasmosis in animals, the risk factors identified in other countries include the presence of definitive hosts (cats), flock size, age, geographical factors, farm management factors and biosecurity (Caballero-Ortega et al., 2008; Stelzer et al., 2019).

No studies on goats and sheep concerning toxoplasmosis have been done in Namibia. Therefore, this study aimed to determine the seroprevalence levels of *T. gondii* in small ruminants (goats and sheep) and
the associated risk factors in the Khomas region of Namibia.

Materials And Methods

Study area

The study area was Namibia's Khomas region, located in the central part of the country (Fig. 1). Namibia's sub-tropical climate varies from arid to semi-arid, and it is the driest country in sub-Saharan Africa (Mwazi and Shamathe, 2007). The country's central highlands receive an annual rainfall of between 300mm and 400mm and have an altitude of up to 1900 metres (Kandiwa et al., 2017). The vegetation is predominantly shrub-veld, and ambient temperatures range from 7°C in winter and up to 33°C in summer (Kandiwa et al., 2019). The Khomas region has approximately 560 livestock farming units, mainly commercial and some resettlement farms and communal settlements (Directorate of Veterinary Services, 2018).

Study animals

The study animals were goats and sheep in the Khomas region of Namibia (Fig. 1). This region has approximately 91 000 sheep and 20 000 goats distributed among an estimated 560 farming establishments (Directorate of Veterinary Services, 2018) that primarily farm with beef cattle. All the animals were reared extensively.

Sampling and data collection

Sample size determination was done according to the calculations done by Pfeiffer (Pfeiffer, 2002). Convenience sampling was used to select the farms because most of the farming establishments in the Khomas region only farm with beef cattle, with only a few having small ruminants. Multistage sampling was then used at the farm level, where only adult sheep and goats were targeted, and individual animals were randomly selected. A questionnaire was administered during sera collection to determine the possible risk factors for *T. gondii* in the Khomas region. Blood was collected from the jugular veins using plain Vacutainer® blood tubes and 20-gauge needles. The blood samples were centrifuged at 6000 rpm for 10 minutes to extract sera which were then stored at -20°C until it was tested.

Serological analysis

An IDEXX Toxotest® (IDEXX Laboratories, Inc, Maine 04092, USA) test kit, an indirect enzyme-linked immunosorbent assay (ELISA), was used to test for specific anti-*T. gondii* IgG antibodies in sheep and goat sera. All reagents were brought to 18-26°C before use and mixed, according to the manufacturer’s instructions. First, diluted positive and negative controls and diluted test sera were added to antigen-coated plates, mixed and then incubated for one hour at 37°C. Next, the solution was removed, and the wells were washed thrice with Wash Solution. Conjugate was then added to all the wells, and the plates were incubated for an additional one hour at 37°C, ensuring that the plates were tightly sealed. The solution was again removed, and the wells were washed thrice with Wash Solution. Next, a TMB
Substrate was added to each well, and the plates were incubated at 18-26°C for 15 minutes. A Stop Solution was then added to each well. Finally, the results were read off at a wavelength of 450 using an ELISA reader within two hours after the addition of the Stop Solution.

The positive and negative controls were then validated and interpreted as follows: a sample to positive control ratio of less than 20% was interpreted as negative; a ratio of at least 20% but less than 30% was suspect; a ratio of at least 30% but less than 100% was interpreted as weak positive. Finally, a ratio of at least 100% was interpreted as positive.

**Data analysis**

The serology and questionnaire data were captured in Microsoft Excel 2013 spreadsheet, in which all the statistical calculations were done. Descriptive statistics were used to calculate herd-level and animal-level prevalence rates. The z-test for comparing two proportions and odds ratios were used to compare the prevalence rates between sheep and goats. Multiple regression analysis and Chi-square test were used to evaluate the risk factors.

**Results**

Eleven of the 299 sheep sera tested from 13 farming establishments were positive, giving an animal-level prevalence of 3.68% (CI 95%: 2.07-6.47) (Table 1). In addition, another five sheep (1.67%; CI 95%: 0.0072-0.0385) were suspicious, giving a combined (positive & suspicious) animal-level prevalence of 5.35% (CI 95%: 0.0332-0.0851). Finally, eight of the thirteen establishments tested had at least one positive sheep, giving a herd-level prevalence of 61.54% (CI 95%: 35.52-82.29) (Figure 2).

| Table 1 |

ELISA results of sheep sera tested from 13 establishments
<table>
<thead>
<tr>
<th>Farm no.</th>
<th>No. of sheep sera</th>
<th>No. positive</th>
<th>No. suspect</th>
<th>% positive</th>
<th>% suspect</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>2</td>
<td>1</td>
<td>13.33</td>
<td>6.67</td>
</tr>
<tr>
<td>2</td>
<td>32</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>3</td>
<td>0</td>
<td>10.00</td>
<td>0.00</td>
</tr>
<tr>
<td>4</td>
<td>29</td>
<td>1</td>
<td>1</td>
<td>3.45</td>
<td>3.45</td>
</tr>
<tr>
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<td>30</td>
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<td>0</td>
<td>3.33</td>
<td>0.00</td>
</tr>
<tr>
<td>6</td>
<td>31</td>
<td>1</td>
<td>0</td>
<td>3.23</td>
<td>0.00</td>
</tr>
<tr>
<td>7</td>
<td>21</td>
<td>0</td>
<td>1</td>
<td>0.00</td>
<td>4.76</td>
</tr>
<tr>
<td>8</td>
<td>24</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>9</td>
<td>18</td>
<td>1</td>
<td>2</td>
<td>5.56</td>
<td>11.11</td>
</tr>
<tr>
<td>10</td>
<td>16</td>
<td>1</td>
<td>0</td>
<td>6.25</td>
<td>0.00</td>
</tr>
<tr>
<td>11</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>12</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>20.00</td>
<td>0.00</td>
</tr>
<tr>
<td>13</td>
<td>46</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td><strong>TOTALS</strong></td>
<td><strong>299</strong></td>
<td><strong>11</strong></td>
<td><strong>5</strong></td>
<td><strong>3.68</strong></td>
<td><strong>1.67</strong></td>
</tr>
</tbody>
</table>

Of the 345 goat sera tested from 19 farming establishments, only one goat was positive, giving an animal-level prevalence of 0.29% (CI 95%: 0.05-1.62) (Table 2). Furthermore, another seven goats (2.03%) were suspicious, giving a combined (positive & suspicious) animal-level prevalence range of 2.32% (CI 95%: 0.012-0.045). Finally, only one of the 19 goat establishments had at least one positive animal giving a herd-level prevalence of 5.26% (CL 95%: 0.009-0.246). Adding suspicious establishments to this figure gives a herd-level prevalence of 31.58% (CI 95%: 0.089-0.511).

Table 2

ELISA results of goat sera tested from 19 establishment
<table>
<thead>
<tr>
<th>Farm no.</th>
<th>No. of goat sera</th>
<th>No. positive</th>
<th>No. suspect</th>
<th>% positive</th>
<th>% suspect</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12</td>
<td>0</td>
<td>1</td>
<td>0.00</td>
<td>8.33</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>0</td>
<td>3</td>
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<td>20.00</td>
</tr>
<tr>
<td>3</td>
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<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
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<td>0.00</td>
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<tr>
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<td>0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>6</td>
<td>19</td>
<td>0</td>
<td>1</td>
<td>0.00</td>
<td>5.26</td>
</tr>
<tr>
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<td>21</td>
<td>1</td>
<td>0</td>
<td>4.76</td>
<td>0.00</td>
</tr>
<tr>
<td>8</td>
<td>19</td>
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<td>0</td>
<td>0.00</td>
<td>0.00</td>
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<td>0</td>
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<td>0.00</td>
</tr>
<tr>
<td>10</td>
<td>18</td>
<td>0</td>
<td>1</td>
<td>0.00</td>
<td>5.56</td>
</tr>
<tr>
<td>11</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>12</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>13</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>14</td>
<td>11</td>
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<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>15</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>16</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>17</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>18</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>19</td>
<td>63</td>
<td>0</td>
<td>1</td>
<td>0.00</td>
<td>1.59</td>
</tr>
<tr>
<td><strong>TOTALS</strong></td>
<td><strong>345</strong></td>
<td><strong>1</strong></td>
<td><strong>7</strong></td>
<td><strong>0.29</strong></td>
<td><strong>2.03</strong></td>
</tr>
</tbody>
</table>

Sheep had a significantly greater individual-level prevalence than goats (p=0.0015) and were 13.14 times more likely to be positive (OR = 13.14; CI 95%: 1.686-102.382). In addition, sheep's herd-level prevalence was significantly greater than for goats (p=0.0005), and sheep farming establishments were 28.8 times more likely to have at least one positive animal than goat flocks (OR = 28.8; CI 95%:2.879-288.103).

Table 3

summary of risk factors investigated
Table 3 shows the list of eight potential risk factors that were investigated using questionnaires. Seropositivity to *T. gondii* was positively associated with the total number of sheep at the farming establishment, a history of abortions and farm size (p<0.05). However, there was no significant association between seropositivity and the number of goats at the establishment, level of Feliformia, the presence of cats (domesticated & strays), and the average rainfall (p>0.05).

### Discussion

To the authors' knowledge, this is Namibia's first study on sheep and goats toxoplasmosis. This study determined animal-level prevalence rates of 3.68% and 0.29% in sheep and goats, respectively, which are very low compared to South Africa, India, Iran and Italy, where seroprevalence rates ranging from 27–64.46% were determined (Gazzinos et al., 2015; Sharif et al., 2015; Bachan et al., 2018; Tagwireyi et al., 2019). These low prevalence rates in Namibia might be due to the tropical semi-arid climate in the country, which is characterised by hot and dry summers interspaced with erratic rainfall and predominantly cold and dry weather in winter. These conditions create an unfavourable environment for the *T. gondii* oocysts to sporulate (Yan et al., 2016). Generally, *T. gondii* infections tend to be high in warm and humid environments but low in hot and dry climates (Meerburg and Kijlstra, 2009).

Animals reared under extensive conditions have been found to have lower seroprevalence rates than those reared under intensive or semi-intensive conditions (Tzanidakis et al., 2012; Tagwireyi et al., 2019). All small ruminants in the current study were grazed extensively, which could also have contributed to the low seroprevalence rates observed in the current study. Extensive grazing conditions in central Namibia are generally hot and dry for most of the year, which might interfere with the sporulation of *T. gondii* oocysts and, therefore, infection rates.

Interestingly, a recent study among pregnant women in Windhoek, Namibia, also found a similarly low seroprevalence rate of 2.61% (n = 344); the authors noted that this figure was low compared to other developing countries, and they attributed this to geographical and climatic factors (Colf et al., 2020). Furthermore, in an earlier study by Colf and co-workers, only 0.961% (n = 312) of blood donors in central
Namibia were seropositive to *T. gondii* (Colf et al., 2014). Therefore, the low seroprevalence rates in the current study agree with these findings and demonstrate that *T. gondii* infections in sheep, goats and humans in the Khomas region are very low compared to the rest of the world, in which one-third of the globe's human population is estimated to be infected (Tegegne et al., 2016).

The current study found that sheep had significantly higher animal-level and herd-level prevalences than goats. Similar trends have been reported in many parts of the world, including Northern Italy (Gazzinos et al., 2015), Africa, Caribbean Islands, Greece, Portugal, Spain, Poland (Stelzer et al., 2019) and Colombia (Martínez-Rodriguez et al., 2020). In one study in South Africa, Tagwirei and colleagues also found a higher seroprevalence in sheep than goats (Tagwireyi et al., 2019), although it was not statistically significant. However, studies in the USA and Egypt recorded a higher seroprevalence in goats than sheep (Mishra et al., 2016; Al-Kappany et al., 2018). Therefore, with few exceptions, the current study and reports from other parts of the world seem to indicate that sheep could be more susceptible to *T. gondii* than goats.

The significant association between seropositivity to *T. gondii* and a history of abortions found in the current study is not surprising. For example, in one study in Northern Iraq, sheep with a history of abortion within the previous twelve months were 13.4 times more likely to be seropositive (Al Hamada et al., 2019). One study in Central Ethiopia (Gebremedhin et al., 2013) and a review by Stelzer and colleagues also reported a similar association (Stelzer et al., 2019). However, in one recent report in Egypt by Abdelbaset and co-workers, no such association was found (Abdelbaset et al., 2020).

The total number of sheep at the farming establishment is another significant risk factor determined in the current study. Since sheep appear more susceptible to *T. gondii* infection than goats, as also established in this study, it is logical that the higher the number of sheep at an establishment, the greater the risk of toxoplasmosis. However, this disagrees with an Italian study where seropositivity decreased with increasing flock size (Cenci-Goga et al., 2013). It is worth noting, however, that this Italian research studied large flock sizes numbering 300–400 sheep, unlike the current study in which flock sizes were much smaller. Additionally, climatic conditions in Tuscany, Italy, are very different from the semi-arid Namibian conditions, which could affect the epidemiology of the disease.

Farm size was also positively associated with *T. gondii* seropositivity, implying that larger farming establishments had a significantly higher seropositivity rate. However, this type of relationship was unexpected. Logically, there should be an inverse relationship between farm size and seropositivity because the probability of small ruminants ingesting *T. gondii* sporulated oocysts in a smaller farming establishment would be higher. For instance, Corbellini and co-workers reported an inverse relationship between farm size and *N. caninum* seropositivity (Corbellini et al., 2006). On the other hand, larger farms in Namibia would be expected to have more wildlife carnivores which have been reported to be seropositive to *T. gondii* (Seltmann et al., 2020). Therefore, the chances of such carnivores contaminating the pastures would be higher on larger farms.
In conclusion, small ruminants in the Khomas region have been exposed to *T. gondii*, although the seroprevalence is low compared to other countries. Sheep appear more susceptible to *T. gondii* infections than goats; therefore, any control measures should be first targeted toward this species. However, given the very low prevalence rate found, introducing wholesale control measures is probably uneconomical at this stage. However, *T. gondii* should be included in the abortion screening tests at the Central Veterinary Laboratory. In this way, the country’s toxoplasmosis status can be continuously monitored.

**Declarations**

**Acknowledgement**

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**Funding:** The IDEXX ELISA kits for this study were funded by the Meat Board of Namibia.

**Conflicts of interest:** All the authors declare that they have no conflict of interest.

**Ethics approval:** This study was approved by the University of Namibia Ethics Committee (Reference number: NEC0007) and the University of Pretoria’s Research Ethics Committees (reference numbers REC087-21 and HUM00/0322).

**Consent to participate:** All the respondents in this research signed informed consent to participate in this research.

**Consent for publication:** All the respondents in this research signed an informed consent permitting the publication of this research.

**Availability of data and material**

The data collected for this research is available upon request.

**Code availability:** Microsoft Office 2013

**Authors' contributions:** All authors participated in the study’s conception, design, material preparation and data collection. Alaster Samkange did data analysis and the writing of the first draft of the manuscript. All authors commented on the previous versions and read and approved the final version of this manuscript.

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Figures
Map of Namibia showing its fourteen regions. The Khomas region is located centrally, as shown on the map. Source: https://www.hauniversity.org/en/Namibia-Regions.shtml The map was used with permission.

![Map of Namibia](https://www.hauniversity.org/en/Namibia-Regions.shtml)  

**Figure 2**

Google map showing the location of *T. gondii* positive & suspicious (red markers) and negative (blue) farming establishments for sheep and goats in the Khomas region. The map on the right is a magnified view of one cluster, as shown by the green arrow.