

Short Communication

Sero-prevalence of toxoplasmosis in sheep and goats in El-Gadarif state

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ABSTRACT

Objective: This cross-sectional study was conducted from July to November 2015 to estimate the prevalence of anti-toxoplasma antibodies in sheep and goats in El-Gadarif state.

Material and methods: A total of 400 serum samples comprising of 200 sheep and 200 goats were collected and tested by Toxo-latex agglutination test and indirect enzyme linked immunosorbent assay (iELISA).

Results: The overall sero-prevalence was 52.0% (n=208/400) using Toxo-latex agglutination test and 45.7%(n=42/92) and 27.2%(n=25/92) using iELISA in sheep and goats, respectively. Furthermore, the sero-prevalences among the two investigated species and the two age groups were statistically similar but were different between localities, breeds and sexes. In the univariate analysis, species (P=0.028) and locality (P=0.001) were associated with Toxo-latex agglutination test positive status. Additionally, species (sheep) and locality (Al-Fao, Al-Hawatah, and West El-Gadarif) had increased odds of being Toxo-latex agglutination test positive in the multivariate analysis.

Conclusion: It can be concluded that the prevalence of anti-toxoplasma antibodies is relatively high and there was no between-species variation in seropositivity. These findings warrant further investigations to estimate the burden of the disease and the likelihood of zoonotic transmission.

KEYWORDS

El-Gadarif; Goats, Sero-prevalence, Sheep, Sudan; Toxoplasmosis

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INTRODUCTION

Toxoplasmosis is an anthrozoonic disease caused by the infection with the obligate intracellular parasite *Toxoplasma gondii* and is among the major global health problems (Sarciron and Gherardi, 2000; Kasper, 2005; Petersen et al., 2010; Torgerson and Macpherson, 2011; Gharekhani, 2014; Elhassan et al., 2015). It is also one of the most prevalent parasitic diseases that have medical and veterinary importance. In humans, toxoplasmosis causes a variety of disease syndromes ranging from flu-like symptoms in immunocompetent adults, to severe disseminated disease in immuno-suppressed individuals and birth defects in infants when women get exposed during pregnancy (Radostits et al., 2006; Dubey, 2010; Innes, 2010a). The symptoms of toxoplasmosis in animals illustrate its economic importance. It results in reproductive failure, embryonic death and resorption, fetal death and mummification, abortion, stillbirths, and neonatal death in small ruminants (Marquardt et al., 2000; Dubey, 2009; Dubey, 2010). The severity of infection in sheep is associated with the stage of gestation at which the ewe becomes infected; the earlier in gestation, the more severe the consequences (Dubey, 2009; Innes, 2010b).

In the Sudan, the prevalence of toxoplasmosis varied by animal species and location, as reported before (Khalil and Elrayah, 2011; Elfahal et al., 2013). While in humans the prevalence of toxoplasmosis was as high as 50.0% (Tamomh et al., 2016). However, only a few studies investigated the risk factors that are associated with *T. gondii*-infection (Hammond-Aryee et al., 2014). Besides, there has been an increasing interest in the prevalence of *T. gondii* infection in small ruminants because of their role on the dissemination of this parasite to man through direct contact or by consuming products of animal origin (Cenci-Goga et al., 2013). Therefore, this study was conducted to estimate the prevalence of anti-toxoplasma antibodies in sheep and goats in El-Gadarif state and to investigate individual animal risk factors.

MATERIALS AND METHODS

Study area: El-Gadarif state is located in the north-eastern part of the Sudan and has international borders with Ethiopia. It falls within the Sudano-Sahelian climate zone of Africa. The soil of El-Gadarif is very fertile enabling many agricultural activities in the rainy season between June and September. Temperature ranges from 17°C to 40°C (Sulieman and Buchroithner, 2006). Different breeds of different animals species are raised and produced in El-Gadarif state for both domestic and export markets (ILRI, 2009).

Sampling strategy and study design: A cross-sectional study design, from June to November 2015, employing a multistage sampling method was used in the current survey as described by Thrusfield (2007). Out of the 10 localities of El-Gadarif state, 4 were conveniently selected, namely: Al-Fao, Wasat El-Gadarif, El-Gadarif municipality, and Al-Hawatah. Within the selected localities smaller administrative units and/or villages were further conveniently selected. In each selected administrative unit and/or village sheep and goats flocks and individual animals were randomly or conveniently sampled (Thrusfield, 2007).

Sample size: The sample size for determining the prevalence of anti-toxoplasma antibodies in sheep and goats was calculated using the standard formula of Thrusfield (2007). The required sample size (n) was determined to be 400 animals; 200 sheep and 200 goats.

Samples: Two whole blood samples were taken from the selected animals and serum was separated and kept at -20°C until processed as recommended by OIE (2008).

Latex agglutination test: The Toxo-latex agglutination test kit was obtained from the Spinreact, S.A./S.A.U., Ctra, Santa Coloma, (GI), Spain, and the test was carried out as described by the manufacturer.

Indirect enzyme linked immunosorbent assay (iELISA): The iELISA kit was obtained from the IDvet Innovative Diagnostics, rue Louis Pasteur, Grabeis, France. The kit components were reconstituted as directed by the manufacturer. These included concentrated conjugate (10X), positive and negative control sera, dilution buffer 2 and 3, concentrated wash solution (20X), substrate solution, and stop solution (0.5 M). The test procedure was carried out as per the manufacturer's protocol. A positive/negative cut-off was calculated as S/P% of $\geq 50\%$.

Data analyses: The Statistical Package for Social Sciences (SPSS) for Windows® version 18.0 (SPSS Inc., Chicago, Illinois) was used for all appropriate statistical analyses. Descriptive statistics of the variables were obtained. Frequencies and prevalence were computed for each variable as well as statistical association tests including chi-square and logistic regression.

RESULTS

The overall sero-prevalence: Generally, anti-toxoplasma antibodies were detected in all investigated localities with variations observed in the sero-prevalences between different age groups, breeds, and sexes as

presented in **Table 1**. The overall sero-prevalence was 52.0%(n=208/400) with a 95% CI between 47.1 and 56.9, using Toxo-latex agglutination test. While, the sero-prevalences using iELISA were 45.7%(n=42/92) with a 95% CI from 35.52 to 55.88 in sheep and 27.2%(n=25/92), with a 95% CI from 18.11 to 36.29 in goats.

With exception of between-locations, the seroprevalences between the categories of the investigated variable were not statistically different as presented in **Table 1**.

Univariate associations: The proportions of sero-positive differ between localities, breeds, age groups, and

males and females. In the univariate analysis using chi square, species ($P=0.028$) and locality ($P=0.001$) were significantly associated with Toxo-latex agglutination test positive status for toxoplasmosis. However, age ($P=0.229$), breed ($P=0.164$), and sex ($P=0.285$) were not significantly associated with Toxo-latex agglutination test positive status for toxoplasmosis (**Table 2**).

Multivariate analysis: Results of the logistic regression analysis assessing the combined relationship between species, localities, and breeds with the positive reaction for toxoplasmosis in the Toxo-latex agglutination test are presented in **Table 3**. The regression coefficients (Exp(B)) express ‘odds ratios’ (OR) (=the increased or

Table 1: Estimated sero-prevalences of anti-toxoplasma antibodies by species, locality, breed, age and sex using Toxo-latex agglutination test in El-Gadarif state, from July to November 2015

| Risk factors | No. of tested | No. of positive | % of positive | 95% CI (Lower – Upper) |
|-------------------------|---------------|-----------------|---------------|----------------------------|
| Species | | | | |
| Sheep | 200 | 115 | 57.5 | 50.65 - 64.35 ^a |
| Goats | 200 | 93 | 46.5 | 39.59 - 53.41 ^a |
| Localities | | | | |
| Al-Fao | 100 | 73 | 73.0 | 64.30 - 81.70 ^a |
| Al-Hawatah | 100 | 51 | 51.0 | 41.20 - 60.80 ^b |
| Wasat El-Gadarif | 100 | 54 | 54.0 | 44.23 - 63.77 ^b |
| El-Gadarif municipality | 100 | 30 | 30.0 | 21.02 - 38.98 ^c |
| Breeds | | | | |
| Wateish sheep | 111 | 63 | 56.8 | 47.58 - 66.02 ^a |
| Agbash sheep | 89 | 52 | 58.4 | 48.16 - 68.64 ^a |
| Balady goat | 128 | 58 | 45.3 | 36.68 - 53.92 ^a |
| Nuoby goat | 72 | 35 | 48.6 | 37.06 - 60.14 ^a |
| Age groups (yrs) | | | | |
| ≤ 2 | 227 | 124 | 54.6 | 48.12 - 61.08 ^a |
| > 2 | 173 | 84 | 48.6 | 41.15 - 56.05 ^a |
| Sex | | | | |
| Male | 216 | 107 | 49.5 | 42.83 - 56.17 ^a |
| Female | 184 | 101 | 54.9 | 47.71 - 62.09 ^a |
| Total | 400 | 208 | 52.0 | 47.10 - 56.90 |

Table 2: Univariate association of anti-toxoplasma antibodies positive status with species, locality, breed, age and sex using Toxo-latex agglutination test in El-Gadarif state, from July to November 2015

| Risk factors | No. of tested | No. of positive | % of positive | χ^2 | df | <i>P-value</i> |
|-------------------------|---------------|-----------------|---------------|----------|----|----------------|
| Species | | | | | | |
| Sheep | 200 | 115 | 57.5 | 4.484 | 1 | 0.028 |
| Goats | 200 | 93 | 46.5 | | | |
| Localities | | | | | | |
| Al-Fao | 100 | 73 | 73.0 | 37.260 | 3 | 0.001 |
| Al-Hawatah | 100 | 51 | 51.0 | | | |
| Wasat El-Gadarif | 100 | 54 | 54.0 | | | |
| El-Gadarif municipality | 100 | 30 | 30.0 | | | |
| Breeds | | | | | | |
| Wateish sheep | 111 | 63 | 56.8 | 5.104 | 3 | 0.164 |
| Agbash sheep | 89 | 52 | 58.4 | | | |
| Balady goat | 128 | 58 | 45.3 | | | |
| Nuoby goat | 72 | 35 | 48.6 | | | |
| Age groups (yrs) | | | | | | |
| ≤ 2 | 227 | 124 | 54.6 | 1.450 | 1 | 0.229 |
| > 2 | 173 | 84 | 48.6 | | | |
| Sex | | | | | | |
| Male | 216 | 107 | 49.5 | 1.141 | 1 | 0.285 |
| Female | 184 | 101 | 54.9 | | | |

Table 3: Multivariate association of anti-toxoplasma antibodies positive status of Toxo-latex agglutination test in EL-Gadarif state, from July to November 2015

| Risk factors | No. of tested | No. of positive | % of positive | Exp(B) | P-value | 95% CI (Lower – Upper) |
|-------------------------|---------------|-----------------|---------------|--------|---------|------------------------|
| Species | | | | | | |
| Goats | 200 | 93 | 46.5 | ref | | |
| Sheep | 200 | 115 | 57.5 | 2.00 | 0.022 | 1.105 – 3.528 |
| Localities | | | | | | |
| El-Gadarif municipality | 100 | 30 | 30.0 | ref | | |
| Al-Fao | 100 | 73 | 73.0 | 6.71 | 0.001 | 3.589 – 12.52 |
| Al-Hawatah | 100 | 51 | 51.0 | 2.44 | 0.003 | 1.353 – 4.381 |
| Wasat El-Gadarif | 100 | 54 | 54.0 | 2.87 | 0.001 | 2.868 – 5.170 |
| Breeds | | | | | | |
| Balady goat | 128 | 58 | 45.3 | ref | | |
| Wateish sheep | 111 | 63 | 56.8 | 1.58 | 0.178 | 0.949 – 2.643 |
| Agbash sheep | 89 | 52 | 58.4 | 1.20 | 0.258 | 0.982 – 2.930 |
| Nuoby goat | 72 | 35 | 48.6 | 1.14 | 0.640 | 0.640 – 2.036 |

decreased probability (OR≠1) of sero-positivity occurrence in comparison to the reference (OR=1). The factors that were associated with increased odds of being Toxo-latex agglutination test positive were species (sheep) and locality (Al-Fao, Al-Hawatah, and West El-Gadarif).

DISCUSSION

Toxoplasmosis is one of the most not uncommon parasitic diseases world-wide (Tenter, 2009; Innes, 2010b; Hammond-Aryee et al., 2014). It has been reported in many species of animals including cats, dogs, small and large ruminants and equines. Besides, circa 2 billion people are chronically infected with this disease around the globe (Tenter, 2009; Prandota, 2009; Innes, 2010b; Cenci-Goga et al., 2011; Hammond-Aryee et al., 2014).

The overall sero-prevalence of anti-toxoplasma antibodies reported in this study was considerably high and was comparable to the sero-prevalence reported by Khalil and Elrayah (2011) in sheep but it was higher than the overall sero-prevalences reported by Khalil and Elrayah (2011) and Elfahal et al. (2013) in Khartoum and Al-Gezira. It was also higher than the seroprevalence reported in the southern part of Africa which was found to be 4.5% (n= 600) in sheep. On the other hand, it was lower than the one reported in Zimbabwe (67.9%; n=335) in small ruminants. In general, varying seroprevalences were reported from different countries and different animal species; for examples Hammond-Aryee et al. (2014) found out that 38.2 and 26.8% of the sheep and goats investigated in Ghana were seropositive and as high as 98.4% of the tested sheep in Egypt. Besides to small ruminants, toxoplasmosis has been also detected in horses, donkeys, camels and humans and in wild animals in the Sudan (Elnahas et al 2003; Tenter, 2009; Khalil et al., 2012; Khalil et al., 2013; Shadia et al.,

2013; Abdel-Raouff and Elbasheir, 2014; Gebremedhin et al., 2014; Hammond-Aryee et al., 2014). Density of cat population and the frequency of contact between cats and susceptible animals could probably lead to variations in the prevalences of toxoplasmosis. Many sheep and goat owners keep their animals in the house where they live, hence more contact between these animals and cats and so higher risk of contracting toxoplasmosis.

The results of the present study indicated that there was no between-species differences in sero-positivity and this was disagreeing with the findings of Khalil and Elrayah (2011) and Elfahal et al. (2013) who found out that 57.5% of the surveyed sheep samples were toxoplasmosis- positive and 32.0% and 20.0% of the cattle and camel samples, respectively. Since the infective stage of this protozoan parasite is swallowed with the ingesta, the dissimilarity of feed intake and feeding behaviors of different animal species might likely result in a diverse sero-positivity among these animal species. Grazers, such as cattle, browsers like goats and camels, and sheep which are classified as intermediate have different grazing behaviors of searching for and selecting the type of grasses or feed as well as grasping and intaking of the selected grasses or feed. Cattle are less selective to their forage in comparison to small ruminants and so they eat more dead material. Sheep and goats have narrower mouths and more flexible lips. The different anatomy of the mouthparts makes it more difficult for cattle to select leaves of woody plants (browse) as camels do (Iqbal and Khan, 2001; Mosavat and Chamani, 2013).

Concerning the prevalence in different age groups, the findings of the present study disagreed with the findings of Nematollahi and Moghddam (2008) and Elfahal et al. (2013) who found that the prevalence of *T. gondii*-

infection was significantly higher in young animals than in old animals. [Yin et al. \(2015\)](#) found positive samples in all age groups with variations but the highest prevalence was detected in young animals. This may reflect the dominance of maternally acquired antibodies in this age group or that cattle unless re-infected, deplete antibodies as their age increases. Furthermore, age was an important factor for being seropositive as a measure of the cumulated life-time risk. [Ahmad et al. \(2015\)](#) indicated that the likelihood of infection increased with age in sheep and goats.

There were significant statistical differences between the sero-prevalences of the surveyed localities and this was inconsistent with the findings of [Elfahal et al. \(2013\)](#) who found no significant relationship between antibody prevalence and location and that the prevalence was 12.7% in Khartoum state and 14.9% in Al-Gazira state whereas the by locality prevalences were 12.9%, 14.0%, and 10.3% in Khartoum, Khartoum North and Omdurman localities, respectively, and 25.0% in Alkamleen and 12.8% in Wad Madani localities. The data reported by [Hammond-Aryee et al. \(2014\)](#) suggested a geographical trend of toxoplasmosis in humans and animals. [Elfahal et al. \(2013\)](#) also indicated that geographical variations might occur not only among different countries but also within countries. [Yin et al. \(2015\)](#) reported that the seroprevalence of toxoplasmosis in Tibetan sheep in Tianzhu and Maqu areas were statistically different.

There was significant statistical difference between the sero-prevalences of the different breeds. This was in disagreement with the results of [Elfahal et al. \(2013\)](#) who found no significant relationship between antibody prevalence and breed. [Ahmad et al. \(2015\)](#) found no significant breed-related difference with regard to seropositivity in goats, while it was significantly high in salt range sheep as compared to other breeds of sheep.

There was significant statistical difference between the sero-prevalence reported from males and females. [Elfahal et al. \(2013\)](#) found higher seroprevalence of *T. gondii* in males (30.8%) than in females (11.9%). Moreover, [Yin et al. \(2015\)](#) made the same observation. However, several studies indicated that the prevalence was higher in females than males which was probably due to the lower levels in immune response or antibody persistence of females in some periods of their lives ([van der Puije et al., 2000](#); [Lopes et al., 2010](#); [Kamani et al., 2010](#)). The longer production life span of females in comparison to males together with being subjected to more stressors (pregnancy and lactation) might make females less resistant to toxoplasmosis.

Among the risk factors investigated by [Rêgo et al. \(2016\)](#), extensive rearing system, number of domestic cats on the farm, and domestic and wild dogs access to the water trough used by the sheep were associated with increased risk of toxoplasmosis, in addition to cats feeding on placenta remains, sex, and breeding in the two investigated areas in Brazil.

[Ahmad et al. \(2015\)](#) found that poor hygienic conditions, presence of cats, extensive farming practice, flock size larger than 50 individuals and pregnancy as risk factors related to toxoplasmosis in sheep. Similarly, poor hygienic condition, usage of outdoor water source, presence of cats, extensive farming practice and flock sized larger than 30 and 50 individuals were factors that influence the occurrence of toxoplasmosis in goats. [Yin et al. \(2015\)](#) indicated that numbers of past pregnancies of Tibetan sheep were not a significant risk factors ($P>0.05$) and was left out of the final model, however, season was considered as risk factor for the infection. [Cenci-Goga et al. \(2013\)](#) five variables (flock size, production system, stagnant water, resident cats, access of stray cats to water) were selected for inclusion in the multivariable Poisson model. The final Poisson model contained only two predictors (flock size and access of cats to water).

CONCLUSION

It can be concluded that the prevalence of anti-toxoplasma antibodies is relatively high and species and locality while there was no between-species variation in sero-positivity. These findings warrant further in-depth investigations to be carried out to allow better understanding of the epidemiology of the disease not just in sheep and goats but in other animal species. Molecular characterization of the parasite and to assess the likelihood of zoonotic transmission are of high importance in the Sudan.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

AUTHORS' CONTRIBUTION

SHI, SES and MAA conceptualized and supervised the work. HBA, HHI, and AKM did the field work, sampling

and lab procedures. YAS and SES wrote the draft of this manuscript, analyzed the data. All the authors read and approved the final version of manuscript before submission.

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