

Low seroprevalence of antibodies to *Toxoplasma gondii* in blood donors in central Namibia

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Although emphasis has been placed on research relating to human immunodeficiency virus (HIV), tuberculosis and malaria, several researchers in Africa are focusing on other threats to human health, such as neglected tropical diseases. *Toxoplasma gondii* is a possible neglected tropical disease in Namibia, although the country has a diversity of climate, ranging from tropical in the north to semi-desert in the south. Except for one study in 1978, no recent studies have determined the burden of *T. gondii* infection in Namibia. Three hundred and twelve convenience samples were collected from volunteer blood donors in central Namibia. Donors provided informed consent to participate in the study, and 5 ml blood was collected. Demographic information was collected by means of a questionnaire. Serum was analysed using Captia™ *T. gondii* immunoglobulin G (Ig) G enzyme-linked immunosorbent assay (ELISA) kit. Only samples that tested positive or equivocal for IgG antibodies were then tested for IgM antibodies using Captia™ *T. gondii* IgM ELISA kit. Of the 312 samples, 3 (0.961%) tested positive for IgG antibodies to *T. gondii*. One sample (0.3%) tested positive for IgM antibodies to *T. gondii*. These donors lived in urban areas in central Namibia and interacted regularly with animals, such as cats and dogs. The prevalence of antibodies to *T. gondii* in Namibian blood donors was found to be considerably lower than that reported in other African countries, but comparable to that in a recent report from South Africa. It is notable that most of the donors lived in the arid central regions of Namibia, where the high altitude could also affect parasite survival.

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Introduction

It is estimated that a third of the world's population is seropositive for *Toxoplasma gondii*, indicating that people have been infected with the parasite at some stage of their lives.¹⁻³ Studies to determine the seroprevalence of exposure to *T. gondii* have been conducted in human and animal populations in many countries,⁴ including blood donors.^{2,5-13}

The prevalence of toxoplasmosis has been studied in several African countries, and ranges from 7% in Zambia to 80% in Ethiopia.¹⁴ In Tanzania, occupationally exposed individuals were identified with a seroprevalence of 46%, while 35% and 30.9% of pregnant women were seropositive for *T. gondii* antibodies.¹⁵⁻¹⁷ In Nigeria, a seroprevalence of 20.8% was reported in apparently healthy individuals, and that of 60% in urban and peri-urban Ethiopian residents.^{18,19}

Recent studies in southern Africa have reported lower rates of seroprevalence of toxoplasmosis than previous studies in the rest of the world. While Kistiah et al noted an unexpectedly low prevalence (6.4% in the general population) in neighbouring South Africa, Siteo et al reported 18.7% in Mozambique.^{14,20,21} *T. gondii* is postulated to be one of the neglected tropical diseases in Namibia.²² Except for Jacobs and Mason, no recent studies have determined the burden of *T. gondii* infection in humans in Namibia.⁸

The objective of this study was to evaluate the exposure by blood donors in Namibia to *T. gondii*. Determining the seroprevalence of antibodies to

T. gondii in blood donors might provide an indication of the seroprevalence in the broader Namibian population.^{2,12} Furthermore, it could give an indication of the risk of transfusion-transmitted toxoplasmosis in Namibian blood donations.

Method

Study design and study population

A cross-sectional, prospective study was performed in voluntary blood donors in central Namibia. Three hundred and twenty-nine blood donors volunteered to participate in the study when they donated blood to the Blood Transfusion Service of Namibia between October 2011 to January 2012. An additional 5 ml of blood was drawn in a plain tube for each donor. The serum was aliquot and stored at -20°C before testing. Each participant received an information sheet describing the purpose of the research, as well as a questionnaire in English or Afrikaans, from which demographic data and information on their contact with animals were collected. Only samples with an accompanying survey were used for testing. Informed consent was obtained from the donors, participation was anonymous and voluntary, and donors could opt out of the study at any stage. Permission for the study was granted by the Institutional Research and Publication Committee, the Research Committee of the Ministry of Health and Social Services, and by the management of the Blood Transfusion Service of Namibia.

Data collection

The prevalence of IgG antibodies to *T. gondii* was determined in 312 samples using the Captia™ *T. gondii* immunoglobulin (Ig) G enzyme-linked immunosorbent assay (ELISA) kit (Trinity Biotech, Bray, Ireland). The manufacturer's protocol was followed to determine the outcome

Table I: Characteristics of the study population (n = 312)

Characteristics	n (%)
Gender	
Females	119 (38.1)
Males	193 (61.9)
Age (years)	
20 and younger	38 (12.2)
20-29	105 (33.7)
30-39	93 (29.8)
40-49	54 (17.3)
50 and older	22 (7.1)
Areas*	
North	24 (7.7)
Central	271 (86.9)
South	17 (5.8)
Residence	
Rural	53 (17)
Urban	253 (81.1)
Unknown	6 (1.9)
Interact with animals on a regular basis?	
No	13 (4.2)
Yes	299 (95.8)
Work with animals on a farm?	
No	212 (68)
Yes	100 (32)

*Regions: North (Caprivi, Ohangwena, Oshikoto, Oshana, Omusati and Kunene); central (Otjozondjupa, Erongo, Khomas and Omaheke) and south (Hardap)

Table II: The seroprevalence of immunoglobulin G and immunoglobulin M antibodies to *Toxoplasma gondii* in Namibian blood donors and the corresponding demographic information

IgG	IgM	Age	Gender	Region	Residence	Live with animals	Kinds of animals	Work with animals
Equivocal	Negative	32	Male	Oshikoto	Urban	Yes	Cattle, goats and dogs	No
Positive	Negative	61	Female	Khomas	Unknown	Unknown	Unknown	Unknown
Equivocal	Negative	31	Male	Khomas	Urban	Yes	Dogs	No
Equivocal	Negative	35	Male	Khomas	Urban	Yes	Cattle, goats and dogs	No
Positive	Negative	18	Female	Khomas	Urban	Yes	Cats	No
Positive	Positive	29	Male	Khomas	Urban	Yes	Dogs and cats	No

IgG: immunoglobulin G, IgM: immunoglobulin M

of the test results. Samples with an equivocal IgG result were retested with the same kit and included in the negative results if still equivocal. Serum samples that tested positive or equivocal for IgG antibodies were further analysed using the Captia™ *T. gondii* IgM ELISA kit. Results from the ELISA tests, as well as responses from the questionnaires, were entered into a Microsoft® Excel® spreadsheet. Statistical analysis was performed using SPSS® version 21. An association between the population characteristics in the surveys and the seropositivity results was not calculated owing to a low prevalence of positive results.

Results

Study population

Of a total of 329 samples, only 312 samples were included in the study as accompanying demographic information was lacking for the rest. The majority (61.9%) of donors were male. Their ages ranged from 16-64, with a mean age of 32.2 ± 11.3 years. Most of the donors (75.7%) were aged 40 years and younger. Donors from different regions in Namibia were included in the study, although the majority (86.9%) were from the central region. Most participants (81.1%) indicated that they lived in urban areas, 95.8% with animals and 32% with animals as part of their occupation. 22.1% of the respondents indicated that they owned cats, while 78.2% of respondents were dog owners. Many respondents were farmers who also had contact with other animals, such as cattle, goats and horses. The demographic distribution of the donors and their exposure to animals is summarised in Table I.

Seroprevalence

Of the 312 samples tested, 3 (1%) were positive and three tested equivocal for IgG antibodies to *T. gondii*. All three IgG-positive donors were urban dwellers in central Namibia and had indicated that they lived with animals, such as cats and dogs. Their ages ranged from 18-61. Two of the three IgG-positive donors were women (Table II). Of the six samples tested for IgM antibodies, 1 (0.3% of the total donors) was positive. This donor was a 29-year-old man who owned cats and dogs.

Discussion

The low prevalence of *T. gondii* antibodies in Namibian blood donors was much lower than the 7-80% reported in other African countries.¹⁴ However, the prevalence is comparable to that of toxoplasmosis in blood

donors in China, which varied from 0.4–20.2%.²³ Alvarado-Esquivel et al reported a 7.4% IgG prevalence of *T. gondii* in healthy blood donors in Mexico, with an IgM prevalence of 1.9%.² A low prevalence of toxoplasmosis (3% in healthy individuals) was also reported in Thailand.¹³

While no specific studies have been published on toxoplasmosis in humans in Namibia, one study reported on its prevalence using Namibian serum samples. Jacobs and Mason carried out an indirect fluorescent antibody survey of the prevalence of *Toxoplasma* antibodies using sera from southern Africa (Natal, Eastern Cape and Western Cape, South Africa) and south-west Africa (Namibia) and Botswana.⁸ The overall prevalence in that study was 20% of 3 379 sera tested. Serum samples were obtained from the San (n = 725) in eastern Namibia, Damara speakers (n = 77) in the Erongo region and urban white residents (n = 261) in the Windhoek area, for the Namibian component of the Jacobs and Mason study. Prevalence rates were 12% in the white residents in Windhoek, 27% in the Damara speakers in Damaraland and 9% in the San. The differences in culture were thought to play a role in the variations in the prevalence of toxoplasmosis in the Namibian groups.⁸

In the current study, the low prevalence in central Namibia indicates a substantial decline from that documented by Jacobs and Mason.⁸ This is in line with the trend in Europe and New Zealand, where a decrease in the prevalence of toxoplasmosis has been seen over the past few decades.¹² One of the reasons for this decline could involve the more common use of frozen meat since the consumption of previously frozen meat is associated with a lower risk.^{24,25} In Europe and America, the consumption of raw or undercooked meat, as part of cultural practice, is the main risk factor for toxoplasmosis.^{25,26} This is not the case in certain populations of southern Africa, although some restaurants serve rare or underdone beef and game steak, particularly popular with tourists. Additionally, the results are comparable to those recently published for South Africa, which also describe a lower seroprevalence of *T. gondii* than that previously reported.^{14,21} This could be attributed to common socio-cultural practices and environmental aspects in the region.⁵

Age and the gender distribution of the blood donors compare well to those in other studies. Most of the blood donors were recruited in the Khomas region, where the capital city is 1 650 m above sea level. High altitude is usually associated with a lower prevalence of toxoplasmosis.² Furthermore, the exceptionally dry conditions encountered in the central regions of Namibia could have also contributed to low exposure to *Toxoplasma* spp. as oocysts are sensitive to desiccation.³ However, the situation may differ in other regions, like the Kavango and Caprivi, where warm, humid conditions prevail and communities share water resources with livestock and wild animals.

Five of the possible six *T. gondii*-positive donors engaged in regular interaction with cats and/or dogs, which have been investigated as risk factors for toxoplasmosis, e.g. contact with dogs and farming activities, as well as living with cats at home.^{2,27,25} As toxoplasmosis has been reported in domestic and wild animals in Namibia, there is the potential risk of transmission to humans since the reservoir for the parasite exists in the country.^{28,29} However, it is notable that the majority of respondents in this study were dog and/or cat owners, but their

exposure to toxoplasmosis was very low. This result is supported by that in another study in which an association between the seroprevalence of *Toxoplasma* spp. and cats in the household or contact with dogs was not found.²⁷

One limited but important aspect of this study involves the possibility of the transmission of *T. gondii* via the national blood transfusion service. The transmission of *T. gondii* through blood transfusion has been well documented.³⁰ *T. gondii* may be transmitted through the transfusion of infected blood since trophozoites can survive for several days in blood and blood products, especially leukocytes.^{31,32} The parasite can survive in citrated blood stored at 4°C for 50 days. In India, after malaria, toxoplasmosis is the most important protozoan disease that is transmitted by blood transfusion.⁵ While the overall risk is probably low for a donor transmission event, the donor who tested positive for IgM antibodies could have potentially been at risk of blood safety being compromised through transfusion, especially if the recipient was a pregnant woman or an HIV-positive patient.⁷ *T. gondii* is currently not part of donor blood screening in Namibia, although it has been proposed in other countries, like India, where high rates of toxoplasmosis exist in the population.⁵

The small, non-random sample used for the study made it difficult to identify any regional trends and establish risk factors based on the questionnaire responses. As most of the study population came from the central arid regions, it would be important for future studies to include the northern tropical regions, in order to obtain a holistic epidemiological picture of *T. gondii* in Namibia. However, evaluating the exposure of volunteer blood donors is a valuable indicator of the epidemiology of an infectious disease in the broader population in the same community.² Therefore, the low seropositivity in central Namibia indicates that the broader population in this area probably has limited exposure to *T. gondii*.

Conclusion

This study, while regional in nature, demonstrated that *Toxoplasma* spp. occurs in Namibia. Further studies are needed to evaluate the current status of the parasite in human and animal populations. Further point prevalence studies should be conducted in the country, particularly in the areas of high prevalence reported by Jacobs and Mason. Occupational risks for abattoir workers, especially those handling goats and sheep in the slaughtering process, should also be established. It is anticipated that an increase could be indicative of a warming trend accompanied by higher humidity to keep the oocysts from desiccating. Ideally, the prevalence of toxoplasmosis in at-risk groups, such as pregnant women, their neonates and persons living with HIV, should also be evaluated.

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