

SEROPREVALENCE OF *TOXOPLASMA GONDII* IN NAZARET TOWN, ETHIOPIA

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Abstract

Objective: To determine the seroprevalence of toxoplasmosis, assess its zoonotic importance and identify factors associated with seroprevalence.

Methods: Questionnaire survey was conducted on 65 serum samples collected from male and female urban and peri-urban residents aged between 15 days and 65 years. Main outcome measures were feeding habits, purpose of keeping cats and association with family members. Serologic evidence of toxoplasmosis was conducted by the Modified Direct Agglutination Test (MDA T) and determination of HIV status using the HIV - Spot Test.

Results: Over 50% of the interviewed people had a history of consumption of raw or undercooked mutton and had close contact with cats. 60% of the serum samples analyzed by the MDA T had serologic evidence of *Toxoplasma* infection. Significantly higher MDA T tiers were encountered both in pregnant and immunocompromised individuals. The risk factors associated to *Toxoplasma* infection, i.e. raw or undercooked mutton consumption and presence of cats appeared significant.

Conclusion and recommendations: The significance of toxoplasmosis as a disease of zoonotic importance was demonstrated. Close contact between family members and the consumption of raw or undercooked mutton were the major risk factors in the transmission of the disease. Considering the relatively high prevalence as revealed by this study it would be important to conduct studies on a wider scale. It would also be important to increase public awareness and upgrade the knowledge on congenital toxoplasmosis.

Key words : Seroprevalence, *Toxoplasma gondii*, Nazareth Town, Ethiopia.

Introduction

Toxoplasmosis is a disease caused by an obligate intracellular protozoan organism *Toxoplasma* that infects a wide range of animals, man and birds (1). Although *Toxoplasma gondii* has feline species as definitive hosts, a wide range of animals serve as intermediate hosts contributing to the maintenance of infection in nature through harbouring viable stages of the parasite in tissues.

The transmission of *Toxoplasma gondii* to humans occurs through accidental ingestion of sporulated oocysts or the consumption of raw or undercooked meat. Among meat producing animals pigs, sheep and goats relatively often harbour *Toxoplasma gondii* cysts in edible tissues and, therefore, raw or undercooked meat from these animals constitutes a major risk to humans. In areas where goat milk is utilized, unpasteurized milk from acutely diseased goats is also an important source of infection especially to children (2). *Toxoplasma gondii* can also be transplacentally transmitted from the mother to the offspring if the infection is contracted during pregnancy (2). Congenital toxoplasmosis is a hazard in areas where the disease is prevalent (3, 4).

All forms of toxoplasmosis that occur in normal individuals may also occur in immunocompromised patients. People who have previously acquired toxoplasmosis, with or without manifestation of the disease may suffer a devastating relapse if their immune defences, particularly cell-mediated immunity are impaired as in the case of Acquired Immuno Deficiency Syndrome-AIDS (4).

The prevalence of *Toxoplasma RO/Idii* and the relative contribution of the various routes of transmission in humans in Ethiopia have not been adequately studied. The objectives of this study were to determine the seroprevalence of toxoplasmosis, to assess the zoonotic importance of the disease and to identify factors associated with seroprevalence.

Methods

Study Area:

This study was conducted from November 1999 to March 2000 in Adama Hospital, Nazareth, Ethiopia. Nazareth is a town 90 kms southeast of Addis Ababa, situated in the Rift Valley, 39.117°N and 8.33°E with an altitude of 1622 masl. The town receives an annual rainfall of 400-800mm and temperature ranging from 13.9°C to 27.7°C (5).

Study subjects and variables:

The study subjects consisted of randomly selected male and female individuals aged 15 days - 65 years visiting the Adama Hospital during the study period. Consumption of raw or undercooked mutton, contact with cats and infection with HIV-AIDS infection were considered as the most important study variables on the basis of previous investigations (6, 7).

Study design :

A cross-sectional study was conducted to determine the prevalence of toxoplasmosis among individuals visiting Adama Hospital, for various health problems. After reviewing daily-patient flow to the hospital expected patient population in the study period was taken as a sampling frame. Systematic random sampling method was used to recruit the required sample size over the study period. Simple questioner was administered to get relevant information about the patient. For serological test approximately 4ml of blood was taken from each subject and the serum stored in aliquots at -20°C until tested.

Questionnaire survey:

A questionnaire was designed and instituted to obtain relevant information on the study variables. 65

individuals visiting the Adama Hospital for various health problems were interviewed about their feeding habits to determine the probable source of infection. They were also asked the purpose of keeping cats and the association that cats have with their family members.

Serological Tests:

Modified Direct Agglutination Test: Approximately 4ml of blood was taken from each of the 65 subjects and the serum stored in aliquots at 20°C until tested. Antibodies to

Toxoplasma gondii were determined by the test using a commercially available kit (Antigene Toxo-Screen AD, Biomerieux SA, Leon, France) and the technique described by Patton *et al.*(8). The Modified Direct Agglutination Test (MDAT) employs intact tachyzoites as antigen; hence antibodies (IgG) to surface antigens are detected. The advantage of the MDA T is that it is easy to perform, can be used on any species since specific conjugates are not used and the results obtained strongly correlate with other serological tests (Dubey *et al.* 1990).

HIV-Spot Test: This was applied as a screening test to detect antibodies to either of the two immunodeficiency viruses: HIV -I and / or HIV -2 using the kit made available by Adama Hospital. Though the performance of this test may not be considered ideal by some investigators it is being widely applied in the country. The test employs capturing reagents absorbed on a porous membrane that can trap antibodies to I-II V -I and/or HIV -2. Positive results were indicated by the development of red spots on the membrane (Genelab Diagnostics, Singapore Science Park, and Singapore).

Ethical Considerations:

Attempts were made to address ethical issues as per the guidelines for biomedical research. Hence, informed consent was obtained from patients and for individuals who were not capable of doing so proxy consent from the guardians was obtained. Appropriate counselling and care of HIV positive patients was done as per the routine guidelines of the hospital.

Sample size determination and data analysis:

Sample size was estimated with the assumed toxoplasmosis prevalence of 96.3% on the basis of previous study (6), effect size of 4% and 90% confidence interval. After data collection study subjects were classified by sex, into three age categories and by HIV status. In each of these categories percentage of reactive persons was determined and reactivity versus different dilution of sera was also assessed. Fisher's exact test was used to evaluate association of the MDAT and the two risk factors: ownership of cat and feeding habits. Odds ratio and corresponding 95% Confidence Interval (CI) were used to quantify the degree to which *Toxoplasma gondii* seroprevalence is associated with feeding habits of the study subjects and presence or absence of cats in their house. STATA ver. 6 was used for data analysis.

Results

Questionnaire survey: The survey showed that 86% (56/65) of the interviewed people had a history of consumption of raw or undercooked mutton. No individual consumed raw goats. None of the cats owned by the interviewed people were properly managed but rather left to scavenge and clean-out rodents from the surrounding environment. Cats had close contact with most family members.

Serology

Of the 65 people examined for *anti-Toxoplasma gondii* antibodies by the MDAT, serologic evidence of toxoplasmosis was found in 60% (39/65) of them (Table 1). Although a large number of the seropositive were females (64.1%), the difference was not statistically significant ($p=0.445$). The 65 individuals examined for *anti-Toxoplasma gondii* IgG antibodies were also tested for HIV infection by the HIV-Spot Test. Nineteen of the 65 individuals considered in the study were HIV positive and, of whom 10 were seropositive (Table 2). Almost all the HIV-Spot Test positive individuals were reactive to the MDAT at dilutions greater than or equal to 1:1024. The majority of the Spot negative individuals were reactive at dilutions of less than or equal to 1:512. Five pregnant women were also positive for toxoplasmosis.

Table 1.Total number and percentage of people reactive at different dilutions of sera by sex and age

Sex and age of people tested	No. studies	No. (%) positive					Reciprocal MDA T titers					
Sex	26	14 (53.8)	32	64	128	256	512	1024	2048	4096	8192	
Male	39	25 (64.1)	1	-	1	4	1	2	3	2	-	
Female	65	39 (60)	2	4	4	2	1	2	5	4	1	
Total			3	4	5	6	2	4	8	6	1	
Age (years)												
<21	15	8 (53.3)		-	1	2	-	-	1	2	-	
22 – 43	36		2	4	2	4	1	3	4	4	1	
>44	14	24 (66.7)	1	-	2	-	1	1	3	-	-	
Total	65	7 (50)	3	4	5	6	2	4	8	6	1	

Table 2 Distribution of MDA T titers in Spot + and Spot – individuals

HIV-Spot test	No. studies	No. reactors (%)	Reciprocal MDA T titers								
			32	64	128	256	512	1024	2048	4096	8192
Positive	19	10 (52.6)	-	1	-	-	-	2	3	4	-
Negative	46	29 (63)	3	3	5	6	2	2	5	2	1
Total	65	39 (60)	3	4	5	6	2	4	8	6	1

Table 3 Association of Toxoplasma infection with feeding habit and cat ownership

Characteristic of the interviewed positive for MDA T	No. (%)	OR (95%CI)	P-value
Feeding Habit		16.9 (1.9–145.6)	0.002
Raw/undercooked mutton	38 (67.9)	-	-
Well-served mutton	1 (11.1)	-	-
Presence of cat in the house		5.3 (4.2 – 6.7)	0.002
Present	24 (80.0)		
Absent	15 (42.9)		

The risk factors associated with seropositivity to toxoplasmosis; raw or undercooked mutton consumption and the presence of cats, identified by the questionnaire, were further evaluated. Individuals consuming raw or undercooked mutton were found 16.9 (95% CI: 1.9 - 145.6) times more likely to be positive than those known to consume well-served mutton (Table 3)

Furthermore, a statistically significant association was observed between the presence of cats in the family environment and seropositivity ($p=0.002$). Individuals with a known history of association with cats were 5.3 times more likely to be seropositive than those with no history of such association (Table 3).

Discussion

Several studies conducted so far indicated that *Toxoplasma gondii* infection in humans is widely distributed in most tropical countries (2, 9, 10). The infection rate reported in this study was higher than those reported for other African countries (7,11, 12) The prevalence of toxoplasmosis in Ethiopia varies from 8% to 89% (6, 13-16). One of the causes for this high prevalence is attributed to the close contact of humans with cats. Over 50% of the questionnaire respondents stated that they had close contact with cats, which were kept for mice/rats control. The relatively higher number of cats shedding oocysts in their faeces in the study area (12.5%), as demonstrated in a separate study (17), strongly depicts the probable contamination with cat faeces (17). This has been confirmed by the fact that people with a known history of association with cats showed a higher rate of positivity.

Another aspect of *Toxoplasma* infection is the potential risk of transmission to people by consumption of raw or undercooked meat. Although we did not produce experimental evidence the high prevalence of

Toxoplasma in this study may be partially attributed to the consumption of raw or undercooked meat, a custom very common in some parts of Ethiopia. More than 50% of the people surveyed confirmed the consumption of raw or undercooked mutton. The high prevalence of *Toxoplasma* infection in sheep and goats in the area (17) coupled with viable cysts remaining in organs/tissues for a long period of time and cysts usually being killed at 60°C and -18°C (18,19) strongly suggests that small ruminants represent major source of infection to humans.

The fact that toxoplasmosis was found in 5 pregnant women, though difficult confirm whether this was a primary or resurgent infection, the work has indicated that congenital infection may constitute a hazard in the area as elsewhere in the world(3,4). The reactivity of a significant number of HIV Spot positive individuals -at -higher -dilutions-in-titre-MDA T-in -this study may reflect recrudescence of infection. Toxoplasmosis is an opportunistic infection in immunocompromised individuals. In the context of an infection with the human immunodeficiency virus none of the classical assays differentiate between primary and resurgent *Toxoplasma gondii* infection (7,20).

Despite the limitations of relatively smaller sample size as a result of extreme seroprevalence used to determine the sample size and the HIV Spot Test currently not being found ideal, the study has provided valuable information on a very important problem of zoonotic significance.

Considering the relatively high prevalence of toxoplasmosis as revealed by this study it would be important to increase public awareness about the infection in rural and urban communities. Emphasis should be made on the risks associated with the consumption of raw / undercooked meat and milk and minimize contact with cats especially with regards to pregnant women. It would also be useful to upgrade the knowledge on congenital toxoplasmosis by conducting further studies.

Acknowledgments

We thank Prof. Sharon Patton for providing the MDA T kit and W/o Zenebech Bekele for typing the manuscript. The support and cooperation of the staff of Adama Hospital is greatly acknowledged.

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