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SERO-PREVALENCE OF *TOXOPLASMA GONDII* INFECTION AND ASSOCIATED RISK FACTORS IN HUMAN POPULATION OF KASHMIR, INDIA

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ABSTRACT

The overall aim of this study was to determine the prevalence of the *Toxoplasma gondii* in population of Kashmir valley and to study associated environmental and food habitat risk factors affiliated with infection. A total of 1055 sera samples were collected from three selected areas of Kashmir valley viz. central, south and north Kashmir. Serological results and epidemiological data collected through a standard questionnaire were statistically analyzed. The overall prevalence was 11.27% by using ELISA IgG. The association of the risk factors to the disease discussed in this study. Consumption of raw/undercooked salad, meat and exposure to soil through farming or gardening have been associated with a higher risk of infection. The present study indicates the need for public health education to raise awareness of risk factors for toxoplasmosis.

KEYWORDS

Toxoplasma, Seroprevalence, ELISA and Risk factors.

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INTRODUCTION

Toxoplasma gondii is a zoonotic intracellular protozoan parasite of worldwide distribution¹. The seroprevalence varies widely in different regions of the globe, measuring between 30% and 60% in most countries². *Toxoplasma gondii* is a ubiquitous parasite of warm-blooded animals that causes one of the most common parasitic infections in humans³.

Toxoplasmosis is most dangerous to two populations: immune compromised patients and foetuses whose mothers acquire acute infection during pregnancy⁴. Human infection generally occurs through the ingestion of raw or undercooked meat that contains cysts, through the ingestion of water or food contaminated with oocysts, or

congenitally through trans-placental transmission from a mother infected during pregnancy. The clinical implications of Toxoplasma infection in pregnant patients are manifold. Such patients may have spontaneous abortions, still births or premature delivery in addition to various fetal anomalies⁵. Ideally every woman should know her toxoplasma status before conception. Toxoplasma antibodies may persist in the serum of an asymptomatic people for years at higher titres⁶.

The prevalence changes according to social and cultural habits, geographic factors, climate, transmission route and it typically increases with age^{7,8}. It has been reported that the prevalence is higher in warm and humid areas^{7,9}. Toxoplasmosis is not a reportable disease and disease prevalence is based on regional studies. Serological surveys indicate that about 80% of all primary infections are asymptomatic, due to the effectiveness of the immune system¹⁰. Latent toxoplasmosis, i.e., the lifelong presence of cysts and anamnestic concentrations of anti-*T. gondii* antibodies in immune competent subjects, is considered asymptomatic and harmless. The present work was designed to provide the valuable information on the seroprevalence of *Toxoplasma gondii* in humans with special reference to associated risk factors in the Kashmir valley.

MATERIAL AND METHODS

Study area

The study was carried out between January and December 2015 in three selected areas of Kashmir valley viz. central, south and north Kashmir. Sampling was done in public health centers, private diagnostic laboratories and from the both urban and rural localities by using simple random sampling method.

Study design

A cross-sectional descriptive study involving the human population both male and females of all age groups. Each participant was provided with a questionnaire to promote confidentiality and to know about the associated risk factors. A venous blood sample was drawn from each participant and

was given a unique identifying number for further laboratory processing.

Sample size

The following formula was used for calculating the adequate sample size in prevalence study¹¹.

$$n = \frac{1.96^2 \cdot P_{\text{exp}}(1 - P_{\text{exp}})}{d^2}$$

Where n = required sample size

P_{exp} = expected prevalence= 20%

d = desired absolute precision=5%

Hence, d = 0.05 and p= 0.5 (50%).

The expected prevalence in the study area was considered 20% due to non-availability of previous epidemiological references. Thus the minimum desired annual sample size was calculated to 246.

Data collection method

The study participants were interviewed by administration of questionnaire to obtain influential risk factors considered in the study which included economic status, sources of drinking water and cooking - eating habits such as: eating raw or undercooked meat, tasting raw food while cooking. All the data were then entered into a computer spread sheet, Microsoft Excel® (Microsoft Corporation, USA) followed by descriptive analysis using SPSS Version 16.0 (SPSS, Inc., Chicago, IL, USA).

Sample collection and sample processing

About 5mL of venous blood were collected aseptically from each of the 1055 participants and were tested by commercially available DS-EIA-Anti-Toxo-M-FAST for detection anti-*T.gondii* antibodies according to manufacturer's instructions and results were also interpreted according to the guidelines provided by the manufacturer. Plasma samples were numbered and tested for anti-*T.gondii* antibodies (IgG) using ELISA assays.

RESULTS

A total of one thousand and fifty five (1055) humans (both male and female) aged from 01 to >40 years from three regions of the valley were involved in the study. The overall seroprevalence was found 11.27% (119/1055). Of these, 36.20% (51/382) were from central Kashmir, 32.98% (36/348) from South Kashmir and 30.80% (32/325)

from North Kashmir. The prevalence rate among the three regions remained almost constant with highest (36.20%) in central Kashmir and lowest (30.80%) in north Kashmir. The distribution of various age groups is shown in Table No.1. The results showed 9.67% (03/31), 13.84% (9/65), 7.00% (11/157), 8.17% (21/257), 10.75% (40/372), 17.17% (17/99) and 24.32% (18/74) were in the age brackets upto 15, 16-20, 21-25, 26-30, 31-35, 36-40 and >40 respectively. However, seroprevalance increased with age (figure 1). Prevalence was lower in 21-25 age groups, but steadily increased 24.32% in those who were older than 40 yr. The results were statistically significant ($p < 0.005$) among the three regions.

Most of the population studied (62.93%) belonged to low or low income groups. The majority of people resided in concrete houses but consumed tube well/hand pump water without using any disinfectant/filter or other treatment. The overall difference in the living conditions among the three regions was not statistically significant. However, type of residence showed a marked variation in which in all the three regions people were living in cemented houses. General Socio-economic characteristics and associated risk factors of this population are shown in Table No.2. Most of the population in present study were from the rural population and had contact with domestic animals (cattle, sheep and goat), chicken and the presence of cats and their littered fecal matter in and around houses was also observed. The majority of population is from rural backgrounds who are engaged in mixed farming used livestock manure for cropping with bare hands which is an important factor, as cat faeces might get mixed with livestock manure, thus posing a high risk of transmission of *T. gondii* oocysts. The major socio-behavioral characteristic of the population studied (67.20%) showed consumption of raw salad that included carrots, turnip, cucumber, tomato, cabbage, cauliflower, nelumbo, fruits especially in the house-keeping women having agricultural background. A considerable proportion of the study population 651 (61.70%) reported tap water as the main source of water, whilst 404 (38.29%) obtained water from

Wells/Rivers/Streams/Hand pump most of which water was consumed without treatment or boiling. Level of education and lack of knowledge of the disease might be the major cause of high seroprevalence during their pregnancies.

ELISA test conducted on blood samples to detect anti-*T. gondii* IgG in the three selected study areas showed the seroprevalence of 30.20% (51/382), 32.98% (36/348) and 30.80% (32/325) were positive in Central, North and South Kashmir respectively (Table No.3, Figure No.1).

DISCUSSION

Toxoplasmosis is an environmental disease in which transmission of the infection has been shown to be promoted by poor environmental practices, cat ownership, poverty, poor eating habits, poor hygiene among others^{12,13}. The exposure of *Toxoplasma gondii* most commonly results in an asymptomatic infection but clinical implications of toxoplasma infection in pregnant women and immune compromised persons are manifold.

This study was carried out in Kashmir valley's three main regions with the main objective to determine the seroprevalence of toxoplasmosis and also to investigate any possible associated risk factors. Seroprevalence of *T. gondii* varies indifferent regions of the world¹⁴ and in Asia the seroprevalence ranged from 0.8% (Suwon region, South Korea) to 63.9% (Babol, Iran)¹⁵. Incidence of toxoplasmosis was considered to be very low in India and in Kashmir, as compared to that of Western countries¹⁶. The overall seroprevalence for toxoplasmosis in human population studied was found to be 11.27%. Our findings indicate low *T. gondii* seroprevalence in comparison to previously reported prevalence of 27% in pregnant women in a hospital based study¹⁷ and in one national survey in India seroprevalence for Toxo-Antibodies (IgG) by ELISA were found in 24.3% with lowest seroprevalences in the northern parts of India¹⁸. One plausible explanation for this difference could be that in present study we included human population both male, young and old from all age groups as compared to these hospital based studies were only women from fertile age groups were studied. Our

results are also in agreement with the work were IgM seropositivity was found 49.47% in women with repeated abortions in contrast to 8.88% serum samples were positive for IgM toxoplasma antibody in clinically asymptomatic women¹⁹. However, various studies showed that prevalence of toxoplasmosis in this region is similar to that of other parts of world as those reported in Botswana with a seroprevalence of 11%²⁰. Also, our results are in concordant with results were sero-prevalence of *T. gondii*-specific IgG detected with crude native Type I and type II antigens was 12.2% and 11.3% respectively from 880 clinically healthy individuals in China²¹. A similar study in Zambia found an overall 7% seroprevalence²². In USA 8.9% of seroprevalence was found in women of child bearing age²³. On the other hand higher seroincidence of 25.3% for IgG and IgM was recorded in women's of Bangladesh²⁴. There is a paucity of information on the sero-epidemiology of toxoplasmosis due, in part to an insufficient amount of research into the epidemiology of toxoplasmosis in the study region.

The relatively gradual increase in trend in the seroprevalence of toxoplasmosis with age was observed which suggests that adults may be more prone to infection with *T. gondii* as they would have had more possible encounters with potential sources of infection²⁵. The comparatively high prevalence in the age group 1 to 15 years (9.67%) is suggestive of children engaging in risky behavior such as playing in soil environments which are similar to the findings of other researchers²³.

This behavior was found usually high 13.84% in adolescents (16-20) years which coincides with the findings were overall seropositivity for Toxo IgG was highest in the age group of 15-25 years²⁶. In the age group of >40 years highest infection detection leading us to speculate that immunity may become weak with age which may affect the distribution of toxoplasmosis across ages, with there being a higher risk in the past as an explanation for the age trends, similar trend was recorded in those who were older than 41 years²⁷.

Consumption of raw/undercooked salad, meat and exposure to soil through farming or gardening have been associated with a higher risk of infection in various studies, similar correlation was observed in the present study, but results were statistically insignificant risk factors for toxoplasmosis²⁸⁻³⁰. During the study it was observed that farming practices food, quality of water consumed social considerations and were the most likely factors associated with the prevalence of toxoplasmosis, besides higher age and pregnancies³¹⁻³⁴. Our findings also found that there is no significant association was found between *Toxoplasma* seroprevalence and various possible risk factors^{35,36}. Some disparities in the results may be due to differences in study designs, environmental settings and differences in study populations in which the different studies were carried out. Also, we could not do multiple follow-up sampling to find out true incidence rates in various years and seasons.

Table No.1: Seroprevalence of Toxoplasmosis in humans of three regions of Kashmir valley with their age groups

S.No	Age Group/Study Area	Upto 15 years	16-20 years	21-25 years	26-30 years	31-35 years	36-40 years	>40 years	Total
1	Central Kashmir N=382	14 (3.66%)	24 (6.28%)	61 (15.96%)	98 (25.65%)	122 (31.93%)	36 (9.42%)	27 (7.06%)	(51/382) 36.20%
2	South Kashmir N=348	6 (1.72%)	20 (5.74%)	51 (14.65%)	88 (25.28%)	132 (37.93%)	28 (8.04%)	23 (6.60%)	(36/348) 32.98%
3	North Kashmir N=325	11 (3.38%)	21 (6.46%)	45 (13.84%)	71 (21.84%)	118 (36.30%)	35 (10.76%)	24 (7.38%)	(32/325) 30.80%
4	Total=1055	3/31	9/65	11/157	21/257	40/372	17/99	18/74	119/1055
5	Seroprevalence (%)	9.67	13.84	7.00	8.17	10.75	17.17	24.32	11.27

Table No.2: Socio-economic status and other potential risk factors for acquired Toxoplasmosis in Humans from different regions of Kashmir valley

Socio-economic status/Potential risk factors	N	Farmer/ Gardening	Low/ lower middle	Residence Type		Consume Raw salad/ Undercooked meat	Water Source		IgG prevalence (%)
				Resides in Mud house	Resides in cemented house		Drink Tap water	Drink Well/Rivers /Streams/Hand pump	
Regions of Kashmir									
Central Kashmir	382	256 (67.01%)	230 (68.20%)	31 (8.11%)	351 (91.88%)	283 (74.82%)	208 (54.45%)	174 (45.54%)	51/382 (30.20%)
South Kashmir	348	297 (85.34%)	227 (65.22%)	27 (7.75%)	321 (92.24%)	230 (66.09%)	228 (65.51%)	120 (34.48%)	36/348 (32.98%)
North Kashmir	325	287 (88.30%)	207 (63.69%)	52 (16.00%)	273 (84.00%)	196 (60.30%)	215 (66.15%)	110 (33.48%)	32/325 (30.80%)
Total	1055	840 (79.62%)	664 (62.93%)	110 (10.42%)	945 (89.57%)	709 (67.20%)	651 (61.70%)	404 (38.29%)	119/1055 (11.27%)

Table No.3: Number of cases studied and the respective seroprevalence in three regions of Kashmir Valley

S.No	Study Areas	Central Kashmir	South Kashmir	North Kashmir	Total
1	No. of cases studied	382	348	325	1055
2	No. positive	51	36	32	119
3	Seroprevalence (%)	30.20%	32.98%	30.80%	11.27%

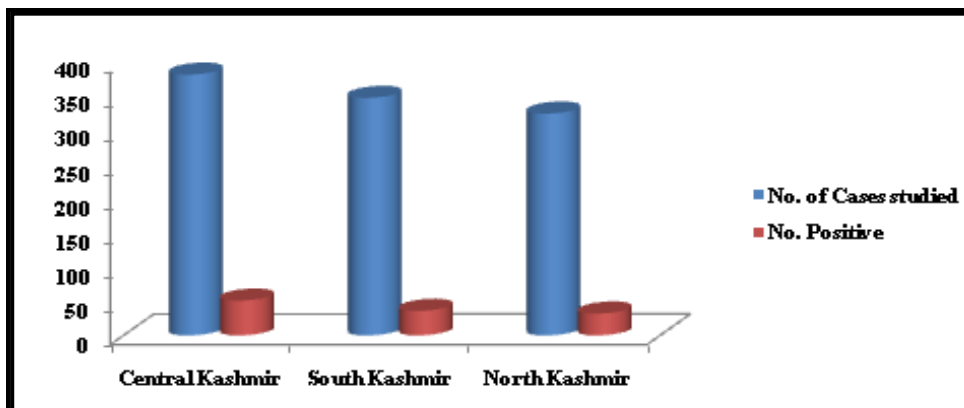


Figure No.1: Number of cases studied and number positive in respective three regions of Kashmir Valley

CONCLUSION

In conclusion, the present study showed the epidemiological seroprevalence of *T.gondii* infection in the human population, for the first time in Kashmir Valley. Suggesting that consumption of raw/undercooked salad, meat and exposure to soil through farming or gardening have been associated with potential risk factor for human infection and indicates the need for public health education to raise awareness of risk factors for toxoplasmosis.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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