SEROEPIDEMIOLOGICAL STUDY OF TOXOPLASMA GONDII INFECTION AMONG WOMEN OF CHILD BEARING AGE.

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ABSTRACT

Sera from 735 Women of Child-bearing age (15-45 years) were tested for the presence of IgG antibodies to toxoplasma gondii by Enzyme-Linked Immuno sorbent Assay (ELISA) technique. In total 263 sera (35.78%) were positive for Toxoplasma antibodies. There was gradual increase in prevalence with advancing age. Highly negative and statistically significant correlation was found between toxoplasma infection and economic status ($r = -0.947$; $p<0.02$). Identical result was revealed between educational status and toxo infection ($P<0.05$). There was no statistically significant difference between married and un married groups as far as prevalence of Toxoplasma antibodies was concerned. prevalence of toxoplasma infection was much higher in women who had a cat as pet at home as compared to those who did not have a pet cat $P<0.05$. This epidemiological model indicates that Faecal-oral spread by oocysts is the main cause of dissemination of infection in Pakistan.

INTRODUCTION

Toxoplasmosis has received world wide attention primarily as a threat to pregnant women, their unborn children and immuno suppressed patient. Toxoplasmosis refer to a clinical disease caused by the sporozoan, Toxoplasma gondii, where as toxoplasma infection refers to the presence of either the tachyzoite form or the cyst form in tissues irrespective of clinical disease[1] The infection may be asymptomatic or may be accompanied by fever or symptoms of lung, liver, heart, brain, lymph node or eye involvement; during pregnancy, foetal infection may result. Immunity is associated with chronic infection, which may recrudesce when a patient is immno suppressed?

Toxoplasma gondii is an obligate intracellular protozoan classified as a coccidian. The two-host coccidian life cycle of Toxoplasma gondii include members of the cat family as definite hosts where the organism has both the enteroepithelial and extra intestinal cycle, while in the incidental (intermediate) hosts such as humans, all orders of mammals, birds and probably reptile as well it exists only in extra intestinal cycle. [1]

The mode of transmission recognized and the stages involved are, first, trans placen-

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tal transmission by tachyzoite, followed by carnivorism via tissue cysts and then faecal-oral spread by oocysts. [2] Toxoplasma infected house mice and other small rodents are important reservoirs of toxoplasma infection. A cat that has eaten a single infected bird or mouse may shed hundreds of thousands of occysts capable of infecting a similar number of intermediate hosts, oocysts persist in moist soil for weeks and months. [3] Human infections originate in several ways: these include the consumption of raw or partially cooked meat containing toxoplasma tissue cysts (bradyzoites); accidental ingestion of food or soil, contaminated with infected feline faeces containing sporulated oocysts or food becomes contaminated with oocysts via cookroaches or flies[4]; consumption of milk containing viable tachyzoies particularly feeding on breast milk of mother infected during puerperium;[5,6] handing infected raw meat and other fresh tissues; through skin penetration in laboratory workers and by transfusion of blood or organ transplant. Blood of asymptomatic carriers is generally safe. [2] The rare routes of transmission include: transmission of tachyzoites by an arthropoda vector like tick; rarely eggs of infected chicken have been found to contain Toxoplasma gondii; and through drinking water contaminated with oocysts.[7]

Frequency of infection varies considerably from one country to another and within a given country. This is due to climactic, soil and other environmental conditions or to some epidemiological features peculiar to the particular regions, such as cultural habits and socioeconomic patterns. It is however, more prevalent in warmer and more moist areas at low altitude[8]. The prevalence of Toxoplasma infection in various part of the world ranges from zero to over 97%. Kean (1972) stated that the Toxoplasma infection involved one-half billion humans. That Toxoplasma infection is a significant public health problem is attested by the fact that the incidence of congenital toxoplasmosis ranges from 1.0 in Montreal, to 7.0 cases per 1000 live births in Vienna.[9] Upto 50% patients with AIDS suffer from fulminating toxoplasmosis.

All the conditions (hot and humid climate, poor socioeconomic conditions, abundance of stray cats, unhygienic habits and taking under cooked meat) conducive to toxoplasma infection exist in Pakistan. Being a global public health problem and particularly due to its grave consequences in congenital toxoplasmosis and in immuno compromised patients, less attention has been focused on the subject in Pakistan. This crossectional sero-epidemiological study was initiated to fig the gap in knowledge regarding local epidemiology of the infection.

MATERIALS AND METHODS

Study Area

This study was carried out in Lahore, the capital of Punjab province and the second largest city of Pakistan, having semi-tropical climate. The annual rainfall is about 490mm and the relative humidity varies between 22 and 90%.

Study Population.

The women of child bearing age i.e. from 15 to 45 years (as on last birthday), irrespective of their martial status were included in study. The sampling frame consisted of the lists prepared on the basis of annual survey conducted by two Maternal and Child Health (M.C.H.) Centres of the College of Community Medicine, Lahore. 735 women of child bearing age were selected by simple random sampling. In case of non availability of a respondent, next women on the sampling frame was included.

Collection, Handling, Transportation and Storage of Specimen.

The data was collected by administering the questionnaire by direct interview. 5ml of blood was taken aseptically avoiding stasis from a superficial vein by a disposable syringe. Blood was transferred to a sterile centrifuge tube, which was then plugged and labelled.

The specimens thus collected were transported to the laboratory in an ice-box. Blood was allowed to clot at room temperature and serum was then separated by centrifugation at 100g for 10 minutes. The serum was transferred in the polystyrene bottles, labelled and stored at -20C in a deep freezer.

Serological Examination:

In this sero-epidemiological study Enzyume-Linked Immuno-Sorbent Assay (ELISA)[10] technique was used to detect IgG-specific antibodies to Toxoplasma gondii from the sera of the sampled population. The kit used for ELISA tests was "Enyzgnost Toxoplasmosis/IgG" (Behring, W. Germany) batch/lot No. 17984. Out of 96 reacting wells in a kit, three were used for negative control sera and one for positive control serum supplied alongwith the kit and remaining 92 wells were used for sera of study population. If the control sera, positive and negative, exhibit an activity given on the respective labels, then the test is valid for evaluation. Visual evaluation was done with the help of plate reading mirror. Results were read by comparing the colour of test wells with the colour of negative control wells. More intense colour of the test wells than of the wells with negative control were considered positive for Toxoplasma antibody. Photometric evaluation was done by reading absorbance in a Titertek Multiskan Photometer (Flow Laboratories) at 405 nanometer (nm).

The results were printed out in absorbance mode through inbuilt facility of the instrument.

Statistical analysis was performed with the help of Department of Biostatistics, College of Community Medicine, Lahore.

RESULTS

1. PREVALENCE AND AGE-WISE DISTRIBUTION OF TOXOPLASMA INFECTION.

735 Sera belonging to women of child bearing age were tested for toxoplasma antibodies. Age of the respondents ranged from 15 to 45 years, the mean age being 27.81 ± 8.15 years. 35.7% (263 out of 735) women were positive for toxo antibody. Distribution of toxoplasma antibody in relation to age groups is shown in table-I. There was a gradual increase in positivity with increasing age. As elicited in Figure-I, the rate of increase in sero positivity with age was 0.176% per year by linear regression of means, between the age of 15 and 45 years. The co-efficient of correlation (r) was 0.642 being statistically significant at $p<0.001$.

2. ECONOMIC STATUS AND TOXOPLASMA INFECTION.

Comparison of toxoplasma infection in different economic groups has been illustrated in table II. Toxoplasma infection was highest in lowest income group and decreased with the increase of income. The difference was statistically significant using chiquare (X2) text $P<0.05$. Linear regression analysis (Figure 2), showed a highly negative correlation ($r = -0.947$; $p<0.02$) of Toxoplasma infection with economic status.

3. RELATIONSHIP OF MARITAL STATUS AND TOXOPLASMA INFECTION.

Table III elucidate the prevalence of Toxoplasma infection in single and married women. The difference was not significant statistically, indicating that martial life has no role in dissemination of toxoplasma infection.

4. EDUCATIONAL STATUS AND TOXOPLASMA INFECTION

Prevalence of toxoplasma infection was highest among illiterate women and less in educated females as explained in Table-IV. There is statistically significant difference in seropositivity among different educated groups ($p<0.05$).

5. CONTACT WITH CATS AND TOXOPLASMA INFECTION:

Prevalence of toxoplasma infection was much higher in women who had a cat as pet in their house as compared to those who had no pet at home (Table-V). The difference in prevalence is significant $P<0.05$. This indicates the importance of cat's role in toxo infection.

DISCUSSION :

Toxoplasma gondii is one of the most successful of parasites. It respects neither the boundaries of geography nor of zoology and causes infection, usually long life, in all the

species of mammals and birds, as far as is known, throughout the world except Antarctica, but the frequency of infection varies from one country to another and also different parts of a given country. In this study, the prevalence rate of 35.78% of Toxoplasma infection among women of child bearing age is quite substantial. Judging from global point of view, the prevalence is rather intermediate, being higher when compared to prevalence of 5% in Navajo Indians, 22% in a London study, 25% in Egypt and 31.2% in Saudi Arabia. [11, 12, 13, 14] However, this prevalence tends to be low when compared to prevalence of 40% in Montreal, 51.5% in Morocco, 58% in Kuwait, and 84% in France [15, 16, 17, 18] The regional experience in Pakistan is, however, a bit different. Toxoplasma prevalence varies from 7.74% to 35.8% in Pakistan as reported in different publications. [19, 20, 21] This difference could be due to geographic distribution of disease or due to different techniques used in different studies.

The high prevalence of infection (33.59%) in younger age group (15-19 years) and slow increase with advancing age reflects the model of transmission through cysts in unhygienic environment and tropical humid climate. In the present study, 64.22% women of child bearing age were free of Toxoplasma antibodies and the calculated incidence rate (sero-conversion rate) is 0.176% per year, which is very low when compared to sero-conversion rate of 2% per year in England and 0.5% per year in Norway [12, 22] The difference may be due to cultural factors e.g. taking undercooked meat and association with cats. etc.

The present study revealed a strong negative correlation with economic status. This result is in conformity with international studies. This observation indicates that Toxoplasma infection is a public health problem of lower income group. People of lower economic status look for daily past-time and hobbies in and around their homes including gardening and sitting outdoors on the ground in the evening. Due to lack of hygienic practices, the transmission of oocysts by flies, cockroaches and other cryptogenic animals through contract with cat faeces, maybe an important mode of transmission in this group.

No significant difference was observed in the prevalence of Toxoplasma infection between married and unmarried females. Information on this aspect was not available in the literature reviewed. However, as stated by Frenkel (1984) transmission through matrimonial activities in unlikely[2].

The difference between prevalence of infection among illiterate and different educated groups in highly striking. Like most of the developing countries, in Pakistan also, educational status is an important indicator of economic status. Poverty and illiteracy go side by side, therefore, all the factors responsible for high prevalence of infection in lower income group are also reasons for high infection among illiterate and less educated class.

The exploration of the life cycle of Toxoplasma gondii has provided sufficiently convincing evidence that the cats and felines are the definite hosts and play an important role in the dissemination of infection. This study revealed the significant difference in prevalence of Toxoplasma infection between group who had cat at pet at home and those

who did not. The data suggests a strong association of Toxoplasma infection with cat contact which is in conformity with the observations of most of the investigators. Wallace (1969) showed a close association of toxoplasma antibody in humans and rats on a small Pacific atoll where cat were present. He also observed the virtual absence of toxoplasma antibody on islands where cats were absent, and thereby established a firm qualitative relationship between cats and Toxoplasma infection.23.

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TABLE-1

PREVALENCE RATES OF TOXOPLASMA ANTIBODIES IN DIFFERENT AGE GROUPS.

Age Group Years	No. Tested	Positive	% Positive
1-19	128	43	33.59
20-24	213	76	35.68
25-29	143	50	34.96
30-34	80	28	35.00
35-39	78	32	41.02
40-44	93	34	36.56
Total	735	263	35.78

TABLE-II

COMPARISON OF TOXOPLASMA INFECTION IN DIFFERENT ECONOMIC GROUP

Family Income (Rs. per month).	No. Tested	Positive	% Positive*
Upto 100	88	38	43.18
1001 - 2000	357	138	38.65
2001 - 3000	147	50	34.01
3001 - 4000	29	7	24.14
4001 and above	114	30	26.32
Total	735	263	35.78

* Test of significance used to determine correlation of economic status with toxoplasma infection was Chi-square test.

x^2 Statistics = 9.7

P $<$ 0.05 (Significant).

TABLE-III

COMPARISON OF TOXOPLASMA PREVALENCE IN RELATION TO MARTIAL STATUS.

Martial Status	No. Tested	Positive	% Positive.
Single	148	55	37.16
Married	587	202	35.43
Total	735	263	35.78

x^2 Statistics = 0.151

P $>$ 0.50 (Not Significant).

TABLE-1V

PREVALENCE OF TOXOPLASMA ANTIBODIES ACCORDING TO EDUCATIONAL STATUS

Educational Status	No. Tested	Positive	% Positive.
Illiterate	313	130	41.53
Primary	244	84	34.42
Matric	110	35	31.82
Graduate	54	12	22.22
Post-graduate	14	2	14.28
Total	735	263	35.78

x^2 Statistics =9.7

P $<$ 0.05 (Significant).

TABLE-V

COMPARISON OF TOXOPLASMA INFECTION IN WOMEN HAVING CAT AS PET AND NO PETS IN HOSUE.

Category	No. Tested	Positive	% Positive.
No pets	507	164	32.35
Cat only	28	15	53.57
Total	735	263	35.78

x^2 Statistics =4.457

P $<$ 0.05 (Significant).

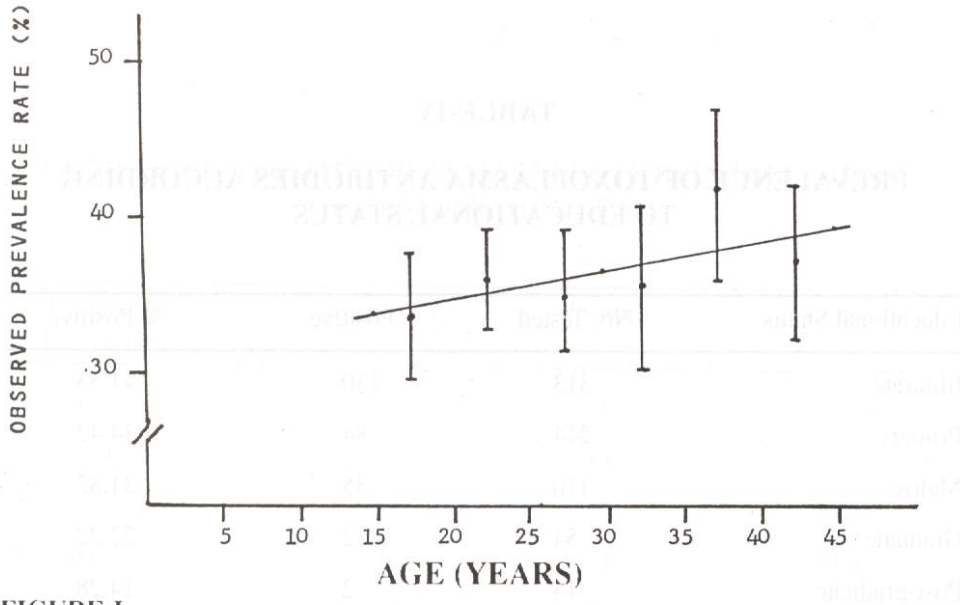

FIGURE-I

Association of age and toxoplasma prevalence. Plotted Points are prevalence rates ± one standard deviation. The regression line is drawn by regression equation (y=a + bx). Different values calculated by Liner regression are :

Constant (a) = 30.85

Regression oefficient (b) = 0.176

Correlation coefficient (r) = 0.642

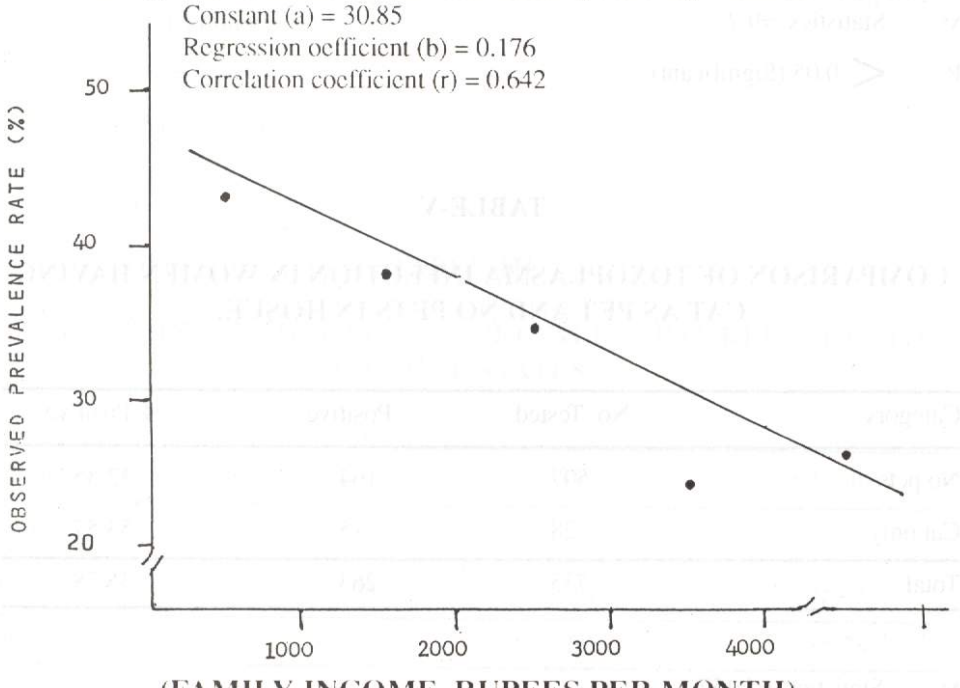

FIGURE-2

Correlation of Toxoplasma Infection with economic status.

REgression Line indicate highly negative correlation ($r = 0.947$; $P < 0.02$) of Toxoplasma infection with economic status.

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