

Seroprevalence of Ocular Toxoplasmosis and the Associated Risk Factors among Pediatric Patients.Archana¹, Raj Nath Singh², Vijay Kumar³¹Senior Resident, Department of Microbiology, Patna Medical College & Hospital, Patna, Bihar, India.²MBBS, MD (Ophthalmology).³Professor & Head, Department of Microbiology, Patna Medical College & Hospital, Patna, Bihar, India.

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Conflict of interest: Nil

Abstract:**Objectives:** The present study was to evaluate the seroprevalence of ocular Toxoplasmosis and the associated risk factors among Pediatric age group patients.**Methods:** All samples were subjected to the enzyme-linked immunosorbent assay (ELISA) for the detection of anti-T. gondii IgG and IgM antibodies. The antigen was prepared from the RH strain of T. gondii tachyzoites harvested from the peritoneal cavity of Swiss albino mice after 3 days of infection. The tachyzoites were purified by differential centrifugation, kept overnight at 4°C and then subjected to sonication and cold centrifugation. Toxoplasma antigen (1 µg/well) was coated on microtitre plates, and ELISA was performed. All the risk factors were assessed by the using the questionnaire to the parents. Socio-economic strata were assessed by using the modified Kuppuswamy scale. All the related factors were noted in the prescribed Performa.**Results:** A total of 206 suspected cases of ocular toxoplasmosis were tested. Rate seroprevalence of ocular toxoplasmosis was 13.59%. out of 206, 64 cases were neonates. Among neonates, 6(9.37%) cases were seropositive. IgG+/ IgM- was 4(6.25%) and IgM+/ IgG+ antibodies was 2(3.12%). Out of 52 infants, 1(1.92%) and 3(5.77%) cases had IgG+/ IgM- and IgM+/ IgG+ antibodies respectively. Out of 47 tested sample of cases age group >1-5 years, seropositivity was seen in 7(14.89%) cases. Similarly, in age group 6-12 years, out of 28 tested sample, seropositivity was seen in 5(17.86%) cases. In age group 13-18 years, out of 22 tested samples, seropositivity was seen in 6(27.27%) cases. Out of 206 tested sample, IgG+/ IgM- was seen in 11(5.34%) cases. And IgM+/ IgG+ was seen in 17(8.25%). Among all tested sample, 13(6.31%) ocular toxoplasmosis were males and 15(7.28%) were females. Majorities of cases were eaten meat (67.85%), kept cattle during the past 5 years 18((64.29%), lived in rural area 20(71.43%), drunk treated water 24(85.71%) and belonged from lower 39(11.29%) and upper lower class 9(32.14%). Most of the parents were illiterate 14(50%) and primary level education 9(32.14%). 75% children were played in open environment with touch soil.**Conclusions:** Ocular toxoplasmosis is greatly seen in neonates, infants, and paediatrics age 1-5 years. Female are more preponderance than male. Poor hygiene, illiteracy, lower socioeconomic status, playing with domestic animals (cat) and touch soil are major risk factors of ocular toxoplasmosis in paediatrics. Hence, we should organise a medical camp in rural as well as urban areas for the awareness of risk factors and early diagnosis and prompt treatment of ocular toxoplasmosis.**Keywords:** Ocular toxoplasmosis, Paediatrics, Risk factors.This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided original work is properly credited.**Introduction**

Ocular toxoplasmosis is a preventable cause of blindness, it is necessary to assess factors that have an impact on human infection. Based on current observations, the risk of acquiring an infection varies geographically and largely depends on control of the release and distribution of oocysts into the environment, the animal reservoir, meat consumption, personal habits, and climatic conditions. In many countries, the prevalence of T. gondii cysts in livestock and the consumption of their contaminated meat are major factors influencing the rate of human infections [1].

Toxoplasma gondii is an obligate intracellular protozoan and the causative agent of toxoplasmosis, one of the most prevalent parasitic infections that affect humans and other warm-blooded animals [2,4] It is estimated that approximately one-third of the human population worldwide has the parasite [3,5].

The prevalence of human Toxoplasma infection varies in different parts of the world, it can range from 10% to 50% in temperate developed countries to over 80% in developing countries of the tropics [6]. The infection is more prevalent in a warm and

humid climate and is lower in cold regions, probably due to the poor survival of the oocyst stage of the parasite under freezing conditions [2, 4,5,7]. Low seroprevalence has been reported in South East Asia, North America, and Europe, whereas high seroprevalence is observed in Latin America and tropical African countries [8]. Central Africa has the highest reported prevalence rates, and the Democratic Republic of Congo (DRC) has the highest rate among these countries [9,10].

Although the seroprevalence of *T. gondii* infection in primary school children has been reported all over the world [11], little is known about the seroprevalence of *T. gondii* infection in primary school children in China [12, 13]).

Two major routes of infection exist. First, humans may become infected by oral ingestion of *Toxoplasma* oocysts, which are produced in the intestine of its specific host, the cat and other Felidae, and released by their faeces into the environment. Second, infection occurs by oral uptake of *Toxoplasma* tissue cysts, which persist in skeletal muscles of intermediate hosts including pigs and sheep. Primary infection during pregnancy may result in fetal infection with fetal death, severe congenital malformation or, especially with infection at later stages of gestation, mild infection of neuronal tissue including the retina. However, more frequently, the pathogen is acquired postnatally, which also results in infection of neuronal tissues and, in most cases, takes a clinically asymptomatic course. It is considered the most frequent foodborne parasitic infection globally [14]. Importantly, ocular involvement is a major pathology following both routes of infection and may cause legal blindness. Objectives of our study was to evaluate the seroprevalence of ocular Toxoplasmosis and the associated risk factors among pediatric age group patients.

Material & Methods

The present study was conducted in the Department of Microbiology with the collaboration of Department of Ophthalmology, Patna Medical College & Hospital, Patna, Bihar during a period from September 2023 to February 2024.

Data was collected with irrespective of age and sex. A total of 206 suspected cases of ocular toxoplasmosis were enrolled in the present study.

Sample Collection

For the detection of anti-*T. gondii* antibodies, blood was aseptically collected by venipuncture in sterile plain vials. The blood was allowed to clot, and following clot retraction, the serum sample was separated and stored at -20°C till further analysis.

Laboratory Procedures

All samples were subjected to the enzyme-linked immunosorbent assay (ELISA) for the detection of anti-*T. gondii* IgG and IgM antibodies as previously reported in the protocol [41]. The antigen was prepared from the RH strain of *T. gondii* tachyzoites harvested from the peritoneal cavity of Swiss albino mice after 3 days of infection. The tachyzoites were purified by differential centrifugation, kept overnight at 4°C and then subjected to sonication and cold centrifugation. *Toxoplasma* antigen ($1\mu\text{g}/\text{well}$) was coated on microtiter plates, and ELISA was performed.

Risk factors were assessed by the using the questionnaire to the parents. Socio-economic status was assessed by using the modified Kuppaswamy scale. All the related factors were noted in the prescribed Performa.

Statistical Analysis

Data was analysed by SPSS software. Chi-square test was applied. P-value was taken less than or equal to 0.05 ($p \leq 0.05$) for significant differences.

Results

A total of 206 suspected cases of ocular toxoplasmosis were tested for seropositivity. Among them, seropositivity was seen in 28 cases. Rate seroprevalence of ocular toxoplasmosis was 13.59%. out of 206, 64 cases were neonates. Among neonates, 6(9.37%) cases were seropositive. 4(6.25%) neonates had IgG+/ IgM- and 2(3.12%) had IgM+/ IgG+ antibodies. Out of 52 infants, 1(1.92%) and 3(5.77%) cases had IgG+/ IgM- and IgM+/ IgG+ antibodies respectively. Out of 47 tested sample of cases age group $>1-5$ years, seropositivity was seen in 7(14.89%) cases. Similarly, in age group 6-12 years, out of 28 tested sample, seropositivity was seen in 5(17.86%) cases. In age group 13-18 years, out of 22 tested samples, seropositivity was seen in 6(27.27%) cases. Thus, out of 206 tested sample, IgG+/ IgM- was seen in 11(5.34%) cases. And IgM+/ IgG+ was seen in 17(8.25%) paediatric age group cases. All the cases of IgG+/ IgM- and IgM+/ IgG+ were not significant differences ($p > 0.05$).

Table 1: Showing the seropositivity for Toxoplasma infection with respect to age

Age group	Total samples tested	IgG+/ IgM-	IgM+/ IgG+	Total seropositive	p-value
Neonates (<1 months)	64	4(6.25%)	2(3.12%)	6(9.37%)	0.403
Infant (>1 month-1 year)	52	1(1.92%)	3(5.77%)	4(7.69%)	0.309
Paediatric (years)					
>1-5	47	2(4.26%)	5(10.64%)	7(14.89%)	0.241
6-12	28	2(7.14%)	3(10.71%)	5(17.86%)	0.642
13-18	22	2(9.09%)	4(18.18%)	6(27.27%)	0.385
Total	206	11(5.34%)	17(8.25%)	28(13.59%)	0.241

In the present study, In neonates, 2(3.12%) cases were males and 4(6.25%) were females. In infants, 1(1.92%) case was male and 3(5.77%) cases were females. In age >1-5 years, 4(8.51%) cases were males and 3(6.38%) were females. In age 6-12

years, 2(7.14%) cases were males and 3(10.71%) cases were females. In age 13-18 years, 4(18.18%) cases were males and 2(9.09%) cases were females. Among all tested sample, 13(6.31%) cases were males and 15(7.28%) cases were females.

Table 2: Showing the seropositivity for Toxoplasma infection with respect to gender

Age group	Total samples tested	Total seropositive	Male	Female	P-value
Neonates (<1 months)	64	6(9.37%)	2(3.12%)	4(6.25%)	0.403
Infant (>1 month-1 year)	52	4(7.69%)	1(1.92%)	3(5.77%)	0.309
Paediatric (years)					
1-5	47	7(14.89%)	4(8.51%)	3(6.38%)	0.695
6-12	28	5(17.86%)	2(7.14%)	3(10.71%)	0.642
13-18	22	6(27.27%)	4(18.18%)	2(9.09%)	0.385
Total	206	28(13.59%)	13(6.31%)	15(7.28%)	0.696

In the present study, majorities of cases were eaten meat (67.85%), kept cattle during the past 5 years 18(64.29%), lived in rural area 20(71.43%), drunk treated water 24(85.71%) and belonged from lower

39(11.29%) and upper lower class 9(32.14%). Most of the parents were illiterate 14(50%) and primary level education 9(32.14%). 75% children were played in open environment with touch soil.

Table 3: Showing the associated risk factors of seropositivity for Toxoplasma infection (N=28)

Risk factors	No. of cases	Percentage
Eating meat		
No	9	32.14%
Yes	19	67.85%
Kept cattle during the past 5 years		
No	10	35.71%
Yes	18	64.29%
Eating raw pork		
No	21	75%
Yes	7	25%
Place of residence		
Urban	08	28.57%
Rural	20	71.43%
Touch soil		
No	07	25%
Yes	21	75%
Presence of cat in the environment		
No	0	0
Yes	28	100%
Drink treated water		
No	04	14.23%
Yes	24	85.71%
Socio-economic status		

Upper class	0	0%
Upper middle	02	7.14%
Lower middle	6	21.43%
Upper lower	09	32.14%
Lower	11	39.29%
Parent Education		
Illiterate	14	50%
Primary	9	32.14%
Secondary	03	10.71%
Graduate	02	7.14%

Discussions

Toxoplasmosis has been described as an environmental disease because transmission has been shown to be promoted by poor environmental sanitation, overcrowding, poverty, certain eating habits and poor hygiene [15,16].

In the present study, overall rate of seroprevalence of ocular toxoplasmosis in paediatric was 13.59% with anti-Toxoplasma IgG in 5.34% and IgM in 8.25%. Another recent study from North India evaluating anti-Toxoplasma IgG and IgM antibodies in women of reproductive age group reported a prevalence of 22.4% and 1.4%, respectively [17]. Studies from North India have documented an anti-Toxoplasma IgG seroprevalence of 15%–51.8% and 2%–5% for IgM antibodies [18,19]. Globally, approximately 25%–30% of humans are infected with *T. gondii*; however, the seroprevalence varies greatly between different countries (10%–80%) and even within countries [20]. This is perhaps due to the differences in exposure to the organism according to the socioeconomic status, cultural beliefs and anthropogenic factors such as handling and owning cats, consumption of meat and unwashed fruits and vegetables handling soil and variations in hand hygiene practices [21]. Other factors such as climate which affects the survival of oocysts in the environment, sanitation and quality of water supply can also impact the seroprevalence trends [22].

The *Toxoplasma* seroprevalence in our study revealed a decline as compared to other study, the exact reasons of which need to be explored. It is plausible that an improvement in socioeconomic status, water and food sanitation, better regulation of indigenous, imported meat quality, avoidance of risk factors during pregnancy and increasing awareness of toxoplasmosis amongst patients and doctors could all have contributed to this. A study from Poland investigating the prevalence of anti-Toxoplasma IgG between 1998 and 2003 reported a decline in seroprevalence from 45.4% to 39.4% [23].

Previous studies have indicated that the parent's occupation is a factor that influences the likelihood that a PSC will be infected with *T. gondii* [24,25]. Generally, children whose parents' occupations are

categorized as unskilled workers tend to be infected more frequently, which could be due to the parents' lacks of sufficient knowledge of personal hygiene. However, in this study, there was significant association between parents' educational level, standard of living, hygiene, and infection. An alternative explanation may be that people acquire *T. gondii* infection through contaminated environments in the community, regarding of their associated risk factors. In the present study, slight gender difference was found associated with the seroprevalence of *T. gondii*, supporting observations made elsewhere [26,27]. This result indicates that many of the children in this community were recently infected with *T. gondii*, which could have occurred through risk factors such as playing in soil, playing with pets (cats and dogs), eating raw and unwashed foods, eating raw/undercooked meat, or drinking of unsafe water [28]. Substantial studies have indicated that the seroprevalence of *T. gondii* infection increases with age [29], and one hypothesis suggests that this outcome is the result of an increasing number of exposure years as the child grows [30].

Among the risk factors analysed in this study, playing/ contact with soil showed higher risk of infection for toxoplasmosis. This route of transmission could explain the similar incidence of seropositivity between boys and girls in this community because both genders are equally exposed to contact with soil. This infection route corroborates the findings of previous studies in urban Brazil [31]. Contact with domestic cats is one of the generally accepted risk factors for infection with toxoplasmosis [2], although no association was observed in this study, in agreement with previous studies [32, 24, 25]. Drinking contaminated water is also a potential source of *T. gondii* infection [33]. A study in Nigeria showed that the seroprevalence rate was higher in pregnant women who drank well water compared with women using packaged water [34]. In the present study, 85.74% of the children reported the use of unsafe water, the association between the source of drinking water and *Toxoplasma* infection was found. The consumption of raw meat also seems to be a risk factor of toxoplasmosis infection. However, eating raw and

unwashed vegetables can serve as a route of infection.

The most common clinical manifestation of toxoplasmosis involves the eye in the form of retinochoroiditis [35]; it is the leading cause of infectious posterior uveitis in non-immunocompromised individuals worldwide [7,36]. Although ocular toxoplasmosis (OT) results from both congenital and acquired infections, eye disease acquired after birth is much more common than congenital infection [7,36,37]. Ocular toxoplasmosis may occur immediately or long after the initial infection or during reactivation [4]. The disability-adjusted life years calculated for toxoplasmosis (both congenital and postnatally acquired) were equivalent to that of tuberculosis [35]. When the disease is acquired after birth, retinochoroiditis accounts for almost the entire burden [35]. Ocular toxoplasmosis is considered a significant cause of uveitis in Africa, but a few studies describe the epidemiology of the disease on this continent. In DRC, toxoplasmosis is the patients' most common aetiology of uveitis [38]. People with OT experienced with worse vision-related quality of life [39]. Retinal toxoplasmosis is recognised as a significant cause of blindness in many parts of the world [5,40]. Overall, 24% of patients with OT developed legal blindness in one eye, generally from retinitis and subsequent scarring within the macula, retinal detachment, or optic atrophy [2]. The prevalence of OT in a population is dependent on the overall prevalence of infection in the population [7]. Due to the severity and irreversibility of ocular lesions caused by *T. gondii*, knowledge about this infection's social and epidemiological factors is essential to apply adequate intervention for prevention. Therefore, the prevention of toxoplasmosis is primarily directed toward health education related to avoiding personal exposure to the parasite.

Conclusions

The present study concluded that the ocular toxoplasmosis is greatly seen in neonates, infants, and paediatrics age 1-5 years. Female are more preponderance than male. Poor hygiene, illiteracy, lower socioeconomic status, playing with domestic animals (cat) and touch soil are major risk factors of ocular toxoplasmosis in paediatrics. Hence, we should organise a medical camp in rural as well as urban areas for the awareness of risk factors and early diagnosis and prompt treatment of ocular toxoplasmosis.

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