Serodiagnosis of toxoplasmosis in Bahrain

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ABSTRACT

Objectives: We report here an analysis of results of anti-*Toxoplasma* immunoglobulin M (IgM) and immunoglobulin G (IgG) measurements reported from the Central Laboratory of Bahrain over a period of 3 years and 9 months.

Methods: This study included all blood samples received at the Salmaniya Medical Complex, Manama, Bahrain serology laboratory for the determination of *Toxoplasma*-specific antibodies during the period of January 2000 to September 2003. A total of 4,739 specimens were assayed for IgG and 1,947 for IgM.

Results: An overall seropositivity of 21.8% for IgG and 10.3% for IgM antibodies were found with no gender differences for either IgG or IgM. In addition, no statistically significant positivity rate differences (p=0.723) were observed between Bahrainis and non-Bahraini residents. The prevalence of *Toxoplasma gondii (T. gondii)*-specific IgG amongst post partum women was 15.8% and 6.3% for IgM, while for women of child-bearing age IgG was higher at 22.3% and 11.6% for IgM. The IgG seropositivity in neonates (<1 month old) was 16.5%, decreasing to 9.3% in preschool children, while for IgM, it was 3.7% at birth increasing to 7.3% in the preschool group. The IgG seroprevalence increased within the first 15 years of life, and leveled thereafter, for IgM however, it was low at birth and increased within the first 12 months of life then leveled-off at the age of 20-40 to approximately 11-14%, with a further increase after 40 years to 17%.

Conclusion: The seropositivity rates of *T. gondii* in the samples examined during the present study fall within the range of other Gulf Cooperative Council countries. True prevalence in the general population may actually be lower.

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Toxoplasmosis, caused by the obligate intracellular protozoan parasite *Toxoplasma gondii (T. gondii)*, is prevalent worldwide. The infection is transmitted to humans through ingestion of infective oocysts excreted in cat feces, which may have contaminated water or foodstuff or by ingestion of tissue cysts in raw or undercooked meat from infected farm animals, including cattle, sheep, pig, goats, camels and poultry.[1,2]

Infection in immunocompetent hosts is, mostly, asymptomatic. However, in approximately 10% of cases it causes a self-limited and non-specific illness, typically manifesting as mild fever with some isolated lymphadenopathy.[1] The severest manifestations are seen following congenital infections,[3] and in immunocompromised patients.[4] While acute toxoplasmosis during pregnancy is asymptomatic in most women, the severity of clinical manifestations in neonate, mainly targeting the CNS and eyes, is linked to the trimester during which the congenital infection occurs.[3,5] Infections

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in immunocompromised individuals are largely related to reactivation of chronic infection.[1] Infection with *T. gondii* in an immunocompetent host results in a strong and persistent T helper 1 response[6] associated with the production of antigen-specific immunoglobulin M (IgM), immunoglobulin G (IgG), immunoglobulin A (IgA) and immunoglobulin E (IgE) antibodies, which confer lifelong immunity against the disease.[7-9]

Routine laboratory diagnosis of toxoplasmosis is carried out using serological tests, or, less frequently, by histological demonstration of the parasite or its antigens, by isolation of the organism, or using PCR.[10] Several methods are employed in the serological diagnosis of toxoplasmosis including the determination of serum IgM and IgG titers, serum IgG avidity and, in the case of congenital infection, comparative western blots of serum from mothers and baby pairs.[10,11] Serum immunoglobulin class and titer is usually the initial step in the diagnosis of suspected cases of toxoplasmosis. It is also utilized for screening pregnant women to assess risk and in newborns suspected of congenital toxoplasmosis.[10,11] The incidence of toxoplasmosis, based on seroprevalence of IgG antibodies in the general population which varies in different countries, does not differ significantly between gender and commonly rises with age.[1] Reported serological prevalence in various countries range from as low as 7.6% in rural Scotland,[12] to 57% in villages in Utter Pradesh in India[13] and the governorate of Qualyobia in Egypt.[14] In the countries of the Gulf Cooperative Council (GCC), reported seroprevalence in Saudi Arabia range between 22.4% and 36%, depending on the group studied.[15-17] In the United Arab Emirates (UAE) seroprevalence in delivering women was reported to be 22.9% and 34% of blood donors in Al Ain.[18,19] Prevalence in school children between the ages of 6-10 years varied in different emirates with the lowest being Dubai with 3.5% and the highest being Sharja with a prevalence of 34.6%.[20] A single report from Bahrain on a small number (n=76) of HIV positive patients showed a seropositivity rate of 20%.[21]

We report here an analysis of results of anti-*Toxoplasma* IgM and IgG seropositivity amongst a selected population of patients.

Methods.

Study population. This laboratory-based study includes all blood samples from either, cases of suspected toxoplasmosis, or healthy persons screened for toxoplasmosis, received at the Salmaniya Medical Complex (SMC) serology laboratory (the Bahrain Central Laboratory), Manama, Bahrain during the period January 2000 to September 2003. A total of 4,739 serum samples, of which 29.9% were from outpatients and 38.4% were from inpatients at SMC, and the remainder (31.7%) were from the different peripheral primary care health centers, were assayed for IgG. A total of 1,947 specimens, similarly drawn from outpatient clinics were 34.5%, from inpatient 25.9% and from different peripheral primary care centers 39.6%, were assayed for IgM.

Serological determination of IgM and IgG. Approximately 5 ml of venous blood was drawn in a plain tube, and dispatched to the laboratory where serum was obtained and refrigerated, to be tested within 24 hours. *Toxoplasma* IgG and IgM antibodies were measured using commercial EIA kits (Toxo IgG EIA II, and Toxo IgM EIA recomb, Roche diagnostics, Mannheim, Germany) as recommended by the kit manufacturer, on the Cobas Core immunoassay analyzer (Roche Diagnostics, Mannheim, Germany). The sensitivity and specificity of the Toxo IgG EIA II, according to the manufacturer, are both 100%, while those for the Toxo IgM EIA recomb are 98.9% sensitive and 99.1% specific. Samples containing 6 IU/ml or higher of IgG were considered positive, while a specimen scoring more than 100% of the kit IgM standard was considered positive.

Statistical analysis. Significance of differences was analyzed using chi-square, and *p* values were calculated using the 2-tailed student's t-test.

Results.

Analysis of samples drawn over a period of 3 years and 9 months showed anti-*Toxoplasma* seropositivity rates of 21.8% for IgG and 10.2% for IgM. There was no significant gender difference for either IgG (p=0.694) or IgM (p=0.733) (**Table 1**). The IgG seropositivity rate of Bahraini nationals was 21.4%, whereas the IgM seropositivity was 10%. Compared to non-Bahraini residents, the IgG seropositivity was 22.8% while IgM was 10.9%. (**Tables 2**). There were, again, no statistically significant gender differences (IgG; Bahraini p=1.000, non-Bahraini p=0.555; IgM, Bahraini p=0.553, non-Bahraini p=0.089) among the 2 populations.

Immunoglobulin G seropositivity according to age ranges was 16.5% in newborns, falling to 13.5% in subsequent months, to reach a low of 1% in the group of 1-5 years, and thereafter rose with age (**Table 3**). Immunoglobulin M seropositivity, on the other hand, did not follow a clear pattern in younger age groups, being 3.7% in newborns, increasing to 7.7% amongst 1-12 month olds, fluctuating between 5.1% and 12.5% before reaching zero in age groups 1-5, 6-10 and 11-15 years. Seropositivity steadily increased with age in subsequent groups reaching a peak of 17% at >40 years (**Table 3**).

Immunoglobulin G seropositivity rates were higher in newborns (<1 month) (16.5%) as compared to preschool children (1 month – 5 years) (9.3%), while IgM followed an opposite pattern, being higher in the preschool group (7.3%) as

Table 1 - Distribution of IgG and IgM seropositivity to *Toxoplasma gondii* among individuals tested in Bahrain between January 2000 and September 2003.

Antibody	Group	N	Positive cases n	Positive cases (%)	*p* value
IgG	Males	538	121	(22.5)	
	Females	4201	911	(21.7)	
	Total	**4739**	**1032**	**(21.8)**	0.694
IgM	Males	432	46	(10.6)	
	Females	1515	153	(10.1)	
	Total	**1947**	**199**	**(10.2)**	0.733

Table 2 - Comparison of Bahraini and Non-Bahraini residents with IgG and IgM seropositivity to *Toxoplasma gondii*.

Antibody	Group	N	Positive cases n	Positive cases (%)	*p* value
IgG	Bahraini males	414	89	(21.5)	
	Bahraini females	3139	672	(21.4)	
	Total	**3553**	**761**	**(21.4)**	**1**
	Non-Bahraini males	124	32	(25.8)	
	Non-Bahraini females	1062	239	(22.5)	
	Total	**1186**	**271**	**(22.8)**	**0.555**
	Overall IgG Total	**4739**	**1032**	**(21.8)**	**0.723**
IgM	Bahraini males	330	30	(9.1)	
	Bahraini females	1141	117	(10.3)	
	Total	**1471**	**147**	**(10)**	**0.553**
	Non-Bahraini males	102	16	(15.7)	
	Non-Bahraini females	374	36	(9.6)	
	Total	**476**	**52**	**(10.9)**	**0.089**
	Overall IgM Total	**1947**	**199**	**(10.2)**	**0.219**

Table 3 - Distribution of IgG and IgM seropositivity to *Toxoplasma gondii* among different age groups in Bahrain from January 2000 to September 2003.

Age Group	Immunoglobulin G N	Immunoglobulin G Positive cases n	Immunoglobulin G Positive cases (%)	Immunoglobulin M N	Immunoglobulin M Positive cases n	Immunoglobulin M Positive cases (%)
< 1 month*	133	22	(16.5)	191	7	(3.7)
1 – 12 months†	208	28	(13.5)	338	26	(7.7)
1 – 5 years†	105	1	(1)	59	3	(5.1)
6 – 10 years	60	5	(8.3)	16	2	(12.5)
11 - 15 years	52	10	(19.2)	20	0	(0)
16 – 20 years	80	16	(20)	54	3	(5.6)
21 – 25 years	224	59	(26.3)	344	37	(10.8)
26 – 30 years	818	182	(22.2)	344	42	(12.2)
31 – 35 years	1074	253	(23.6)	297	41	(13.8)
36 – 40 years	1008	236	(23.4)	167	18	(10.8)
> 40 years	977	220	(22.5)	117	20	(17.1)

* Newborn
† Preschool (combined 1-12 months + 1-5 years).

compared to newborns (3.7%) (**Table 3**). Amongst women of childbearing age, 22.3% were positive for IgG and 11.6% for IgM. Postpartum women had lower positivity rates for both IgG (15.8%) and IgM (6.3%) antibodies (**Table 4**).

Statistically significant IgG differences (p=0.002) were found among non-Bahraini residents. Nationals of Yemen had the highest IgG seropositivity (36.7%), followed by nationals of the Eastern Mediterranean region (34.8%), and Egypt (33.7%). The lowest seropositivity was amongst nationals of the Indian subcontinent (20.6%) and in non-Bahraini GCC nationals (16.7%) (**Table 5**).

Discussion. This retrospective study was based on selected blood samples referred from SMC and peripheral health centers for either prenatal or postnatal screening; or for suspicion of clinical toxoplasmosis. The data measured therefore, does not reflect population prevalence rates. However, the large numbers of specimen tested over a long period, provides indicators for presumptive rates of toxoplasmosis in the different population groups. True seroprevalence rates in the general population would thus, be expected to be lower than these reported rates of presumptive infection.

Our results showed an IgG seropositivity rate of 21.8%, with no significant differences between genders, and is consistent with an earlier report from a small group of HIV positive patients in Bahrain.[21] Regionally, it fits within the range of reported seroprevalence in Saudi Arabia[16,17,22] and in delivering women (22.9%) in Al Ain, UAE;[18] but is lower than those reported amongst blood donors (34%) in UAE.[19] The IgM seropositivity, suggesting current or recent infection, was 10.2% in our overall sample, again with no significant gender differences, while it was 6.3% in women in maternity wards and 3.7% in newborn infants in our study, suggesting a significant risk level of congenital infection, though the reported incidence of clinical toxoplasmosis in Bahrain remains relatively small. Immunoglobulin M antibodies develop early, and in most cases their titers become negative within a few months.[1] However, positive titers are maintained during the chronic phase of the infection, and may persist after acute infection.[23] Thus, IgM positivity is not necessarily an indication of a recent infection. Few surveys of IgM seroprevalence are available from the region; however, a report showed a comparable IgM prevalence of 3% in delivering women in Al Ain, UAE.[18]

Sources of *T. gondii* infection in Bahrain are unknown. Raw or undercooked meat is not a part of the Bahraini diet, though consumption of raw or unpasteurized milk is a possible source. Cats, which are common in Bahrain, may be the principal source of infection. The distribution of IgG seropositivity

Table 4 - The IgG and IgM seropositivity to *Toxoplasma gondii* among delivering women and child-bearing age women in Bahrain.

Group	Antibody	N	Positive cases n	(%)
Child-bearing age*	IgG	3499	779	(22.3)
	IgM	1064	123	(11.6)
Delivering	IgG	146	23	(15.8)
	IgM	32	2	(6.3)

* Age 20-40 years.

Table 5 - The IgG seropositivity to *Toxoplasma gondii* of non-Bahraini nationals by geographic area of origin.

Group	N	Positive cases n	(%)	*p* value
Non-Bahraini GCC	72	12	(16.7)	
Indian Subcontinent*	717	148	(20.6)	
Egypt	86	29	(33.7)	
Eastern Mediterranean†	66	23	(34.8)	
Yemen	79	29	(36.7)	
Total	**1020**	**241**	**(23.6)**	**0.002**

*India, Pakistan and Bangladesh, †Jordan, Lebanon, Palestine and Syria, GCC - Gulf Cooperative Council.

observed in the present study, according to age groups, follows the worldwide pattern. It is initially high in newborns below 1 month of age, decreases to a minimum at 1-5 years, consistent with the loss of maternally-acquired antibodies, and then rises again with age. In the present study, IgM seropositivity was 3.7% in newborns, rising to 7.7% amongst 1-12 month olds; and then generally rising with age.

Amongst the non-Bahraini residents, there was a significant difference in the IgG seropositivity rates between the various national groups (p=0.002). Individuals from Yemen had the highest rates with 36.7%. Nationals of Eastern Mediterranean countries, where consumption of raw meat and liver is common, showed infection rates, which were second highest at 34.8%. Human seroprevalence ranges in Jordan were reported between 22 and 26% and in Syria, the seroprevalence in the local Awassi sheep was 44.6%, and in some herds up to 100%.[24,25] Seropositivity in individuals from Egypt was also high at 33.7%, which is within the reported seroprevalence rates of 27.8-57.9%.[14,26] Indian subcontinent nationals, showed a lower seropositivity rate of 20.6%, which is much lower than those reported on the subcontinent of 30-57%.[13,27,28] This may be related to the different food habits in this group. Non-Bahraini GCC nationals had the lowest seropositivity of 16.7%.

In conclusion, it appears that the seropositivity rates of *T. gondii* in the selected sample are within the range of other GCC countries. True prevalence in the general population may actually be lower.

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