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K. Kraivichiam

C. Saksiridsampant

B. Vivalvakin

C. Saksiirisampant

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Seroprevalence of Anti-*Toxoplasma gondii* antibodies in Thai people detected by ELISA

Kanyarat Kraivichian* Wilai Saksirisampant*

Boosba Vivatvakin** Charnchai Saksirisampant***

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Objective : *To study the seroprevalence of Toxoplasma gondii infection in Thai people*

Design : *Descriptive cross-sectional study*

Setting : *Department of Parasitology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand*

Subjects : *450 sera from normal healthy Thai people from the well-baby clinic (King Chulalongkorn Memorial Hospital), Undergraduate students (Chulalongkorn University) and blood donors (Thai Red Cross Society).*

Methods : *All sera were determined for the presence of anti-Toxoplasma gondii IgG and IgM antibodies by enzyme linked immunosorbent assay (ELISA)*

Results : *The seroprevalence of IgG-Ab to Toxoplasma gondii in normal Thai people was 5.8 % (26/450). Of these 450 sera, all were negative for IgM-Ab. Our results show that IgG-Ab tends to rise with the increasing age in the population but there is no statistically significant relationship between titre and age. However, the seroprevalence of this infection in Thai people is lower than that of some other countries.*

* Department of Parasitology, Faculty of Medicine, Chulalongkorn University

** Department of Pediatric, Faculty of Medicine, Chulalongkorn University

*** Chulavej Hospital

Conclusion : *These data of Anti-Toxoplasma gondii antibodies in different age groups in normal Thai people provide information for further epidemiological study. They show low prevalence when compared to people from western countries. The titres of IgG-Ab tended to rise with the increasing age of the population. These also provide the base line for diagnostic evaluation in patients with Toxoplasmosis.*

Key words : *Toxoplasma gondii, Antibodies, ELISA*

Reprint request : Kraivichian K, Department of Parasitology, Faculty of Medicine,
Chulalongkorn University, Bangkok 10330, Thailand.

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กัญญรัตน์ ทรัพย์วิเชียร, วิไล ศักดิ์ศิริสัมพันธ์,บุษบา วิวัฒน์เวคิน, ชาญชัย ศักดิ์ศิริสัมพันธ์.
การตรวจหาความชุกของแอนติบอดีต่อเชื้อท็อกโซพลาสมา กอนดิโอในกลุ่มคนไทยที่มีสุขภาพ
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วัตถุประสงค์	เพื่อศึกษาความชุกของการติดเชื้อท็อกโซพลาสมาในคนไทยที่มีสุขภาพดี
รูปแบบการวิจัย	การศึกษาเชิงพรรณนาแบบ cross-sectional
สถานที่ทำการศึกษา	ภาควิชาปรสิตวิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพฯ
ประชากรที่ศึกษา	ตัวอย่างน้ำเหลือง จำนวน 450 ตัวอย่าง เก็บจากคนไทยที่มีสุขภาพดี มีอายุ ตั้งแต่แรกเกิดจนถึง 60 ปี โดยแบ่งเป็นเพศชาย 232 ราย และเพศหญิง 218 ราย คือ จากกลุ่มเด็กสุขภาพดีที่มารับการตรวจสุขภาพใน Well baby clinic (ร.พ.จุฬาลงกรณ์) นิสิตปริญญาตรี (จุฬาลงกรณ์มหาวิทยาลัย) และ ผู้บริจาคโลหิตของศูนย์บริจาคโลหิต (สภากาชาดไทย)
วิธีการศึกษา-วัดผล	ทำการตรวจหาแอนติบอดีจำเพาะต่อเชื้อท็อกโซพลาสมา กอนดิโอ ชนิด IgG และ IgM จากตัวอย่างน้ำเหลืองโดยวิธีอีไลซา
ผลการศึกษา	จากการศึกษาตัวอย่างน้ำเหลือง 450 ตัวอย่าง มีเพียง 26 ตัวอย่างที่ให้ผล บวกหรือคิดเป็น 5.8 % (26/450 ราย) โดยไม่พบว่ามีรายใดที่ให้ผลบวกของ แอนติบอดีชนิด IgM เลย ผลการศึกษานี้เห็นแนวโน้มว่าในประชากรที่อายุ มากขึ้น มีโอกาสพบแอนติบอดีชนิด IgG เป็นบวกมากขึ้น แต่จากการคำนวณ ทางสถิติ ไม่พบมีนัยสำคัญ อย่างไรก็ตามความชุกของการติดเชื้อ ชนิดนี้มี ระดับต่ำมากในคนไทย เมื่อเทียบกับข้อมูลจากต่างประเทศ
วิจารณ์และสรุปผล	ผลของระดับแอนติบอดีต่อเชื้อท็อกโซพลาสมา กอนดิโอ ในคนไทยปกติที่มี กลุ่มอายุแตกต่างกันนี้ เป็นข้อมูลส่วนหนึ่งของการศึกษาระบาดวิทยา ของโรค ส่วนความชุกของแอนติบอดีที่พบในคนไทยปกติพบว่ามีระดับต่ำ กว่าที่พบในชนชาติตะวันตก ระดับของ IgG แอนติบอดีมีแนวโน้มสูงขึ้น ตามอายุ ข้อมูลเบื้องต้นเหล่านี้ อาจเป็นประโยชน์ ใช้เปรียบเทียบระดับ แอนติบอดีในคนไข้โรคนี้ต่อไปได้

Toxoplasma gondii has a worldwide distribution. Serologic surveys in many countries have produced a range of prevalence rates (0 – 68 %).⁽¹⁾ Chronic infection had been reported in more than ten million people.^(1,2) Normally, this infection does not cause life-threatening disease except when the host is immunocompromized or when primary infection during pregnancy is transmitted transplacentally by the mother to fetus.

The systemic infection remains latent in the body after an acute stage of disease. During the acute stage there is a strong, specific antibody response. Antibodies persist life long at a low level. Transmission of *T. gondii* to humans occurs by two routes which are by ingestion of tissue cysts in raw or undercooked meat or by accidental ingestion of oocysts from soil contaminated with cat feces.

From different parts of Thailand there have been reports range of seroprevalence of toxoplasma ranging from 4.6 % to 13 % in the general population and 2.3% to 12% in healthy pregnant women.⁽³⁻⁷⁾ These previous surveys have used IHA and IFA screening tests.

We attempted to determine the seroprevalence of toxoplasma antibodies in the general population with a different serological method. The IgG and IgM antibodies of *Toxoplasma gondii* in healthy people in Bangkok were detected by ELISA.

Materials and Methods

Study groups

A total of 450 sera from various age groups of people were randomly collected from the following sources : normal healthy Thai adult blood donors, undergraduate students from Chulalongkorn University

and healthy babies from the well baby clinic of King Chulalongkorn Memorial Hospital. The information of age and sex was recorded.

Anti Toxoplasma IgG and IgM antibodies detection

Anti-IgG and Ig-M antibodies to *Toxoplasma gondii* were detected by the ELISA method. The reagent –kit of Toxonostika IgG (T₁) and Toxonostika IgM (T₂) were purchased from Organon Teknika, Holland. This is a micro-enzyme immunoassay based on the sandwich and double sandwich methods. *Toxoplasma gondii* IgG antibodies in the serum bind to the antigen-coated surface of the tested plate wells. Then, the anti-human IgG Horseradish peroxidase conjugate binds to these Ag-Ab complexes. The *Toxoplasma gondii* IgM antibodies in the samples were captured by sheep anti-human IgM-coated surface of the solid phase. The added Ag will react with the IgM-Ab and then anti-*Toxoplasma gondii*-antibodies conjugated with horseradish peroxidase will bind to these complexes. After adding the substrate, an enzymatic reaction results in color production and sulphuric acid is added to terminate the reaction. The OD result is read at 450 nm within one hour. Standard curve was plotted between the OD and titre of the standard negative and positive control sera. The cut-off value of IgG-Ab and IgM-Ab titers were calculated according to manufacturer's recommendation which were those equal to or more than 1:100. In the recommended system, sample should be diluted to 1:100 before adding in the tested plate well. If the sample had lower OD than the standard positive control, it would be negative result. In our experiment, The tested serum with dilution of 1:5 was each carried out. The final titre should be divided by 20. The positive

result of IgG-Ab indicates that the person had *Toxoplasma gondii* infection, either recent infection or past infection. A positive result of IgM-Ab means that the person is actively infected. Interpretation of results of *Toxoplasma* IgG and IgM antibodies are summarized in Table 1.

Statistical analysis

The data were analysed statistically by the SPSS computer program. ANOVA and LSD were used to analyses the difference of the titre's in each age group.

Result

A total of 450 sera were obtained from 3 different groups of healthy people. Group 1 was healthy babies from the well baby clinic King Chulalongkorn Memorial Hospital. The second group was blood donors from the Thai Red Cross Society and the last group was undergraduate students from Chulalongkorn University. There were 232 males and 218 females. All sera were tested for the presence of IgG and IgM antibodies of *Toxoplasma gondii* by using the ELISA method. The characteristic of population are depicted in Table 2.

Table 1. Test concept for screening, diagnosis and testing of samples with unknown character.

Test specimens in IgG-Ab and IgM-Ab by ELISA						
Screening or unknown	1.	IgG	+	IgM	-	immune
	2.	IgG	-	IgM	-	non-immune
	3.	IgG	-	IgM	+	early acquired toxoplasmosis
	4.	IgG	+	IgM	+	recently acquired toxoplasmosis
diagnosis	1.	IgM	-			no recently acquired toxoplasmosis
	2.	IgM	+	IgG	-	early acquired toxoplasmosis
				IgG	+	recently acquired toxoplasmois

Table 2. The characteristic of the population.

Group	Total	Group 1	Group 2	Group 3
Total	450	110	241	99
Age range (yr.)	4.4 – 60	25 day – 15	26 – 60	19 – 25
Mean age (yr.)	24.4	4.4	47.9	20.9
Female	218	54	118	46
Age range (yr.)	4.7 – 60	30 day – 15	26 – 60	19 – 25
Mean age (yr.)	23.8	4.7	46.1	20.6
Male	232	56	123	53
Age range (yr.)	4.05 – 60	25 d – 15	26 – 60	19 – 23
Mean age (yr.)	25.05	4.05	49.8	21.3

IgG antibody study

IgG antibody was found at low levels in all 450 serum specimens. The titre was range from 1:2-1:256. The most frequently titre was 1:8 which accounted for 30 % (136/450) as shown in Table 3. Statistical analysis was performed using ANOVA and LSD to determine the difference of the titre in each age group. IgG antibody titre tended to rise with the increasing age of the population. Using regression analysis, $r = 0.482$ and slope = 132×10^{-12} , thus IgG antibody showed no relationship with increasing age.

When only the female group was analysed, the antibody titre ranged from 1:2 – 1:256, which was similar to the male population as shown in Table 3. The most frequently found titre was also 1:8. These was also no relationship in the titre and the age found when using regression analysis ($r = 0.249$ and slope = 6.42×10^3).

Significant Toxoplasma antibody titre ($1 > 100$) was detected in only 26 persons which accounted for 5.8 %.

Table 3. Age and sex distribution of Toxoplasma ELISA IgG-Ab titre in Thai population.

Age Group (years)		No tested	<i>Toxoplasma gondii</i> IgG titre								Mode
			1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	
0-1	M	12	1	6	2	3	-	-	-	-	1:4
	F	11	1	2	5	3	-	-	-	-	1:8
2-5	M	13	1	5	5	2	-	-	-	-	-
	F	5	-	3	2	-	-	-	-	-	1:4
6-15	M	31	-	11	12	3	2	2	1	-	1:8
	F	38	1	7	16	7	2	2	2	1	1:8
15-25	M	49	-	6	11	17	5	5	1	4	1:16
	F	49	-	10	19	11	6	2	-	1	1:8
26-35	M	46	-	3	14	16	6	6	1	-	1:16
	F	37	-	3	14	10	5	4	1	-	1:8
36-45	M	35	-	1	6	14	11	1	2	-	1:16
	F	40	1	-	9	11	6	9	2	2	1:16
46-55	M	18	-	1	3	6	6	2	-	-	-
	F	25	-	1	14	6	4	-	-	-	1:4
56-60	M	29	-	-	-	5	9	7	5	2	1:32
	F	13	-	-	4	1	5	2	-	1	1:32
Total	M	232	2	33	53	66	39	23	10	6	1:16
	F	218	3	26	83	49	28	19	5	5	1:8
Total		450	5	59	136	115	67	42	15	11	1:8
Percentage		100	1.11	13.11	30.22	25.56	14.87	9.33	3.33	2.44	-

IgM antibody study

IgM antibody was found in 428 specimens from 450 specimens (95 %) with a titre ranging from negative to 1:8 as shown in Table 4. The most prevalent IgM-Ab titre in this study was 1:4 in males and 1:2 in females. If a titre of 1:100 or more was considered positive as recommended by the commercial kit, none of sera were positive. The IgM antibody result did not depend on age in either group ($r = 0.267$, slope = 0.69).

Discussion

Toxoplasma gondii antibodies among Thai people have been reported ranging from 2.3 % to 13%.⁽⁸⁻⁷⁾ These are a low prevalences when compared to people from western countries such as the reported from United State have produce a range of estimate (10-60%) of the prevalence of infection in adult.⁽⁸⁻¹⁰⁾ The highest prevalence in Thailand was found in rural villagers of Phayao province, Northern-west Thailand (13%).⁽⁴⁾

Table 4. Age and sex distribution of *Toxoplasma* ELISA IgM-Ab titre in Thai population.

Age Group (years)		No tested	- ve	<i>Toxoplasma gondii</i> IgM-Ab Titre			Mode
				1:2	1:4	1:8	
0 - 1	M	13	1	7	5	-	1:2
	F	11	-	7	4	-	1:2
2 - 5	M	13	-	9	3	1	1:2
	F	5	-	2	3	-	1:4
6 - 15	M	32	1	22	9	-	1:2
	F	38	4	23	11	-	1:2
16 - 25	M	50	-	17	30	3	1:4
	F	49	2	33	14	-	1:2
26 - 35	M	46	-	3	30	13	1:4
	F	37	-	19	18	-	1:2
36 - 45	M	30	-	5	24	1	1:4
	F	40	6	15	19	-	1:4
46 - 55	M	19	-	7	11	1	1:4
	F	25	8	12	5	-	1:2
56 - 60	M	29	-	10	17	2	1:4
	F	13	-	6	7	-	1:4
Total	M	232	2	80	129	21	1:4
	F	218	20	117	81	-	1:2
Total		450	22	197	110	21	1:2

In this study, we analysed 450 serum specimens from the healthy Bangkok population using an ELISA technique which was claimed to be a convenient and reliable way to detect IgG and IgM antibodies to *Toxoplasma gondii*.⁽¹¹⁾ The presently available combination of the IgG and IgM ELISA is a powerful tool in *Toxoplasma* serology which can be used in screening and diagnosis. When the two antibodies are found to be negative, the person is unprotected. When IgM antibodies are found but no IgG antibodies, a very early acute infection is detected. If the two antibodies are found (IgG and IgM antibodies positive) a recent or acute infection is occurring. When only IgG antibodies are found, we can conclude the person is immune due to previous exposure.

Persons with sera with IgG but without IgM antibodies are extremely unlikely to have acquired toxoplasmosis recently. In our study, significant toxoplasma IgG antibody titre (1:>100) was detected in only 5.8% - which indicated recent exposure. Even though we used a different method to detect the infection, the prevalence was still low, similar to previous studies.^(3,5-7) An interesting finding was no evidence of exposure among children below the age of 6 years, indicating little transmission is occurring by oocysts. This is probably because these children were in the middle and upper economic class levels. Persons who had a titre of 1:64 or 1:32 probably also had experienced infection, but lower titres are difficult to interpret because they probably indicate a recent infection, post-infection or a nonspecific response to the antigen. Detection of single IgG-Ab titre may not be sufficient to make the diagnosis of toxoplasmosis. Paired sera detection is preferred since it has more

accuracy to document the infection. In our study, the data were based on a single collection of blood from each individual, we did not carry out follow-up studies on the individuals found with a positive IgG-Ab titre.

IgG Ab tended to rise with the increasing age in both male and female groups but there is no statistically significant relationship in the titre and the age. The same results have been observed in many studies - presumably due to accumulated opportunities for infection.⁽¹²⁻¹³⁾ Increasing of age may have greater chance to be exposed to *Toxoplasma gondii* than the younger population and the more susceptibility of the older population to infection by *Toxoplasma gondii* may be the explanation of the difference in the titre among the age groups.

People in Bangkok have been exposed to *Toxoplasma gondii* from two major modes: occasional consumption of undercooked or raw infected meat (eg. Lamb, Naem, hamburger) and ingestion of food and water contaminated by oocysts which are shed into environment by cats.

The main diet in Bangkok is rice, fish, chicken, pork and vegetable. However, food is generally well cooked, in contrast to the rural villages of Phayao province, North-west Thailand. The habit of consuming raw or undercooked meat is potentially a significant source of *Toxoplasma* in this group. Furthermore, cats are more common domestic animals in these villages, so this population may have higher risk than the Bangkok population to exposure to the organism.

In conclusion, the study showed that the seroprevalence of *Toxoplasma* antibodies in Bangkok was 5.8%. It was lower than Phayao province and Krabi province but higher than Chiang Mai province. Children over 6 years old show a greater exposure to

Toxoplasma gondii in both sexes.

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