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The incidence of *Toxoplasma* antibodies among people in Korea, as revealed by hemagglutination test

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Toxoplasma gondii is an obligatory intracellular protozoa which has a world wide distribution in mammals including man and sometimes in birds. However, as it is difficult to isolate the parasite from the host except for the cases of acute stage of the infection, the serological tests as well as isolation of the parasite are generally used in diagnosis of the disease. It is no wonder that isolation of the parasite from patients is very difficult, because their cysts remain latent and inactive stage in central nervous system and muscle of the patients in the chronic stage. In this stage, serological tests show always positive reaction except for some of the cases who are supposed to have an inhibitory factor against the reaction. Positive reaction in serological tests of toxoplasmosis indicates the active or inactive stage of the infection in the host and also indicates the pre-existing infection in which the parasites are recently eradicated from the host.

In Korea only one study on *Toxoplasma* has been reported by Soh *et al.* (1960) regarding toxoplasmin skin tests of special group. The present authors examined for the blood from randomly selected inhabitants of Seoul and rural areas of Southern Korea using hemagglutination test by Hanaki-Nobuto-Sato's method (1963). In addition, the relationship of hemagglutination (HA) titer between new-born babies and their mothers, the HA titer of mental retarded children and some other specific groups were also reported herewith.

Materials and Methods

The blood samples used were randomly collected from inhabitants of Seoul and rural areas in Korea. Blood samples of new-born infants were collected from umbrical cord just after delivery at the Department of Obstetrics of Seoul National University Hospital. Peripheral blood from the ear lobe, venous blood or umbrical cord blood was taken and absrobed into the filter paper. These samples were examined for HA titer by Hanaki-Nobuto-Sato's method. Before reading titer, the settled tray was put in the incubator at 37°C for 3 to 4 hours and afer that the titer was read (Nakayama, 1969). The limit of titer examined was $\times$ 4,096. The titer $\times$ 256 or more was determined to be positive. The results of reaction were compared with positive and negative controls in every series of the examinations.

Results

1) *Reproducility of hemaggulutination titer in human toxoplasmosis by Hanaki-Nobuto-Sato's method*

The Hanaki-Nobuto-Sato's method was originally developed to examine the sera of swine toxoplasmosis. In order to examine the reliability of this method for human toxoplasmosis, a long-term observation was carried out on five HA-positive persons with the interval of one month. In the case 1 and 2 among them, HA titers

were × 256 to × 1,024 during ten times of examination performed in the periods of 238 days. In the case 3 and 4, HA titers were × 1,024 to × 4,096 after 5 and 7 times of examination performed in the periods of 128 to 247 days. In the case 5, HA titers were × 4,096 by two times examination. According to these findings, this test is believed to be reliable in human toxoplasmosis.

2) *HA titers in the inhabitants of Korea*

Out of 1,990 samples from the inhabitants in Seoul and rural areas, the positive rate of HA titer was 14.3 per cent (Table 1). By age group, the positive rate of new-born infants and the children aged 0-9 were 17.1 per cent and 14.5 per cent respectively. That of the age group from 10 to 29 years was around 11 per cent showing slightly decreasing tendency compared with younger age group. There was a tendency that the positive rate became gradually higher with the increase of ages. There was no significant differences by sex in HA-positive rate ; *i.e.* the positive rate of males was 13.8 per cent in 1,207 persons and that of females was 14.9 per cent in 783 persons.

The test was performed on urban and rural inhabitants. The positive rate turned out was 16.3 per cent in 1,013 urban people, in contrast to 10.4 per cent in 347 rural people (Table 2 and 3). The difference between these two groups was statistically significant ($x^2=7.189>x_0^2=6.63$, $p=0.01$). The sexual difference of positive rate in urban residents was not recognized showing in 16.1 per cent in 720 males and in 16.7 per cent in 293 females. The sexual difference of positive rate in rural residents was not also recognized, showing the positive-rate of 9.4 per cent in 223 males and 12.1 per cent in 124 females. The positive rate in urban children aged 0-9 years was 32.2 per cent and that of rural children was 10.4 per cent. Both of them present higher positive rate than that of age group of 10-29 years. When the age group of 0-9 years was subdivided into the 0-4 years and 5-9 years groups, the positive rate in urban children aged 0-4 years showed high rate of 58.8 per cent whereas that of 5-9 years age group was 21.4 per cent. This difference is statistically significant ($x^2=7.89>x_0^2=6.63$, $p=0.01$). In rural children, 0-4 years age group and 5-9 years age group showed the positive rate of 13.9 per cent and 8.3 per cent respectively (Table 4).

3) *Relationship of HA titer between the new-born babies and their mothers*

Because of high HA-positive rate in age group of 0-4 years as presented above, the transference of antibody through the placenta from mothers to their babies was postulated. Therefore, the HA titer of the mother was compared with that of her baby using the

Table 1 HA titer of inhabitants in Korea

Age	No. of inhabitants exam.	HA titer					
		<64	64	256	1,024	4,096	posi. %
New born	123	73	29	15	5	1	17.1
0- 9	275	171	64	32	6	2	14.5
10-19	573	341	169	56	7	0	11.0
20-29	534	301	170	54	8	1	11.8
30-39	274	140	80	44	10	0	19.7
40-49	86	48	18	16	4	0	23.3
50-59	94	54	25	10	2	3	16.0
60-76	31	12	11	5	1	2	25.8
Total	1,990	1,140	566	232	43	9	14.3

HA titer≧1 : 256 was determined to be positive.

Table 2 HA titer of urban inhabitants in Seoul

Age	No. of inhabitants exam.	HA titer					
		<64	64	256	1,024	4,096	posi. %
0- 9	59	18	22	16	2	1	32.2
10-19	281	171	74	34	2	0	12.8
20-29	348	195	110	37	5	1	12.4
30-39	187	92	61	27	7	0	18.2
40-49	56	27	15	11	3	0	25.0
50-59	63	34	16	10	2	1	20.6
60-76	19	7	6	4	1	1	31.6
Total	1,013	544	304	139	22	4	16.3

HA titer ≧1 : 256 was determined to be positive.

Table 3 HA titer of rural inhabitants in Korea

Age	No. of inhabitants exam.	HA titer					
		<64	64	256	1,024	4,096	posi. %
0- 9	96	65	21	6	3	1	10.4
10-19	79	52	22	4	1	0	6.3
20-29	66	45	15	5	1	0	9.1
30-39	39	29	4	6	0	0	15.4
40-49	24	16	3	4	1	0	20.8
50-59	31	20	9	0	0	2	6.5
60-76	12	5	5	1	0	1	16.7
Total	347	232	79	26	6	4	10.4

HA titer≧1 : 256 was determined to be positive.

Table 4 HA titer in children aged 0 to 9 years in Seoul

Inhabitancy	Age	No. of child. exam.	HA titer					
			<64	64	256	1,024	4,096	posi. %
Urban	0-4	17	3	4	8	1	1	58.8
	5-9	42	15	18	8	1	0	21.4
	Total	59	18	22	16	2	1	32.2
Rural	0-4	36	26	5	2	2	1	13.9
	5-9	60	39	16	4	1	0	8.3
	Total	96	65	21	6	3	1	10.4

HA titer≧1 : 256 was determined to be positive.

umbrical cord blood in urban area of Seoul. The result showed that the agreement in both mother and her baby was obtained in 80.2% of 116 couples, whereas disagreement appeared in remaining 19.8% (Table 5). The positive reactions were observed in 19 (16.4%) out of 116 cases of new-born babies. Among them, 11 cases were from HA-

positive mothers and the remaining 8 cases from negative mothers. Among 11 couples having positive titer in both mother and her baby, 8 cases were found to have same titer level in both of them and the remaining 3 disagreed.

Table 5 Relationship of HA titer between mothers and their new born babies in Seoul

HA titer of mother	HA titer of baby	No. of couples exam.	%	Remarks
+	+	11	80.2	*M=**B 8 M> B 1 M< B 2
−	−	82		
+	−	15	19.8	
−	+	8		
Total		116		

HA titer≧1 : 256 was determined to be positive.
*M and **B indicate positive titer of mother and new born baby respectively.

In order to observe the relationship between the premature delivery or abortion and the HA titer of mother and baby, 108 pairs whose history on that matter were obvious were selected (Table 6). The HA positive new-born babies from 39 mothers who had the history of premature delivery or abortion were 7 (17.9%), and those from 69 mothers who had no such a history were 12 (17.4%), and no significant difference in positive rates was recognized between them. The HA-positive baby from mothers who had the history of abortion or premature delivery and negative HA titer was only one case in this study, but those babies from the HA-negative mothers who did not have a past history were 7 in number.

A comparison was undertaken between the body weight of babies at delivery and the positivity of HA reaction in number of 110 babies. The average weight of 20 HA-positive babies was 3.16 kg, in contrast to 3.22 kg of 90 HA-negative babies. There is no significant difference between them.

Table 6 HA titer in babies born of mothers having or not having history of abortion and premature delivery

HA titer of mother	HA titer of baby	mother having history of abortion	mother having no history of abortion
+	+	6 *M=**B 4 M> B 1 M< B 1	5 M=B 4 M<B 1
−	−	25	52
+	−	7	5
−	+	1	7
% of positive baby		17.9	17.4

HA titer≧1 : 256 was determined to be positive.
*M and **B indicate positive titer of mother and new-born baby respectively.

An observation was made on correlation of mother's age and their babies in HA titer (Table 7). From 64 mothers aged 20–29 years, 10 (15.6%) HA-positive babies were born and 10 (23.3%) from 42 mothers aged 30–39 years. However, the difference between these positive rates was not statistically significant.

Table 7 Relationship between positive HA titer in new-born baby and age of mother

Age of mother	No. of baby exam.	Positive baby No.	Positive baby %
20–29	64	10	15.6
30–39	42	10	23.3
40–42	2	0	0

HA titer≧1 : 256 was determined to be positive.

4) *HA titers in specific groups*

a) *Mentally deficient group :* The HA test was carried out on the blood samples from 303 mental deficiencies whose ages ranged from 6 to 49 years. The positive reactions were observed in 31 (10.2%) cases (Table 8): No significant difference was recognized by age groups.

In order to compare the results in Korea with those in Japan, the HA titer was

Table 8 HA titer in feebled-minded persons

Age	No. of persons exam.	HA titer					
		<64	64	256	1,024	4,096	posi. %
6- 9	40	27	9	4	0	0	10.0
10-19	199	106	72	17	4	0	10.6
20-29	55	23	27	4	1	0	9.1
30-39	5	5	0	0	0	0	0
40-49	4	3	0	1	0	0	25.0
Total	303	164	108	26	5	0	10.2

HA titer≧1 : 256 was determined to be positive.

Table 9 HA titer of children in Shimada Hospital in Tokyo where feebled-minded and deformity children are accommodated

Age	No. of child. exam.	HA titer					
		<64	64	256	1,024	4,096	posi. %
0- 4	23	11	10	2	0	0	8.7
5- 9	49	19	21	9	0	0	18.4
10-19	108	38	40	25	5	0	27.8
20-27	6	1	4	0	1	0	16.7
Total	186	69	75	36	6	0	22.6

HA titer≧1 : 256 was determined to be positive.

Table 10 HA titer examined at pediatric hospital for orphans

Age	No. of child. exam.	HA titer					
		<64	64	256	1,024	4,096	posi. %
0- 4	56	41	10	4	1	0	8.9
5- 9	24	20	2	2	0	0	8.3
10-16	14	12	1	1	0	0	7.1
Total	94	73	13	7	1	0	8.5

HA titer≧1 : 256 was determined to be positive.

examined for the 186 mentally deficient or congenitally malformed children aged 0-27 years in Shimada Hospital in Tokyo (Table 9). The positive rate was 22.6 per cent in total, being 15.6 per cent in 90 female and 29.2 per cent in 96 male children. In this series of examination, the age group of 0-9 years showed lower positive rate than that of 10-19 years.

b) *Patients in Pediatric Hospital for Orphans:* Orphans admitted in Pediatric Hospital in Seoul for the treatment of various medical and surgical condition and new-bron infants admitted for the pediatric protection were examined for HA titers of toxoplasmosis. Positive reaction appeared in only 8 (8.5%) out of 94 orphans (Table 10). The higher positive rate was not recognized in

Table 11 HA titer in special groups

Group	No. of persons exam.	Positive HA titer	
		No.	%
Blood donors	27	2	7.4
Soldiers	45	3	6.7
Positive case of Paragonims skin test	55	4	7.2
Chlonorchiasis patients	4	1	25.0
Rural sclool children	50	3	6.0
Children in T.B. sanatrium	69	8	11.6
Bilateral uveitis patient	1	0	0
Total	251	21	8.4

HA titer≧1 : 256 was determined to be positive.

lower age group in contrast to general population in Korea as stated above.

c) *Other groups:* These groups include people infected with helminths, children in Tuberculosis Sanatorium and others. Out of 251 persons, 21 (8.4%) were HA-positive and any group did not show significantly higher positive rates (Table 11).

Discussion

Among the various methods of hemagglutination tests in toxoplasmosis, Hanaki-Nobuto-Sato's method was developed originally for the detection of swine toxoplasmosis. Recently its applicability was expanded to human toxoplasmosis, because the technics are rather simple and the sensitized red cells are commercially available in Japan. Nakayama (1969) examined this methods in rabbits and mice infected with *Toxoplasma* and concluded that Hanaki-Nobuto-Sato's method was reproducible in toxoplasmosis of these experimental animals. In the present test in human toxoplasmosis, also the satisfactory result was obtained in the reproducibility of HA titer.

In our survey applying this method of test for the randomly selected 1,990 Korean inhabitants of Seoul and rural areas, the positive rate was 14.3 per cent and no sexual difference in the rate was observed. By the report of Soh *et al.* (1960), the positive rate among specific groups were 5.6 per cent in 373 persons by the toxoplasmin skin test in Korea. Using the hemagglutination test, Zaman (1968) reported 17.2 per cent of positive rate in the inhabitants of Singapore. In Japan, Hasegawa *et al.* (1954) reported 7.2 per cent of positive rate in 483 people, using the dye test when the titer of 1 : 16 or more was determined to be positive. Using the dye test, Shimizu *et al.* (1958) reported 6.2 per cent of positive rate in 512 adults in Japan and concluded that no sexual difference in positive rate existed.

Recently, toxoplasmosis in Japan has attracted many clinical attentions especially in the fields of opthalmology, obstetrics and pediatrics. Many kinds of investigations on this problem has been undertaken. Because of the simplicity of practice, skin test which is delayed type hypersensitivity reaction (Sabin *et al.*, 1942; Frenkel, 1948) has been applied widely. Using this skin test, the positive rates of toxoplasmosis has been reported in Japan as 19 per cent by Tominaga *et al.* (1961), 7.6 per cent and 13.6 per cent by duplicated examinations in 340 inhabitants in Okinawa by Uehara *et al.* (1965), 11. 1per cent in 958 school children by Hongo *et al.* (1965) and 13 per cent by Wada *et al.* (1965). Using the same kind of method, Tsunematsu (1967) reported that the positive rate of persons aged under 20 years was less than 10 per cent and the rates rose with the increase of age, attaining to 40 per cent in 50 or older age group. By the report of

Hanada *et al.* (1967), only 1.5 per cent in 3,475 babies at the age of 3 years were found to be positive by skin test. As *Toxoplasma* infection is postulated to be transmitted through the mediation of pork, some series of surveys were undertaken for the butchers and workers in slaughter house. By the results of Wada *et al.* (1965), the skin test positives were 67–80 per cent and that by Kawado *et al.* (1966) was 17.9 per cent and 44 per cent by Imamura *et al.* (1966). Kosaka *et al.* (1967) reported 22.7 per cent of skin test positive rate in 1,301 butchers. Discrepancies in the results might be explained by variability in infection status in different areas, difference in examined groups, and some technical factors in the skin test.

Many survey reports by the hemagglutination test using *Toxoplasma* sensitized red blood cells have appeared recently. In the reports of Tsurumi *et al.* (1967), Suda (1967) and Sato *et al.* (1967), the positive rates were 7.7, 20.6 and 31 per cent respectively. Ouchi *et al.* (1969) examined 2,454 pregnant women and reported the positive rate as 23.8 per cent. Tsunematsu (1967) observed that the HA-positive rates were below 10 per cent in the group of persons aged less than 29 years and increased up to 39 per cent in 60-years or older age group using Lewis and Kessel method (1961). The results of present study in Korea showed the same tendency with that of Tsunematsu, except for the appearance of high positive rates in the lower age group under 10-years including the new-born babies. Although the sample size was small, it turned out that the positive rate was higher in age group of under 4 years than in 5–9 years both in urban and rural areas, and further observations are necessitated to confirm it. However, the reason of higher positive rate in yonger children does not seem to be caused by transfer of the antibody from mother, because it is generally accepted that the antibody from the mother does not persist in the child more than one year. It seems to offer some possibilities such as early infection or other factors.

As the transfer of the antibody from mother to fetus through placenta is postulated, 116 pairs of mother and her baby were examined for HA titer. Agreement of the results in both mother and her baby was obtained in 80.2 per cent, whereas disagreement appeared in remaining 19.8 per cent of the cases tested. Among the cases which showed disagreement, 8 cases were positive neonatal babies from mothers with negative titer. Only one was a baby whose mother had the history of abortion, and mothers of the remaining 7 babies had no such histories. The reasons why the babies with positive reactions come from the HA negative mothers can not be explained.

The relations between the positive HA reaction and the history of premature delivery or abortion in mothers were reported by various workers. Hanazawa (1966) reported that the incidence of positive skin test were twice in women with history of habitual abortion than in women without such history. Tanaka *et al.* (1967) reported that the HA positive rate in normal women was 10.8 per cent, but that of the women with habitual abortion were 23.8 per cent. The significant difference was recognized between them. Ouchi *et al.* (1969) examined 2,454 pregant women finding HA-positive cases in 23.8 per cent. They stated that the higher rate was observed in a group with a history of abortion or premature delivery. Kobayashi *et al.* (1967) observed the relation between the HA-positive rates and number of abortions in 510 women. They reported that the HA-positive rates were 17.6 per cent, 24.2 per cent and 42.8 per cent in women who had such histories once, twice and three times or more respectively. On the other hand, Yatsugami *et al.* (1965) reported that among 150 women experienced habitual abortion, the HA-positive rate was 20.0 per cent, but the rate in 50 normally pregnant and non-pregnant women was 20.3 per cent. Another example was the report of Oniki (1965), showing that HA-positive rate was 20 per cent in normal pregnant women, 22 per cent in women with the history of abor-

tion or premature delivery, and 22 per cent in normal non-pregnant women. Tsunematsu (1967) reported that the HA-positive rates were 21 per cent in normal pregnant women and 28 per cent in 102 women with habitural abortion. He concluded that the difference was not singinficant, as the age factor influenced on the positive rate. Koga *et al.* (1968) also reported that no significant difference was observed between the normal women, women with histories of abortion, and with histories of premature delivery, because they obtained the date of HA-positive rates as 15.5 per cent, 13.3 per cent and 16.4 per cent respectively.

Hongo *et al.* (1965) reported that 11.1 per cent of 958 school children was positive by skin test, while 10.8 per cent of 46 mental dificiencies was positive. Torii *et al.* (1965) also reported that in 59 mental deficiencies the rate was 16.9 per cent. In our study it could be hardly stated that the positive rate of mental deficiency is higher than that of normal population.

In the examinations of children in Tuberculosis Sanatorium, helminths-infected group and military personnels, the positive rates obtained were lower than that of normal populations. Therefore, it appears that the cross reaction by this test with other kinds of infection does not occur.

Conclusion

In order to evaluate the status of *Toxoplasma* infection in Korean people, randomly selected from Seoul and rural districts were examined by Hanaki-Nobuto-Sato's hemagglutination test, and the following results were obtained.

1) The HA-positive rates: The overall positive rate was 14.3 per cent in 1990 persons and difference of the rate by sex was not observed. The higher rate was obtained in age group 0-9 years and new-bron infants. Although the rates were slumped in age group of 10-29 years, they gradually increased with ages. Statistically singnificant difference in positive rate were observed between the rural and urban people, showing 10.4 per cent and 16.3 per cent respectevely. The highest rates 32.2 per cent were observed in urban children aged 0-9 years. Subdividing the group into the 0-4 and 5-9 years, positive incidence of the former was 58.8 per cent and the latter was 21.4 per cent, indicating a significant difference. This tendency was observed in rural children also.

2) Correlation of HA titers between mothers and their babies: 116 pairs of mothers and their new-born babies were concomitantly examined for *Toxoplasma* HA test. Result of the examination agreeded in 80.2 per cent of the pairs tested including 11 paris of positives and 82 pairs of negatives. No correlation between previous abortions of the mother and positive baby was recognized. No difference was present in body weight of the positively and negatively reacted babies. No statistically significant differences was observed between the positive rates of babies and the ages of mothers.

3) HA reactions in mental deficiency: 303 mental deiciencies in various institutes in Korea were examined and 10.2 per cent of positive rate was obtained. It was not different from that of normal inhabitants.

4) HA reactions in some specific groups: After the examination of children in tuberculosis sanatorium, helminths-infected group and military personnels, the positive rate was rather lower than that of normal inhabitants.

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韓国における感作血球凝集反応によるヒト・トキソプラスマ抗体の出現状況

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無作意に採血したソウル市街地および韓国農村の住民について，花木，信藤，佐藤法によつて Tp 抗体値を検した．予め東京において5名の抗体値陽性者を選び長期にわたつて本法による抗体値の再現性を検し，満足すべき成績が得られた．抗体値≧1：265を陽性値とした場合，韓国住民1,990名の陽性率は14.3%であり，性別による差異は認められなかつた．このうち新生児および0～9歳児の陽性率はそれぞれ17.1%および14.5%で10～19歳児になると陽性率低下し，以後は年齢の進むにつれて陽性率増大の傾向を示した．これら被検者のうち生育，居住地の明確なものを選び市街地および農村住民にわけて検した陽性率はそれぞれ16.3%および10.4%で推計学上有意の差のあることを知つた．市街地住民0～9歳児の陽性率は32.2%で特に高率を示したので，年齢を更に0～4歳と5～9歳に細分して陽性率をみると，前者は58.8%の高率を示し，後者は21.4%を示し，推計学上有意差を示した．

そこで，116例の母とそれらの新生児の抗体値を検した結果は母子とも陽性11例，両者陰性82例であり，一致率は80.2%であつた．不一致例について母陽性で新生児陰性は15例で，この逆は8例であつた．これらの8例の陰性母から陽性の新生児が出現した理由については説明し難い．なお，母の早流産既往の有無と新生児抗体値との関係をみたが，既往母39名からの新生児陽性率は17.9%で非既往の母69名からのそれは17.4%で両者に全く差異がなかつた．陽性および陰性新生児の平均体重はそれぞれ31.6 kgおよび3.22 kgで，これまた両者間に有意差は認められなかつた．母の年齢と新生児陽性との関係についても検したが，20～29歳の母からの新生児は64例中15.6%に，30～39歳のそれは42例中23.3%に陽性児が認められたが，推計上学の有意差ではない．

精薄者および小児病院収容児の陽性率はそれぞれ303名中10.2%および94名中8.5%であつた．なお，特殊群として検した蠕虫寄生者，結核児等251名の陽性率は8.4%でむしろ低かつた．