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Indirect Haemagglutination and Indirect Fluorescent Test Antibodies against *Toxoplasma gondii* among Blood Donors in Sri Lanka

by

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SUMMARY Of 1069 sera from predominantly male blood donors aged between 17 and 48 years, 641 (60.0%) were negative for IHA antibodies against *Toxoplasma gondii* and 428 (40.0%) were positive in titres ranging from 1:2 to 1:32, 768. In the IFA, 463 (43.3%) were negative and 606 (56.7%) were positive in titres ranging from 1:2 to 1:4096. The titre of the highest frequency of distribution for the IHA was 1:128 and for the IFA was 1:64. With the IHA there were 180 sera at and above the titre of the highest frequency of distribution and with the IFA there were 176 such sera, giving prevalence rates of 16.8% and 16.5% respectively.

Although there was a statistically significant increase in the results of the IHA at a titre of 1:16 and above in the age group 36-40 years compared to those of the age group 17-20 years, this was not considered to show an increase in the infection rates with age. There were also statistically significant increases in the prevalence rate and rates at a titre of 1:16 and above in the IHA in the present study compared to those of an earlier study on neonates. These differences and differences in the prevalence rates of toxoplasmosis in other parts of the world are discussed.

INTRODUCTION

Studies on the frequency of distribution of antibody titres in normal adult populations have been carried out throughout the world using the Sabin-Feldman dye test and complement fixation tests. These have recently been made using the indirect haemagglutination test (IHA) of Jacobs and Lunde (1957). Such surveys in addition to providing epidemiological information regarding the prevalence of toxoplasmosis also provides clinicians with information on the significance of the antibody status of their patients. This is of extreme importance in a common infection like toxoplasmosis which may be asymptomatic or with protean clinical manifestations. Though a study has been made to obtain such data by de Silva, Kulasiri, Sugathapala and Amarasinghe (1972) in neonates in Sri Lanka using the IHA, the corresponding values for the adult population are not available.

The indirect fluorescent antibody test (IFA) of Kelen, Ayllon-Leindl and Labzoffsky (1962) is now coming into prominence for laboratory diagnosis of toxoplasmosis due to the safety and simplicity in carrying out the test and may even replace the classical dye test in serological investigations of clinical toxoplasmosis. The IFA antibodies have now been shown to be different from the IHA antibodies. Therefore the present investigation was undertaken to obtain these basic data on the distribution pattern of the IHA and IFA antibodies in the normal adult population.

MATERIALS AND METHODS

Samples of blood from donors calling at the Blood Bank, General Hospital, Colombo were collected from May to August 1971. With a few exceptions the age and sex of the donors were noted at the time of collection. The day's collection of samples were stored in the refrigerator overnight. The sera were separated on the following day and stored in the deep freeze (-20°C) till required for examination. Some of the samples of sera were stored as long as 6 months or more before testing or retesting in cases where a large discrepancy occurred between the results of the IHA and IFA.

The micro-modification method (Kulasiri, 1970; Kulasiri and Amarasinghe, 1970) of the indirect haemagglutination test of Jacobs and Lunde (1957) was performed. Two-fold serial dilutions were made using Takatsy microdiluters. Sera showing "heterophile" antibodies were reabsorbed with larger quantities of sheep erythrocytes till the sample was clear of such antibodies. Those samples where this could not be carried out after a few attempts were discarded from the series. The *Toxoplasma* haemagglutination antigen was prepared from mouse ascitic fluid by the method of Lunde and Jacobs (1964).

The IFA (Kelen, Ayllon-Leindl and Labzoffsky, 1962) was used with some modifications. The antigen was prepared from *Toxoplasma* from mouse ascitic fluid, fixed in 1% formalin in phosphate-buffered saline of pH. 7.2 (PBS) for 30 mins. Further processing of the antigen was as described by Sulzer and Hall (1967). Antigen slides were prepared from the *Toxoplasma* suspension by applying small drops of the suspension in 4 spots along either edge of microscopic slides and allowing them to dry in the air. Thus each slide would have 8 smears, each smear about 1.5 mm. in diameter. The smears were individually ringed with a "diamond" pencil to indicate the location of the smear during processing and examining. The prepared antigen slides and the bulk antigen in small quantities were stored in the deep freeze at -20°C.

The anti-human conjugate used in this study was fluorescein-labelled anti-human sheep immunoglobulins prepared by Wellcome Reagents Limited. The contents of each vial of freeze-dried conjugate were dissolved in 1 ml. of distilled water as recommended and then made upto the optimum dilution in 1:2000 solution of Evans blue in PBS. Each batch of conjugate was titrated for optimum activity using human positive and negative sera of known titre. The conjugate functioned satisfactorily at a dilution of 1:100 but throughout this study a dilution of 1:50 was used. According to the manufacturers the anti-human conjugate contained antibodies against IgM, IgG and IgA immunoglobulins.

In the performance of the IFA the sera were not inactivated prior to use. Two-fold serial dilutions were made in PBS using Takatsy microdiluters as before. Starting with the highest dilution a drop of each of the serum dilutions was applied on the corresponding smear on the antigen slide with a Pasteur pipette. The smears were then incubated in a moisture chamber placed in an air incubator at 37°C. for 30 mins. After

the incubation the smears were washed first with distilled water, then with PBS, dried by gentle blotting and drops of anti-human conjugate in the optimum dilution applied to the smears. The smears were further incubated for 30 mins. at 37°C. as before. They were washed in distilled water, PBS, dried by gentle blotting and mounted in glycerol containing 10% of phosphate buffer of pH 8.0.

The slides were examined under a Leitz "Dialux" microscope adapted for fluorescent microscopy. The source of ultra-violet light was a high pressure mercury lamp "HBO 200" used with a BG 12 exciter filter and BG 38 ultra-violet filters. The microscope was equipped with a dark ground condenser, fluorite dry high power objective (x42), a monocular tube (x1.25) and an ocular (x12) fitted with a K 530 ocular filter.

The sera were first screened from dilution 1:2 to 1:256 and those showing positivity above 1:256 were repeated starting at an initial dilution of 1:32 and going upto 1:4096. Those showing still higher positivity were again retested starting at a still higher initial dilution. With every batch of specimens known positive and negative controls were used. The positive control was intially diluted up to 2 dilutions below the known titre and two-fold dilutions carried out from this initial dilution to a dilution above the known titre. The negative control was diluted from 1:2 to 1:16 in two-fold dilutions. The positive serum dilutions were applied to the smears on one side of the control antigen slide and those of the negative serum applied to the smears on the other edge. A batch of tests was acceptable only if there was not more than a difference of one two-fold dilution from the known titre in the positive and negative controls. The examination under the fluorescent microscope was carried out within 24 hours of the preparation of the slides. Where the examination could not be done immediately the slides were left in the refrigerator.

Sera showing a negative in the IHA with a positive in the IFA were repeated with the IHA after kaolin absorption to remove haemagglutination inhibitors as recommended by Lunde and Jacobs (1963). Those sera that showed a wide discrepancy in the results of the two tests were repeated with both tests.

The results obtained with the two tests in the various age groups were compared with those obtained in the age group 17 - 20 years at two levels. At the first level the titre 1:16 was considered as the base and comparisons were made between the values for titres below 1:16 and for those for titres of 1:16 and above. At the second level the titre of the highest frequency of distribution was taken as the base. Thus for the IHA the comparisons were made among those values for titres below 1:128 and those of titres 1:128 and above. Similarly for the IFA the comparisons were made between values for titres below 1:64 and those for titres of 1:64 and above. The statistical analyses were carried out by determining the X^2 using the "fourfold" table with Yates correction. As the number of individuals in the age group 46-48 years was small, its results were not statistically analysed. The results obtained in the present study were also compared using the X^2 test with those obtained in the survey of IHA antibodies in neonates by de Silva *et al.* (1972). The analyses were carried out at both levels i. e. 1:16 and above and 1:128 and above.

RESULTS

A total of 1069 donor sera were examined by the IHA and the IFA. Of these 26 were females and 964 were males. In 79 individuals the sex was not recorded. The ages of the donors ranged from 17 years to 48 years, more than half of the number falling between 21 and 30 years of age. Of the 156 donors aged between 17 and 20 years, only one donor was aged 17 years and of the 10 donors between the ages of 46 and 48 years, 3 donors were 46 years old, 4 were 47 years old and the other 3 were 48 years old. All these donors were males. In 68 persons, the age was not recorded.

Table 1 shows the distribution of the titres among the various dilutions tested and their percentages of the total number of sera examined. Graph I shows the frequency of the percentage distribution of the positive titres in the two tests. It is seen from these that the titre of the highest frequency of distribution in the IHA is 1:128. Although in the IFA the number of positives in the titres 1:2 and 1:4 is higher than that in the next peak at titre 1:64, it is seen from Graph 1 that there is a progressive reduction in the frequency of distribution from the titre 1:2 to titre 1:16 from which it rises again to reach the maximum at 1:64. The titre 1:64 is considered as the titre of the highest frequency of distribution. In the IHA there were 180 sera at and over the titre of the highest frequency of distribution for this test namely 1:128 and in the IFA there were 176 sera at and over the highest frequency of distribution for this test namely 1:64.

TABLE 1

The distribution of antibody titres of the IHA and the IFA

Titre	*IHA*		*IFA*	
	No.	*Percent.*	*No.*	*Percent.*
Negative	641	60.0	463	43.3
1:2	19	1.8	121	11.3
1:4	25	2.3	105	9.8
1:8	26	2.4	79	7.4
1:16	32	3.0	52	4.9
1:32	71	6.6	73	6.8
1:64	75	7.0	84	7.9
1:128	96	9.0	58	5.4
1:256	38	3.6	21	2.0
1:512	24	2.2	8	0.7
1:1024	11	1.0	2	0.2
1:2048	9	0.8	2	0.2
1:4096	—	—	1	0.1
1:8192	1	0.1	—	—
1:16382	—	—	—	—
1:32768	1	0.1	—	—

Graph 2 shows the percentage distribution of the IHA results of the blood donor population alongside that of the neonate population studied by de Silva *et al.* (1972). As the two curves overlap each other between titres 1:8192 and 1:16,384 only the curve obtained with the blood bank sera is shown. Again the two curves follow similar patterns from the titre 1:16 except for an inversion at titre 1:64.

Table 2 shows the titres of the IHA in relation to those of the IFA. If titres below 1:16 are considered unimportant, then of the 1069 sera examined with the two tests, 711 (66.5%) of the sera were below a titre of 1:16 in the IHA and 358 (33.5%) of the sera were positive at a titre of 1:16 or higher. Similarly in the IFA 768 (71.8%) of the sera were below a titre of 1:16 and 301 (28.1%) were at a titre of 1:16 or higher. It is also seen from Table 2 that the majority of the titres obtained with one test falls within ± of a two-fold dilution of the corresponding titre of the other test. This is especially noticeable when sera with high titres are considered.

TABLE 2.

The titres of the indirect haemagglutination antibody test (IHA) in relation to those of the indirect fluorescent antibody test (IFA). The reciprocals of the titres are given.

		IFA TITRES												
		0	2	4	8	16	32	64	128	256	512	1024	2048	4096
	0	430	105	66	34	4	1	1	—	—	—	—	—	—
	2	12	3	1	1	2	—	—	—	—	—	—	—	—
I	4	9	3	7	3	2	1	—	—	—	—	—	—	—
H	8	4	3	5	5	3	5	—	1	—	—	—	—	—
A	16	2	5	9	9	4	1	1	1	—	—	—	—	—
	32	4	2	14	13	18	10	8	1	1	—	—	—	—
T	64	2	—	1	5	9	24	24	6	4	—	—	—	—
I	128	—	—	2	6	9	23	29	21	5	1	—	—	—
T	256	—	—	—	2	—	5	11	13	7	—	—	—	—
R	512	—	—	—	1	1	1	7	7	2	4	—	1	—
E	1024	—	—	—	—	—	2	1	4	1	2	1	—	—
S	2048	—	—	—	—	—	—	2	4	1	1	1	—	—
	4096	—	—	—	—	—	—	—	—	—	—	—	—	—
	8192	—	—	—	—	—	—	—	—	—	—	—	1	—
	16384	—	—	—	—	—	—	—	—	—	—	—	—	—
	32768	—	—	—	—	—	—	—	—	—	—	—	—	1

Tables 3 and 4 show the respective distribution of IHA titres and IFA titres according to the ages of the donors. When the results of the IHA of the age group 17 - 20 years were compared with the other age groups at the first level namely the number of sera below the titre 1:16 and that at titre 1:16 and higher a statistically significant increase in the number of sera showing titres of 1:16 and higher was seen in the results of the age group 36-40 years ($X^2 = 5.804$, probability <0.02). In the comparisons at the second level i. e. the number of sera showing titres below the titre of the highest frequency of distribution and that of the titre of the highest frequency and above no statistically significant difference was seen. In the IFA results none of the comparisons showed any statistically significant differences with age.

TABLE 3.

The distribution of indirect haemagglutination antibody titres according to the age groups of the donors

Age in years	Number of donors	RECIPROCALS OF TITRES															
		0	2	4	8	16	32	64	128	256	512	1024	2048	4096	8192	16384	32768
		No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
17-20	156	107	3	3	2	2	8	13	7	6	2	2	—	—	—	—	1
21-25	299	182	5	9	7	9	14	21	29	9	6	2	5	—	1	—	—
26-30	260	148	3	8	8	7	21	16	23	15	4	5	2	—	—	—	—
31-35	136	83	1	3	4	6	11	10	9	2	6	1	—	—	—	—	—
36-40	104	54	3	1	3	5	12	4	16	1	2	1	2	—	—	—	—
41-45	36	22	2	1	2	1	3	1	3	—	1	—	—	—	—	—	—
46-48	10	4	—	—	—	—	1	2	—	2	1	—	—	—	—	—	—
Total	1001	600	17	25	26	30	70	67	87	35	22	11	9	—	1	—	1

TABLE 4.

The distribution of indirect fluoroscent antibody titres according to age groups of the donors

Age in years	Number of donors	RECIPROCALS OF TITRES															
		0	2	4	8	16	32	64	128	256	512	1024	2048	4096	8192	16384	32768
		No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
17-20	156	85	14	11	12	7	6	7	6	5	2	—	—	1	—	—	—
21-25	299	129	40	22	20	15	24	20	15	7	4	2	1	—	—	—	—
26-30	260	100	28	35	21	9	17	26	17	5	1	—	1	—	—	—	—
31-35	136	55	15	20	11	9	7	8	7	2	2	—	—	—	—	—	—
36-40	104	44	9	9	8	5	11	12	5	1	—	—	—	—	—	—	—
41-45	36	14	6	2	5	4	3	2	—	—	—	—	—	—	—	—	—
46-48	10	5	—	—	—	—	2	1	1	—	1	—	—	—	—	—	—
Total	1001	432	112	99	77	49	70	76	51	20	10	2	2	1	—	—	—

In the comparisons of the results of the blood bank sera with those of the neonate sera (de Silva *et al.*, 1972) at the first level 358 (33.5%) of the blood bank sera showed positive while 172 (23.4%) of the neonate sera were positive. These values show a statistically high significance ($X^2 = 20.722$, probability < 0.01). At the second level 180 (16.8%) of the blood bank sera showed positive while 97 (13.2%) were positive in the neonate sera. These results are statistically significant ($X^2 = 4.112$, probability < 0.05).

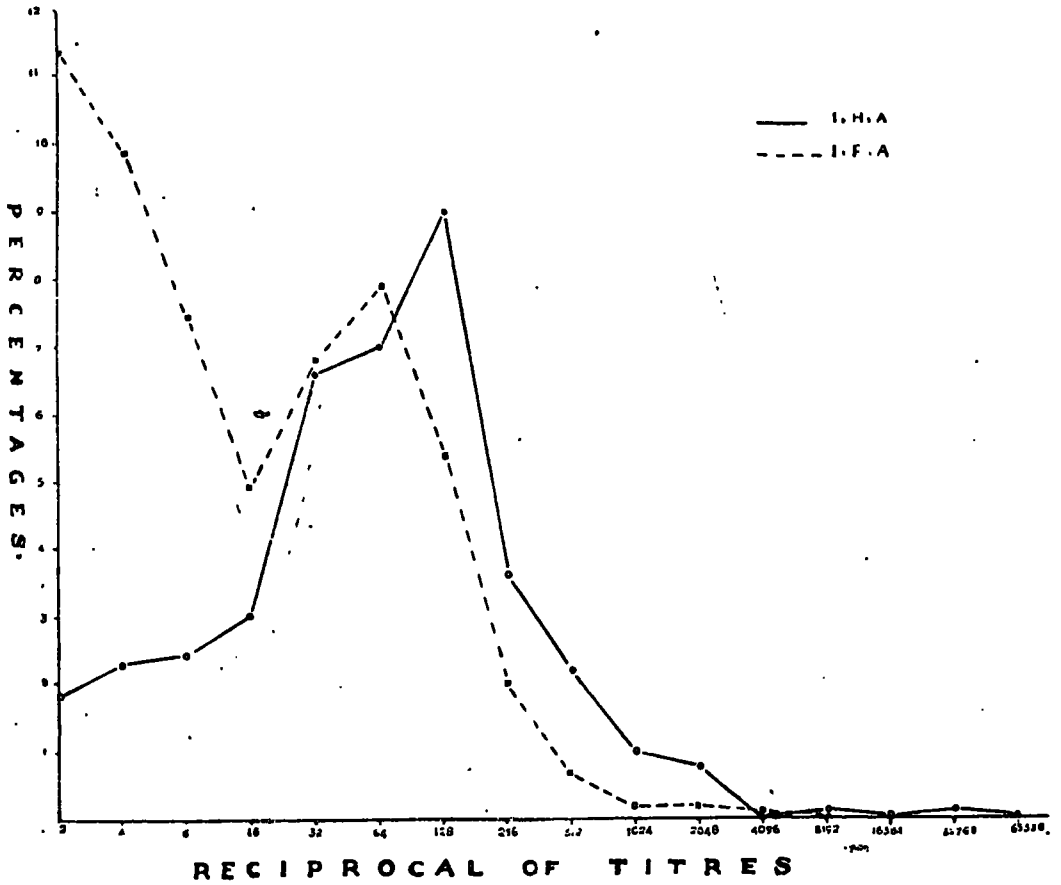
DISCUSSION

The sample of the population that was examined was predominantly male and between the ages of 18 and 46 years, more than half of them being between 21 and 30 years. Therefore the results obtained in this survey should strictly apply to males of age between 18 and 46 years.

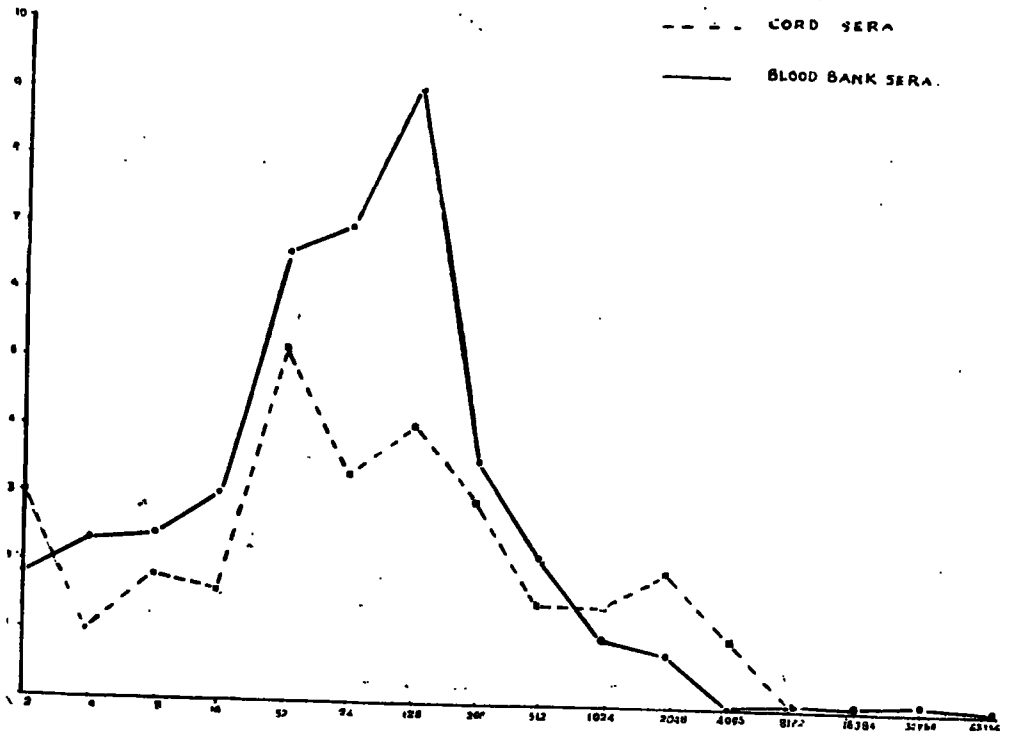
In the population studied there was a wide disparity in the percentages of the IHA and IFA titres among the lower dilutions i. e. titres below 1:16 (Table 1). Primarily the number of negatives in the IHA was larger than in IFA. On sudying the IFA results of the 641 sera showing an IHA negative reaction 430 sera were also negative in the IFA (Table 2). Of the remaining 211 sera showing a negative IHA reaction, 171 sera showed titres up to 1:4 in the IFA making a total of 601 sera within one fourfold dilution of the negative reaction in the IHA. A difference of this order is acceptable in comparing two different serological reactions with different characteristics.

The curves showing the percentage distribution of IHA and IFA positive titres (Graph 1) converged to titre 1:16 from widely separated rates at titre 1:2. In the IHA there was a progressive rise in the curve from titre 1:2 to titre 1:16 and then to the titre of the highest frequency of distribution. On the other hand in the IFA there was a progressive drop in the curve to the titre 1:16 from which there was a rise in the curve to reach its titre of the highest frequency of distribution. The IHA and IFA curves above the titre 1:16 ran more or less parallel. However the IHA curve at titre 1:32 showed a distinct displacement as was seen in the previous study (Graph 2) on the IHA antibodies in neonates (de Silva *et al.*, 1972). The IFA curve also showed a similar displacement but to a lesser extent. The titres of the highest frequency of distribution for the IHA and IFA were 1:128 and 1:64 respectively. After these titres, the frequencies of distribution decreased. Up to the titre of 1:128 the number of positives was greater in the IFA than in the IHA for each titre while at the titre 1:128 and above there were more positives in the IHA than in the IFA. In the acute phase of toxoplasmosis the IFA antibodies appear earlier than the IHA antibodies and reach higher levels faster, while in the convalescent phase the IFA antibodies begin to disappear earlier than the IHA antibodies. Further, as seen in this study the IHA antibodies reach a higher level than the IFA antibodies. They also disappear slower. Because of this lag in the appearance and disappearance of the two types of antibodies and the level to which they are formed, the two curves would be separate from each other. The distribution of antibody titres observed can be assumed to be the result of such differences in the two types of antibodies. The consistent displacement of the curves at titre 1:32 cannot be explained. It is also seen from this study that no useful purpose is served by the examination of sera below the titre 1:16.

The number of sera at and above the respective titres of the highest frequency of distribution for the two tests was practically the same, 180 for the IHA and 176 for the IFA. The similarity of the curves and these figures indicated that the two tests were similar and reliable in their performance. This study also revealed that because of the lag in time in the appearance and disappearance of the two types of antibodies the two tests are complementary to each other. Further it revealed that an infection with *Toxoplasma* did not necessarily cause acute disease as one of the donors had an IFA titre of 1:4096 and an IHA titre of 1:32,768 suggesting a very recent infection without the patient being aware of it.



Graph 1. The percentage distribution of IHA and IFA titres in the 1069 sera examined.



Graph 2. The percentage distribution of IHA titres in the blood bank sera and the neonate sera.

Jennis (1963) did not find an increasing prevalence of antibodies in the IHA with increasing age in New South Wales, Australia but Nakayama *et al.* (1970) found statistically significant increases in the IHA at titres of 1:256 and above among age groups 40-49 and 60-76 years compared with that of the age group 10 - 19 years. In Sri Lanka a statistically significant increase was recorded only in the titres of 1:16 and above in the age group 36-40 years compared with the age group 17-20 years. This increase in the low titres of this age group cannot be considered as an increase in the infection rates with age as seen among the Koreans as such increases were not recorded in the titres of 1:128 and higher or in the titres of the IFA. The differences in the prevalence rates among the people of Australia and Sri Lanka and those of Korea may be due to different food habits of the people concerned and their associations with the reservoir hosts.

The present study also confirmed the titre of the highest frequency of distribution of the IHA namely titre 1:128 obtained by de Silva *et al.* (1972). However the percentage of positive titres in the various dilutions in the previous study was lower than those of the present study up to the titre 1:512 and it was higher in the previous study above this titre. But there was a statistically significant increase in the positives at and above 1:16 and at and above 1:128 in the present study. The similarity of the two curves indicated that the two populations, the adult and neonate whose antibodies represent those mainly obtained from their mothers were exposed to the name extent. The differences seen in the percentages of distribution may be due to one or more of three causes. A differential in the antibody titres of the mother and her neonate could cause such a variation. A difference in the percentage distribution of antibody in the male and female populations may also cause such a difference. Finally different age compositions of the blood donors and the mothers of the neonates could influence the percentage distribuion. Jennis (1963) and Nakayama *et al.* (1970) did not find any statistically significant differences between the sexes in the incidence of positive reactions and the latter workers could not record a significant difference between the antibody levels of the mothers and their respective babies. Though Nakayama *et al.* (1970) found a significant increase in the prevalence rate of *Toxoplasma* antibodies in the older age groups, such increases were not seen in Australia and Sri Lanka as discussed earlier. Therefore further studies on the distribution of antibodies especially among females are indicated to understand the differences in the distribution patterns of antibodies in the neonates and the adult population in Sri Lanka.

As mentioned earlier the titre of the highest frequency of distribution for the IHA in Sri Lanka was 1:128. This value is lower by a twofold dilution than those obtained for U. S. military recruits (Walls *et al.*, 1967), and for military recruits from Brazil (Walls and Kagan, 1967). However the same value of 1:128 was obtained by Jennis (1963) for blood donors in New South Wales, Australia, by Kagan and Walls (1968) for military recruits from Colombia and by Walls and Kagan (1967) for military recruits from some parts of Brazil, namely Piaui, Ceará, Paraiba, Pernambuco and Bahia.

Although the values of the titre of the highest frequency of distribution were the same in the latter group, the percentages of positives at each dilution were different. Therefore the curve of the percentage distribution of positive sera was different for each country depending on those values. The curve for the blood donors of New South Wales, Australia (Jennis, 1963) was very similar to that for Sri Lanka while those obtained for military recruits of Brazil (Piaui, Ceará Paraiba, Pernambuco and Bahia) and Colombia were basically different.

The prevalence rates i. e. the percentages of positives at and above the titre of the highest frequency of distribution also differed for the countries studied. The values for Australia, the U. S. and Sri Lanka for the adult population were 13.6%, 14.8% and 16.8% respectively while that for the neonate population of Sri Lanka was 13.2%. In contrast the overall prevalence rates for Brazil and Colombia were 35% and 35.7% respectively. These are much higher than those for the other countries. In Brazil itself there was a wide variation in the prevalence rates, the highest being for the warm humid lowlands and the lowest for the arid area. In Korea Nakayama *et al.* (1970) adopting the titre of 1:256 as "positive" obtained a prevalence rate of 16.3% for the urban population of Seoul and 10.4% for the rural population while that for the entire population was 14.3%. These values are similar to those of the former countries.

According to Walls and Kagan (1967) the percentage distribution curves with sharp peaks at the titre of the highest frequency of distribution indicated an efficient transmission while a low peak at this titre suggested a less efficient transmission of the disease. A value of high magnitude (e. g. 1:256) of the titre of the highest frequency of distribution indicated an effective exposure whereas a value of low magnitude (1:128) indicated a less effective exposure. The percentage distribution curve for Sri Lanka was peaked at the titre of the highest frequency of distribution to the same level as those of the U. S. and New South Wales, Australia, but at a lower level than that for Brazil indicating that the transmission in Sri Lanka is about the same level as in the countries mentioned except Brazil. The titre of the highest frequency of distribution for Sri Lanka is lower than those for other countries mentioned except Australia and Colombia. This pointed to a less effective exposure of the population in these three countries.

The titre of the highest frequency of distribution for the IFA for Sri Lanka was 1:64, a value one dilution lower than the corresponding titre in the IHA. The prevalence rate measured from values obtained with the IFA was 16.5% a slightly lower figure than that with the IHA. As no epidemiological studies have yet been carried out with this test in other countries such comparisons are not possible. However it is seen from Graph 1 that some of its parameters such as the prevalence rate and the shape of the curve are similar while others like the magnitude of the percentage distribution and the titre of the highest frequency of distribution differed. These differences could be expected in the comparison of the two tests which measure different antibodies appearing and disappearing at different rates.

In applying the results of these investigations to clinical practice, the physicians should obtain results of both tests whenever possible. In this laboratory the IFA and IHA values are always provided when a short clinical history is given in the request form indicating the necessity for the test. When the specimen is supplied without a clinical history only the IHA is carried out. The results of both tests are required to evaluate the clinical status of the patient regarding toxoplasmosis. If the titres in both tests are below the respective titres of the highest frequency of distribution the infection can be considered to be at its early stages (especially when the value of the IFA is higher than that of the IHA, a stage hardly met with in practice) or it has spent itself the antibodies being the only evidence of previous contact with the organism, a more likely stage. If the values are at or above the respective titres of the highest frequency or if the IFA value is greater than the IHA titre then a further antibody determination a few weeks later is indicated. An increase of at least a fourfold dilution in the second determination, 4 weeks apart in the IFA or both would indicate acute toxoplasmosis while such an increase in the IHA alone would reveal only a recent infection, the degrees of the two values and their difference giving an index to the time lapse after the exposure. Values of both IFA and IHA over 1:4096 would indicate an acute or convalescent phase. If the IFA and IHA values 4 weeks apart in neonates show an increase or are maintained at the same level clinical toxoplasmosis is indicated whether the infection is apparent or not while a reduction in the antibody level indicates the disappearance of maternal antibodies. In children up to one year a lower antibody level in either of the tests compared to the antibody level of the mother in the respective test would indicate the presence of antibodies of maternal origin and not clinically significant. In children over one year the presence of antibodies would indicate a congenital or acquired toxoplasmosis, apparent or inapparent, depending on the respective values of the IFA and IHA to be interpreted as discussed earlier. Determinations of both IFA and IHA titres are especially indicated in diseases of the eye for differential diagnosis and in patients subjected to immuno-supressive therapy as latent toxoplasmosis could flare up under such trearment.

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