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A Survey of Indirect Haemagglutination Test Antibodies against *Toxoplasma gondii* in Neonates in Ceylon

by

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SUMMARY 520 (70·8%) of 734 cord bloods obtained from the de Soysa Hospital for Women, Colombo were negative for *Toxoplasma* antibodies by the indirect haemagglutination test of Jacobs and Lunde. The remaining 214 (29.2%) bloods were positive at titres of 1 : 2 to 1 : 8192. The frequency of distribution curve showed 3 peaks at titre 1 : 32, 1 : 128 and 1 : 2048. The peak at 1 : 128 is considered to be the highest frequency in the normal neonate population and that at 1 : 2048 to show infections during the course of the pregnancy. Neonates with titres of this order are at risk of congenital infection with *Toxoplasma*. No gross abnormalities were observed in 34 neonates showing titres of this order.

In the follow-up of 15 neonates with titres from 1 : 2 to 1 : 8192, the haemagglutination antibodies were usually lost within 3 months, probably much earlier. The titre at birth had no relationship to the length of persistence of the antibodies. Seven neonates had antibodies from 1 : 2 to 1 : 2048 while the mothers were negative. One infant showed a titre of 1 : 512 over 37 days after an initial fourfold increase in titre in 20 days.

INTRODUCTION

Yahashi (1960), de Rover-Bonnet (1968) and Kimball, Kean and Fuchs (1968) carried out surveys to assess the *Toxoplasma* antibody levels in the neonates using the dye test, the complement fixation test and the intra-dermal test. Tanaka (1967) studied the antibody levels of the mother and the neonate using the indirect haemagglutination test, the dye test and the intra-dermal test. The present study was undertaken to evaluate the *Toxoplasma* antibody levels in neonates in Ceylon, their significance and the duration for which they remained detectable.

MATERIALS AND METHODS

Samples of blood from some infants born in the wards of the de Soysa Hospital for Women in Colombo were collected between March 1969 and February 1971. The blood was obtained from the umbilical cord as soon as it was severed from the placenta and the blood brought to the laboratory on the following day. The sera were separated and stored in the deep freeze at -20°C. till required for examination. Due to the lack of mice for antigen preparation some of the specimens were tested as late as 6 months after

the collection. Further samples of blood were obtained from those infants whose sera were tested and found to be positive for antibodies a few days after the receipt of the cord blood. Samples of mothers' blood, and in some cases the fathers' blood were also collected at the same time.

The indirect haemagglutination test of Jacobs and Lunde (1957) was performed according to the method adopted by Kulasiri (1970) and Kulasiri and Amarasinghe (1970). Antigen was prepared by the method of Lunde and Jacobs (1964). Those sera that showed "heterophile" antibodies were reabsorbed with larger quantities of sheep erythrocytes till the samples showed a negative reaction in the "heterophile" control. In a few cases it was not possible to completely remove such antibodies even after several absorptions. These were not included in this study. Antigen from 10 batches was used.

RESULTS

A total of 734 specimens of cord blood was examined by the indirect haemagglutination test. Of these 520 (70.8%) were negative for haemagglutination at a dilution of 1 : 2, the lowest dilution tested. These were considered negative while the other 214 (29.8%) were considered positive. The distribution of the positive titres among the various dilutions tested and their percentages of the total number of sera examined are shown in Table 1. Figure 1 shows these values in the form of a histogram where the number of positives are shown against the reciprocal of their respective titres.

TABLE 1

The distribution of indirect haemagglutination test titres in the 734 cord blood specimens.

Titre	No. of specimens	Percentage of total
Negative	520	70.8
1 : 2	22	3.0
1 : 4	07	1.0
1 : 8	13	1.8
1 : 16	12	1.6
1 : 32	38	5.2
1 : 64	25	3.4
1 : 128	30	4.1
1 : 256	22	3.0
1 : 512	11	1.5
1 : 1024	11	1.5
1 : 2048	15	2.0
1 : 4096	07	1.0
1 : 8192	01	0.1

TABLE 2

The indirect haemagglutination test results in the follow-up cases.

Number	Titre of cord blood	Titre of second specimen	Interval between the 2 specimens in days	Titre of mother	Titre of father
A 518	1 : 128	1 : 4	94	1 : 256	—
A 519	1 : 8	Negative	80	1 : 8	—
A 539	1 : 2	Negative	100	1 : 256	Negative
A 552	1 : 32	Negative	88	1 : 256	—
A 574	1 : 2	Negative	76	Negative	Negative
A 576	1 : 32	Negative	71	1 : 128	1 : 128
A 578	1 : 32	Negative	75	Negative	Negative
A 579	1 : 8	Negative	73	Negative	1 : 128
A 711	1 : 128	1 : 64	6	1 : 128	—
A 771	1 : 128	1 : 512	20	1 : 1024	Negative
		1 : 512	37	1 : 1024	—
A 826	1 : 2	Negative	13	Negative	—
A 851	1 : 1024	1 : 512	16	Negative	Negative
A 894	1 : 256	Negative	10	Negative	Negative
A 904	1 : 2048	Negative	13	Negative	—
A 914	1 : 8192	1 : 8192	9	1 : 16384	—

Table 2 shows the results of the haemagglutination tests on the second specimens of sera obtained from 15 infants giving a positive reaction, their mothers' sera and their fathers' whenever obtainable. In the case of the neonate A 771 two repeat specimens were obtained within a period of 57 days (an interval of 20 days between the cord blood and the second sample and 37 days between the second and the third samples). The cord blood gave a titre of 1:128 which increased in 20 days to 1:512. This titre was maintained for another 37 days. The mother's titre was 1:1024. In the neonate A 914 the cord blood gave a titre of 1:8192 which remained unchanged 9 days later. The mother's titre was 1:16,384, the highest titre we have obtained in this series. Further investigations could not be carried out in both these cases. However no obvious signs of infection were seen in these infants at the time the last samples of blood were drawn from them. In neonates A 574, A 578, A 579, A 826, A 851, A 894 and A 904 the cord blood showed titres ranging from 1:2 to 1:2048 with the mothers' blood showing a negative reaction. The second samples of blood from these infants were negative except that from A 851 which was still reacting at a titre of 1:512 at the end of 16 days.

DISCUSSION

It has been shown that when the number of positives is plotted against their respective antibody titres in surveys of normal populations, a curve with a single peak is obtained (Mitchell and Green, 1960; Walls and Kagan, 1967; Walls, Kagan and Turner, 1967). The titre with the highest frequency will vary in different populations depending on the prevalence of the infection in the population concerned and secondarily on the method used in the estimation of the antibody levels. Any value over this titre with the highest frequency

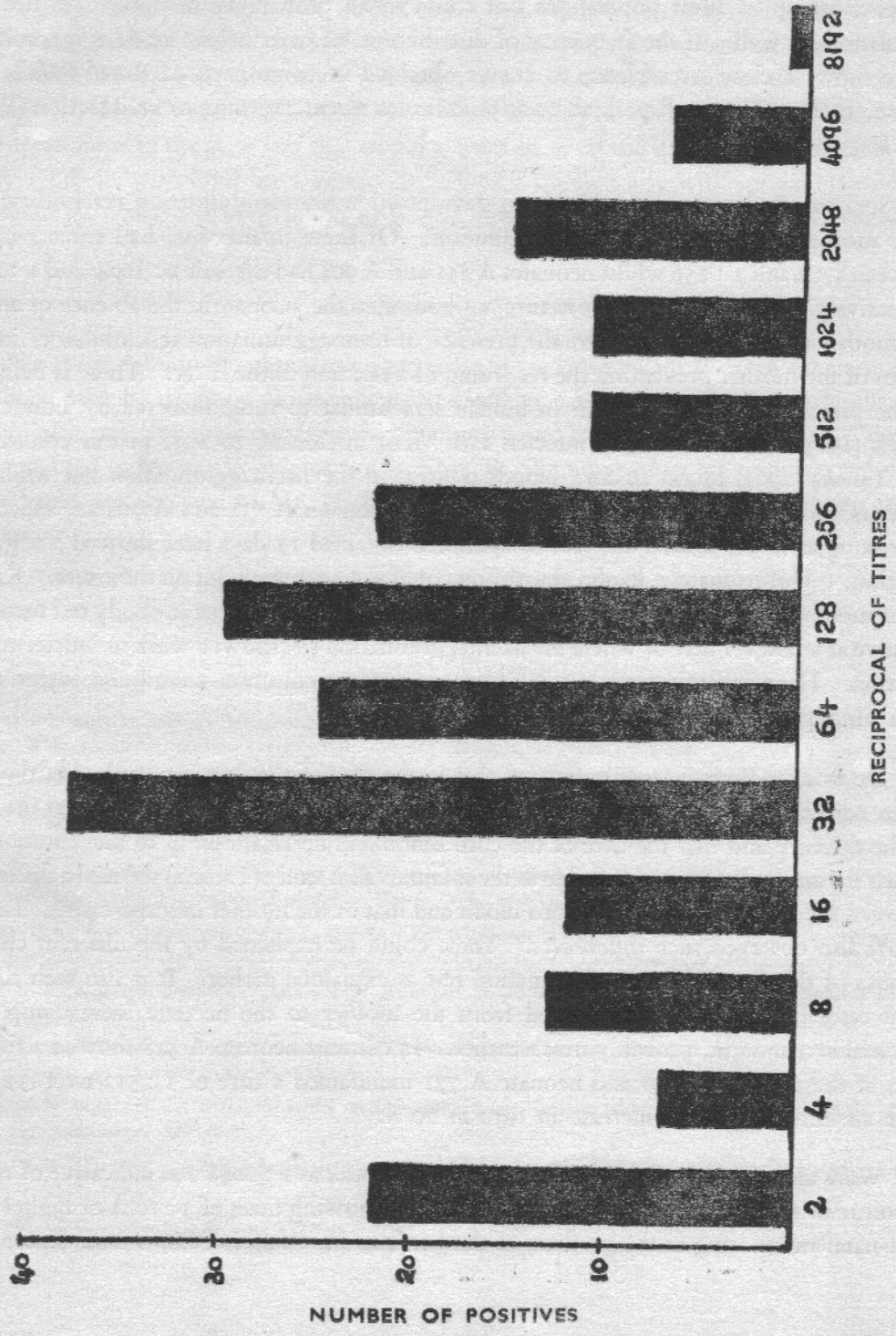
is considered significant in clinical cases in that population. In the present study 3 such peaks, one at a titre of 1:32, another at a titre of 1:128 and a third at a titre of 1:2048 were seen (Figure 1).

A possible explanation for the presence of the peak at the titre 1:32 is that the test is non-specific at low titres. Chordi, Walls and Kagan (1964) considered titres of 1:200 and below as non-specific while Walls *et al.* (1967) considered titres of even 1:200 as specific. According to Chordi *et al.* (1964) false positives may arise due to the presence of "heterophile" antibodies in the sera and mouse proteins and non-specific antigens in the *Toxoplasma* antigen. As the sera used in this study had previously been absorbed overnight with sheep erythrocytes for "heterophile" antibodies and appropriate controls instituted the low titres could not be due to such antibodies. Lunde and Jacobs (1967) did not find mouse antigens in sufficiently large amounts in their *Toxoplasma* antigen to give a positive haemagglutination reaction with sheep erythrocytes sensitised with *Toxoplasma* antigen and tested against goat anti-mouse serum. The different results obtained by Chordi *et al.* (1964) and Lunde and Jacobs (1967) may be due to the different methods of preparation of antigens, the former by the method of Lunde and Jacobs (1959) and the latter by the method of Lunde and Jacobs (1964). As the method for the preparation of antigen adopted in this study was that of Lunde and Jacobs (1964) our antigen could be expected to perform in the same way as that tested by Lunde and Jacobs (1967). Further, Kulasiri (1970) did not find "H" and / or "O" antibodies against *Salmonella typhi* and / or "H" antibodies against *Salmonella paratyphi A* interfering in the performance of the haemagglutination test, an important consideration when applying serological diagnostic tests under conditions prevailing in developing countries. On the other hand Lunde and Jacobs (1965) found cross reactions between *Besnoitia jellisoni* and *Toxoplasma* in the indirect haemagglutination test in sera from rabbits immunized with the two organisms but the homologous titres were always higher than the heterologous ones. This was confirmed by Suggs, Walls and Kagan (1968) using *B. jellisoni, B. panamensis* and some *Toxoplasma* isolates. Lunde and Jacobs (1965) were able to demonstrate precipitin lines in common with both systems using agar-gel diffusion. The possibility is therefore present that these and other antigenically related organisms could influence the haemagglutination test results especially among the low titres. However this is rather unlikely as no human infections with *Besnoitia* have so far been detected.

We are unable to give a satisfactory explanation for the presence of the peak at the titre of 1:32.

The peak at titre 1 : 128 represents the highest frequency of distribution of titres. All titres over this value are considered clinically signfiicant. Although this value in this survey is lower than those reported by Mitchell and Green (1960), Chordi *et. al.* (1964) and

FIGURE 1. Histogram showing the frequency of distribution of haemagglutination antibody titres in the positive cord bloods.



Walls *et al.* (1967), Walls and Kagan (1967) found that in certain parts of Brazil the highest frequency of occurance in adult population was a titre of 1 : 128. These studies relate to the distribution in adult populations but could in all probability be applied to neonate populations as well. If the frequency of distribution of titres below 1 : 64 is ignored the curve shows a close resemblance to curves obtained in some parts of Brazil (Walls and Kagan, 1967). The small peak at 1 : 2048 indicates recent exposure or re-infection (Walls and Kagan, 1967).

Sera from seven neonates (Table 2) gave positive haemagglutination reactions whilst their mothers were negative at 1 : 2 dilution. Of these infants five had titres ranging between 1 : 2 and 1 : 256 whilst neonates A 851 and A 904 had titres of 1 : 1024 and 1 : 2048 respectively. The presence of low titre antibodies in the neonate in the absence of any in the mother could be explained by the presence of haemagglutination test inhibitors in the serum of the mother preventing the recording of a reaction in the latter. There is evidence of the presence of such inhibitors in human sera similar to those detected by Lunde and Jacobs (1963) in expermentally infected rats. It is interesting to note in this connection that Tanaka (1967) found 10 cord bloods positive in the haemagglutination test while the mothers' blood was negative. The results of the neonates A 851 and A 904 are especially interesting as in the former the second specimen obtained 13 days later showed a negative reaction. Unfortunately, kaolin absorption could not be carried out on these sera. Kaolin absorption is now being done routinely where the indirect fluorescent antibody test records a positive at whatever titre or where the haemagglutination test shows a weak or indeterminate reaction. Haemagglutination test inhibitors are more common round the lower titres than among the higher ones.

De Roever-Bonnet (1968) reported that in the case of the dye test antibodies the titre of the cord blood was exactly the same as that of the venous blood of the mother at the time of the delivery and that the titre of the cord blood had no relationship to the duration for which the antibodies were detectable in the infant. This study (Table 2) showed a difference between the antibody titre of the cord blood and that of the mother in some cases. Tanaka (1967) also observed such differences. These could be explained by the different characteristics of the dye and haemagglutination test as explained earlier. It is also seen that in most cases the antibodies transferred from the mother to the neonate were completely lost within 3 months, probably much earlier. In contrast neonate A 518 showed a titre of 1 : 4 at the end of 94 days and neonate A 771 maintained a titre of 1 : 512 over 37 days after an initial four-fold increase in titre in 20 days.

Walls and Kagan (1967) believed that the small peak at 1 : 2048 was indicative of recent exposure or reinfection. On this ground neonates showing titres of 1 : 1024 or higher need post-natal monitoring as the mothers of these infants have had infections with *Toxoplasma*

during some part of the pregnancy and hence the neonates are at risk of infection. There were 34 such infants (Table 1) and of these we were able to observe even partly only 4 cases namely A 771, A 851, A 904 and A 914. It is regrettable that further investigations could not be carried out as these cases were not available for detailed clinical examination. However, no gross pathological signs were observed in them at the time the last specimens of blood were obtained. A more detailed study involving laboratory and clinical investigations is in progress and this would answer some of the questions presented by this study. Infants with high titres would be followed up clinically with special attention to occulopathy which has been found to be the most common residual sign of *Toxoplasma* infection.

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REFERENCES

CHORDI, A., WALLS, K. W. & KAGAN, I. G. (1964). Studies of the specificity of the indirect hemagglutination test for toxoplasmosis. *Journal of Immunology*, **93**, 1024-1033.

DE ROEVER-BONNET, H. (1968). Toxoplasmosis in pregnant women and their children. Data obtained from a paper presented at the Eighth International Congresses on Tropical Medicine and Malaria, Teheran.

JACOBS, L. & LUNDE, M. N. (1957). A hemagglutination test for toxoplasmosis. *Journal of Parasitology*, **43**, 308-314.

KIMBALL, A. C., KEAN, B. H. & FUCHS, F. F. (1968). Toxoplasmosis in obstetrical patients in New York. Data obtained from a paper presented at the Eighth International Congresses on Tropical Medicine and Malaria, Teheran.

KULASIRI, C. DE S. (1970). Specificity of the haemagglutination test for toxoplasmosis with reference to infections with *Salmonella typhi* and *Salmonella paratyphi*. *Ceylon Journal of Medical Science*, **19**, 15-17.

KULASIRI, C. DE S. & AMARASINGHE, D. K. C. (1970). Indirect haemagglutination test antibodies against *Toxoplasma gondii* (Nicolle & Manceaux, 1908) in cases of lymphadenopathy in Ceylon. *Ceylon Journal of Medical Science*, **19**, 41-46.

LUNDE, M. N. & JACOBS, L. (1959). Characteristics of the Toxoplasma hemagglutination test antigen. *Journal of Immunology*, **82**, 146-150.

LUNDE, M. N. & JACOBS, L. (1963). Toxoplasma hemagglutination and dye test antibodies in experimentally infected rats. *Journal of Parasitology*, **49**, 932-936.

LUNDE, M. N. & JACOBS, L. (1964). Properties of Toxoplasma lysates toxic to rabbits on intravenous injection. *Journal of Parasitology*, **50**, 49-51.

LUNDE, M. N. & JACOBS, L. (1965). Antigenic relationship of *Toxoplasma gondii* and *Besnoitia jellisoni*. *Journal of Parasitology*, **51**, 273-276.

LUNDE, M. N. & JACOBS, L. (1967). Differences in *Toxoplasma* dye test and hemagglutination antibodies shown by antigen fractionation. *American Journal of Tropical Medicine and Hygiene*, **16**, 26-30.

MITCHELL, R. G. & GREEN, C. A. (1960). The haemagglutination test for Toxoplasma antibodies. *Journal of Clinical Pathology*, **13**, 331-335.

SUGGS, M., WALLS, K. W. & KAGAN, I. G. (1968). Comparative antigenic study of *Besnoitia jellisoni*, *B. panamenis* and five *Toxoplasma* isolates. *Journal of Immunology*, **101**, 166-175.

TANAKA, A. (1967). Serological survey of Toxoplasma in obstetrical and gynecological diseases and isolation of Toxoplasma from the placenta. *Journal of the Osaka City Medical Center*, **16**, 529-555 (In Japanese. Read in abstract only).

WALLS, K. W. & KAGAN, I. G. (1967). Studies on the prevalence of antibodies to *Toxoplasma gondii*. 2. Brazil, *American Journal of Epidemiology*, **86**, 305-313.

WALLS, K. W., KAGAN, I. G. & TURNER, A. (1967). Studies of the prevalence of antibodies to *Toxoplasma gondii*. 1. U. S. military recruits. *American Journal of Epidemiology*, **85**, 87-92.

YAHASHI, H. (1960). A study of toxoplasmosis in new borns especially premature infants. *Journal of the Osaka City Medical Center*, **9**, 2547-2557 (In Japanese. Read in abstract only).