A REPORT ON THE PREVALENCE OF TOXOPLASMIC ANTIBODIES IN SINGAPORE

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INTRODUCTION

The laboratory of diagnosis of *Toxoplasma* is generally based on the detection of *Toxoplasma* antibodies in the serum, as the isolation of the parasite is not always possible. Many serological tests have been employed for this purpose and include the Sabin-Feldman dye test (Sabin and Feldman, 1948) and the indirect haemagglutination reaction (Lunde and Jacobs, 1959).The dye test is more widely used but suffers from two main disadvantages in that it requires the use of live organism and a heat labile factor found in some human sera known as the "accessory factor". These two disadvantages, on the other hand, are eliminated in the indirect haemagglutination (IHA) reaction. This test is highly sensitive and had given results comparable to the dye test (Lunde and Jacobs, 1958).

The IHA test has been routinely used since 1963 for the detection of *Toxoplasma* antibodies in the sera sent to the Department of Parasitology from various hospitals in Singapore. This is a report of the results obtained with these sera from 1963 to this date. Some apparently normal sera have also been included to give a general idea of the prevalence of the parasite in the Singapore population.

MATERIALS AND METHODS

The IHA reaction is based on the agglutination of sensitized (antigen-coated) red blood cells in the presence of antibodies. In a positive reaction, the red blood cells form a diffuse carpet-like pattern at the bottom of the tube. In a negative reaction they settle down to form a sharply defined button-like clump (Plate I). The reaction could be performed by cells of human or animal origin. In our studies, sheep red blood cells have been used. These cells were obtained from sheep killed at the Singapore abattoir and were brought to the laboratory in Alsever's solution. These cells were then formalized and tanned according to the method described by Stein and Desowitz (1964) in their haemagglutination studies with malaria.

The antigen for sensitizing the cells was prepared according to the method employed by Jacobs and Lunde (1957). The parasites were obtained from mice previously infected with the Rh strain of *Toxoplasma*. The Rh strain is highly virulent to mice and the trophozoites are found in large numbers in the peritoneal cavity of the animal, three to four days after the date of infection. The peritoneal fluid from infected mice was pooled and washed at least twice in cold physiological saline by centrifugation. The supernatant was then discarded and the sediment containing parasites was then resuspended in ten times its volume of chilled distilled water. The tube was kept overnight at 3-4°C to produce lysis of the parasites. The next day an equal volume of chilled 1·7% saline was added to restore the mixture to isotonicity. It was re-

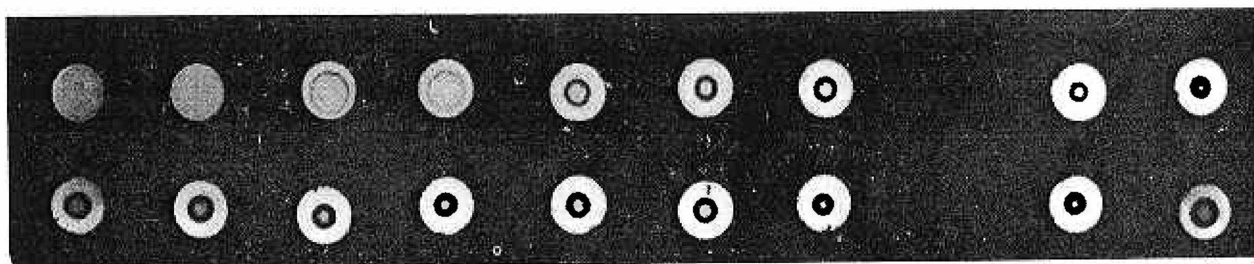

Plate I. Shows the pattern of cell agglutination at the bottom of tubes in the indirect haemagglutination reactions. The bottom row of tubes shows two-fold dilutions of a negative serum with the clumping of cells as a small red button. The top row shows a positive serum with agglutination up to four tubes. The two tubes at extreme right are the serum controls and those adjacent to these are the diluent controls.

centrifuged and the clear supernatant was collected and stored at -20°C. This represented the stock antigen solution at 1:20 dilution.

All sera were inactivated at 56°C for 30 minutes and absorbed overnight in tanned sheep red blood cells before testing. Controls of tanned, unsensitized cells in test sera and tanned sensitized cells in diluent were always run together with test dilutions of sera. The diluent used was 0·125% crystalline bovine albumin in phosphate-buffered saline (pH 7·2).

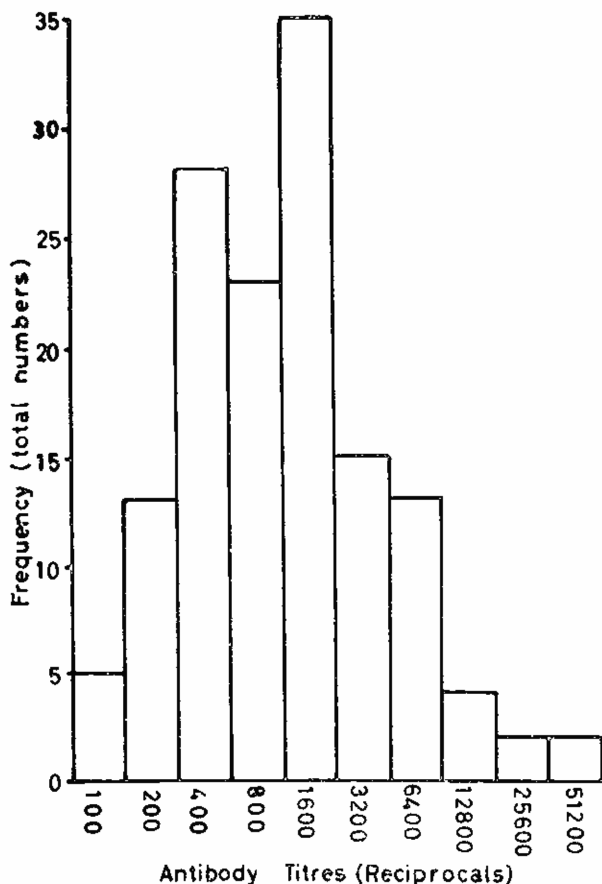

Fig. 1. Shows the frequency of antibody titres in sera of clinically suspected cases.

RESULTS

During the course of this study 339 samples of sera from clinically suspected cases were tested by means of the IHA technique. Out of these 140 showed antibody titres of 1/100 and above. A serum was considered positive if a dilution of 1/100 or more agglutinated the antigen-sensitized cells. From Figure 1 it is noted that most of the positive cases lie between the titre range of 200 to 6,400. Data correlating the distribution of titres in various age groups is shown in Table 1. Although antibody titres were found in all age groups, the higher age group included most of the positive cases. Over 40% of the positive cases were found in people over 30 years. There was no significant difference between the sexes. The IHA test was also performed on 169 sera from apparently healthy

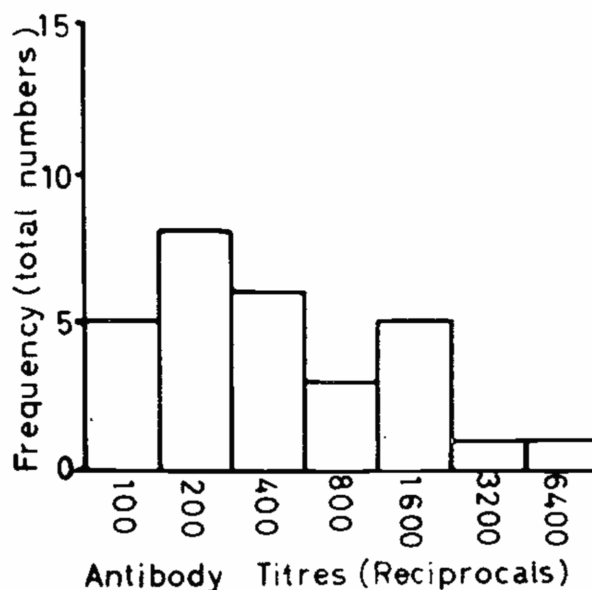

Fig. 2. Shows the frequency of antibody titres in sera of apparently healthy individuals.

TABLE 1

DISTRIBUTION OF ANTIBODY TITRES IN VARIOUS AGE GROUPS*

Age Group (in yrs.)	100	200	400	800	1,600	3,200	6,400	12,800	25,600	51,200	Total No. Positive
< 1	1	-	-	2	2	-	-	-	-	-	5
> 1 - 5	-	-	3	7	4	1	1	-	-	-	16
> 5 - 10	1	1	1	2	3	1	2	-	-	1	12
> 10 - 20	1	3	3	2	1	3	-	1	-	-	14
> 20 - 30	2	2	2	4	3	-	1	-	-	1	15
> 30 - 50	1	4	3	4	5	3	2	2	-	-	24
> 50	-	1	4	4	8	4	1	1	-	-	23

*Includes only those sera where relevant data on age was known.

individuals. Out of these 29 were positive for haemagglutinating antibodies. A lower range of titres was seen in this group (Fig. 2) compared to the suspected cases. 19 of the positive sera had titres ranging from 100 to 400 only.

DISCUSSION

Although the IHA test is quite specific in the detection of *Toxoplasma* antibodies, there is some difference of opinion as to which is the minimum significant titre. Chordi, Walls and Kagan (1964) regarded titres of 1/200 and more as significant or specific for *Toxoplasma.* Jennis (1963), however, includes an HA titre of even 1/8 as positive. We have taken titres of 1/100 and above as positive. Using this criterion, a rather high positive rate of 41·3% was obtained in the sera of clinically suspected cases. Even in the apparently healthy individuals 17·2% were found to be positive for *Toxoplasma* antibodies. These figures agree with the results of other workers who have also found a high prevalence of *Toxoplasma* antibodies in the tropics (Lunde and Jacobs, 1958; Kessel, Lewis and Jacobs, 1965). So far there has been no clear explanation for the apparently high prevalence rates in the tropics.

It is obvious from the results presented in the survey that infection with *Toxoplasma gondii* is of a considerable importance in Singapore. As the parasite is widely distributed in nature and shows little host specificity, the human infection could be acquired from a wide range of animals. The Department of Parasitology is presently engaged in a survey of the animal population of Singapore to assess the relative importance of various animals as a source of human infection. The details of this survey are being published at a later date.

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