



## Indirect Haemagglutination Test Antibodies against *Toxoplasma gondii* (Nicolle & Manceaux, 1908) in Cases of Lymphadenopathy in Ceylon

by

C. DE S. KULASIRI AND D. K. C. AMARASINGHE  
*Medical Research Institute, Colombo, Ceylon.*

It is well known that lymphadenopathy is one of the many manifestations of acquired toxoplasmosis. According to Siim (see Beverley and Beattie, 1958) about 5% of the cases of lymphadenopathy of unknown aetiology in Denmark was due to toxoplasmosis. In Britain, Beverley and Beattie (1958) thought that about 7% of the unexplained lymphadenopathies might be due to this disease. Fleck and Ludlam (1965) suggested that the cases of lymphadenopathy of uncertain origin should be investigated for toxoplasmosis. In Ceylon, Jayawera (1969) was able to demonstrate *Toxoplasma gondii* as the aetiological agent of lymphadenopathy in a case suspected of tuberculosis or Hodgkin's disease. It was therefore decided to study, with this indirect haemagglutination test, the antibody levels against *T. gondii* in sera sent to the Institute from patients suspected of infectious mononucleosis. Sera from patients suspected of syphilis, sent during the same period were also studied. In addition, the results obtained in examining sera from cases of lymphadenopathy and fever of unknown origin suspected of toxoplasmosis are included in this report.

### MATERIALS AND METHODS

Samples of sera from blood sent for the Paul-Bunnell test for infectious mononucleosis from November 1968 to February 1970 were obtained from the serology laboratory of the Institute. They were stored in the deep freeze at  $-20^{\circ}\text{C}$  till required for the examination. The short clinical history when supplied in the "Request Form" accompanying the blood was noted. A similar number of sera from blood sent for the V.D.R.L. test for syphilis was also obtained from the same laboratory, each day the Paul-Bunnell sera were collected. These, too, were stored at  $-20^{\circ}\text{C}$  till required. The results of the Paul-Bunnell and the V.D.R.L. tests of the respective sera were obtained from the serology laboratory.

The haemagglutination test of Jacobs and Lunde (1957) was performed as described earlier (Kulasiri, 1970) except that the antigen was prepared in phosphate-buffered saline of pH 6.4 and the erythrocyte suspension reduced to 2%. The tanned sheep erythrocytes for use in the serum controls were incubated for 15 mins at  $37^{\circ}\text{C}$  in phosphate-buffered saline of pH 6.4 and subjected to the same treatment as the sensitised cells. An equal number of sera from the Paul-Bunnell and the V.D.R.L. groups were preliminarily screened in each batch up to a dilution of 1:256 starting at a dilution of 1:2, in twofold dilutions. Those sera that showed a positive reaction at 1:256 were re-tested up to a maximum dilution of 1:8192 starting at a dilution of 1:16 in twofold dilutions in subsequent batches of tests. Haemagglutination antigen from 6 batches were used in these tests.

The sera from the blood sent to the parasitology laboratory for serological diagnosis of toxoplasmosis were separated immediately on receipt. They were also stored at  $-20^{\circ}\text{C}$  till required. The period of storage did not usually exceed seven days. They were tested in the same manner as the previous sera by the haemagglutination test. The specimens included in this study were received between November 1968 and September 1970. Antigen from 20 batches were used in their examination.

#### RESULTS AND OBSERVATIONS

A total of 150 sera sent for the Paul-Bunnell test for infectious mononucleosis was examined with the haemagglutination test for toxoplasmosis. The results obtained are summarized in Table 1. None of the sera sent during this period was positive in the Paul-Bunnell test when absorbed with the guinea-pig kidney suspension. Table 2 shows the haemagglutination test results of cases of recorded lymphadenopathy and continued fever among the 150 cases shown in Table 1. The rest of the sera could not be categorised as no clinical histories were available. Of the 5 high titre sera shown in Table 1, two were from cases of lymphadenopathy with titres of 1:1024 and 1:2048, one serum showing a titre of 1:1024 was from a case of continued fever while one of the remaining sera with a titre of 1:1024 was from a case with suspected glandular fever, the other showing a titre of 1:2048 was from a case of suspected leukaemia. The signs and symptoms together with the serological results suggested that these cases were acute toxoplasmosis. Therefore the percentage of infection can be assumed to be 3.3%. In the 49 cases where lymphadenopathy was definitely indicated, the percentage of infection was 4.1%. In the 27 cases where the sign was only continued fever the percentage of infection was 3.7%.

TABLE 1

Haemagglutination test results of 150 sera sent for the Paul-Bunnell test for infectious mononucleosis

TEST	NEGATIVE	POSITIVE		
		1:2 — 1:64	1:128 — 1:512	1:1024 — 1:4096
PAUL-BUNNELL	150	—	—	—
HAEMAGGLUTINATION	120	18	7	5

TABLE 2

Haemagglutination test results of sera sent for the Paul-Bunnell test for infectious mononucleosis and showing lymphadenopathy or continued fever

Haemagglutination Test titre	Lymphadenopathy	Continued fever
Negative	37	24
1:2 — 1:64	9	1
1:128 — 1:512	1	1
1:1024 — 1:4096	2	1
TOTAL	49	27

A total of 150 sera sent for the V.D.R.L. test for syphilis was examined with the haemagglutination test for toxoplasmosis. The results obtained are shown in Table 3. Very few clinical details were provided with these specimens but most of these were from patients suspected of venereal disease. It is interesting to note that the 8 sera that reacted with the V.D.R.L. antigen did not show any reactivity even at low titre in the haemagglutination test. The titres of the 2 sera showing high values were 1:1024 and 1:4096. In all probability these 2 patients would have had acute toxoplasmosis showing a percentage of infection of 1.3%.

TABLE 3

Haemagglutination test results of 150 sera sent for the V.D.R.L. test for syphilis

TEST	NEGATIVE	POSITIVE		
		1:2 — 1:64	1:128 — 1:512	1:1024 — 1:4096
V.D.R.L.	142	8	—	—
HAEMAGGLUTINATION	109	28	11	2

TABLE 4

Haemagglutination test results of sera of cases of suspected toxoplasmosis with lymphadenopathy

Age	Duration of lymphadenopathy	Negative	1:4 — 1:64	1:128 — 1:512	1:1024 — 1:4096
< 16 years	< 2 weeks	1	—	—	—
	> 2 weeks	3	—	—	1
	Unknown	28	6	2	5
> 16 years	< 2 weeks	—	—	—	—
	> 2 weeks	1	3	1	1
	Unknown	48	10	9	3
Unknown	< 2 weeks	1	—	—	—
	> 2 weeks	1	—	—	—
	Unknown	19	3	2	1
Total		102	22	14	11

A total of 149 sera from patients with lymphadenopathy suspected of toxoplasmic origin was examined by the haemagglutination test. The results obtained are categorised in Table 4 according to the age of the patients and the duration of the lymphadenopathy. Of the sera that gave high titres, 9 were from patients whose duration of lymphadenopathy was not indicated. However, the clinical histories of the 11 patients with titres of 1:1024 and higher suggested an infection with *Toxoplasma*. Thus the percentage of infection could be considered to be 7.4%. Of the 14 patients with titres between 1:128 and 1:512, the duration of lymphadenopathy of 13 was unknown while that of the remaining one was over 2 weeks. These clinical histories together with the serological evidence suggested acute toxoplasmosis. A subsequent examination would have indicated an active infection, at least in some, by a rise in titre. Thus 25 out of 149 cases had a possible toxoplasmosis aetiology for the lymphadenopathy. It is also seen that 6 out of the high titre sera were from children under 16 years of age.

A total of 148 sera from patients with continued fever was examined with the haemagglutination test. The results obtained are summarized in Table 5. Seven sera gave titres of 1:1024 and over. Only one of these patients had fever of less than 2 weeks and in the others the duration of fever was not indicated. The fever of these patients might have been due to acute toxoplasmosis giving a percentage of infection of 4.7% in this group. Of the 16 patients whose sera showed a titre between 1:128 and 1:512, one had fever of

TABLE 5

Haemagglutination test results of sera of cases of suspected toxoplasmosis with fever

Age	Duration of fever	Negative	1:4—1:64	1:128—1:512	1:1024—1:4096
< 16 years	< 2 weeks	1	—	—	—
	> 2 weeks	7	1	—	—
	Unknown	25	4	2	1
> 16 years	< 2 weeks	4	—	1	—
	> 2 weeks	23	1	3	—
	Unknown	32	5	8	3
Unknown	< 2 weeks	2	—	—	1
	> 2 weeks	3	—	—	—
	Unknown	14	3	2	2
Total		111	14	16	7

less than 2 weeks, 12 had fever of unknown duration and 3 of more than 2 weeks duration. The serological results suggested contact with *Toxoplasma* during recent times but whether the clinical signs observed were due to acute toxoplasmosis could only have been ascertained by the examination of subsequent samples of sera.

#### DISCUSSION

The results obtained by a single serological examination provided only limited information. Only a change in titre is significant diagnostically. However, certain interpretations of titres are applicable to the results of single serological examinations. The following interpretations of titres are applicable to the haemagglutination test of Jacobs and Lunde (1957) for toxoplasmosis. A titre between 1:16 and 1:64 may reflect a past exposure to the disease or the early stages of the disease where a rise in titre would confirm it. A titre between 1:128 and 1:512 signifies a recent exposure to the disease and again a rise in titre is diagnostic. A titre of 1:1024 or higher is very significant if the clinical signs and symptoms suggest a diagnosis of toxoplasmosis. At this level of antibodies it is rarely that a rise in titre is observed. The only confirmatory evidence in such cases is the isolation of the causative organism and/or the clearing of the pathological symptoms or lesions on treatment with chemotherapeutic drugs. Usually in practice by the time toxoplasmosis is suspected the disease has progressed beyond the stage where a rise in titre could be observed. However, it should be remembered that the antibody titres do not drop fast and that perfectly healthy persons who have had recent contact with the organism would show titres of this magnitude. Thus in recording the percentage of infection in various groups that have been considered, only those cases that showed titres of 1:1024 and higher were reckoned although this may be a conservative estimate. Some of the others may well have been cases of acute toxoplasmosis especially those showing lymphadenopathy of over 2 weeks duration. Those cases of lymphadenopathy of unknown duration can safely be assumed to be of over 2 weeks duration, especially in adults, as the patients would not have consulted medical opinion if it had been of a lesser period. In this connection it is interesting to note that cases of acute toxoplasmosis have been observed with titres of 1:512 (Remington, Barni t, Meikel and Lunde, 1962; Lake, 1953) and 1:256 (Remington *et al.*, 1962; Fairchild, Greenwald and Decker, 1967). At the time *Toxoplasma* was isolated by us from the case reported by Jayawera (1969) the haemagglutination titre was only 1:512. No earlier or later serological examinations were carried out in this case.

Quite understandably the infection rate was below 3.5% except in cases of lymphadenopathy and of continued fever. For instance among sera sent from cases of suspected toxoplasmosis the rate for lymphadenopathy cases was 7.4% and for cases of continued fever, 4.7%, and amongst the sera sent for the Paul-Bunnell test with lymphadenopathy or continued fever the values were similar to those of the toxoplasmosis group.

The results presented here clearly indicate that in cases of lymphadenopathy and continued fever of over 2 weeks duration and unknown aetiology it is important to institute the appropriate tests for toxoplasmosis to exclude a possible toxoplasmic origin of these conditions.

## SUMMARY

150 sera sent for the Paul-Bunnell test for infectious mononucleosis and 150 sera sent for V.D.R.L. test for syphilis were examined with the haemagglutination test for toxoplasmosis. Assuming a conservative titre of 1:1024 or higher with the clinical signs and symptoms as indicative of acute toxoplasmosis, the percentages of infection were 3.3 and 1.3 respectively. The percentage of infection in the former group with indicated lymphadenopathy was 4.1 and that with indicated continued fever was 3.7.

149 sera from cases of lymphadenopathy suspected of toxoplasmosis and 148 sera from patients with continued fever suspected of toxoplasmosis were also examined with the haemagglutination test. Also assuming a conservative titre of 1:1024 or higher as suggestive of acute toxoplasmosis the percentage of infection in the former group was 7.4 and in the latter group was 4.7. The significance of the titres between 1:128 and 1:512 in these cases is discussed.

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