

## Prevalence of toxoplasma antibodies in sera from Greece and Africa

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The first serological survey for toxoplasma antibodies in Greece, was carried out on a selected population of different ages by Mercier, Tzamouranis & Crimbitis (1961). Using mainly the dye test they found that 29 of 170 patients had serum titres greater than 1 in 64. Walters (1957) expressed the view that toxoplasmosis, as judged by serological and other evidence, may be widely endemic in W. Africa. Middlemiss (1957), from radiological data, reported that the disease was present in Sierra Leone, Nigeria and N. Rhodesia. Orio, Depoux, Heuls & Ceccaldi (1958) used the complement fixation test to carry out a survey on unselected patients of different age groups in the Middle Congo, and found a relatively high incidence. Positive results at 1/8 were found in 7.4% of those aged 5–15 years, in 12% at 15–30 years and in 9% at above 30 years. De Jongh & de Jager (1959) reported the first case of toxoplasmosis in Liberia. From the results of the dye test French (1962) concluded that the human disease was prevalent in Ghana. Of 63 sera, 31 had dye test titres of 1/16 or greater. Ludlam (1965) using the same test found a high incidence of toxoplasma antibodies among adult Africans of both sexes in the Niger Delta with a much higher incidence of dye test antibodies (83.3% at 1/8) in men in the southern part of the Niger Delta than in men from the northern area (52.6%).

### MATERIAL AND METHODS

We have examined sera from healthy members of three different tribes, the Baganda, Masai and Bondei, in East Africa as well as from unselected healthy Negroes from Harbel, Liberia, and in Greeks from the island of Syros and the Greek mainland. Titres were determined by the direct-agglutination (Fulton & Turk, 1959) and dye tests (Sabin & Feldman, 1948; Fleck & Payne, 1963), and are reported below. Large numbers of arthropods obtained from Greece were examined to find if they were harbouring toxoplasma.

*Antigen.* The RH strain (Sabin, 1941) of the parasite was used in both tests.

*Dye test.* Sera were stored at  $-20^{\circ}\text{C}$ . and heated at  $56^{\circ}\text{C}$ . for 30 min. just before testing.

*Direct agglutination test.* A formalized suspension of *Toxoplasma gondii* free from other cells served as antigen.

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*Arthropods.* Six hundred mosquitoes, either culicine or anopheline (*Anopheles maculipennis*, *A. superpictus*, *A. saccharovi* and *A. hyrcanus*), as well as 260 bed-bugs and 400 ticks were obtained from various malaria stations on the Greek mainland. They were ground in 10% rabbit serum in normal (0.85%) saline and the suspensions were examined microscopically for the presence of *T. gondii* and injected intraperitoneally into mice. The peritoneal contents of the mice were examined at 4 days and again at 8 days.

## RESULTS

Table 1 shows the titres of 228 sera grouped according to age, from an unselected population of Greeks living on the island of Syros and on the mainland. The sera were obtained from volunteer army recruits, healthy pregnant women, hospital staff and school-children. They are now grouped together since the titres found for both communities were approximately the same. The rising incidence of anti-

Table 1. *Dye tests on sera from a healthy Greek population*

	Age in years					Totals
	0-10	- 20	- 30	- 40	40+	
Sera positive at						
1/1024	2	1	0	1	0	4
1/256	3	12	25	12	2	54
1/64	1	7	15	7	12	42
1/16	1	2	12	14	8	37
1/4	0	6	5	1	7	19
Sera negative	9	26	22	12	3	72
Total						228
Percentage positive at 1/16 or more	44	41	66	72	69	60

Table 2. *Dye tests on sera from a healthy Liberian population*

	Age in years			Totals
	11-20	21-30	30+	
Sera positive at				
1/1024	2	1	0	3
1/256	5	8	2	15
1/64	7	21	9	37
1/16	3	15	11	29
Sera negative	9	30	10	49
Total				133
Percentage positive at 1/16 or more	65	60	69	63

body with increase in age is indicated and is commonly found in surveys of this nature. There is a levelling from the age of 30 years onwards. The incidence of antibody in this population is higher than that found in Britain for all age groups (Beattie, 1958; Fleck, 1963).

The results obtained with 133 Liberian sera are given in Table 2. The population was healthy and again unselected. The incidence of antibodies was high under the

age of 20 years and did not rise with increase in years—an unusual finding. The numbers of sera from 30 years upwards are too few to allow speculation.

The results for seventy-two sera of three East African tribes shown in Table 3 are too few in number to allow any conclusion to be drawn other than that antibodies were present among these populations, with only a moderate increase in incidence with age.

Table 3. *Dye tests on sera from healthy East Africans*

	Bondei infants (½–2 years)	Bondei adults (over 20)	Masai infants	Baganda adults
Sera positive at				
1/256	0	0	0	3
1/64	1	3	1	7
1/16	1	0	2	10
1/4	1	0	0	3
Sera negative	10	4	6	20

Table 4. *Comparison of dye test and direct agglutination titres in sera from Greece and Liberia*

Direct agglutination titre	1/5120 or more	0	0	0	0	2
	1/1280–1/2560	0	1	8	16	2
	1/320–1/640	4	13	68	6	1
	1/80–1/160	46	106	40	1	0
	Neg. to 1/40	149	61	7	1	0
		Neg. to 1/8	1/16–1/32	1/64–1/128	1/256–1/512	1/1024 or more
		<span style="border-top: 1px solid black; display: inline-block; width: 80%; margin: 0 auto;"></span>				
		Dye-test titre				

In Table 4 the results obtained by the direct agglutination and dye tests for a total of 532 Greek and Liberian sera are compared. The extra 171 sera in this analysis were obtained from Greek hospital patients. There is reasonably good correlation between the tests, the direct test giving titres approximately 4–5 times that obtained in the dye test, corresponding to a difference of 1 dilution used in the latter test. All the sera found negative in the dye test gave direct agglutination titres of 1/10 to 1/40. The agglutination test has a number of advantages over the dye test in that it is macroscopic in character, avoids the need for live parasites and accessory factor, and can be rapidly performed with an antigen which remains stable over 6 months.

In the examination of arthropods from Greece for the presence of toxoplasma a total of 639 mice were used for direct inoculation with ground-up arthropod material or for subinoculation with a brain suspension when the presence of infection in the original mice was suspected. Direct agglutination tests performed on the sera of forty-nine inoculated mice and on controls indicated that infection was absent. Difficulty was experienced when the suspension became infected with bacteria or fungi. This was prevented by storing the suspension in 1:200 formol saline.

## DISCUSSION

It is clear from the results recorded that antibodies to toxoplasma are present among all the populations examined. It was not possible to select the samples of sera so that the infection rates in different classes of these populations could be determined as a help in providing clues to the possible mode of spread of the disease. In the past, serological surveys of this nature have shown that antibodies have a widely varying incidence throughout the world. In one area in Guatemala it was 96 %, in Tahiti 68 % and 30 % in Europe and N. America, but only 4 % among Navajo Indians in Arizona. The reason for the considerable variation in incidence is not understood, especially as the mode of spread of the disease has so far eluded investigators. Differences in climatic conditions such as rainfall and temperature may exert an influence on the proportion of the population affected, so also may closeness of association with wild or domestic animals. In both these groups the disease is highly endemic and is regarded as a zoonosis. Arthropods, however, have not yet been incriminated as vectors in nature, and our results support earlier findings. Recent work by Hutchison (1965) has indicated a possible method of spread of the disease which may be applicable to man. It is known, for example, that infection can be transmitted in animals by eating infected food. This author has suggested that *T. gondii* free in the intestine from food or from lesions may associate with nematode ova and thus pass to the exterior. Critical experiments are now being carried out to find if the infective material was indeed transmitted by helminth ova.

## SUMMARY

Sera from healthy Greeks, Liberians and East Africans were tested for dye test and direct agglutinating toxoplasma antibodies. At a titre of 1/16 or more 137/228 Greeks, 84/133 Liberians, 2/13 Bondei infants, 3/7 Bondei adults, 3/9 Masai infants and 20/43 Baganda adults gave a dye test positive result. This confirms the widespread occurrence of this infection.

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