HUMAN TOXOPLASMA INFECTION

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(With 2 Figures in the Text)

INTRODUCTION

Toxoplasma infection is not a common cause of disease in man, but, perhaps, not so uncommon as is sometimes thought. Bamatter (1952) has analysed 457 reports from many countries and concludes that the diagnosis has been established on parasitological grounds in seventy-five cases, on serological evidence in 140 more and can be admitted in a further 170 showing cerebral calcification. In this country since 1948 there have been reports of about fifty cases of possible, probable or proved clinical toxoplasmosis.

By contrast it seems probable that subclinical toxoplasmosis is common. That it exists is shown by the fact that mothers who give birth to congenitally infected babies do not themselves show signs of illness. That it may be frequent is shown by the results of antibody surveys.

The most widely used method for demonstrating the presence of toxoplasma antibodies is the cytoplasm-modifying (dye) test of Sabin & Feldman (1948). Using this method von Zeipel & Linder (1951), in Sweden, found the sera of 37 % of blood donors to give a positive reaction in a titre of 1/10 or more. Feldman, quoted by Weinman (1952), in Pittsburgh, taking a 1/16 titre as significant, obtained the following percentages of positive results: 5 % at 0–4 years, 10 % at 5–9 years, 25–30 % at 10–29 years, 45 % at 30–39 years, 65–70 % at 40 years and over. In Holland, Verlinde & Makstenieks (1951) found 63 % of positive reactions (titre less than 1/100) in the sera of 853 persons.

Complement-fixation tests have given lower results since complement-fixing antibodies disappear more rapidly than those measured by the dye test. In this country Macdonald (1950) found 5 % of sera from normal adults positive at a titre of 1/10 or more. Cathie & Dudgeon (1949) found three of 200 Wassermann sera with titres between 1/4 and 1/16, and the writers (unpublished) found three of 230 with titres between 1/5 and 1/10. Using a new type of antigen, which gave 94 % correlation with the dye test at a titre of 1/50 or more, Westphal & Bauer (1952) found 18 % of positive reactions in sera from residents of North Germany.

Another method used to demonstrate infection is the skin test with toxoplasmin (Frenkel, 1948). Using this method Gard (1951) obtained 46 % of positive results in 1894 persons in Stockholm. His percentages were from 3 % under the age of 5 to 50 % at the age of 40. Similar results were obtained by Thalhammer (1951) in Austria, whose percentage positive in 1006 persons was again 46 %, rising from

0 % under the age of 5 to 79 % between the ages of 40 and 50. These authors found close correlation between positive skin tests and positive dye tests. In the United States, Feldman & Sabin (1949) found 31 % of 142 persons positive, rising from 0 % under 5 to 65 % between 40 and 50. Negative skin tests were found in five persons whose sera reacted positively in the dye test.

In this country Fisher (1951) examined 901 hospital in-patients with 1 % of positive reactions under the age of 4, 4·3 % from 5 to 9, 7 % from 10 to 14 and 17·5 % over 15.

'Toxoplasmosis is not normally a disease' (Westphal & Bauer, 1952). Rather it seems to be a symbiotic condition in which many animals of different species, including man and some birds, perhaps even insects, are hosts. Indeed *Toxoplasma* appears to be one of the most successful of parasites. Rarely the balance is tipped to the detriment of the host. This is sometimes due, as in laboratory infections (Magnusson, 1951; Ström, 1951; Sexton, Eyles & Dillman, 1953), to prolonged contact with heavy concentrations of parasites or to their entry by an unusual route, such as a finger prick. It may be due, as suggested by Franke & Horst (1952), to lowering of resistance by other disease or excessive strain. Sometimes the balance is never established, as in the non-immune foetus of an infected, but immune, mother, so that congenital disease follows (Weinman, 1952).

That toxoplasma seldom produces disease does not make the quest for sources and modes of human infection less interesting; congenital infection accounts for only a small proportion of all infections.

It is possible that infection may come from man himself. Kemp (1950) suspected this in the case of the nurse of one of her patients.

Man's domestic animals, particularly dogs and cats, have frequently been suspected. Toxoplasma in dogs may produce ulceration of the intestine and be discharged in the faeces; it may also be discharged in the urine, and Olafson & Monlux (1942) found infection to spread rapidly among puppies. Siim (1950) found that the sera of 10 of 54 dogs in Copenhagen reacted in the dye test to a titre of 1/250 or higher; and Otten, Westphal & Kajahn (1951), taking a titre of 1/25 as indicative, considered 2–5 % of Hamburg dogs to be infected. They also found that 23 of 30 persons intimately associated with infected dogs gave dye test titres of from 1/25 to 1/400. Feldman (1953) found 30 of 51 dogs, 15 of 44 cats, 12 of 25 goats, 22 of 75 swine but no cattle out of 76 to react to a titre of 1/16 or more. Thalhammer (1951), however, found 5 of 34 cattle positive (method of testing not mentioned), and suggested that infection might be spread by milk. Olafson & Monlux (1942) described toxoplasmosis in sheep. Farrell, Docton, Chamberlain & Cole (1952) have isolated the parasite from swine. (For further references see Winsser, 1952.)

Toxoplasma infection has been found in wild rats. Perrin, Brigham & Pickens (1943), by the method of guinea-pig inoculation, found it in 8·7 % trapped in Savannah, and Eyles (1952), by mouse and guinea-pig inoculation, found it in over 3 % of Memphis rats. Using the dye test he obtained positive results in 20 of 100 rats and made the interesting observation that the inoculation of tissues from rats whose sera gave a negative dye test could produce infection in animals.

Of all birds pigeons alone have been shown to be hosts to a toxoplasma capable of infecting mammals, and Feldman & Sabin (1949) found high titre antibodies in the sera of five park keepers who handled pigeons. Epizootics of toxoplasmosis have been described in pigeons (Wiktor, 1950; Winsser, 1952). Jacobs, Melton & Jones (1952) found, as did Eyles with rats, that the tissues of pigeons in which no antibodies could be found might cause toxoplasmosis on inoculation into mice. Of 80 pigeons examined 10 were shown to be infected, 4 by animal inoculation and 7 by the dye test (titre of over 1/16). Only one was positive by both methods.

Toxoplasma has frequently been found in hares and rabbits.

Christiansen & Siim (1951) reported it as the cause of an epizootic among hares in Denmark. Hulphers, Lilleengen & Rubarth (1947) found it in 27 of 840 dead hares in Sweden. Wiktor (1950) found it in an epizootic in rabbits in the Belgian Congo. Marotel & Pierron (1943) reported it as the cause of death in 30% of rabbits in a breeding station. Sabin (1942) mentions that he obtained positive neutralization tests with sera from rabbit handlers.

Natural infection has recently been found in dog ticks (Giroud, Jadin & Reizes, 1951) and in mites on African rodents (Giroud & Le Gac, 1952).

Several modes of infection have been considered.

At first sight the alimentary route would appear probable, but, although possible, it is not easy to infect mice and guinea-pigs in this way. Weinman (1952) and Kozar, Wysacka & Sikorska (1952) have shown that gastric juice impairs the virulence of the parasite. Jacobs (1953) suggests that the pseudocysts may be more resistant. As a route of entry the alimentary canal cannot be excluded.

Infection is more easily produced through surface mucous membranes (Mesnil & Sarrailhe, 1913; Cowen & Wolf, 1950). The writers have infected mice readily by nasal instillation, either with or without ether anaesthesia, so that droplet infection appears possible, as Pinkerton & Henderson (1941) suggested when they found toxoplasma-laden macrophages in the alveoli of two adult patients with pulmonary lesions.

The parasitaemia which occurs in the experimental disease suggests transmission by blood-sucking insects. In some cases there has been a story of an insect bite before the onset of the disease in man. Numerous workers (Laven & Westphal, 1950; Van Thiel, 1950; Blanc, Bruneau & Chabaud, 1950; Piekarski, 1949; Havlik, 1952) have tried to transmit infection by fleas, lice, ticks, bed bugs and blood-sucking flies. It is true that these insects become infected when they feed on infected mice, and the infection can be transmitted to other mice by inoculating them with the ground-up bodies of the insects or by getting the mice to eat them. So far, however, no one has succeeded in showing transmission by the bite of a blood-sucking insect.

PRESENT INVESTIGATION

Apart from the investigations by Macdonald (1950) and Cathie & Dudgeon (1949) with complement fixation and by Fisher (1951) with the skin test, little is known about the prevalence of antibodies to toxoplasma, and hence, presumably, of latent toxoplasmosis in this country.

For this reason sera from 'normal' persons in Sheffield have been examined. The sera came from blood donors, children undergoing tonsillectomy, patients attending ophthalmic out-patient departments for removal of foreign bodies or for refraction errors (none of whom had chorio-retinitis or uveitis), and laboratory workers who had not worked with toxoplasma. The method used was the dye test of Sabin & Feldman (1948), as modified by Beverley & Beattie (1952).

The percentages of persons in the various age groups whose sera gave positive reactions to various titres are shown in Table 1 and Fig. 1.

Table 1. *Percentage of general population: dye test*

	Age in years					
Titres	0–9	10–19	20–29	30–39	40–49	50+
⩾1/4	2	11	25	25	21	29
⩾1/8	1	10	12	16	12	13
⩾1/16	1	4	4	1	4	2
⩾1/32	1	0	2	1	0	1
⩾1/64	0	0	0	0	0	0
Totals at risk	108	55	101	105	108	104

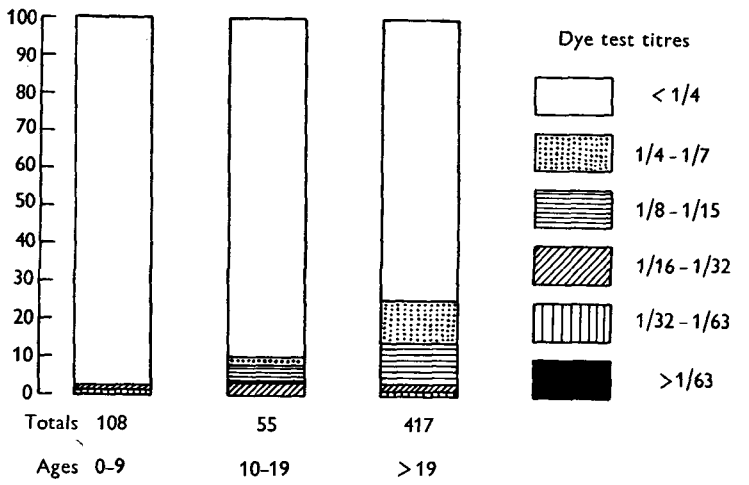

Fig 1.

The χ^2 test showed no significant difference at a titre of 1/4 or more between the age groups from 20 onwards ($\chi^2 = 1{\cdot}62$, $n = 3$), but a significant difference between the age group ⩾ 20 and the two lower groups ($\chi^2 = 29{\cdot}9$, $n = 2$). For this reason all under 20 have been excluded from further analyses. The χ^2 tests also showed no significant difference between the two sexes ($\chi^2 = 2{\cdot}17$, $n = 1$).

A quarter of the adult population had antibodies for toxoplasma.

If these antibodies arise, as is believed, from infection whence does the infection come?

The discovery of very high titre antibodies (1/12,800) in the serum of a butcher's boy suffering from glandular enlargement (Skipper, Beverley & Beattie, submitted for publication) seemed to offer a clue, which became stronger when it was found

that his employer's serum reacted to a titre of 1/1600, the serum of his employer's dog to a titre of 1/80 and that of a cat which scavenged scraps from the shop to a titre of 1/200; while his father, mother and sister, with whom he lived, an uncle and aunt with whom he spent his week-ends, and his pet cat which he kept at home, all gave negative reactions. (One of Verlinde & Maksteniek's (1950) patients was the son of a butcher, but no antibodies were found in his father's serum.)

Contacts with meat or animals were thought of as likely sources.

Table 2. *Percentage of occupational groups: dye test*

Titres	General population, males over 20 years	Abattoir workers	Veterinary surgeons	Rabbit handlers	Rabbit trappers
⩾1/4	27	34	34	64	79
⩾1/8	14	25	24	56	71
⩾1/16	2	12	12	43	67
⩾1/32	1	3	4	25	42
⩾1/64	—	1	2	12	21
Totals at risk	229	146	50	142	24

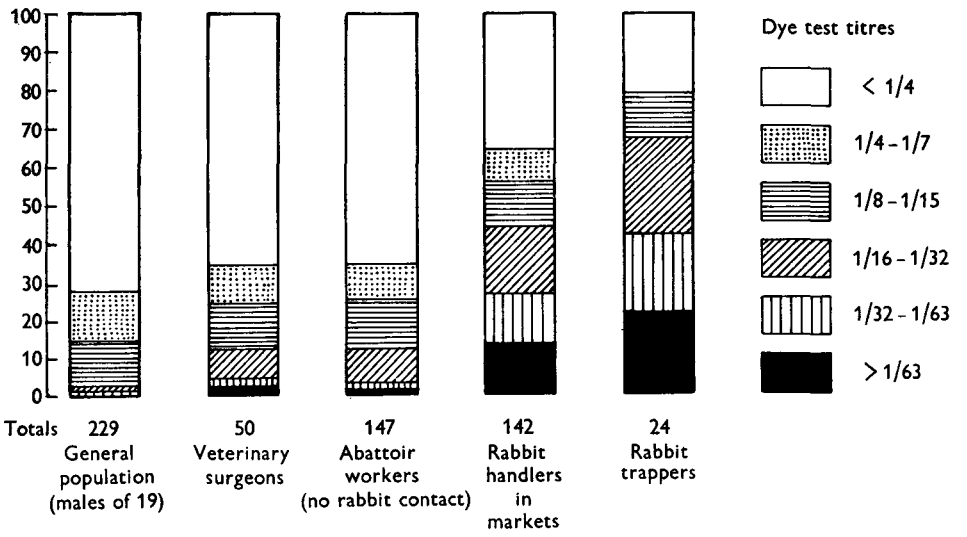

Fig. 2.

The sera of abattoir workers, veterinary surgeons, rabbit handlers and rabbit trappers were, therefore, examined with the results shown in Table 2 and Fig. 2.

It will be seen that at all titres there is an increase from left to right. At a titre of 1/4, however, the differences between veterinary surgeons, abattoir workers and controls are not significant. In order to utilize the whole range of titre values, thus giving a true picture of each occupational group, a ranking system was used as follows:

Titre	<1/4	1/4–1/7	1/8–1/15	1/16–1/31	1/32–1/63	>1/64
Rank	0	1	2	3	4	5

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Using these ranks the mean values of the occupational groups were calculated and are shown below:

	Control group	Abattoir workers	Veterinary surgeons	Rabbit handlers	Rabbit trappers
Mean value	0·44	0·75	0·76	2·04	2·83

There is a steady rise in the mean values. An analysis of variance of the results showed a difference between the groups, the control group being significantly lower than any of the others. (See Appendix.)

There was no difference between the abattoir workers and veterinary surgeons, but each of these groups was significantly lower than the rabbit handlers and trappers whilst the rabbit trappers had a significantly higher level than the rabbit handlers.

Skin test with toxoplasmin were also made. In 340 persons 94 % agreement was found between a positive skin reaction and a dye test positive to a titre of 1/4 or more. The discrepancies were six with positive skin test and a negative dye test and 14 with a positive dye test and a negative skin test. Of these 14, one had a titre of 1/34, one of 1/15 and 12 of less than 1/8.

An attempt was made by questioning the Leeds and Manchester abattoir workers and the Manchester rabbit handlers to obtain further information on the importance of dogs as a source of infection and also on the part played by pigeons. Of 144 who had not kept dogs 57 % showed dye test titres of 1/4 or more. Of 84 who had kept dogs 46 % had dye test titres of 1/4 or more. As far as it goes, this does not support the view that dogs are an important source of infection, but should not be taken to mean that a person who cares for a dog ill with toxoplasmosis is not liable to contract infection. Only three men kept pigeons. One of them had a serum titre of 1/12, the other two of less than 1/4.

The next step was obviously to examine the sera of native wild rabbits. Frozen imported rabbits were left out of consideration as it is known that freezing kills toxoplasma.

Rabbit sera and the trapper's sera were obtained in the Ceiriog Valley in Denbighshire in March 1953. Blood samples were taken from freshly killed rabbits either by cutting the blood vessels in the neck or from the inferior vena cava. The samples were taken daily to Llangollen where the sera were separated and stored in an ice-cream refrigerator. When all the samples had been collected they were taken in boxes insulated with kapok to the laboratory in Sheffield where they were tested by the dye test and also by the complement-fixation test (Sabin, 1949). The results are shown in Table 3.

The dye tests on the rabbit sera began at a higher dilution than did those on human sera, because it was desired, by looking for higher titres, to obtain more definite evidence of infection. To have set up longer series of dilutions to detect lower titres would have introduced dilution errors. Low dilutions of rabbit sera may contain non-specific antibodies (Sabin & Feldman, 1948). Therefore it was not considered expedient, especially with such large numbers of sera, to carry out preliminary tests in low dilutions and then to repeat the tests in a higher range,

even though there may have been some cases of specific low titre antibody which would not be detected.

At first sight the dye test and complement-fixation test results in the highest dilutions appear to agree, but, in fact six sera which gave dye test titres of 1/160 or more proved negative in the complement-fixation test. Among the lower titres, of those positive at 1/80 in the dye test, 18 were negative in the complement-fixation test and, at 1/40, 59.

In six instances positive complement fixation (five at 1/5 and one at 1/10) was accompanied by a negative dye test.

Table 3. *Rabbits*

Titres	Dye test (%)	Complement-fixation test (%)
⩾ 1/5	Not tested	10
⩾ 1/10	Not tested	7
⩾ 1/20	Not tested	4
⩾ 1/40	34	4
⩾ 1/80	12	4
⩾ 1/160	5	3
Totals at risk	321	300

Twenty-one sera proved anticomplementary and are not included.

DISCUSSION

It is possible, though it seems highly improbable, that the antibodies reacting with toxoplasma antigens and the allergic skin reactions are due to the stimulus of antigens other than toxoplasma. So far the only organisms said to be antigenically related to toxoplasma are sarcosporidia (Muhlpfordt, 1951). The writers have examined the serum of a man known to be infected with sarcosporidia without finding any evidence of antibodies to toxoplasma.

Doubts have been expressed about the value of the skin test with toxoplasmin. It is true that it is sometimes negative in the presence of positive dye test. The results it gives are, moreover, only qualitative. For these reasons its diagnostic value is limited, but the results obtained in the present investigation seem to confirm its value as an easy and sufficiently reliable method for epidemiological surveys.

If, as is generally assumed, these antibodies indicate infection, past or present, with toxoplasma, then one must conclude that infection is very common. In Sheffield it is rare in early life, but increases with age until, by the age of 20, one-quarter of the population have been infected. From other countries higher figures have been given and incidence has risen in each decade. In Sheffield there was no significant rise in the decades over 20, nor was any significant difference found between the incidences in men and in women.

When the strength as well as the number of positive reactions in the dye test were considered (by means of a ranking system) veterinary surgeons and abattoir workers were significantly and equally more positive than the general population.

This supports the view that man's domestic animals are a source of human infection.

Still more positive were rabbit handlers and, most of all, rabbit trappers.

Wild rabbits appear to be frequently infected, for a third of the sera tested have antibodies active in the dye test to a comparatively high titre of 1/40 or more. The complement-fixation test confirmed this by giving 10 % of positive results with titre of 1/5 or more.

Unfortunately it was not possible, in the remote valley where the samples were obtained, to inoculate mice with tissue from rabbits, nor were parasites resembling toxoplasma found in sections of a few diseased livers.

There is a strong correlation between contact with wild rabbits and the presence of antibodies to toxoplasma. This correlation is probably true, because it is difficult to think of any other causative factor common to workers in markets and to trappers in the open.

How infection can spread from rabbit to man is not known. If from the flesh of the animals, women, who more frequently prepare rabbits for cooking, should show a higher incidence of antibodies than men, but they do not.

Toxoplasma is possibly transmitted from rabbit to rabbit by an ecto-parasite. This ecto-parasite may survive in the fur of the animal after it has been killed. Hence those who handle unskinned rabbits, trappers, wholesalers and retailers, may be particularly liable to infection.

SUMMARY

1. By the dye test, toxoplasma antibodies in titres of 1/4 or more, were found in the sera of 25 % of a sample of adult population of Sheffield.

2. Significantly higher, when strength as well as number was considered, were the reactions of the sera of veterinary surgeons and abattoir workers.

3. Still higher were the reactions of the sera of those who handled rabbits. Highest of all were those of rabbit trappers.

4. Toxoplasma antibodies active to a titre of 1/40 in the dye test were found in 34 % of wild rabbits and, to a titre of 1/160, in 5 %.

Thanks are due to the hundreds of volunteers who made the survey possible. The Medical Officers of Health of Sheffield, Leeds and Manchester and the veterinary officers, superintendents of markets and food inspectors in these cities made admirable arrangements, which are gratefully recorded, for the obtaining of specimens. Valuable advice and help in obtaining samples from rabbits were given by officers of the Pests Control Division of the Ministry of Agriculture and Fisheries and by their trappers. Dr Marmion supplied some of the sera from veterinary surgeons. The assistance of the technical staff of the Bacteriology Department, University of Sheffield, is gratefully acknowledged. The work was assisted by a grant to C.P.B. from the Medical Research Council.

APPENDIX

Analysis of variance

The results of the analysis of variance (Table 2) show that there is a significant overall difference between the occupation groups when the variance between occupation groups is compared with the variance within groups. The best estimate of the standard error is the one based on the 'within occupation groups' variance and the standard errors of all the differences have been calculated from this (1·55).

The t values for each pair of results which were calculated from

$$t = \frac{\text{difference between means}}{\text{S.E. of difference}}$$

are as follows, the group with the lowest mean value being shown first. All the differences were significant except the one between the abattoir workers and veterinary surgeons.

Difference to be tested	t	Degrees of freedom	Significance
Controls and			
Abattoir workers	2·34	373	$0{\cdot}01 < P < 0{\cdot}02$
Veterinary surgeons	2·01	277	$0{\cdot}02 < P < 0{\cdot}5$
Rabbit contacts	10·61	469	$P < 0{\cdot}001$
Rabbit trappers	8·89	251	$P < 0{\cdot}001$
Abattoir workers and			
Veterinary surgeons	0·04	194	$P > 0{\cdot}1$
Rabbit contacts	8·74	286	$P < 0{\cdot}001$
Rabbit trappers	7·54	168	$P < 0{\cdot}001$
Veterinary surgeons and			
Rabbit contacts	6·21	190	$P < 0{\cdot}001$
Rabbit trappers	6·65	72	$P < 0{\cdot}001$
Rabbit contacts and			
Rabbit trappers	2·86	164	$0{\cdot}001 < P < 0{\cdot}005$

Source of variance	D.F.	Variance	Variance ratio	Significance
Between occupational groups	4	82·2	53·0	$P < 0{\cdot}001$
Within occupational groups	586	1·55		
Total	590			

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