

Some observations on the epidemiology of toxoplasmosis in Canada

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SUMMARY

Between 1961 and 1974, 11934 samples of serum were tested by the Sabin-Feldman Dye test for the presence of antibodies to *Toxoplasma gondii*.

Analysis of high-titred sera suggested that a 6-year cycle of high disease prevalence occurred across Canada. In addition, a decline in the percentage of positive reactions occurred each year in the Fall. The suggestion that this decline was due to dry conditions during the summer months was supported by the observation that differences in the prevalence of toxoplasma infection in ten Canadian cities were related to their average summer rainfall. The significance of these observations in relation to the epidemiology of toxoplasmosis in this country is discussed. The influence of patient age on the prevalence of infection was also investigated; the results obtained suggested that at least 75% of infants with high antibody titres against *T. gondii* had obtained these antibodies by placental transfer from their mothers.

INTRODUCTION

In spite of several surveys (Ffrench & Fish, 1961; Seah, 1973; DeSavigny, 1974) the status of toxoplasmosis in the population of Canada is still unclear. While the prevalence of positive serological reactions in Montreal (Seah, 1973) and Toronto (Ffrench & Fish, 1961; DeSavigny, 1974) is known, there is little information available on the incidence of the disease, on the major sources of infection or on the geographical distribution of infection. There are several reasons for this – in particular the indirect fluorescent antibody (I.F.A.) test has only recently become recognized as being comparable to the Sabin-Feldman dye test (S.F.D.T.) in sensitivity and specificity (DeSavigny, 1974; Walton, Benchoff & Brooks, 1966). The other serological tests available – the complement fixation test (C.F.T.) and the indirect haemagglutination test (I.H.A.) – are considerably less sensitive (Ffrench & Fish, 1961; DeSavigny, 1974) and reliable conclusions on toxoplasmosis prevalence cannot be derived by the use of these tests. Before the introduction of the I.F.A. test the S.F.D.T. was the only accurate method available for the detection of antibodies to *Toxoplasma gondii*.

Sabin-Feldman dye tests have been performed at the Ontario Veterinary College since 1959. In this paper the results of tests performed on over 11,000 serum samples submitted since January 1961 have been analysed.

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These samples were submitted by physicians in each of the provinces excluding Saskatchewan and Manitoba. They were derived from patients with suspected toxoplasmosis. They do not represent a random sample of the Canadian population, consequently no quantitatively accurate statements as to the prevalence of toxoplasma infection may be derived from them. We do assume, however, that these samples are selected randomly in relation to time of sampling, geographical location, and patient's sex and age. Consequently the relation of these factors to toxoplasmosis prevalence is investigated.

The nature of immunity to *T. gondii* is such that individuals, once infected, remain carriers for the duration of their life-span (Frenkel, 1971). In consequence antibodies tend to persist for a considerable period and their presence implies only that infection has occurred. The titre of these antibodies is determined both by the time of testing relative to acquisition of infection and by the size of the infecting dose.

Normally the s.f.d.t. is performed only to a serum dilution of 1/1024. Samples with such titres are considered to reflect a relatively recent infection (Robertson, 1961). Such samples therefore provide an indication of the incidence of infection. The percentage of samples positive at lower titres gives an indication of prevalence. For historical and comparative reasons a positive reaction at 1/16 has been considered here to represent a minimal positive reaction. Since however many of the initial reservations concerning the specificity of the s.f.d.t. have been shown to be unwarranted (Feldman, 1968) the use of a titre of 1/16 as a minimal positive reaction is no longer valid. The presence of a positive reaction at any titre is indicative of infection.

METHODS

The Sabin-Feldman dye test

The serological tests were performed as described by Sabin & Feldman (1948). Toxoplasma trophozoites of the RH strain were passaged at 3-day intervals in mice. For test purposes they were aspirated from the peritoneal cavities of infected mice and washed once. Freshly isolated trophozoites were incubated for one hour in a mixture of the serum under test and 'accessory factor' (a complement-like component of human serum.) Methylene blue dye was added to the mixture and the organisms examined after a further 30 min.

Normally trophozoites stain deeply with methylene blue. After incubation in antibody-containing serum in the presence of accessory factor only the nuclear endosome stains. Serum was inactivated at 56° C. for 30 min. before testing at dilutions of 1/16, 1/64, 1/256 and 1/1024. The titre of the serum was considered to be that dilution at which 50% of the trophozoites were unstained. At least 100 trophozoites were counted at each dilution.

Analysis of results

From the records available certain data were numerically coded and transferred to punched cards, one card being used per sample. Duplication of cases was

Table 1. Case data coded for in analysing S.F.D.T. results

1. Year and month of testing	4. Submitting physician in selected cases*
2. Case number	5. Age and sex
3. Province and city or area of origin	6. Titre

* Only the 13 major sources of samples were coded.

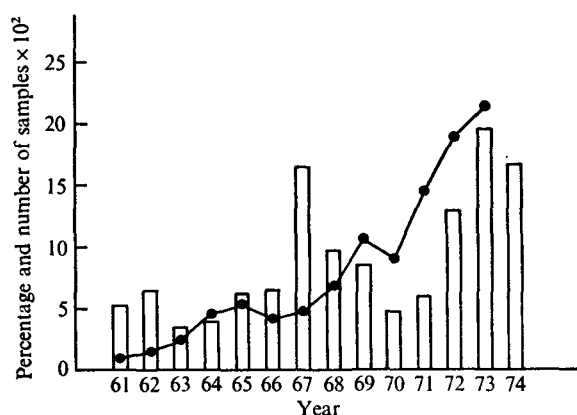


Fig. 1. The percentage of samples reacting at 1/1024 and the number of samples ($\times 10^3$) submitted for testing for toxoplasmosis from 1961 to 1974. \square , Percentage of high titred samples; \bullet — \bullet , number of samples ($\times 10^3$).

avoided. The data coded are listed in Table 1. Details of all tests submitted between 1 January 1961 and 31 August 1974 were transferred to cards in this way and the information subsequently stored on magnetic tape.

The stored data were analysed and cross-tabulated using the set of computer programs SPSS5, 'A statistical package for the Social Sciences' (Nie, Bent & Hull, 1970). The computation was performed on an IBM 370/155 computer of the Institute of Computer Science at the University of Guelph.

Age adjustment of results

In order to eliminate differences caused by the different age distribution of the sample population in different provinces, results were adjusted to conform to the overall Canadian age distribution in 1970 (Census of Canada, 1971).

RESULTS

Between January 1961 and August 1974, 11,934 samples of serum were tested for the presence of antibodies to *Toxoplasma gondii*. Of these samples 45.5% were positive at a dilution of 1/16 and 11.5% were positive at 1/1024. The number of samples tested annually increased from 111 in 1961 to 2148 in 1973.

Samples showing a positive reaction at 1/1024 were cross-tabulated according to the year in which the test was performed (Fig. 1). It was observed that the percentage of sera reacting at this dilution appeared to follow a cyclical pattern.

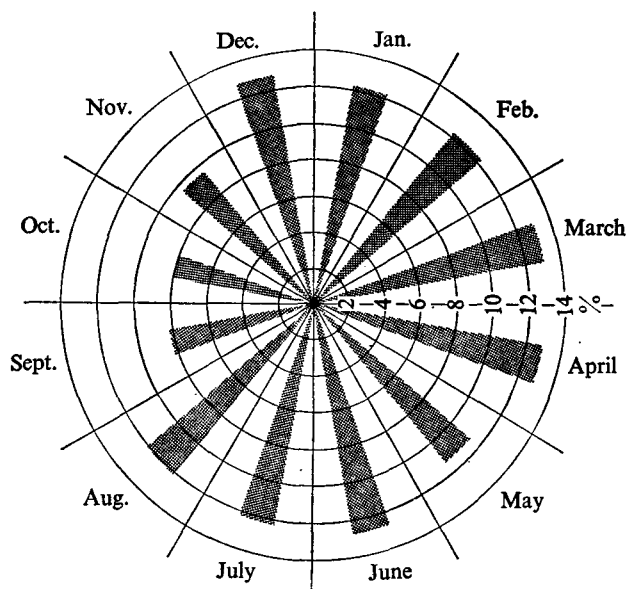


Fig. 2. Pooled monthly percentage of samples reacting at 1/1024 in the Sabin-Feldman dye test, 1961-74.

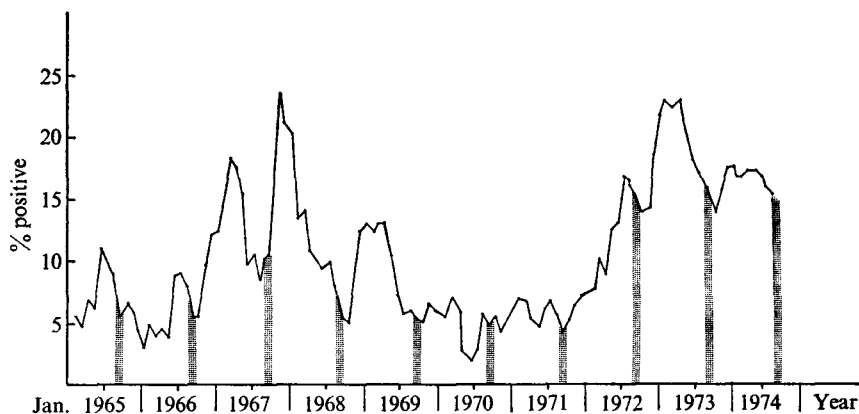


Fig. 3. A 3-month running average of the percentage of samples reacting at a titre of 1/1024. The shaded bars indicate the month of September. Note the consistent presence of a decline in the percentage of reactive sera in the Fall of each year - 1970 excluded.

A relatively low percentage of sera reactive at 1/1024 was found in 1963, 1964 and 1970. High values were observed in 1967 and 1973 with a lesser peak in 1962. Analysis of these results on a provincial basis showed that peak prevalence rates were seen in Ontario in 1962, 1967 and 1974. Samples from Quebec and British Columbia showed peak rates in 1967 and 1973. Insufficient samples were submitted from these provinces before 1965 so that it was not possible to determine whether a third peak occurred in these provinces in 1962.

Examination of results on a pooled monthly basis (Fig. 2) indicated that the percentage of high titred serum ($> 1/1024$) remained relatively constant between

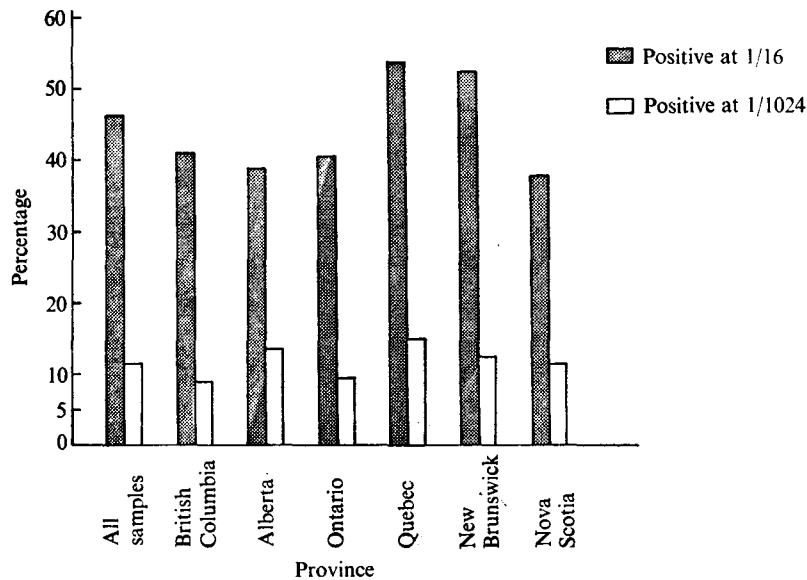


Fig. 4. Results of the Sabin-Feldman dye test by province of origin on an age-adjusted basis.

December and August at approximately 12%. However, in September, October and November this percentage dropped to around 8%. This drop is statistically significant at the 1% level. A less-marked decline was observed in samples reacting at 1/16. Analysis of samples submitted between January 1965 and August 1973 by plotting a 3-month running average (Fig. 3) demonstrated that the Fall decline was an annual event. Only in 1970, a year of very low toxoplasma antibody levels, did this decline fail to materialize. The month of minimum prevalence over the whole country varied between August and November. The Fall decline was observed to occur in samples submitted from British Columbia, Alberta, Ontario and Quebec. The month of minimum prevalence varied between September in Ontario and November in British Columbia. Analysis of samples reacting at 1/16 also showed indications of a decline during the Fall. In 1967, 1968, 1969 and 1973 a definite spring rise was also observable (Fig. 3).

Geographical variations in the percentage of reactive samples

The classification of results by province of origin (Fig. 4) showed that the highest percentage of positive results (on an age adjusted basis), were found in samples from Quebec and New Brunswick at 54.3% and 51.0% respectively. Samples from British Columbia, Alberta, Ontario and Nova Scotia were approximately 40% positive. Further examination of samples submitted from the province of Quebec (Fig. 5) indicated that samples from Trois Rivieres and the South bank of the St Lawrence had the highest percentage of high titred samples (18.5% and 19.5% respectively at 1/1024).

Samples from Ontario were 40.57% positive on an age adjusted basis. The range of results varied considerably; Hamilton had an unusually low percentage of

Table 2. Key to Fig. 5, and the number of samples submitted from each area in Ontario, Quebec and the number of submitting sources

City	Number of samples	Number of sources
1 Windsor and Sarnia	95	2
2 London	290	4
3 Kitchener-Waterloo and Guelph	165	4
4 Toronto	3460	13
5 Hamilton	716	8
6 Niagara Falls and St Catharines	253	5
7 Peterborough	29	1
8 Kingston	172	4
9 Ottawa	252	2
10 Montreal	2271	21
11 Trois Rivières	160	3
12 Quebec	1470	6
13 North Shore P.Q.	520	9
14 South Shore P.Q.	707	13

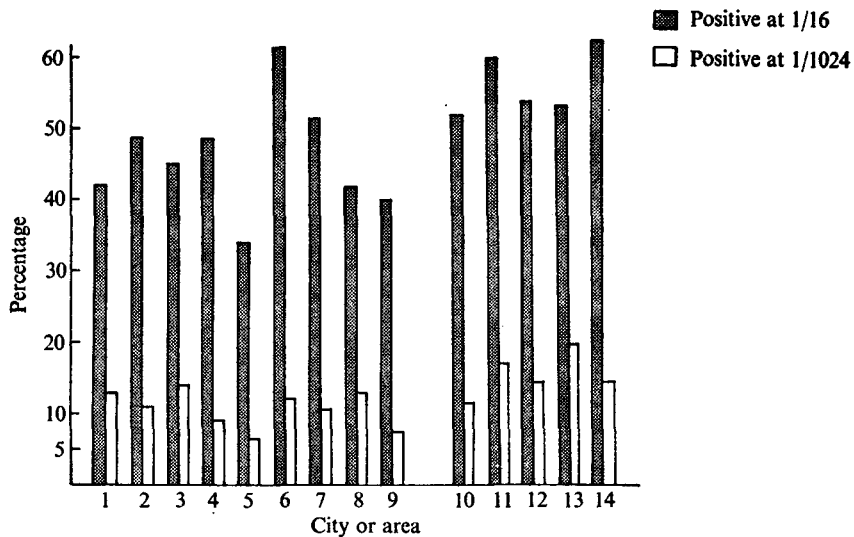


Fig. 5. Results of the Sabin-Feldman dye test by city or area of origin (see Table 2 for key to cities).

positive samples (33.8%), St Catharines, only 39 miles away, had an unusually high percentage of positive samples (67.6%). However, these samples were submitted by a single physician. Subsequent random sampling has revealed a normal percentage of positive reactions from this area (unpublished observations).

Six-yearly cyclical variations in the annual percentage of high-titred serum was observed in samples from both Toronto and Montreal.

The relation of patient's age to antibody prevalence

Ffrench & Fish (1961) demonstrated that the prevalence of anti-toxoplasma antibodies rose steadily with increasing age to reach a maximum value of 35% at

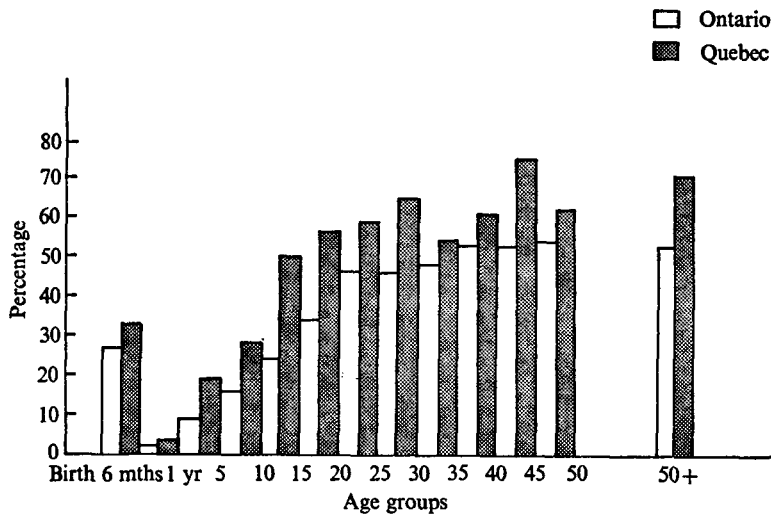


Fig. 6. Age-specific prevalence rates of anti-toxoplasma antibodies (titre $\geq 1/16$) in samples from Ontario and Quebec.

Table 3. Age-adjusted prevalence rates (data based on the age distribution of the Canadian population as reported by Census of Canada (1971))

Age group (years)	Percentage of population showing a positive reaction in the Sabin-Feldman dye test		
	Quebec	Ontario	Canada
1-5	0.66	1.53	1.10
6-10	3.07	1.64	2.05
11-15	5.47	4.28	3.99
16-20	5.49	3.69	4.49
21-25	5.11	3.94	4.65
26-30	4.76	3.56	4.17
31-35	3.40	2.85	3.13
36-40	3.60	3.06	3.33
41-45	4.38	2.82	3.63
46-50	3.58	3.12	3.30
51+	14.33	10.08	12.18
Total	53.85	40.57	46.02

age 50. The results derived from our data (Fig. 6) also show a steady increase of age specific rate with increasing age. Overall, 60% of samples from patients 50 years of age or older were positive. This figure was higher for samples from Quebec and correspondingly lower in samples from Ontario. Of 951 samples obtained from children less than 6 months old, 28.1% were positive at 1/16 and 3.2% positive at 1/1024. In contrast 4.8% of 330 samples from children between 6 months and 1 year old were positive at 1/16 and 2.2% were positive at 1/1024. We interpret this to indicate that most of the antibodies present in children under 6 months of age were maternally derived. The antibodies present in the 6-months to 1-year-old group, however, possibly represent the response of these children to

infection. The age adjusted prevalence rates (Table 3) show that most toxoplasmosis in the Canadian population is in individuals between 11 and 25 years of age.

The relation of patient's sex to antibody prevalence

There was no significant difference in antibody prevalence between the sexes. In Ontario, males were 38% positive and females 39% positive at 1/16. In Quebec, males were 51.6% positive while females were 56.4% positive. This difference is not statistically significant.

DISCUSSION

It is currently considered that toxoplasmosis in man may be acquired by three routes: by the ingestion of undercooked infected meat (Kean, Kimball & Christenson, 1969); by the accidental ingestion of oocysts shed by cats and other felidae (Hutchinson, Dunachie, Work & Siim, 1971) and by transplacental passage (Couvreux & Desmonts, 1962). Although the first two routes probably account for most human infection it is difficult to measure the relative risks involved. This may be because it is neither uncommon to ingest 'rare' meat nor to come into relatively close contact with cats. Nevertheless, certain features of these modes of transmission may be reflected in incidence and prevalence data.

Infection through ingestion of uncooked meat would be directly influenced by meat-eating patterns, which in turn tend to be influenced by economic and particularly ethnic considerations. Seasonal variations in meat ingestion patterns probably do exist. Using the number of animals slaughtered for human consumption as a prime indicator (Livestock Market Reviews, 1971-4), seasonal variations are seen in the killing of hogs, sheep and lambs. These variations do not, however, appear to relate to the prevalence of toxoplasmosis. Similarly, the annual variation in the numbers of animals killed for meat between 1961 and 1974 bore no apparent relationship to the variations in toxoplasmosis prevalence. Little evidence exists for differences in disease prevalence between major ethnic groups (Ffrench & Fish, 1961; Kimball *et al.* 1960), although Seah (1974) has reported that Chinese in Montreal have a much-reduced toxoplasma antibody prevalence rate.

Infection by accidental ingestion of oocysts derived from cats depends upon the degree of exposure to these animals. This too may be influenced by ethnic considerations. However, in the densely populated areas of southern Canada the degree of exposure of humans to felines is probably very uniform. In this case a major factor in the transmission of disease to man could be climatic. Oocysts become highly infectious within 24 hr. of being passed in cat faeces. They can remain viable in moist soil for long periods of time and the only environmental factor which significantly affects this viability is drying (Frenkel & Dubey, 1972). It might therefore be expected that drying of soil during the warm, dry, summer months would have an influence on toxoplasmosis occurring in the Fall, if oocysts are a major source of infection of humans. That this hypothesis is probably

Table 4. *The relation between the prevalence of antibodies to Toxoplasma gondii and climatic conditions based upon results from ten Canadian cities*

Variable 1	Variable 2	Correlation coefficient
Positive samples at 1/16 (%)	Average annual rainfall	-0.08057
	Average August rainfall	0.399
Positive samples at 1/1024 (%)	Average annual rainfall	0.13482
	Average August rainfall	0.7069*
	Minimum summer rainfall	0.715*
	Average August rainfall/average August temperature	0.712*
	Maximum annual rainfall	-0.2126

* Significant at the 5% level.

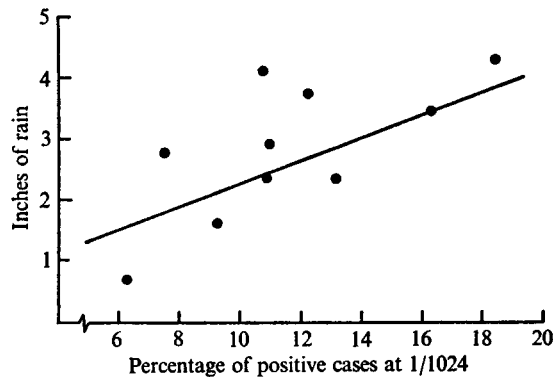


Fig. 7. The relationship between August rainfall in inches and the percentage of samples reacting at a titre of 1024 for ten Canadian cities. The correlation coefficient r between these two variables is 0.7069.

correct is reflected in our observation that the percentage of positive cases shows a decline in the Fall of each year.

It is difficult to account for the cyclical variation in positive dye test levels between years. We hypothesize that this may be related to cycles in the rodent or wild-bird populations. There is evidence available to suggest that rodent cycles are related to toxoplasma infection (Elton, 1942). Changes in the toxoplasma infection status of rodents or wild birds could be readily and rapidly transmitted to the human population through cats. Experiments are under way to confirm or refute this hypothesis.

If, as suggested, drying is of importance in regulating the spread of toxoplasmosis to the human population, then differences in prevalence of infection between geographical areas may reflect their climatic status. When the prevalence of antibodies to *T. gondii* in ten Canadian cities was related to the degree of dryness during the late summer (Table 4, Fig. 7), a significant degree of correlation was found. Cities with relatively dry summers such as Victoria have a relatively low prevalence of toxoplasma infection while the situation in Quebec is the reverse – wet summers and a high prevalence of infection (Table 5).

Table 5. *The association between late summer rainfall and the percentage of high-titred serum samples*

City	Prevalence of antibodies at 1/1024 (%)	August rainfall (in.)*
Halifax, N.S.	10.8	4.06
Fredericton, N.B.	12.2	3.78
Montreal, P.Q.	16.3	3.48
Quebec, P.Q.	18.5	4.41
Ottawa, Ont.	7.5	2.78
London, Ont.	11.0	2.97
Toronto, Ont.	10.9	2.39
Edmonton, Alta.	13.3	2.27
Vancouver, B.C.	9.2	1.63
Victoria, B.C.	6.3	0.65

* Canadian Almanac and Directory for 1974.

Certain other features of our data tend to support the hypothesis that climatic factors are of major significance in influencing the prevalence of toxoplasmosis. For instance, the low proportion of positive reactions in samples from Hamilton may perhaps be ascribed to the low rainfall in that city. Being in the shadow of the Niagara Escarpment, Hamilton receives rather less rainfall than the surrounding areas (Ontario Ministry of Agriculture and Food, 1973). Finally, additional evidence as to the significance of climate may be derived from the observation that the prevalence of antibodies to *T. gondii* is extremely low in Navajo Indians as compared with other centres across the United States (Feldman & Miller, 1956). These Indians live in one of the most arid parts of the U.S., where oocyst survival would be very brief. (See also Kimball *et al.* 1960.)

The 6-year cycle of toxoplasmosis prevalence shows little relation to any climatic feature. Similarly it has not been possible to show drops in disease prevalence in individual dry years such as 1973.

Since climatic conditions appear to exert a significant influence over the prevalence of toxoplasmosis, we conclude that oocyst derived infection is probably of major significance in the spread of toxoplasmosis in Canada.

The high prevalence of antibodies to *T. gondii* in infants under 6 months relative to the prevalence after 6 months implied that most of these antibodies are maternally derived. Since 28.1% of young infants possessed antibodies, it might be expected that approximately 28% of mothers also possessed antibodies. DeSavigny (1974) using the I.F.A. test to screen 500 randomly selected maternal sera showed 28.2% of these to be positive. Since our selected samples do not differ from this random sample, we conclude that physicians are relatively unsuccessful in the selection of infants with congenital toxoplasmosis. The small number of positive samples after 6 months possibly reflected the proportion of those infants who were congenitally infected and consequently mounted an immune response of their own. These results emphasized again the importance of determining whether the antibodies found in infants are of the IgG type and therefore probably maternally derived or of the IgM type and thus synthesized by the infant in response to infection.

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