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Toxoplasmosis and the wild rabbit *Oryctolagus cuniculus* in Victoria, Australia with suggested mechanisms for dissemination of oocysts

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SUMMARY

Sera or serum eluates from 1697 wild rabbits [*Oryctolagus cuniculus* (L)], collected over the past 10 years from 24 sites in 5 geographic regions of Victoria, were examined for antibodies to *Toxoplasma gondii*. Sera generally were classified into three broad groups, negative (titre < 10), weakly reactive (titre 10 to 100) and strongly positive (titre > 1000). Strongly positive sera came from rabbits collected in a small number of localized areas, most significantly the Melbourne and Metropolitan Board of Works (MMBW) Sewerage Farm at Werribee and Mud Island in Port Phillip Bay.

INTRODUCTION

Toxoplasmosis is a protozoan zoonosis caused by *Toxoplasma gondii* (Dubey, 1977). It has a world wide distribution in terrestrial and aquatic homeothermic animals although Felidae are the only known definitive host. The disease shows a varying severity in different animal species. In man it is recognized generally as a mild, afebrile illness although recent work with mice suggests that latent infection may be associated with impaired mental performance (Piekarski, Zippelius & Witting, 1978; Hutchison *et al.*, 1980). It is also considered a cause of congenital abnormality (Szabo, 1974). Toxoplasmosis is a major cause of ovine (Hartley & Moyle, 1974) and caprine (Munday & Mason, 1979) abortion and can cause a severe, sometimes fatal illness in some wild animals e.g. the hare, *Lepus europaeus* (Pallas) (Christiansen & Siim, 1951). With the exception of a small study on wild animals in Tasmania (Munday, 1972), there is little knowledge of the distribution of *T. gondii* in the wild in Australia. In this study, wild rabbit and hare sera were screened for antibodies to *T. gondii*. The results suggest a strong correlation between toxoplasmosis in wild rabbits and at least one method of treatment of sewage effluent.

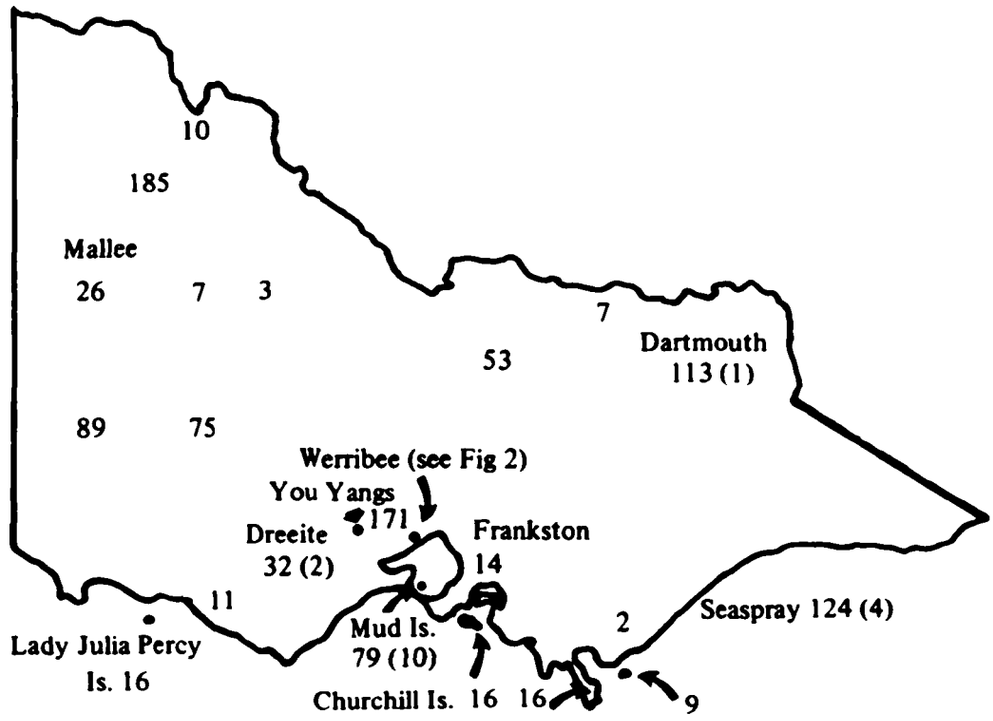

Fig. 1. Sites from which wild rabbit sera were collected in Victoria. Figures refer to number of individual sera collected, figures in brackets state number found to be seropositive.

MATERIALS AND METHODS

Sera

Serum samples from 1697 wild rabbits were collected from 24 sites throughout Victoria from 1971 to 1980. Of these samples, 1556 were collected as whole blood samples and subsequently separated and stored as sera, the remainder (141 samples) were absorbed on Ekwip No. 8 filter paper and stored and tested as eluates. Collection areas are shown in Fig. 1. The 3 areas at the Melbourne and Metropolitan Board of Works (MMBW) Farm collectively labelled as Werribee in Fig. 1 are shown in detail in Fig. 2. The collection areas included the Mallee region, a semi-arid area in north-west Victoria, the dry coastal region at Seaspray, the MMBW Farm at Werribee, and a wet sub-alpine area at Dartmouth. Four island populations were also sampled. The 45 hare sera were collected from Central Victoria and the Mallee.

Growth of T. gondii

T. gondii strain RCH-Vic, supplied by Mr Ian Jack, Royal Children's Hospital, Melbourne, was grown in a diploid foetal human tongue cell line CSL 300 developed at the Commonwealth Serum Laboratories and used at about its 50th cell culture passage. *T. gondii* was stored under liquid nitrogen at −196 °C as infected cell cultures, and co-seeded with uninfected cells to establish a new infection. Cell culture methods were based on those decribed by Hayes & Stirling (1975). Cell cultures were grown on Eagle's Basal Medium supplemented with 10% (v/v) unheated foetal calf serum and 10 mM HEPES buffer. *T. gondii* was passaged in cell cultures by infecting confluent monolayers with tachyzoites released into the

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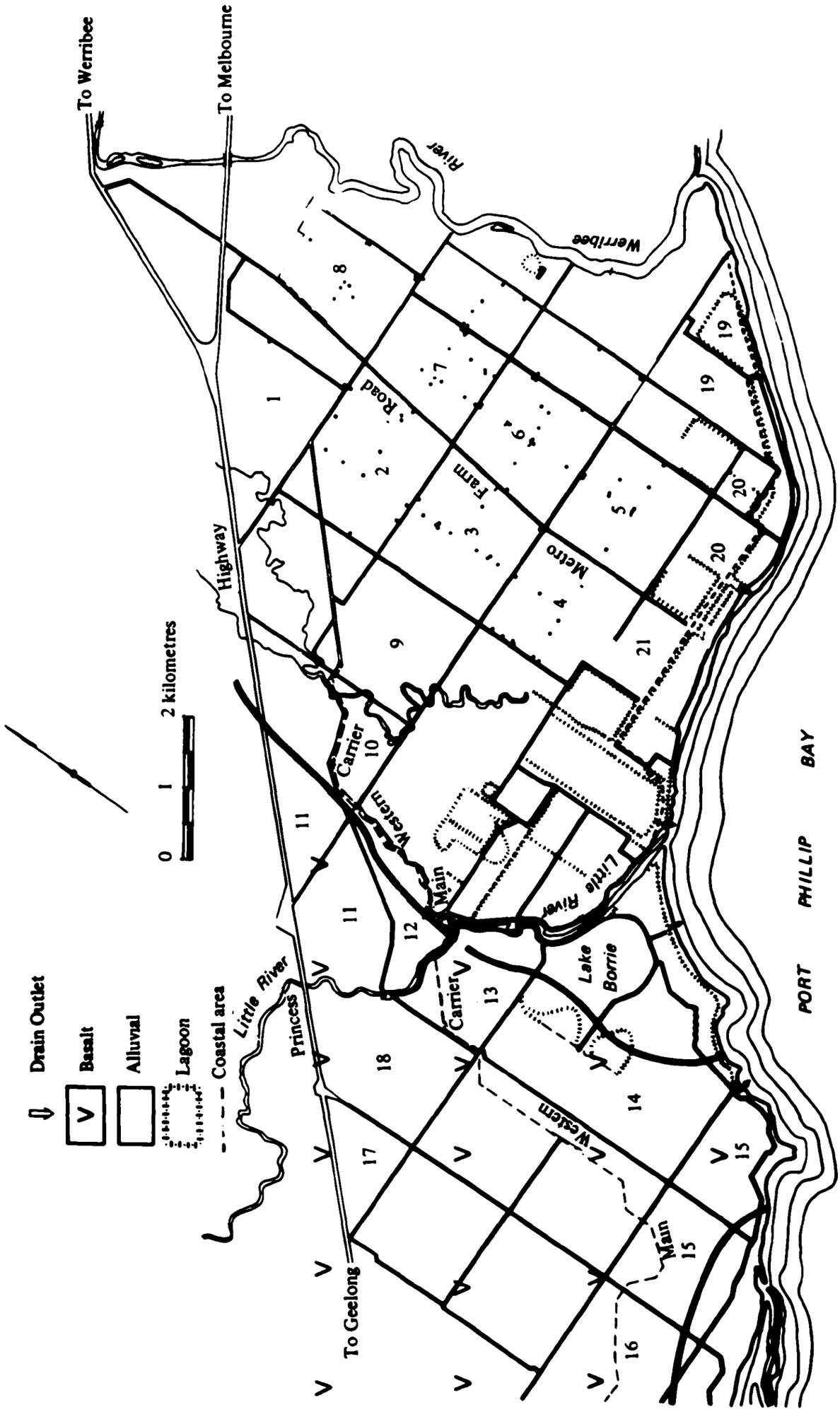

Fig. 2. Detailed map of collection sites on the MMBW Sewage Farm at Werribee. Numbers refer to areas where rabbits were collected, dots show the exact location of collection for seropositive rabbits.

Table 1.

Key to Fig. 2. Area map for the MMBW Sewage Farm at Werribee showing number of rabbits collected and the number seropositive for each area.

Area	1*	2*	3*	4*	5*	6*	7*	8*	9*	10*	11†
Number of rabbits											
total	20	36	64	57	40	71	88	41	1	3	43
positive	2	13	16	13	12	23	22	10	0	0	0
Area	12†	13‡	14†	15†	16†	17†	18†	19‡	20§	21§	Coast
Number of rabbits											
total	25	4	4	5	9	7	3	13	6	1	98
positive	0	0	0	0	0	0	0	0	0	0	0

* Land filtration irrigated pastures.
† Dryland.
‡ Varied region; rabbits from dryland or lagoon banks only.
§ Dryland and lagoon banks.
Positive sera are those with a titre greater than 100.

supernatant fluid from cultures in which approximately 50% of the cells were infected. Infected cells were maintained on Medium 199 supplemented with 5% (v/v) unheated foetal calf serum and 10 mM HEPES buffer.

Preparation of antigen

Supernatants from cell cultures that were heavily infected but not grossly degenerate were centrifuged at 3000 *g* for 10 min. The tachyzoites were resuspended to the original volume with PBS (phosphate buffered saline −0·01 M sodium phosphate (Na_2HPO_4 and NaH_2PO_4), 0·145 M sodium chloride pH 7·2) and 8 smears, each of 5 μl, were made on glass slides. Slides were air-dried, fixed in acetone at room temperature for 5 min and stored at −20 °C.

Immunofluorescence

Sera were tested by indirect immunofluorescence (Nairn, 1976) for the presence of antibodies to *T. gondii* at dilutions of 1 in 10 and 1 in 50. Positive sera were titrated at doubling dilutions from the appropriate $\log_{10}$ dilution. Titres were expressed as the reciprocal of the highest serum dilution giving bright uniform staining of the *T. gondii* tachyzoites. The conjugate used was a fluorescein-conjugated sheep anti-rabbit immunoglobulin (Wellcome Reagents Ltd, Beckenham, Kent, England).

RESULTS

Rabbit sera could be classified into 3 distinct groups. The majority of rabbit sera showed no significant antibody level to *T. gondii* i.e. titre < 10. About 15% of rabbit sera showed a weak reactivity (titre 10–100) with the *T. gondii* tachyzoites. Rabbit sera from all districts sampled showed this reactivity although the incidence varied from around 5% in rabbits from the You Yangs to 50% of the small sample from Frankston. The third group of sera showed strong reactivity with *T. gondii* (titre above 100). Antibody titres were generally above 1000 and frequently in excess of 10000. The presence of high titre antibodies to *T. gondii* correlated

strongly with the site of collection. High titre sera were found in only 5 of the 24 collection areas. These were Dartmouth (0·9% of sera), Seaspray (3·2%), Dreeite (6·3%), Mud Island (13%) and the land filtration area of the MMBW Farm at Werribee (26%). The overall results from Victoria are summarized in Fig. 1. The detailed results for the MMBW Farm in Fig. 2 and Table 1 illustrate clearly the geographical location of seropositive rabbits. The area of the MMBW Farm is about 11000 ha; all seropositive rabbits came from the pasture areas of the farm used for land filtration of sewage. No rabbits from the immediately adjacent coastal, dryland or lagoon banks were seropositive.

All 45 hare sera tested were seronegative.

DISCUSSION

The wild rabbit *Oryctolagus cuniculus* (L) in Australia is a very useful animal for the epidemiological study of toxoplasmosis because of its relatively sedentary habits, wide distribution and limited size of home territorial range. In the absence of adverse stimuli, rabbits on the MMBW Farm rarely stray more than 400 m from their warren systems (Shepherd, unpublished data).

There have been a number of epidemiological studies of toxoplasmosis in wild animals, including rabbits. Munday (1972) reported that 12 of 69 Tasmanian wild rabbits showed an immunofluorescence titre of 8. Munday (1969) also reported the isolation of *T. gondii* from 3 of 34 rabbits but did not present serological data for these rabbits. Beverley, Beattie & Roseman (1954), in a study of English wild rabbits, found 34% to have a dye test titre of 1 in 40 and 5% to have a titre of 1 in 160. However, Lainson (1955) failed to isolate *T. gondii* from 122 English wild rabbits. He suggested that either the distribution of *T. gondii* in the wild was very localized or that the dye test titres reported by Beverley *et al.* (1954) indicated 'experience' with *Toxoplasma* antigen rather than actual infection. Riemann *et al.* (1978) stated that 'high antibody responses (indirect haemagglutination titres of 1 in 4016) are a result of recent, first-time exposure, chronic infection or repeated exposure to infection'. Jones (1980) showed that following an intramuscular injection of attenuated *Toxoplasma*, mice retained a dye test titre in excess of 1000 for at least 11 months. These and other results suggest that, generally, there may be a real difference in the infection status of animals with high and low anti-*Toxoplasma* titres. One exception to this would be when the low titre is predominantly IgM thus indicating recent infection.

In the study reported here, rabbits with a low serum reactivity to *T. gondii* were found in all areas. The prevalence of reactors seemed to be influenced by climate and proximity to human population, however, in general, there was little divergence from the average of 15%. In contrast, rabbits with high-titre antibodies to *T. gondii* were found in only five localized areas, and two of these areas accounted for 121 of the 128 rabbits with high titres. The epidemiology of these two areas, the MMBW Sewage Farm land filtration area and Mud Island, differs markedly from all other reported surveys of toxoplasmosis in wild herbivores. The only tenable explanation is an abnormally high contamination of the pastures in these areas with oocysts. Another explanation, a high level of congenital infection, would have required the high levels of pasture contamination to generate the initial

difference from other areas of Victoria. Additionally, because *Toxoplasma* places the host at a biological disadvantage for survival one would expect the disease to be self-limiting in the absence of high levels of pasture contamination. Such a mechanism was postulated by Cox *et al.* (1980) to explain the absence of *Encephalitozoon cuniculi* in Victorian wild rabbits.

The cause of the high level of pasture contamination at the MMBW Farm land filtration area is unlikely to be the immediate presence of cats. *T. gondii* infection of cats has been demonstrated in the Mallee region (14% of 59) and North Central Victoria (44% of 16) (Coman & Jones, personal communication) where no rabbits tested were seropositive. Domestic and feral cats are only present in 'normal' numbers on the MMBW Farm and their wide-ranging habits should lead to *Toxoplasma*-infected rabbits on the adjacent dry land and coastal areas. Frenkel, Ruiz & Chinchilla (1975) have demonstrated the long soil-survival time of *Toxoplasma* oocysts even in relatively dry sites. Furthermore, toxoplasmosis in the wild rabbits on Mud Island could not be caused by feral cats for there has been no authenticated sighting of a cat on Mud Island in the past 10 years (Mr David Venn, Fisheries and Wildlife Division, Department of Conservation, Victoria, personal communication). We therefore assume that the sewage effluent itself is contaminated with *Toxoplasma* oocysts. These presumably originate from the large number of flat-dwelling or house-contained cats in Melbourne. It is well established that many cat-owners use the sewage system to dispose of the soiled absorbent cat litter and in fact this method has been advocated recently (Stevenson & Hughes, 1980).

Sewage arriving at the MMBW Farm is either treated directly by land filtration or, following sludge settlement, is subjected to grass filtration or passed through settling lagoons. The effluent then discharges into Port Phillip Bay near Werribee. Two possible procedures for the dissemination of *Toxoplasma* oocysts from the MMBW Farm to Mud Island can be postulated. Water movement within the bay is clockwise, thus sewage discharged near Werribee would pass the bay-side beaches of Melbourne and eventually leave the bay at Port Phillip Heads. Mud Island lies within the bay near this narrow exit and owes its existence to particulate matter being trapped within this clockwise stream. Thus one hypothesis is that viable *Toxoplasma* oocysts are discharged into the bay, carried in a clockwise stream past the bay-side beaches and finally discharged from the bay at Port Phillip Heads or collected as a sediment on Mud Island.

An alternative hypothesis is that certain birds, in particular seagulls, might act as passive carriers of the *Toxoplasma* oocysts by feeding upon areas contaminated with oocysts during the day and then at night returning to their various roosting sites. Mud Island is recognized as a major nesting and roosting site for seagulls. The suggestion of passive transfer of oocysts by seagulls, though at present unsubstantiated, has a precedent in the work of Beverley *et al.* (1975) who found that viable oocysts were present in the faeces of some sheep and lambs that had previously been fed cat-derived oocysts.

Both hypotheses raised in this discussion are unsubstantiated and considerable experimentation is required to determine which, if either, is correct. Questions of major importance are the stability of *Toxoplasma* oocysts in salt water, whether viable oocysts can pass through an avian gut without excysting, and what are the actual levels of oocysts in sewage effluent when it reaches the MMBW Farm, when

it is discharged into the bay and on the land filtration pastures. Present evidence (Table 1) also suggests that significant levels of viable oocysts may be associated with the land filtration areas of the Farm only. Further serum samples will be required from rabbits in the other areas, especially grass filtration, before this result could have statistical significance.

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