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Potential contamination of drinking water with *Toxoplasma gondii* oocysts

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SUMMARY

The world's first documented toxoplasmosis outbreak associated with a municipal water supply was recognized in 1995 in Victoria, British Columbia, Canada. It was hypothesized that domestic cat (*Felis catus*) or cougar (*Felis concolor*) faeces contaminated a surface water reservoir with *Toxoplasma gondii* oocysts. An extensive investigation of the Victoria watershed 1 year following the outbreak documented the presence of an endemic *T. gondii* cycle involving the animals inhabiting the area. Cats and cougars were observed throughout the watershed. Serological evidence of *T. gondii* infection was demonstrated among domestic cats living in the Victoria area. Cougars were found to shed *T. gondii* oocysts. Serological evidence of *T. gondii* infection in deer mice living in the riparian environments of the watershed suggested that *T. gondii* oocysts were being shed near the water edge. Contamination of Victoria's water supply with *T. gondii* oocysts potentially occurred during the study period and future waterborne toxoplasmosis outbreaks in this and other communities are possible.

INTRODUCTION

Toxoplasma gondii is a protozoan capable of infecting a variety of animal species [1]. Felids, both domestic and wild, are capable of serving as definitive hosts, shedding *T. gondii* oocysts in their faeces. All warm-blooded animals, including humans, can potentially serve as intermediate hosts, harbouring *T. gondii* in the form of tissue cysts. For the most part, infection causes only mild clinical disease in humans, and serosurveys across North America suggest that approx. 30% of the human population has been exposed [1]. Serious clinical disease occasionally occurs when organisms cross the placenta in women and infect the foetus [2], in immunocompromised individuals, particularly AIDS patients [3], and rarely in immunocompetent individuals, leading to a variety of syndromes including neuroretinitis [4] and encephalitis [5]. It is because of these sequelae that toxoplasmosis is a serious public health concern.

People acquire toxoplasmosis postnatally by ingesting *T. gondii* oocysts from contaminated environments or by consuming *T. gondii* tissue cysts in

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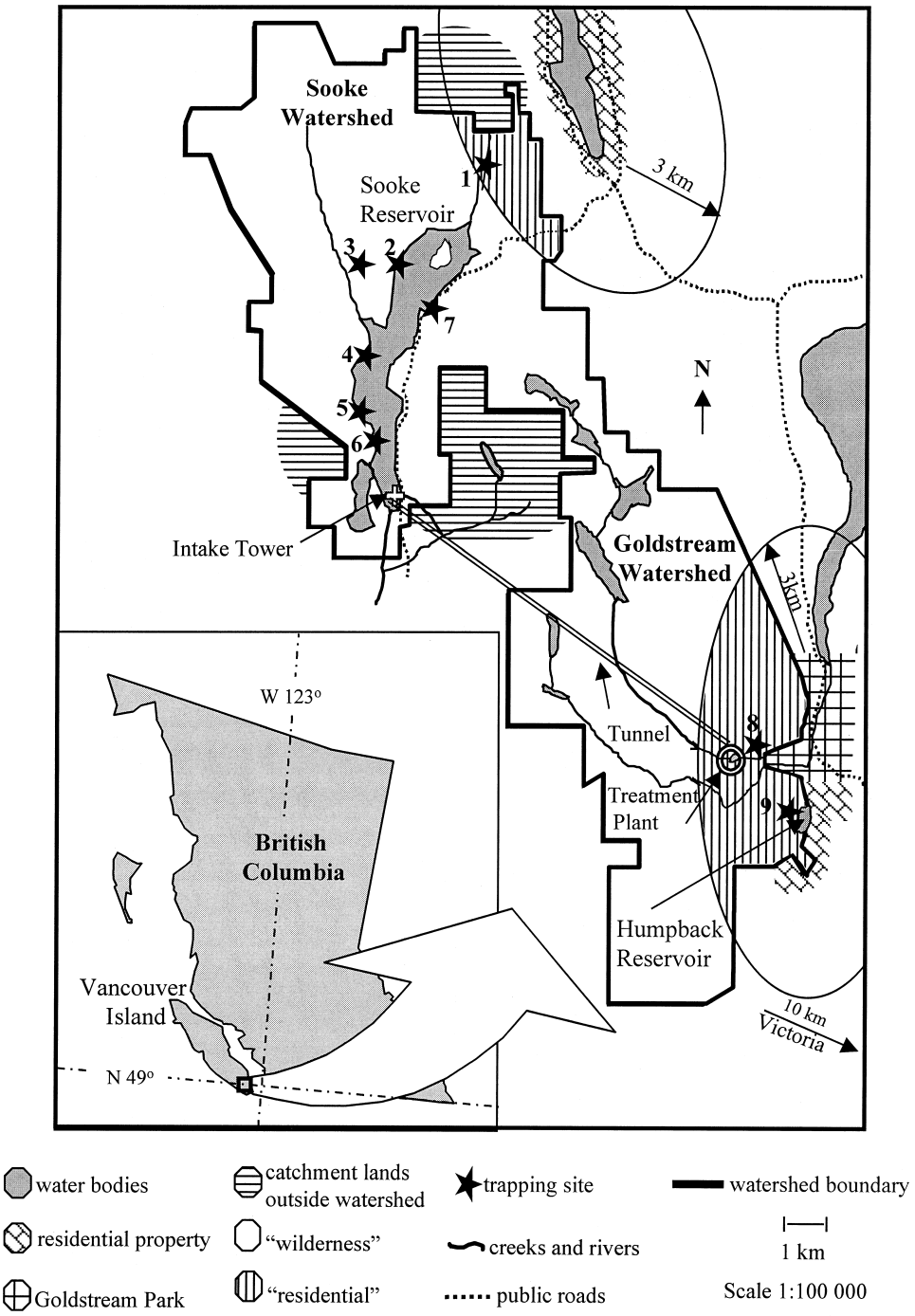

Fig. 1. Victoria watershed.

inadequately prepared meat products [1]. Although a previous outbreak of waterborne toxoplasmosis has been documented among US military personnel partaking in exercises in the Panama jungle [6], waterborne transmission has generally not been considered an important mechanism of disease transmission, until recently.

In the fall of 1994, the world's largest recorded human toxoplasmosis outbreak, and the only outbreak associated with a municipal water supply, began to unfold in the city of Victoria, on Vancouver Island, British Columbia, Canada [7, 8]. By the summer of 1995, it was estimated that between 2900 and 7700 individuals had been infected, representing 0·9–2·0% of Victoria's population [8]. An epidemiological investigation at that time led to the hypothesis

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that the most probable source of infection was a surface water reservoir known as Humpback Reservoir (Fig. 1). Although conclusive evidence was lacking, the authors speculated that faeces from domestic cats (*Felis catus*) or cougars (*F. concolor*) entered the reservoir or one of its feeder streams, resulting in contamination of the water supply with *T. gondii* oocysts [7, 8]. Humpback reservoir was decommissioned following the outbreak.

The objective of the present study was to assess the potential for contamination of the Victoria water supply with *T. gondii* oocysts. We evaluated the original hypothesis incriminating a water reservoir contaminated by cats or cougars as a possible cause of the Victoria toxoplasmosis outbreak, and assessed the present and future likelihood of waterborne transmission. Methods included the sampling of domestic cats and cougars, the only felids on Vancouver Island, and assessing the potential for water contamination with feline faeces. The latter was investigated by studying domestic cat and cougar activities in the area, and by conducting a serological survey of deer mice (*Peromyscus maniculatus*) inhabiting the riparian environments of the watershed for anti-*T. gondii* antibodies. Findings in this study demonstrated that *T. gondii* cycles among the wild and domestic animals frequenting the Victoria watershed.

MATERIALS AND METHODS

Assessing domestic cat and cougar activities in the Victoria watershed

From April 1996 to April 1997, the watershed for the city of Victoria was extensively surveyed for evidence of domestic cat and cougar activities. An experienced animal tracker (and Greater Victoria Water District employee) provided information with respect to cougar activity in the area and assisted with the tracking of cougars. All Greater Victoria Water District (GVWD) staff members were asked to record domestic cat and cougar sightings on a standardised form. A study of domestic cat demographics in the two municipalities bordering the Victoria watershed was undertaken by reviewing 1996 animal control data provided by the Victoria Society for the Prevention and Cruelty of Animals (SPCA) and the Capital Regional District (CRD) Animal Control Division. Domestic cat populations in these municipalities were estimated using published formulae [9] and were based on 1996 household data provided by the CRD Regional Planning Services.

Sampling cougars for anti-*T. gondii* immunoglobulin G (IgG) and *T. gondii* oocysts

Between April 1996 and April 1997, 16 cougars on Vancouver Island that were killed as a result of a predator control programme were necropsied within 12 h of death [10]. Provincial conservation officers estimated an age for each cougar based on animal size and teeth characteristics. At necropsy, faeces were collected from the rectum and colon, and blood was collected from the heart. Blood was centrifuged and sera removed and stored at −20 °C. Faeces (20–50 g) were preserved in 2% H_2SO_4 and stored at 4 °C.

Attempts were also made to collect cougar faeces from within the Victoria watershed. Cougar faeces were relatively difficult to locate because, like domestic cats, cougars routinely bury their faeces [11]. We observed that cougars occasionally defecated on roads of the watershed, facilitating collection. Cougar faeces were differentiated from other animal faeces, primarily wolf (*Canis lupus*), based on scat and animal track characteristics described by Murie [11]. Seven fresh (moist and not weathered) cougar faecal samples were collected from the roads of the watershed.

Sampling domestic cats for anti-*T. gondii* IgG and *T. gondii* oocysts

Fresh faeces of 23 stray domestic cats were opportunistically collected between January and April 1997 from an animal shelter operated by the Victoria SPCA. Faeces were also collected in the summer of 1996 from three cats that had accompanied owners to Goldstream Provincial Park. During 3 days in August, a campsite by campsite survey was conducted in the park, and patrons who were camping with their cats were requested to collect faeces into plastic bags that were collected the following day. Goldstream Provincial Park bordered the eastern boundary of the Goldstream watershed (Fig. 1). Sera were opportunistically collected from 73 cats brought to a veterinary clinic in the winter and spring of 1997. All cats were having blood drawn for either routine presurgical screening or for diagnostic purposes. The clinic was located approx. 5 km southeast of the Victoria watershed. Cat sera and faeces were handled in the same manner as were the cougar samples.

The results of anti-*T. gondii* IgG testing of 221 cats conducted by a private veterinary laboratory in British Columbia between January 1996 and April 1997 were reviewed. Sera were submitted by practising

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veterinarians from throughout British Columbia who had specifically requested *T. gondii* testing. The laboratory used a commercially available immunofluorescence assay (IFA) (VMRD, Inc., Pullman, Washington, USA) to test for anti-*T. gondii* IgG; titres of $\geqslant 16$ were considered positive.

Sampling deer mice for anti-*T. gondii* IgG

Deer mice (*Peromyscus maniculatus*) were live trapped using box-type Sherman traps (H. B. Sherman Traps Inc., Florida, USA). Nine trapping sites were established in the riparian environments of the Victoria watershed in a variety of habitat types (Fig. 1). Traps were baited with portions of oats, peanut butter, carrot and wiener. Trapping took place eight times throughout the year, once per month from April to August 1996, then once each in October 1996, January 1997, and March 1997. Each trapping session involved 40–60 traps divided among two to four trapping sites. Sessions lasted 2–3 days and traps were left open between trapping sessions. In total, 1227 trap nights took place between April 1996 and April 1997.

All deer mice were anaesthetized with methoxyflurane, individually identified by ear notching, orbitally bled, and released following recovery. Mice were only bled once per trapping session even if recaptured. Approximately 0·25 ml of blood was collected from each mouse. Following centrifugation, sera were decanted and stored at -20 °C.

Laboratory testing for anti-*T. gondii* IgG and *T. gondii* oocysts

Faecal and serum samples from all species sampled were sent for *T. gondii* examination to the United States Department of Agriculture Parasite Biology and Epidemiology Laboratory, Beltsville, MD, USA. Sera were examined for anti-*T. gondii* IgG with a modified direct agglutination test (MAT) using formalin fixed tachyzoites [12]. The MAT was chosen because of its proven high sensitivity and specificity. Desmonts and Remington [13] demonstrated the high sensitivity and specificity of the MAT compared to the Sabin–Feldman dye test (DT) with respect to the detection of IgG antibodies to *T. gondii* in humans. Numerous studies since then have shown that the MAT is most sensitive for detecting anti-*T. gondii* IgG in animals compared to several other available diagnostic tests (including the DT) [14–17]. In an extensive study of toxoplasmosis in pigs using the isolation of viable *T. gondii* from tissues as the definitive test, the MAT was 82·9% sensitive and 90·29% specific [17].

For the MAT, sera were diluted 1:25, 1:50 and 1:500. Results of $\geqslant 25$ were selected as the threshold titre for determining seropositive status based on experience with agglutination testing with human and animal sera [1]. Cougar and domestic cat faeces were tested for *T. gondii* oocysts by a sensitive and specific mouse bioassay technique, described elsewhere [1, 10].

Statistical analysis

Statistical analyses were done with the help of a microcomputer statistical software package (Epi Info, Version 6.03, Epidemiology Program Office, Center for Disease Control and Prevention, Atlanta, Georgia, USA). Exact binomial confidence intervals were calculated for anti-*T. gondii* IgG seroprevalences of cats and mice [18]. An uncorrected chi-square was used to compare mice seroprevalences among different trapping sites [19]. A Fisher's exact test was used to compare the proportion of *T. gondii* IFA seropositive cats from Victoria with those from the rest of the province as the expected frequency of seropositive cats from Victoria was less than five [18].

RESULTS

Victoria watershed description

The Victoria watershed was situated approximately 10 km northwest of the city of Victoria. The area consisted of two main watersheds that drained into a series of reservoirs (Fig. 1). Sooke Reservoir, situated in the 7100 ha Sooke watershed, provided Victoria's primary water supply. Four reservoirs in the 6600 ha Goldstream watershed acted as the city's backup water supply. The Victoria watershed was comprised of forest stands of various ages, wetlands, brush and meadows, suitable habitats for a number of wild animal species. The boundaries of the Victoria watershed encompassed the majority of the water catchment lands with the exception of three parcels of land that extended out from the Sooke watershed (Fig. 1).

Although much of the land bordering the Victoria watershed was forested, areas of residential development existed adjacent to the eastern boundary of the Goldstream watershed and along the northern reaches of the Sooke watershed (Fig. 1). Recreational

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Table 1. *Necropsied Vancouver Island cougar* (Felis concolor vancouverensis) *modified agglutination test anti*-T. gondii *immunoglobulin G titres and* T. gondii *oocyst shedding status*

Location of animal collection	Estimated age (years)	Sex	Antibody titre	Faecal oocyst shedding
North Island*				
Gold River	2·5	M	> 500	Neg
Gold River	0·5	M	< 25	Neg
Sayward	1·5	F	50	Neg
Mid Island†				
Courtenay	4	M	> 500	Neg
Courtenay	1·5	M	50	Pos
Nanaimo	2	M	50	Neg
Parksville	5	F	50	Neg
Parksville	1	M	50	Neg
Parksville	1	F	50	Neg
Port Alberni	5	M	50	Neg
Qualicum	2	M	< 25	Neg
Qualicum	2	F	> 500	Neg
South Island‡				
Ladysmith	0·8	M	50	Neg
Port Renfrew	1	F	50	Neg
Port Renfrew	1	M	50	Neg
Sooke§	2	F	> 500	Neg

* > 160 km from the Victoria watershed.
† 60–160 km from the Victoria watershed.
‡ < 60 km from the Victoria watershed.
§ Cougar killed within the Victoria watershed.

facilities, including campgrounds, abutted both the Sooke and Goldstream watersheds.

Domestic cat activities in the Victoria watershed

GVWD staff members recorded 10 domestic cat sightings during the study period. Eight sightings occurred near the northern reaches of Sooke Reservoir, one cat was sighted in the southern region of the Sooke watershed, and one cat was sighted near the water treatment plant in the Goldstream watershed (Fig. 1). We also observed three cats during a 3-day survey of Goldstream Provincial Park in the summer of 1996. When questioned, park caretakers reported that it was not uncommon for cats to accompany patrons to the park. They also reported that cats had occasionally gone missing from the campsite and feral cats lived in the area.

The numbers of domestic cats living in the two municipalities bordering the Victoria watershed (Langford and Highlands) in 1996 were estimated at 4678. During this same time, SPCA and CRD animal control records indicated that 123 stray cats from these municipalities had been impounded. Impounded stray cats represented 2·6% of the total estimated cat population.

Cougar activities in the Victoria watershed

GVWD staff members documented 11 cougar sightings during the study period. Six sightings occurred in the northern regions of the Sooke watershed, one in the southern half of the Sooke watershed, and four in the Goldstream watershed, including one near Humpback Reservoir. Reports included the sightings of larger males, smaller females and kittens of various ages.

We found what we believed to be seven fresh cougar faecal samples on roads of the watersheds, and old cougar faeces were observed adjacent to ditches and streams that eventually drained into the reservoirs. With the help of a GVWD employee (an experienced animal tracker) cougar tracks were observed along the roads in both the summer and winter throughout the watershed. In the winter, when snow covered the ground, many tracks were followed off the roads and into the forests. Based on the sizes of the prints, the tracker estimated that larger males, smaller females

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Table 2. *Victoria veterinary clinic domestic cat* (Felis catus) *modified agglutination test anti*-T. gondii *immunoglobulin G titres*

Titre	Number (% of total) Total $n = 73$	Average age (years)
< 25	57 (78·1%)	5·4
25	2 (2·7%)	8·0
50	3 (4·1%)	5·3
500	11 (15·1%)	9·8
Total seropositive	16 (21·9%; 95% CI: 13·4–33·4%)	8·7

Table 3. *Modified agglutination test anti*-T. gondii *immunoglobulin G seroprevalence of deer mice* (Peromyscus maniculatus) *living in riparian environments of the Victoria watershed*

Trapping site	Total tested	Positive titre (% of total)‡
Wilderness*		
2	10	1 (10·0%)
3	12	1 (25·0%)
4	43	3 (9·3%)
5	4	0
6	5	0
7	6	0
All	80	5 (6·3%; 95% CI: 2·1–14·0%)‖
Residential†		
1	5	2 (40·0%)
8	21	3§ (14·3%)
9	45	6 (13·3%)
All	71	11 (15·5%; 95% CI: 8·0–26·0%)‖
Residential and wild	151	16 (10·6%; 95% CI: 6·2–16·6%)

* Wilderness: farther than 3 km from residential property (Fig. 1).
† Residential: within 3 km of residential property (Fig. 1).
‡ All titres were 50 unless noted.
§ One mouse had a titre of 500.
‖ Chi-square test for homogeneity of wilderness and residential seroprevalences: $\chi^2 = 3{\cdot}39$; $P = 0{\cdot}066$.

and their kittens all frequented the area. He also suggested that the watershed was home to about six cougars on an annual basis, including one or two mothers with kittens.

Laboratory findings

The origin of each necropsied cougar, estimated age, sex, anti-*T. gondii* IgG titre and oocyst shedding status are listed in Table 1. Only one of the 16 cougars was from within the Victoria watershed. All cougars appeared in relatively good body condition, and many had partially digested portions of domestic animals in their stomach including goat, pig, goose and dog. The cougar shedding oocysts had faecal soiling around the rectum and tail and very loose watery colonic and rectal contents. Of seven suspected cougar faecal samples collected from within the watershed, one contained *T. gondii* oocysts. Quantitative analysis revealed that faeces from the necropsied cougar shedding *T. gondii* contained $2{\cdot}5 \times 10^6$ oocysts/g of faeces, and the positive faecal sample collected from within the Victoria watershed contained 1×10^4 oocysts/g of faeces [10].

All 26 domestic cat faecal samples collected from the animal shelter and from Goldstream Provincial Park were negative for *T. gondii* oocysts by mouse bioassay. Of the 73 sera collected from domestic cats visiting the nearby veterinary clinic and tested by MAT, 21·9% [95% CI: 13·4–33·4%] were seropositive for anti-*T. gondii* IgG (Table 2). The average

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age of seronegative cats was 5·4 years, and 8·7 years for seropositive cats.

A review of the private veterinary laboratory *Toxoplasma* testing records revealed that of the 221 sera tested by IFA, 17 cats originated from the Victoria area and 204 were from the rest of the province. Of the 17 cats from the Victoria area, 5 (29·4%) were positive for anti-*T. gondii* IgG, and of the remaining 204 cats, 45 (22·1%) were seropositive. These seroprevalences were not significantly different ($P = 0{\cdot}54$). The overall seroprevalence for anti-*T. gondii* IgG by IFA was 22·6% [95% CI: 17·3–28·7%].

During 1227 trap nights, 185 blood samples were collected from 151 deer mice. Of 17 mice captured more than once, 16 were negative for anti-*T. gondii* IgG by MAT and did not seroconvert on subsequent sampling. The remaining mouse was seropositive in June of 1996, then tested negative four times, including during the final trapping session in March of 1997. Mice were stratified into two groups: 'wilderness' (trapping sites farther than 3 km from residential property) and 'residential' (trapping sites within 3 km of residential property) (Fig. 1). Deer mouse MAT results are provided in Table 3.

DISCUSSION

Field observations together with laboratory analyses demonstrated that both cougars and domestic cats could act as sources of *T. gondii* oocysts in the Victoria watershed. Mouse serology suggested that *T. gondii* oocysts were being shed into the riparian environments or were carried there by insects or by water run-off. The above observations suggested the presence of an endemic *T. gondii* cycle among the animals of the Victoria watershed. Given that the method of water treatment used in Victoria (chloramination) was probably ineffective at removing viable oocysts from the water [8], it is possible that people consuming Victoria's water were exposed to *T. gondii* oocysts during the study period.

Our findings gave biological support to Bell and colleagues [7] hypothesis that incriminated domestic cats or cougars as probable sources of the Victoria waterborne toxoplasmosis outbreak in 1994/5. However, it is difficult to accurately predict the ongoing and future risks of water contamination with *T. gondii* oocysts because information is still lacking in several areas. More complete information on cougar and domestic cat demographics in the Victoria watershed are needed. The levels of oocysts shed by both cougars and cats frequenting the area are not known. Water samples are not routinely tested for *Toxoplasma* oocysts. Finally, accurate estimates of the incidence and risk factors of human toxoplasmosis in the communities served by the Victoria water supply are not available.

In this study, the *T. gondii* seroprevalence of domestic cats sampled from a veterinary clinic near the Victoria watershed (Table 2) and the seroprevalence of cats tested by a diagnostic laboratory in British Columbia, were both approx. 22%. Among the cats tested by the private laboratory, the seroprevalences of cats living in the Victoria area and cats throughout the rest of the province were not significantly different. As expected, the average age of seropositive cats by MAT was older than the average age of seronegative cats, and is likely attributable to the longer time available for exposure. Anti-*T. gondii* IgG seroprevalences among domestic cats reported here are similar to those reported in other surveys conducted across North America [1], suggesting that the domestic cat populations of Victoria and the rest of the province had a *T. gondii* infection prevalence similar to cats in other communities throughout North America.

Although antibody levels in cats sampled from Victoria demonstrated the presence of *T. gondii* infection in the local cat population, sampling did not necessarily indicate the infection level among cats that frequented the Victoria watershed. Ideally, we would have preferred to have tested stray and feral cats, as they are more representative of the cat population that could have visited the watershed. Unfortunately, SPCA regulations would not permit sampling of animals sheltered in their facility. A survey by Dubey [20] in Kansas City demonstrated a higher *T. gondii* seroprevalence in stray adult cats (57·9%) compared to domiciled adult cats (37·5%). This is expected as the diet of stray cats likely includes a larger proportion of rodents (common intermediate hosts of *T. gondii*) than that of domiciled cats.

Neither the *T. gondii* oocyst shedding status of domestic cats, nor the potential for future re-infection and oocyst shedding, can be confidently predicted by anti-*T. gondii* IgG titres. Dubey [21] demonstrated that seronegative domestic cats experimentally infected with *T. gondii* tissue cysts generally shed a total of $>$ 20 million oocysts over 5–14 days following initial infection. Anti-*T. gondii* IgG are detectable by MAT 10–14 days after exposure, and remain elevated

for at least 5 years [22]. Although it is generally believed that once cats have shed *T. gondii* oocysts they become immune to the re-shedding of oocysts [1], Dubey [21] observed the re-shedding of oocysts in 4 of 9 cats inoculated with *T. gondii* tissue cysts 77 months after primary infection, albeit in lower numbers than at primary infections. All four cats had detectable anti-*T. gondii* IgG titres prior to the re-feeding of *T. gondii* tissue cysts. Two of the four cats demonstrated an anamnestic antibody response and two did not. Similarly, while it is generally believed that when *T. gondii*-specific antibodies are detected in feline serum it is likely that the cat has already finished shedding oocysts [22], Ruiz and Frenkel [23] detected oocyst shedding in 20 of 109 seropositive cats from Costa Rica.

T. gondii oocysts were not found in the faeces of any of the 26 domestic cats tested in this study; however, given the small sample size, this was not unexpected. In previous studies of domestic urban and rural cats, it is reported that the proportion of domestic cats excreting oocysts at any one time is not high, usually being $< 2\%$ in most countries [1, 20, 24]. This figure can be used to estimate that, of approximately 4678 cats that lived in the two municipalities bordering the Victoria watershed in 1996, < 94 ($< 2\%$) of these cats were likely shedding *T. gondii* oocysts at any given time.

The high cougar seroprevalence of *T. gondii* infection, together with the observation that cougars do shed *T. gondii* oocysts, suggested that the cougar may serve as an important source of *T. gondii* on Vancouver Island and elsewhere. Fourteen of the 16 cougars necropsied had antibodies to *T. gondii* (Table 1). One of 16 necropsied cougars was shedding large numbers of oocysts at the time of necropsy, and cougar faeces collected from within the watershed also contained oocysts. Although only one necropsied cougar was killed within the Victoria watershed, a review of cougar home range size by Lindzey [25] suggests that cougars from anywhere on Vancouver Island could potentially migrate into the area. Tracking of 11 male and 2 female cougars on Vancouver Island demonstrated average home ranges of 137 km^2 and 55 km^2 for males and females respectively (personal communication, D. Janz, British Columbia Ministry of Environment).

Although the life expectancy of cougars is difficult to estimate, it has been suggested that the annual survival rate for cougars 1–13 years old is 88–95% [25]. Wooding [26] and Forsyth [27] reported approximate life spans for cougars of 8–15 years. A 19-year-old female cougar was found on Vancouver Island (personal communication, D. Janz, British Columbia Ministry of Environment). Given the mean estimated age of cougars sampled in this study (2 years) and the high seroprevalence of *T. gondii* (88%), it appears that most cougars on Vancouver Island are infected at a young age. If toxoplasmosis in cougars behaves as it does in domestic cats, with limited re-shedding of oocysts following subsequent exposure [1], young cougars represent the greatest risk to Victoria's water supply. However, Ruiz and Frenkel [23] suggested that wild cats may be adapted to *T. gondii* transmission and oocysts may be shed intermittently, similar to other feline parasites. If this is the case, all cougars, including seropositive adults, could pose a threat.

The documentation of cougar and domestic cat activities in the Victoria watershed demonstrated the potential for exposure of the water supply to cougar and cat faeces. Tracking of cougars showed that they travelled throughout the entire watershed, including the riparian environments. Cougars are plentiful on Vancouver Island and the island may be home to one of the highest cougar densities in North America at 1·9–7·3 cougars/100 km^2 (personal communication, D. Janz, British Columbia Ministry of Environment) [25]. Many domestic cats lived in the two municipalities bordering the Victoria watershed and additional cats accompanied their owners during visits to Goldstream Provincial Park. Although domestic cat faeces were not found in the watershed, defecation in the area probably occurred.

The serological survey of deer mice living in the riparian environments allowed us to investigate further the epidemiology of *T. gondii* in the Victoria watershed. Deer mice were selected because of their relative abundance, ease of capture, feeding habits and small home range. Most adults stay in the same general area throughout their lives and home ranges of *Peromyscus* spp. have been reported to vary in size from approx. 0·1–10 acres [28]. Few *Peromyscus* spp. survive for more than 12 months [28]. Deer mice are omnivores, feeding primarily on seeds, berries, nuts, green vegetation and invertebrates [28, 29]. Deer mice have also been shown experimentally to consume water and soil [29, 30]. The presence of anti-*T. gondii* IgG in deer mouse serum suggests *T. gondii* oocyst consumption either directly from the environment, or in association with an invertebrate transport host. Invertebrates, including earthworms and cockroaches,

can mechanically transport *T. gondii* oocysts and when consumed, can infect a warm-blooded animal [31, 32]. Deer mice do not appear to develop serious disease following infection with *T. gondii* [33], making then useful environmental sentinels for *T. gondii*.

The seroprevalence of *T. gondii* infection in deer mice in this study (10·6%) was higher than previously reported in North America. Brillhart and colleagues [34] found anti-*T. gondii* IgG in 3/56 (2·5%) deer mice by MAT in Kansas, USA. Dubey [33] found all of 99 deer mice negative by mouse bioassay in Montana. Smith and colleagues [35] found all 21 deer mice captured from Iowa, USA swine farms negative by MAT. Finally, Dubey and colleagues [24] found 3/61 (4·1%) deer mice from Illinois, USA swine farms seropositive by MAT. The seroprevalence among deer mice in the present study suggested higher levels of *T. gondii* oocysts in the riparian environments of the Victoria watershed compared to other areas in North America where deer mice have been sampled. The high density of cougars on Vancouver Island, a relatively high density of black-tailed deer (a preferred prey species of cougars) within the protected watersheds (personal observation), and the presence of domestic cats within the watershed, may have all contributed to the high deer mouse anti-*T. gondii* IgG seroprevalence.

Residential development within close proximity to Victoria's water supply may have increased the potential for *T. gondii* contamination. When mice were stratified into two groups depending on the distance of the trapping sites to nearby residential development (Fig. 1, Table 3), the anti-*T. gondii* IgG seroprevalences of mice captured in the three trapping sites closest to human development were higher. This higher seroprevalence could indicate that domestic cats and cougars are attracted to areas adjacent to residential property. Populations of domestic animals around residential centres, including cats, dogs, goats and fowl, may attract cougars. Cougars on Vancouver Island hunt a number of domestic animal species as illustrated by the stomach contents of the necropsied cougars.

Deer mouse serology, although useful in a comparative sense, must be interpreted with caution for two reasons. First, deer mice can act as intermediate hosts for *Hammondia hammondi*, a coccidia in which the definitive host is the cat [36]. Using the Sabin–Feldman dye test, it has been demonstrated that infection with *H. hammondia* can result in antibodies that cross-react to those of *T. gondii*, producing false-positive results [37]. Cross-reactivity using the MAT has not been investigated to our knowledge. Therefore, an anti-*T. gondii* IgG titre indicates infection with *T. gondii*, or potentially, *H. hammondi*, both parasites known to be shed only by cats. Thus, antibodies to either parasite suggest contamination of the environment with feline faeces. Second, repeated congenital infections with *T. gondii* in laboratory mice (*Mus musculus*), without re-exposure, has been demonstrated [38, 39]. However, the ability to serologically detect congenitally infected mice has been questioned [24, 40]. Jacobs [40] demonstrated congenital infections in laboratory mice without detectable antibodies, and Dubey and colleagues [24] found that of nine naturally infected rodents determined by bioassay, including one deer mouse, six had no detectable antibodies to *T. gondii* in their sera by MAT. We are not aware of any report of congenital transmission of *T. gondii* in deer mice.

The findings of this study suggested the presence of an endemic *T. gondii* cycle among the animals of the Victoria watershed. Domestic cats and cougars were observed throughout the watershed, including adjacent to Victoria's primary water reservoir. Serological evidence of *T. gondii* infection was demonstrated in the domestic cat population living in the Victoria area, and cougars were observed to shed *T. gondii* oocysts. Serological evidence of *T. gondii* infection in deer mice living in the riparian environments of the Victoria watershed suggested that *T. gondii* oocysts were being shed next to both Humpback Reservoir and Sooke Reservoir. Water contamination of Victoria's water supply with *T. gondii* oocysts was potentially occurring during the study period, and future waterborne toxoplasmosis outbreaks in this and other communities where domestic and wild cats frequent are possible. Routine sampling of untreated water for *T. gondii* oocysts with sensitive techniques, accurate surveillance for cases of human toxoplasmosis, and an ongoing assessment of water consumption as a risk factor in endemic human toxoplasmosis in the Victoria area would contribute to a better understanding of the public health significance of our findings.

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REFERENCES

1. Dubey JP, Beattie CP. Toxoplasmosis of animals and man. Boca Raton, FL: CRC Press, 1988: 220.
2. Guerina NG. Congenital infection with *Toxoplasma gondii*. Pediatr Ann 1994; **23**: 138–51.
3. Gellin BG, Soave R. Coccidian infections in AIDS – toxoplasmosis, cryptosporidiosis, and isosporiasis. Med Clin North Am 1992; **76**: 205–34.
4. Fish RH, Hoskins JC, Kline LB. Toxoplasmosis neuroretinitis. Ophthalmology 1993; **100**: 1177–82.
5. Hollins PJ, Hoffbrand BI, Haffajee IM. *Toxoplasma* encephalitis in a raw steak eater. Postgrad Med J 1972; **48**: 384–5.
6. Benenson MW, Takafuji ET, Lemon SM, Greenup RL, Sulzer AJ. Oocyst-transmitted toxoplasmosis associated with ingestion of contaminated water. N Engl J Med 1982; **307**: 666–9.
7. Bell A, Gill R, Isaac-Renton J, et al. Outbreak of toxoplasmosis associated with municipal drinking water – British Columbia. Can Commun Dis Rep 1995; 21–18: 161–4.
8. Bowie WR, King AS, Werker DH, et al. Outbreak of toxoplasmosis associated with municipal drinking water. Lancet 1997; **350**: 173–7.
9. Gehrke BC. Results of the AVMA survey of US pet-owning households on companion animal ownership. J Am Vet Med Assoc 1997; **211**: 169–70.
10. Aramini JJ, Stephen C, Dubey JP. *Toxoplasma gondii* in Vancouver Island cougars (*Felis concolor vancouverensis*); serology and oocyst shedding. J Parasitol 1998; **84**: 438–40.
11. Murie OJ. A field guide to animal tracks. Boston, MA: Houghton Mifflin Company, 1982: 375.
12. Dubey JP, Desmonts G. Serological responses of equids fed *Toxoplasma gondii* oocysts. Equine Vet J 1987; **19**: 337–9.
13. Desmonts G, Remington JS. Direct agglutination test for diagnosis of *Toxoplasma* infection: method for increasing sensitivity and specificity. J Clin Microbiol 1980; **11**: 562–8.
14. Dubey JP, Desmonts G, Antunes F, McDonald C. Serologic diagnosis of toxoplasmosis in experimentally infected pregnant goats and transplacentally infected kids. Am J Vet Res 1985; **46**: 1137–40.
15. Dubey JP, Desmonts G, McDonald C, Wallis KW. Serologic evaluation of cattle inoculated with *Toxoplasma gondii*: comparison of Sabin-Feldman dye test and other agglutination tests. Am J Vet Res 1985; **46**: 1085–8.
16. Dubey JP, Thulliez P. Serologic diagnosis of toxoplasmosis in cats fed *Toxoplasma gondii* tissue cysts. J Am Vet Med Assoc 1989; **194**: 1297–9.
17. Dubey JP, Thulliez P, Weigel RM, et al. Sensitivity and specificity of various serologic tests for detection of *Toxoplasma gondii* infection in naturally infected sows. Am J Vet Res 1995; **56**: 1030–6.
18. Rosner B. Fundamentals of biostatistics, 3rd ed. Boston, MA: PWS-Kent Pub. Co., 1990: 655.
19. Hennekens CH, Buring JE, Mayrent SL. Epidemiology in medicine. Boston, MA: Little, Brown and Company, 1987: 383.
20. Dubey JP. Feline toxoplasmosis and coccidiosis: a survey of domiciled and stray cats. J Am Vet Med Assoc 1973; **162**: 873–7.
21. Dubey JP. Duration of immunity to shedding of *Toxoplasma gondii* oocysts by cats. J Parasitol 1995; **81**: 410–5.
22. Dubey JP, Lappin MR, Thulliez P. Long-term antibody responses of cats fed *Toxoplasma gondii* tissue cysts. J Parasitol 1995; **81**: 887–93.
23. Ruiz A, Frenkel JK. *Toxoplasma gondii* in Costa Rican cats. Am J Trop Med Hyg 1980; **29**: 1150–60.
24. Dubey JP, Weigel RM, Siegel AM, et al. Sources and reservoirs of *Toxoplasma gondii* infection on 47 swine farms in Illinois. J Parasitol 1995; **81**: 723–9.
25. Lindzey F. Mountain lion. In: Novak M, Baker JA, Obbard ME, Malloch B, eds. Wild furbearer management and conservation in North America. Ontario: Ontario Ministry of Natural Resources, 1987: 657–68.
26. Wooding FH. Wild mammals of Canada. Toronto, Ontario: McCraw-Hill Ryerson, 1982: 272.
27. Forsyth A. Mammals of the Canadian wild. Candem East, Ontario: Candem House Publishing Ltd., 1985: 351.
28. King JA, ed. Biology of *Peromyscus* (Rodentia). Stillwater, OK: The American Society of Mammalogists, 1968: 593.
29. Deavers DR, Hudson JW. Water metabolism and estimated field water budgets in two rodents (*Clethrionomys gapperi* and *Peromyscus leucopus*) and an insectivore (*Blarina brevicauda*) inhabiting the same mesic environment. Physiol Zoology 1979; **52**: 137–52.
30. Beyer WN, Connor EE, Gerould S. Estimates of soil ingestion by wildlife. J Wildlife Man 1984; **58**: 375–82.
31. Wallace GD. Intermediate and transport hosts in the natural history of *Toxoplasma gondii*. Am J Trop Med Hyg 1973; **22**: 456–63.
32. Ruiz A, Frenkel JK. Intermediate and transport hosts of *Toxoplasma gondii* in Costa Rica. Am J Trop Med Hyg 1980; **29**: 1161–6.
33. Dubey JP. *Toxoplasma gondii* infection in rodents and insectivores from Montana. J Wildl Dis 1983; **19**: 149–50.
34. Brillhart DB, Fox LB, Dubey JP, Upton SJ. Seroprevalence of *Toxoplasma gondii* in wild mammals in Kansas. J Helminth Soc Washington 1994; **61**: 117–21.
35. Smith KE, Zimmerman JJ, Patton S, Beran GW, Hill

HT. The epidemiology of toxoplasmosis on Iowa swine farms with an emphasis on the roles of free-living mammals. Vet Parasitol 1992; **42**: 199–211.
36. Frenkel JK, Dubey JP. *Hammondia hammondi*: a new coccidium of cats producing cysts in muscle of other mammals. Science 1975; **189**: 222–4.
37. Christie E, Dubey JP. Cross-immunity between *Hammondia* and *Toxoplasma* infections in mice and hamsters. Infect Immun 1977; **18**: 412–5.
38. Beverely JKA. Congenital transmission of toxoplasmosis through successive generations of mice. Nature 1959; **183**: 1348–9.
39. Remington JS, Jacobs L, Melton ML. Congenital transmission of toxoplasmosis from mother animals with acute and chronic infections. J Infect Dis 1961; **108**: 163–73.
40. Jacobs L. The occurrence of *Toxoplasma* infection in the absence of demonstrable antibodies. Proceedings of the First International Congress of Parasitology 1964; **1**: 176–7.