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Case-control studies of sporadic cryptosporidiosis in Melbourne and Adelaide, Australia

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SUMMARY

Few studies have assessed risk factors for sporadic cryptosporidiosis in industrialized countries, even though it may be numerically more common than outbreaks of disease. We carried out case-control studies assessing risk factors for sporadic disease in Melbourne and Adelaide, which have water supplies from different ends of the raw water spectrum. In addition to examining drinking water, we assessed several other exposures. 201 cases and 795 controls were recruited for Melbourne and 134 cases and 536 controls were recruited for Adelaide. Risk factors were similar for the two cities, with swimming in public pools and contact with a person with diarrhoea being most important. The consumption of plain tap water was not found to be associated with disease. This study emphasizes the need for regular public health messages to the public and swimming pool managers in an attempt to prevent sporadic cryptosporidiosis, as well as outbreaks of disease.

INTRODUCTION

Outbreaks of cryptosporidiosis have been studied extensively. The largest reported outbreak occurred in Milwaukee in 1993, when a drinking water supply contaminated by *Cryptosporidium* was estimated to have infected 403000 people [1]. Because of drinking waters' propensity to rapidly infect large numbers of people the primary focus of international and Australian research to date has been the cause of outbreaks of cryptosporidiosis rather than factors contributing to sporadic disease. Sporadic disease however, may be numerically more common than outbreaks. A number of epidemiological studies suggest that the incidence of gastroenteritis in developed nations is around 0·7 episodes per person per year [2–6]. On this basis it can be estimated that in the United States during 1993 waterborne gastroenteritis from recognized outbreaks accounted for less than 0·25% of all gastroenteritis, despite the occurrence of over 400000 cases of illness from the Milwaukee *Cryptosporidium* outbreak [7]. It is important to establish risk factors for sporadic cryptosporidiosis and examining the role of drinking water as a risk factor for sporadic disease is considered particularly important.

We aimed to examine risk factors for sporadic cryptosporidiosis amongst the general communities of

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both Melbourne and Adelaide, Australia. These two cities were chosen because they represent two extremes in raw water quality and treatment in Australia. Melbourne has high quality source water from highly protected catchments which undergoes only minimal treatment with chlorination [8], whilst Adelaide has poor quality source water from unprotected catchments which undergoes full conventional water treatment including coagulation, sedimentation, filtration and chlorination [9]. A case-control study design was used to examine risk factors for sporadic cryptosporidiosis.

METHODS

Case-control studies were conducted in Melbourne from June 1998 to May 2001 and in Adelaide from November 1998 to May 2001. Ethical approval was obtained from the local institutional review committees. Participants were recruited from the general communities of both cities. Controls were directly matched to cases according to age and sex, with four controls matched to each case.

We defined a case as having *Cryptosporidium* oocysts detected in a faecal specimen by an accredited pathology laboratory, the onset of any diarrhoea or vomiting within 8 weeks before the administration of questionnaire, residence in a household with a fixed telephone connection and the ability to speak English. Participating pathology laboratories sent reports of newly diagnosed cases of cryptosporidiosis to the Departments of Human Services in Victoria and South Australia and these were forwarded to the Department of Epidemiology and Preventive Medicine (DEPM). With the permission of the case's attending doctor, cases were recruited by telephone calls. A maximum of nine telephone calls were made to recruit individual cases. On contacting the case, or their main care-giver for children less than 12 years old, the interviewer requested their consent to participate. If consent was given, the computer assisted telephone questionnaire (CATQ) was administered immediately or at a mutually convenient time.

We defined a control as not having diarrhoea or vomiting in the 2 weeks before the onset of the matching case's illness, residence in a household with a fixed telephone connection and the ability to speak English. Controls were matched to cases by sex and the following age brackets: < 1 year old, $1-< 5$ years old, $5-< 12$ years old, $12-< 18$ years old, $18-< 40$ years old, $40-< 65$ years old and $\geqslant 65$ years old. Telephone sampling using the Electronic White Pages (EWP) residential telephone directory was used to recruit controls [10]. Prior to contacting a randomly generated telephone number an introductory letter was first sent to the household to improve participation rates [11]. Approximately 2 weeks after the introductory letter, telephone calls to these numbers were then made, and a database of willing potential controls was created in advance of cases over the course of the study.

After a case was recruited and had the CATQ administered, random potential controls of the same sex and age bracket were automatically generated from this database. If a suitably matched control was available the CATQ was administered, but if not then a new randomly selected potential control was generated until a control was eventually recruited. Four matched controls were interviewed for each case. We attempted to administer the control questionnaires within 3 working days of a case questionnaire. Only one control was recruited from each household for each case, and the control was then retained in the database of potential controls for possible selection at a later time. Controls were retained to prevent possible selection bias by their exclusion from the database [12–14].

The CATQ inquired into demographic information, clinical details of the case's illness, education level, employment, the consumption of tap water, the consumption of particular food groups, recreational water activities, the presence of immunological impairment, the consumption of regular medication, contact with persons who may pose a risk of cryptosporidiosis, animal contact, rural or overseas travel and exposure to child-care or breast feeding.

To isolate apparently sporadic cases, clusters of cases (and their matched controls) were removed from the database for analysis. The removal of clusters was done by firstly identifying the symptom onset date of primary cases and then removing subsequent cases who had their symptom onset within a 14 day period if they either: resided within the same household, attended the same swimming pool or attended the same childcare centre.

Data analysis

The power of the study was calculated using drinking water as the most important exposure to be assessed.

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For Melbourne with 201 cases and 795 controls the study had 80% power, at 2-sided 5% significance level, to detect a trend in odds ratio (OR) of 1·22 per quartile increase in the exposure [15]. For Adelaide with 134 cases and 536 controls the study had 80% power, at 2-sided 5% significance level, to detect a trend in OR of 1·28 per quartile increase in the exposure.

Detectable OR (with 80% power) for binary exposures in the Melbourne study ranged from 1·6 for an exposure with 50% prevalence among controls to 1·9 for 10% prevalence. The corresponding detectable OR for Adelaide were 1·8 for 50% prevalence to 2·2 for 10% prevalence.

Data were analysed using Stata [16]. The standard analysis method for matched case-control studies is conditional logistic regression when the matched sets are independent. However, in this study controls from the same household could be selected at multiple times for different cases, thereby inducing a correlation in the exposures across different matched sets, which needed to be accounted for in the analysis. Standard conditional logistic regression was formulated as a stratified Cox proportional hazard regression mode, with the matched sets as strata [17]. This allowed an extension to accommodate the clustering effect of individuals from the same household using the 'cluster' option in the 'stcox' command in Stata. In this way the Cox proportional hazards model was used to calculate crude and adjusted odds ratios for the various exposures examined, together with 95% confidence intervals (CI). Linear trend per unit being measured (e.g. per glass of water consumed) or per category being measured (e.g. per two glass categories of water consumed) was also calculated, together with 95% CI. Linear trend was used to evaluate a dose–response relationship when this was relevant.

All exposures could not be adjusted for, because many were highly correlated, making such an analysis numerically unstable. Because of the importance of examining plain drinking water as a risk factor for sporadic cryptosporidiosis, this exposure was the main focus of the adjusted analysis. Individual confounding was sought by adjusting plain water against each exposure separately. Biologically plausible associations between exposures and plain drinking water, and exposures and cryptosporidiosis were considered, particularly those that were statistically significant. Where possible there needed to be sufficient numbers of participants in the exposed group to achieve numerical stability. Finally, when a number of related exposures were examined from the same group the most important single exposure based on published evidence was chosen.

RESULTS

Melbourne

There were 271 cases reported in Melbourne during the study period. Of those, 201 (74·2%) had the CATQ administered and were included in the analysis (Table 1). Twenty-four (8·9%) had the CATQ administered but were retrospectively excluded from the analysis because they were found to form disease clusters, 14 (5·2%) had the CATQ administered but were retrospectively excluded because it was administered more than 8 weeks after their symptom onset, 7 (2·6%) cases refused to participate, 4 (1·5%) doctors refused permission, 11 (4·1%) where case notification to the DEPM failed, 3 (1·1%) cases were unavailable, 1 (0·4%) did not have a fixed telephone connection, 1 (0·4%) had an incorrect telephone number recorded and 5 (1·8%) could not be contacted within 8 weeks of symptom onset.

Of the residential telephone numbers from which the control sample was generated, letters were sent to 5691 households. Of these, 3191 were contacted by telephone and were eligible to participate in the study, with 2177 (68·2%) agreeing to participate. Of those households agreeing to participate, 4322 individual potential controls were recruited for the database, with 795 CATQ administered to controls and included in the analysis, with 90 controls having conducted more than one CATQ. For the 2500 households that were either not contactable or ineligible to participate, 54 (0·9%) letters were returned as the address was incomplete, 230 (4·0%) had the maximum of 9 telephone calls exhausted without contact, 36 (1·0%) were ineligible because of a household member with a chronic gastrointestinal illness, 302 (5·3%) had insufficient English, 968 (17·0%) had an incorrect phone number recorded, 126 (2·2%) returned the letter as there was a new resident, 773 (13·6%) had not had all 9 calls exhausted but calling was ceased because sufficient numbers had been recruited and 11 (0·2%) were unavailable.

The median age for recruited cases was 11 years (range 0–81 years) and for controls 11 years (range 0–83 years). One hundred and seven cases (53·2%) were male (Table 1).

Cryptosporidiosis was not associated with the consumption of plain tap water. Neither the crude nor

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Table 1. *Crude analysis with 95% confidence intervals (CI)*

	Melbourne			Adelaide		
	Cases	Controls	Crude OR*, 95% CI	Cases	Controls	Crude OR*, 95% CI
Number	201	795		134	536	
Median age in years (range)	11 (0–81)	11 (0–83)		10 (0–83)	11 (0–86)	
Male	107 (53·2%)	428 (53·8%)		67 (50%)	268 (50%)	
Plain water						
No	37 (18·4%)	177 (22·3%)	1·0	52 (38·8%)	218 (40·7%)	1·0
Yes	164 (81·6%)	618 (77·7%)	1·3 (0·9–1·9)	82 (61·2%)	318 (59·3%)	1·1 (0·7–1·6)
Plain water (glasses/day) (four categories)						
None	37 (18·4%)	177 (22·3%)	1·0	52 (38·8%)	218 (40·7%)	1·0
0·01–2·00	64 (31·8%)	225 (28·3%)	1·4 (0·9–2·1)	32 (23·9%)	117 (21·8%)	1·2 (0·7–1·8)
2·01–4·00	42 (20·9%)	216 (27·2%)	0·9 (0·6–1·5)	29 (21·6%)	98 (18·3%)	1·2 (0·8–2·0)
> 4·00	58 (28·9%)	177 (22·3%)	1·6 (1·0–2·5)	21 (15·7%)	103 (19·2%)	0·9 (0·5–1·5)
Linear trend per one glass increase			1·05 (0·99–1·11) $P = 0·10$			0·98 (0·92–1·04) $P = 0·47$
Linear trend per one category increase (four categories)			1·11 (0·96–1·28) $P = 0·15$			0·98 (0·84–1·15) $P = 0·85$
Education level						
0–12 years or schooling	33 (16·5%)	125 (15·7%)	1·0	27 (20·2%)	118 (22·0%)	1·0
Trade qualification	61 (30·5%)	304 (38·2%)	0·7 (0·5–1·2)	51 (38·1%)	186 (34·7%)	1·2 (0·7–2·0)
University	106 (53·0%)	366 (46·0%)	1·1 (0·7–1·7)	56 (41·8%)	232 (43·3%)	1·1 (0·7–1·7)
Work with sewage						
No	94 (94·0%)	360 (91·6%)	1·0	59 (93·7%)	237 (94·1%)	1·0
Yes	6 (6·0%)	33 (8·4%)	0·7 (0·3–1·6)	4 (6·4%)	15 (6·0%)	1·1 (0·4–3·0)
Work in child-care						
No	93 (93·0%)	375 (95·4%)	1·0	59 (93·7%)	241 (95·6%)	1·0
Yes	7 (7·0%)	18 (4·6%)	1·5 (0·7–3·5)	4 (6·4%)	11 (4·4%)	1·6 (0·6–4·3)
Work with animals						
No	95 (95·0%)	377 (95·9%)	1·0	58 (92·1%)	229 (90·9%)	1·0
Yes	5 (5·0%)	16 (4·1%)	1·2 (0·5–2·9)	5 (8·0%)	23 (9·1%)	0·9 (0·4–2·1)
Uncooked carrots						
No	88 (43·8%)	270 (34·0%)	1·0	55 (41·1%)	166 (31·0%)	1·0
Yes	113 (56·2%)	525 (66·0%)	**0·6 (0·5–0·9)**†	79 (59·0%)	370 (69·0%)	**0·6 (0·4–0·9)**†
Uncooked carrots						
None	88 (43·8%)	270 (34·0%)	1·0	55 (41·0%)	166 (31·0%)	1·0
Monthly	26 (12·9%)	110 (13·8%)	0·7 (0·4–1·1)	18 (13·4%)	88 (16·4%)	**0·6 (0·3–1·0)**†
Weekly	55 (27·4%)	279 (35·1%)	**0·6 (0·4–0·8)**†	44 (32·9%)	199 (37·1%)	**0·6 (0·4–1·0)**†
Daily	32 (15·9%)	136 (17·1%)	0·7 (0·4–1·1)	17 (12·7%)	83 (15·5%)	0·6 (0·3–1·0)
Linear trend per one category increase			**0·85 (0·73–0·98)**† **$P = 0·02$**			**0·82 (0·70–0·99)**† **$P = 0·03$**

Unpasteurized milk products						
No	194 (97·5%)	784 (98·7%)	1·0	130 (97·0%)	531 (99%)	1·0
Yes	5 (2·5%)	10 (1·3%)	2·0 (0·7–5·5)	4 (3·0%)	4 (0·8%)	**3·9 (1·2–12·4)**†
Swimming in public pool						
No	103 (51·2%)	550 (69·2%)	1·0	97 (72·4%)	411 (76·7%)	1·0
Yes	98 (48·8%)	245 (30·8%)	**2·5 (1·8–3·4)**†	37 (27·6%)	125 (23·3%)	1·3 (0·9–2·0)
Swimming in public toddlers' pool						
No	163 (81·1%)	710 (89·3%)	1·0	122 (91·0%)	509 (95·0%)	1·0
Yes	38 (18·9%)	85 (10·7%)	**2·3 (1·5–3·4)**†	12 (9·0%)	27 (5·0%)	**1·9 (1·0–3·6)**†
Separate days in incubation period using public toddlers' pool						
No days	163 (81·1%)	710 (89·3%)	1·0	122 (91·0%)	509 (95·0%)	1·0
1 day	15 (7·5%)	33 (4·2%)	**2·3 (1·3–4·1)**†	2 (1·5%)	10 (1·9%)	0·9 (0·2–3·4)
> 1 day	23 (11·4%)	52 (6·5%)	**2·2 (1·4–3·7)**†	10 (7·5%)	17 (3·2%)	**2·7 (1·3–5·4)**†
Linear trend per 1 day increase			**1·18 (1·08–1·30)**†			**1·24 (1·05–1·45)**†
			***P* = 0·0001**			***P* = 0·019**
Swimming in public adults' pool						
No	109 (54·2%)	574 (72·2%)	1·0	99 (73·9%)	421 (78·5%)	1·0
Yes	92 (45·8%)	221 (27·8%)	**2·5 (1·8–3·4)**†	35 (26·1%)	115 (21·5%)	1·3 (0·9–2·1)
Separate days in incubation period using public adults' pool						
No days	109 (54·2%)	574 (72·2%)	1·0	99 (73·9%)	421 (78·5%)	1·0
1 day	26 (12·9%)	56 (7·0%)	**2·7 (1·7–4·3)**†	7 (5·2%)	27 (5·0%)	1·1 (0·5–2·5)
2 days	25 (12·4%)	101 (12·7%)	1·4 (0·9–2·3)	13 (9·7%)	49 (9·1%)	1·2 (0·6–2·1)
> 2 days	41 (20·4%)	64 (8·1%)	**3·7 (2·5–5·5)**†	15 (11·2%)	39 (7·3%)	1·8 (0·9–3·3)
Linear trend per 1 day increase			**1·18 (1·12–1·24)**†			**1·12 (1·04–1·22)**†
			***P* = 0·0001**			***P* = 0·004**
Immune system illness						
No	193 (96·0%)	792 (99·6%)	1·0	126 (94·0%)	530 (99·1%)	1·0
Yes	8 (4·0%)	3 (0·4%)	**14·0 (3·5–55·2)**†	8 (6·0%)	5 (0·9%)	**7·5 (2·7–20·8)**†
Children at home in nappies						
No	139 (69·2%)	620 (78·0%)	1·0	91 (68·4%)	436 (81·3%)	1·0
Yes	62 (30·9%)	175 (22·0%)	**1·6 (1·2–2·3)**†	42 (31·6%)	100 (18·7%)	**2·2 (1·4–3·2)**†
Children < 6 yr at home with diarrhoea						
No	169 (84·5%)	769 (97·2%)	1·0	112 (84·9%)	522 (97·8%)	1·0
Yes	31 (15·5%)	22 (2·8%)	**7·3 (4·2–12·5)**†	20 (15·2%)	12 (2·3%)	**7·1 (3·8–13·3)**†
Children < 6 yr at home attending childcare						
No	152 (75·6%)	691 (87·1%)	1·0	105 (78·4%)	476 (89·0%)	1·0
Yes	49 (24·4%)	102 (12·9%)	**2·2 (1·6–3·1)**†	29 (21·6%)	59 (11·0%)	**2·4 (1·5–3·8)**†
Persons > 5 yr at home with diarrhoea						
No	169 (84·5%)	726 (91·7%)	1·0	103 (76·9%)	491 (91·6%)	1·0
Yes	31 (15·5%)	66 (8·3%)	**2·1 (1·4–3·2)**†	31 (23·1%)	45 (8·4%)	**3·4 (2·2–5·3)**†
Animal contact at home						
No	101 (50·3%)	311 (39·1%)	1·0	54 (40·3%)	162 (30·2%)	1·0
Yes	100 (49·8%)	484 (60·9%)	**0·6 (0·4–0·8)**†	80 (59·7%)	374 (69·8%)	**0·6 (0·4–0·9)**†

[*continued overleaf*]

Table 1 (*cont.*)

	Melbourne			Adelaide		
	Cases	Controls	Crude OR*, 95% CI	Cases	Controls	Crude OR*, 95% CI
Dog contact at home						
No	128 (63·7%)	445 (56·0%)	1·0	75 (56·0%)	259 (48·3%)	1·0
Yes	73 (36·3%)	350 (44·0%)	**0·7 (0·5–1·0)**†	59 (44·0%)	277 (51·7%)	0·7 (0·5–1·1)
Cat contact at home						
No	161 (80·1%)	587 (73·8%)	1·0	97 (72·4%)	347 (64·7%)	1·0
Yes	40 (19·9%)	208 (26·2%)	**0·7 (0·5–1·0)**†	37 (27·6%)	189 (35·3%)	0·7 (0·5–1·0)
Animal contact away from home, e.g. pet stores, farms, zoos						
No	99 (49·3%)	359 (45·2%)	1·0	65 (48·5%)	197 (36·8%)	1·0
Yes	102 (50·8%)	435 (54·8%)	0·8 (0·6–1·1)	69 (51·5%)	338 (63·2%)	**0·6 (0·4–0·9)**†
Dog contact away from home						
No	131 (65·2%)	420 (52·8%)	1·0	83 (61·9%)	245 (45·7%)	1·0
Yes	70 (34·8%)	375 (47·2%)	**0·6 (0·4–0·8)**†	51 (38·1%)	291 (54·3%)	**0·5 (0·4–0·7)**†
Cat contact away from home						
No	160 (79·6%)	622 (78·4%)	1·0	117 (87·3%)	398 (74·3%)	1·0
Yes	41 (20·4%)	171 (21·6%)	0·9 (0·7–1·3)	17 (12·7%)	138 (25·8%)	**0·4 (0·2–0·7)**†
Calf contact away from home						
No	184 (91·5%)	770 (97·0%)	1·0	129 (96·3%)	528 (98·5%)	1·0
Yes	17 (8·5%)	24 (3·0%)	**3·1 (1·7–5·4)**†	5 (3·7%)	8 (1·5%)	2·5 (1·0–6·4)
Lamb contact away from home						
No	191 (95·0%)	776 (97·7%)	1·0	132 (98·5%)	521 (97·2%)	1·0
Yes	10 (5·0%)	18 (2·3%)	**2·3 (1·2–4·6)**†	2 (1·5%)	15 (2·8%)	0·5 (0·1–2·1)
Drink unboiled water from a country river, lake or dam within Australia						
No	182 (90·6%)	749 (94·3%)	1·0	123 (91·8%)	517 (96·5%)	1·0
Yes	19 (9·5%)	45 (5·7%)	**1·8 (1·1–3·0)**†	11 (8·2%)	19 (3·5%)	**2·4 (1·2–4·6)**†
Travel overseas						
No	174 (86·6%)	781 (98·2%)	1·0	127 (94·8%)	531 (99·1%)	1·0
Yes	27 (13·4%)	14 (1·8%)	**8·6 (4·8–15·6)**†	7 (5·2%)	5 (0·9%)	**5·6 (2·1–14·6)**†
Consume unboiled water, ice cubes or salad overseas						
No	174 (86·6%)	785 (98·7%)	1·0	130 (97·0%)	534 (99·6%)	1·0
Yes	27 (13·4%)	10 (1·3%)	**12·9 (6·4–26·1)**†	4 (3·0%)	2 (0·4%)	**8·0 (1·9–33·9)**†
Participant breast fed if < 3 years old						
No	41 (91·1%)	99 (86·1%)	1·0	22 (100·0%)	67 (89·3%)	1·0
Yes	4 (8·9%)	16 (13·9%)	0·6 (0·2–2·1)	0 (0%)	8 (10·7%)	–
Participant in child-care if < 6 years old						
No	35 (54·7%)	127 (48·5%)	1·0	18 (45·0%)	82 (51·6%)	1·0
Yes	29 (45·3%)	135 (51·5%)	0·7 (0·4–1·4)	22 (55·0%)	77 (48·4%)	1·4 (0·7–3·0)

* Adjusted for matching factors (age, sex) and family clustering.

† Bold type indicates statistically significant at $P \leqslant 0{\cdot}05$ level.

Table 2. *Adjusted analysis with 95% CI**

	Melbourne	Adelaide
Plain water		
No	1·0	1·0
Yes	1·3 (0·9–2·1)	1·0 (0·7–1·6)
Pain water (glasses/day) (four categories)		
None	1·0	1·0
0·01–2·00	1·4 (0·9–2·4)	1·1 (0·7–2·0)
2·01–4·00	1·0 (0·6–1·7)	1·2 (0·7–2·0)
> 4·00	1·5 (0·9–2·6)	0·8 (0·4–1·3)
Linear trend per one glass increase	1·04 (0·98–1·10)	0·96 (0·90–1·02)
	$P = 0·25$	$P = 0·21$
Linear trend per one category increase (four categories)	1·10 (0·95–1·28)	0·95 (0·80–1·12
	$P = 0·21$	$P = 0·52$
Educational level		
0–12 years or schooling	1·0	1·0
Trade qualification	0·7 (0·4–1·3)	1·6 (0·9–2·9)
University	1·0 (0·6–1·6)	1·2 (0·7–2·1)
Uncooked carrots		
No	1·0	1·0
Yes	**0·6 (0·4–0·9)**†	**0·6 (0·4–0·9)**†
Swimming in public pool		
No	1·0	1·0
Yes	**2·7 (1·9–3·8)**†	1·2 (0·8–1·9)
Children < 6 yr at home with diarrhoea		
No	1·0	1·0
Yes	**7·4 (4·0–13·8)**†	**8·6 (4·8–15·6)**†
Persons > 5 yr at home with diarrhoea		
No	1·0	1·0
Yes	**1·8 (1·1–2·9)**†	**3·7 (2·2–6·2)**†
Animal contact at home		
No	1·0	1·0
Yes	**0·6 (0·4–0·8)**†	**0·6 (0·4–0·9)**†
Calf contact away from home		
No	1·0	1·0
Yes	**2·9 (1·5–5·7)**†	**5·1 (1·5–17·3)**†
Drink unboiled water from a country river, lake or dam within Australia		
No	1·0	1·0
Yes	1·5 (0·8–2·7)	**3·1 (1·5–6·5)**†

* Adjusted for matching factors (age, sex), family clustering, plain drinking water (as dichotomous variable).
† Bold type indicates statistically significant at $P \leqslant 0·05$ level.

adjusted OR for drinking any plain tap water compared to no plain tap water was statistically significant. Similarly, there was no significant dose–response relationship using a linear trend (Table 1).

For the crude analysis, statistically significant risk factors associated with sporadic cryptosporidiosis included swimming in public pools (both toddler's and adult's), immune system impairment, having children at home in nappies, having contact with children or adults with diarrhoea and having children at home attending childcare. Other risk factors included calf and lamb contact away from home, the consumption of unboiled water from a river, lake or dam within rural Australia, overseas travel and the consumption of unboiled water, ice-cubes or salad overseas. Statistically significant protective factors included the consumption of uncooked carrots, contact with any animal at home, dog contact at and away from home, and cat contact at home.

After performing an adjusted analysis on a limited number of exposures (Table 2), swimming in public pools, contact with persons with diarrhoea, and calf contact away from home all remained statistically significant risk factors, while the consumption of unboiled rural water from a river, lake or dam within Australia was no longer significant. Contact with any

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Table 3. *Clinical features of cases*

Clinical feature	Melbourne	Adelaide
Duration of illness (days)		
Median (range)	21 (1–100)	15 (2–120)
Mean (range)	22·4 (1–100)	19·4 (2–120)
Hospital admission for ⩾ one nights	14 (7·0%)	16 (11·9%)
Median number of nights (range)	2 (1–6)	2 (1–4)
Visits to the doctor		
Median number of total visits (range)	2 (0–16)	2 (0–8)
Median number of visits before the visit at which the faecal specimen was collected (range)	1 (1–5)	1 (1–6)
Symptoms		
Diarrhoea present	198 (98·5%)	132 (98·5%)
Median stools per day when worst (range)	9·5 (1–40)	7 (1–40)
Mean stools per day when worst (range)	11·3 (1–40)	10·3 (1–40)
Watery motions	195 (98·5%)	129 (96·3%)
Bloody motions	17 (8·5%)	8 (6·0%)
Medication to stop diarrhoea	62 (31·3%)	31 (23·5%)
Vomiting	111 (55·2%)	95 (70·9%)
Abdominal pain	182 (90·5%)	121 (91·7%)
Felt hot	81 (40·3%)	51 (38·1%)
Time lost from study or work if participant 6 years old or more	101 (77·6%)	59 (44·0%)
Median number of days lost (range)	5 (1–21)	5 (1–77)
Time lost from study or work by family member other than case	76 (37·8%)	52 (38·8%)
Median number of days lost (range)	5 (1–28)	5 (1–77)

animal at home and the consumption of uncooked carrots all remained statistically significant protective factors.

The clinical details of cases are described in Table 3. The duration of the illness was a mean of 22·4 days (range 1–100 days). Fourteen (7·0%) cases required hospital admission for 1 night or more, with a median stay of 2 nights (range 1–6 nights). Cases visited a doctor a median of 2 times (range 0–16 visits). The faecal specimen was usually collected on the first visit to the doctor (median 1, range 1–5 visits). Diarrhoea was present in 198 cases (98·5%). The number of stools passed per day when the diarrhoea was at its worst was a median of 9·5 and mean of 11·3 (range 1–40 stools/day). One hundred and ninety-five (98·5%) reported watery motions and 17 (8·5%) bloody motions. Sixty-two (31·3%) reported the use of medication to stop their diarrhoea. For other symptoms, 111 (55·2%) reported vomiting, 182 (90·5%) reported abdominal pain and 81 (40·3%) reported feeling hot. Of those aged 6 years old or more, 101 (77·6%) lost time from study or work. The time lost from study or work was a median of 5 days (range 1–21 days). Of all cases, 76 (37·8%) had another family member lose time from study or work so they could be cared for. The time lost from study or work by another family member was a median of 5 days (range 1–28 days).

Adelaide

There were 173 cases reported in Adelaide during the study period. Of those, 134 (77·5%) had the CATQ administered and were included in the analysis (Table 1). Twenty-two (12·7%) had the CATQ administered but were retrospectively excluded from the analysis because they were found to form disease clusters, 5 (2·9%) had the CATQ administered but were retrospectively excluded because it was administered beyond 8 weeks of their symptom onset, 1 (0·6%) case refused to participate, 2 (1·2%) doctors refused permission, 3 (1·7%) cases were unavailable, 2 (1·2%) didn't have a fixed telephone connection, 1 (0·6%) had an incorrect telephone number, 1 (0·6%) could not be contacted within 8 weeks of symptom onset and 2 (1·2%) had insufficient English.

Of the residential telephone numbers from which the control sample was generated, letters were sent to 4492 households. Of these, 2558 were contacted by telephone and eligible to participate in the study, with 1766 (69·0%) agreeing to participate. Of those

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households agreeing to participate, 3362 individual potential controls were recruited for the database, with 536 CATQ administered to controls and included in the analysis, with 49 controls having conducted more than one CATQ. For the 1934 households that were either not contactable or ineligible to participate, 49 (1·1%) letters were returned as the address was incomplete, 159 (3·5%) had the maximum of 9 telephone calls exhausted without contact, 23 (0·5%) were ineligible because of a household member with a chronic gastrointestinal illness, 192 (4·3%) had insufficient English, 696 (15·5%) had an incorrect phone number recorded, 198 (4·4%) returned the letter as there was a new resident, 613 (13·6%) had not had all 9 calls exhausted but calling was ceased because sufficient numbers had been recruited and 4 (0·1%) were unavailable.

The median age of recruited cases was 10 years (range 0–83 years) and for controls 11 years (range 0–86 years). Sixty-seven cases (50·0%) were male (Table 1).

Cryptosporidiosis was not associated with the consumption of plain tap water. Neither the crude nor adjusted odds ratio for drinking any plain tap water compared to no plain tap water were statistically significant. Similarly, there was no significant dose–response relationship using a linear trend (Table 1).

For the crude analysis, statistically significant risk factors associated with sporadic cryptosporidiosis included the consumption of unpasteurized milk products, swimming in a public toddlers' pool, immune system impairment, having children at home in nappies, having contact with persons with diarrhoea and having children at home attending childcare. Other risk factors included the consumption of unboiled water from a river, lake or dam within rural Australia, overseas travel and the consumption of unboiled water, ice-cubes or salad overseas. Statistically significant protective factors included the consumption of uncooked carrots, contact with any animal at or away from home, as well as dog and cat contact away from home.

After performing an adjusted analysis on a limited number of exposures (Table 2), contact with children and adults with diarrhoea, and the consumption of unboiled rural water within Australia remained statistically significant risk factors, whilst calf contact away from home became statistically significant. Contact with any animal at home and the consumption of uncooked carrots remained statistically significant protective factors.

The clinical details of cases are described in Table 3. The duration of the illness was a mean of 19·4 days (range 2–120 days). Sixteen (11·9%) cases required hospital admission for 1 night or more, with a median stay of 2 nights (range 1–4 nights). Cases visited a doctor a median of 2 times (range 0–8 visits). The faecal specimen was usually collected on the first visit to the doctor (median 1, range 1–6 visits). Diarrhoea was present in 132 cases (98·5%). The number of stools passed per day when the diarrhoea was at its worst was a median of 7 and a mean of 10·3 (range 1–40 stools/day). One hundred and twenty-nine (96·3%) reported watery motions and 8 (6·0%) bloody motions. Thirty-one (23·5%) reported the use of medication to stop their diarrhoea. For other symptoms, 95 (70·9%) reported vomiting, 121 (91·7%) reported abdominal pain and 51 (38·1%) reported feeling hot. Of those aged 6 years old or more, 59 (44·0%) lost time from study or work. The time lost from study or work was a median of 5 days (range 1–77 days). Of all cases, 52 (38·8%) had another family member lose time from study or work so they could be cared for. The time lost from study or work by another family member was a median of 5 days (range 1–77 days).

DISCUSSION

This is one of the first case-control studies to examine risk factors for sporadic cryptosporidiosis in an industrialized country. Other studies have focused on outbreaks of disease. Drinking water was not associated with sporadic disease which is consistent with findings from a randomized clinical trial recently conducted in Melbourne [6]. This study examined gastroenteritis rates in families receiving either plain or filtered tap water and found no evidence of waterborne gastrointestinal disease. We found that the risk factors for sporadic infection were generally similar for both Melbourne and Adelaide despite vastly different water supplies. These included risk factors commonly associated with outbreaks of disease, most importantly, swimming in public pools and person-to-person transmission. These results suggest that drinking water is unlikely to cause a significant amount of sporadic cryptosporidiosis in major Australian cities.

There were several specific exposures within the swimming related group that were associated with sporadic cryptosporidiosis. The most consistent fea-

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ture for the two cities was an association with public toddlers' pools, which can be used by large numbers of children. This source of recreational water activity was a biologically plausible risk factor for cryptosporidiosis, considering its faecal-oral transmission route, the chlorine resistant nature of oocysts and the generally poorer hygiene habits of younger children which increase the risk of a faecal accident. In addition, public pools have been associated with numerous previous outbreaks [18], including several outbreaks in Australian cities [19–22].

Person-to-person transmission was a risk factor for sporadic cryptosporidiosis in both Melbourne and Adelaide. All types of exposures examined were associated with disease. The strongest risk factor within this group of exposures was having young children at home with diarrhoea. This is not surprising and is biologically plausible considering the route of transmission. Furthermore, it is well recognized as a source of secondary transmission according to previous research [22–24].

Calf and lamb contact away home were found to be statistically significant risk factors in Melbourne, whilst only the former risk factor significant in Adelaide. Calves are reservoirs for *Cryptosporidium* oocysts and contact with these animals has been associated with cryptosporidiosis previously [25, 26]. In contrast, various types of animal contact at home were found to be protective factors. Other investigators have suggested companion animals pose a small risk [27]. A possible explanation for these findings could be that animals kept at home shed oocysts to which occupants are regularly exposed, which could confer active immunity. Adding to the complexity is that some types of animal contact away from home were also found to be protective, whereas presumably this type of contact is more infrequent. This area requires further attention to be more fully understood.

Several food-related exposures were examined including some meats (sausage meat, hamburgers, offal), lettuce and other leafy vegetables, uncooked berries, uncooked mushrooms and uncooked carrots, but none were identified as risk factors and uncooked carrots were associated with a protective effect against sporadic cryptosporidiosis. The protective effect of regularly consumed raw vegetables has been identified in unpublished outbreaks in the United Kingdom according to Casemore [27]. The reasons behind the protective effect of only raw carrots remain speculative.

Unpasteurized milk products such as cheese were significantly associated with sporadic cryptosporidiosis for Adelaide but not for Melbourne. The small numbers in the exposed groups make it difficult to make deductions about differences between the two cities because of the limited statistical power. Unpasteurized milk products are biologically plausible as a risk factor and have been associated with cryptosporidiosis previously [28, 29].

The consumption of unboiled water from a rural river, lake or dam within Australia was a statistically significant risk factor for Adelaide only. It is a biologically plausible risk factor since the microbiological quality of country water sources is highly variable in Australia. These water sources are not necessarily maintained in accord with drinking water quality guidelines, and may have livestock grazing along their banks, with such animals having been implicated in the contamination of water sources overseas [30].

Immune system illness, overseas travel and the consumption of unboiled water, ice-cubes and salad overseas were strongly associated risk factors for sporadic cryptosporidiosis. Although biologically plausible and having been associated with cryptosporidiosis previously, their OR are also likely to be inflated due to the ascertainment bias of cases. This may be because overseas travellers who present to their doctor with diarrhoea are more likely to have a faecal specimen requested, than those with diarrhoea who haven't travelled overseas. Secondly, Melbourne and Adelaide pathology laboratories are more likely to test for *Cryptosporidium* oocysts in an overseas traveller (unpublished data).

We did not identify plain tap water as a risk factor for sporadic cryptosporidiosis in either Melbourne or Adelaide. The case-control studies allowed the opportunity to evaluate water supplies from different ends of the water quality spectrum. Melbourne has high quality source water from protected catchments that undergoes very little treatment, whereas Adelaide's has poor quality source water that undergoes extensive treatment. Although drinking water has been associated with many past outbreaks of cryptosporidiosis, Melbourne's drinking water supply was not considered to be a likely source of disease prior to this study because of its highly protected catchment areas, with no public access or farming. In contrast, Adelaide's drinking water supply was considered to pose a greater risk of being associated with disease, because of its poor quality source water with few

limitations on public access or farming. The results of this study however, suggest that the water treatment processes currently in place in Adelaide are adequate to prevent waterborne sporadic cryptosporidiosis.

The type of symptoms experienced by cases for Melbourne and Adelaide were typical of those reported by other investigators [31, 32], with the vast majority of participants reporting watery diarrhoea and abdominal pain. Symptoms were often severe and protracted, with a mean duration of 22·4 days (range 1·0–100·0 days) and 19·4 days (range 2·0–120·0 days) for Melbourne and Adelaide respectively. The mean number of stools passed per day when the diarrhoea was at its worst was high; 11·3 (range 1·0–40·0 stools) and 10·3 stools (range 1·0–40·0 stools) for Melbourne and Adelaide respectively.

The severity of disease reported by cases in this study was attributable to the selective effect of the passive surveillance system by which cases were recruited using laboratory notification. Such cases are likely to represent only a small fraction of all cryptosporidiosis cases in the community as supported by overseas research [33], since only those with relatively severe symptoms are likely to seek medical attention and have a faecal specimen examined. In contrast, studies in volunteer subjects and outbreak investigations with active case finding have reported less severe clinical symptoms and a shorter duration of disease. In the volunteer study by DuPont [31] the mean duration of illness was 3·1 days (range 2·4–3·6 days), with a mean of 6·4 unformed stools per day (range 4·0–11·0 stools) when the diarrhoea was at its worst. In the Milwaukee outbreak [1] people who had clinical cryptosporidiosis which was not laboratory confirmed had a mean duration of illness of 4·5 days (range 1·0–38·0 days), and a mean of 7·7 unformed stools per day (range 1·0–60·0 stools) when the diarrhoea was at its worst. For the 285 laboratory confirmed Milwaukee cases the disease was more severe with a mean duration of 12·0 days (range 1·0–55·0 days), with a mean of 19·0 unformed stools per day (range 1·0–90·0 stools) when the diarrhoea was at its worst.

A methodological limitation of case-control studies is recall bias of exposures, particularly when reliant on human memory [34]. Self-reported estimates for exposures investigated such as drinking water intake were likely to result in random rather than systematic error, which would have the effect of underestimating the association between the exposure in question and cryptosporidiosis. However, biased over-reporting of estimates was possible for cases who suspected that a particular exposure was responsible for their illness. Such a situation was only likely to occur on a large scale if publicity was heightened at the time the study was conducted, which linked cryptosporidiosis to the exposure in question. In July to September 1998 Sydney's drinking water supply received substantial local and international media attention when *Cryptosporidium* and *Giardia* oocysts were reportedly detected, with three boil water advisories issued to the public. This adverse publicity therefore adds further weight to the study finding that cryptosporidiosis is not associated with the consumption of tap water. In early 1998 prior to the commencement of the case-control studies, several Melbourne swimming pools were closed due outbreaks of cryptosporidiosis connected with the pools in question [19]. Considering most Melbourne cases were recruited at the end of 2000 and the early part of 2001 it is unlikely that biased reporting ensued. Furthermore, there were no swimming pool closures in Adelaide over the course of the study yet swimming in a public toddlers' pool was still found to be a statistically significant risk factor.

The large numbers of exposures examined meant that some of the factors associated with cryptosporidiosis might have been due to chance. To prevent 'data trawling' however, we only asked about exposures that were biologically plausible, or exposures that had been previously associated with cryptosporidiosis.

A weakness of this study was that due to its relatively small size it did not have the statistical power to detect associations for uncommon exposures or exposures with small OR. For example, working with sewage may be a significant risk factor but due to only 6% of Adelaide controls reporting this exposure an OR of at least 1·9 would have needed to have been detected.

In summary, this study suggests that drinking water is unlikely to be a major cause of sporadic cryptosporidiosis in metropolitan cities of Australia. It emphasizes three areas of public health importance. Firstly, public swimming pools are an important potential source of large numbers of cryptosporidiosis cases. This therefore reinforces the need for appropriate and regular public health messages to the public and swimming pool managers, in an attempt to prevent both sporadic disease and outbreaks. It also reinforces the need for close surveillance of swimming pools by health departments to minimize outbreaks. Secondly, education of the public about general

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hygiene measures to limit person-to-person transmission needs to be undertaken. Thirdly, due to the severity of illness in immunocompromised people, this group should be cautioned about public swimming pool attendance, as well as being made aware of other important risk factors identified from this study.

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