

1 **Epidemiology and associated risk factors of giardiasis in a**
2 **peri-urban setting in New South Wales Australia**

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15 Running head: EPIDEMIOLOGY AND ASSOCIATED RISK FACTORS OF GIARDIASIS.

16 SUMMARY

17 Giardiasis is one of the most important non-viral causes of human diarrhoea. Yet, little is
18 known about the epidemiology of giardiasis in the context of developed countries such as
19 Australia and there is limited information about local sources of exposure to inform
20 prevention strategies in New South Wales. This study aimed to (1) describe the epidemiology
21 of giardiasis and (2) identify potential modifiable risk factors associated with giardiasis that
22 are unique to south-western Sydney, Australia. A 1:2 matched case-control study of 190
23 confirmed giardiasis cases notified to the South-Western Local Health District Public Health
24 Unit from January to December 2016 was employed to investigate the risk factors for
25 giardiasis. Two groups of controls were selected to increase response rate; Pertussis cases and
26 neighbourhood controls. A matched analysis was carried out for both control groups
27 separately. Variables with a significant odds ratio (OR) in the univariate analysis were placed
28 into a multivariable regression for each matched group respectively. In the regression model
29 with the neighbourhood controls, age and sex were controlled as potential confounders.
30 Identified risk factors included being under five years of age (aOR = 7.08; 95% CI 1.02 –
31 49.36), having a household member diagnosed with a gastrointestinal illness (aOR = 15.89;
32 95% CI 1.53 – 164.60) and having contact with farm animals, domestic animals or wildlife
33 (aOR = 3.03; 95% CI 1.08 – 8.54). Cases that travelled overseas were at increased risk of
34 infection (aOR = 19.89; 95% CI 2.00 – 197.37) when compared with Pertussis cases. This
35 study provides an update on the epidemiology and associated risk factors of a neglected
36 tropical disease, which can inform enhanced surveillance and prevention strategies in the
37 developed metropolitan areas.

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39 **Key words:** Epidemiology, Giardiasis, Surveillance, diarrhoea, transmission, control

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41 **INTRODUCTION**

42 *Giardia duodenalis* (also known as *Giardia lamblia* or *Giardia intestinalis*) is one of the
43 most common enteroparasites affecting humans with an estimated 280 million people being
44 infected each year, around the world [1]. It is a protozoan parasite that causes infection in the
45 bowel and clinically manifests as a diarrhoeal illness. Additionally, giardiasis has been
46 associated with the development of chronic diarrhoea or irritable bowel syndrome (IBS),
47 debilitating fatigue and reactive arthritis [2]. Giardiasis is not a life-threatening disease
48 however infections may often go unnoticed due to many cases having a lack of symptoms. If
49 left without treatment, the infection can become serious; impairing the development of
50 children and resulting in a failure to thrive [3]. Certainly, giardiasis contributes negatively to
51 public health development of endemic countries and causes devastating socio-economic loss.
52 In 2004, *G. duodenalis* was officially included in the WHO Neglected Diseases Initiative [4].
53 Meanwhile in Australia, giardiasis is a notifiable disease in several states and territories
54 including New South Wales (NSW) [5].

55 Giardiasis is the most common notifiable parasitic infection in NSW. While the burden of
56 disease is greater in developing settings with poor access to water, sanitation, and hygiene
57 (WASH) facilities, sporadic cases occur in developed countries including Australia and
58 outbreaks are not uncommon [6]. In 2014, nearly 3000 cases were notified by laboratories in
59 NSW [7], and 3,434 cases reported in 2015 [7]. South Western Sydney (SWS) accounts for
60 approximately 6% of cases state-wide. Amongst hospitalised patients, giardiasis was the
61 second most commonly identified enteric protozoa, affecting mainly school age and young
62 children [8]. In Australia, giardiasis is frequently associated with waterborne infections, day
63 care centre disease outbreaks, and travel-associated diarrhoea.

64 Few Australian studies have documented the prevalence of giardiasis; however, there are no
65 recent studies that have examined the risk factors that drive local transmission of giardiasis
66 [9, 10]. The aim of this study was to describe the epidemiology of giardiasis and to identify
67 the risk factors and sources of exposure associated with the disease in SWS region of NSW.
68 The study provides information on the impact of giardiasis on human health in SWS and a
69 better understanding of the epidemiology and associated risk factors that can inform public
70 health control strategies.

71 **MATERIALS AND METHODS**

72 **Study site**

73 The South-Western Sydney Local Health District (SWSLHD) was the research site. The
74 SWSLHD includes seven Local Government Areas (LGA): Bankstown, Camden,
75 Campbelltown, Fairfield, Liverpool, Wingecarribee and Wollondilly (see Supplementary
76 Figure S1).

77 The SWSLHD is the largest and fastest growing District in metropolitan Sydney. It has a
78 large population of approximately 900,000, has a diverse geography, including significant
79 populations in both rural and urban areas, and approximately 46% of the population speak a
80 language other than English at home. Public Health surveillance data can provide an example
81 of what could be occurring across the NSW state.

82

83 **Study design and data collection**

84 *Case-control survey*

85 A 1:2 case-control study of risk factors was designed with prospective recruitment of cases
86 and controls. Cases were all confirmed cases of giardiasis notified to the SWSLHD PHU

87 from 1 January 2016 to 31 December 2016. A study questionnaire was developed based on a
88 comprehensive review of the literature and was used to collect data from all cases and
89 controls who agreed to participate in the study. Both case and control questionnaires are
90 accessible online as supplementary material on the Cambridge Core website. The
91 questionnaire asked about various socio-demographic features, self-reported clinical
92 symptoms, information about care seeking behaviour and treatment received, the number of
93 household members or other close contacts with similar symptoms, and a range of exposures
94 experienced 3 weeks before illness onset (for cases) or a similar time frame for controls.
95 Enhanced data collection for this study also included additional details on potential
96 confounders including: country of birth, language spoken at home, highest educational
97 attainment, and occupation of the parents (for cases residing with their parents).

98

99 *Recruitment and selection of participants*

100 Laboratories are required under the *NSW Public Health Act 2010* to notify PHUs of cases of
101 giardiasis. As per the NSW Control Guideline protocols for investigation, once a giardiasis
102 case was notified to the SWSLHD PHU, staff contacted the diagnosing doctor of the
103 giardiasis case to request permission to contact the case or the parent or guardian (for persons
104 under 16 years old), to interview the case.

105 *Cases*

106 A “case” was a person who had laboratory definitive evidence for the detection of *G.*
107 *duodenalis* cysts or trophozoites in stool samples or samples of duodenal contents. Informed
108 consent was provided by the case or their parent (for persons under 16 years); with parents/
109 guardians asked to complete the responses on behalf of children 12 years old or younger and
110 to provide consent for children 13 to 15 years to answer their own questions.

111 *Controls*

112 A “control” was defined as a person resident in SWSLHD, and who did not have a history of
113 a positive *Giardia* test in the previous 3 months (due to the possibility of chronic infection
114 with *Giardia*). In order to improve the response rate and reduce selection bias, three different
115 sets of controls were identified for the study.

116 (1) Control group 1: Neighbourhood controls (NBH):

117 Confirmed giardiasis cases were grouped into (i) urban and (ii) regional areas based on
118 Australian Bureau of Standards regional classification. The aim was to identify ten (10)
119 controls for each case to increase the likelihood of at least one household responding to the
120 questionnaire. The following sampling strategy was employed.

121 i. Urban: A list of all addresses in SWSLHD geocoded to latitude longitude coordinates
122 was obtained from the Geocoded National Address File. This dataset is available for
123 free from “Public Sector Mapping Agencies” Australia. A 500m radius buffer (due to
124 the dense population in urban areas) was drawn around each case’s address using
125 Geographic Information System (GIS) tools (For an example see Supplementary
126 Figure S2). Ten houses were then randomly selected from the list of addresses for
127 each buffer.

128 ii. Rural: The procedure followed was the same as for urban areas, except that 5 km
129 buffers were used to account for population sparseness.

130 A letter with the Patient Information Statement (PIS) and control questionnaire was
131 sent to the selected household, with a request that the person with the next birthday in
132 the household complete the questionnaire. The completed questionnaire was to be
133 returned by post in the self-addressed envelope provided.

134 (2) Control group 2: Pertussis case:

135 Confirmed Pertussis cases notified in the same year, within the same age range (± 5
136 years), residing within the same LGA but not on the same street as the corresponding
137 giardiasis case were identified. If there were two or more persons meeting the criteria,
138 one would be selected by simple random sampling using a random sampling function
139 in Excel. Where no age match was available for the same LGA, one was selected
140 from the closest LGA. Each control was contacted by telephone and once consent
141 was obtained, the individual was interviewed with the standardized control
142 questionnaire. If the person refused to participate in the study, or was uncontactable
143 after three phone calls, then the person was listed as a non-response.

144 (3) Control group 3: Friend Control:

145 This recruitment method yielded no controls and was not considered further.

146 *Sample Size*

147 Based on surveillance data, it was estimated that the SWSLHD PHU received an average of
148 147 giardiasis notifications annually between the years 2012 and 2015. In a 1:3 unmatched
149 design with a two-sided confidence level of 95% ($z_{\alpha/2}=1.96$) with power ($z_{\beta}=0.80$) of 80%
150 and an estimated prevalence of a risk factor of 17% in controls and 40% in cases, at least 35
151 cases and 105 controls were needed to detect a significant risk of exposure (odds ratio >3.25)
152 [11]. Oversampling of cases and controls was performed to accommodate for any non-
153 responses or incompleteness in the data. As such, a total of 50 cases and 150 controls were
154 needed.

155 *Matched case-control analysis*

156 Survey data was entered into an outbreak questionnaire developed using the Notifiable
157 Conditions Information Management System (NCIMS) and analysed using IBM SPSS
158 Statistics version 23.0 [12]. Pertussis cases were matched to cases by age (± 5 years) and
159 location; NBH controls were matched to cases by location (urban or rural). Univariate
160 analysis was carried out to compare cases with each control group separately and an adjusted
161 estimate of the Odds ratio (OR) and their 95% confidence intervals (CI) were calculated from
162 matched pairs of cases and controls for various risk factors.

163 For each case control group, variables with a significant OR in the univariate analysis were
164 placed into a multivariable regression for each matched group respectively. No potential
165 confounders were identified in the regression model with the Pertussis cases. In the
166 regression model with the neighbourhood controls, age and sex were controlled as potential
167 confounders. A backward stepwise elimination process was employed, using a likelihood
168 ratio test to produce the most parsimonious model [13]. All variables with a Wald χ^2
169 statistically significant at the *P*-value of <0.05 were considered significant. Odds Ratios (OR)
170 and 95% confidence intervals (95%CI) for the association were reported. Cases for whom we
171 could not identify suitable matching control subjects were excluded from the matched case-
172 control analysis.

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175

176 **RESULTS**

177 Of the 217 giardiasis cases invited to participate in the study, 68 (31.3%) consented to be
178 interviewed for the study (see Fig. 1). Letters were mailed to 1,983 randomly selected
179 households residing in the same neighbourhood as cases (Fig. 1). Of these, 113 controls
180 (5.7%) returned a completed questionnaire and were included in the study. A total of 75
181 Pertussis cases were selected from NCIMS and contacted via telephone. Of these, 36 (48.0%)
182 agreed to be interviewed for the study and, 26 (34.7%) could not be contacted after three
183 telephone call attempts. To reduce the risk of selection bias, two separate matched analyses
184 were done: one which combined 21 cases and 36 Pertussis cases and the other matched 68
185 cases and 68 neighbourhood controls.

186 *Demographic characteristics*

187 The distribution of the cases and controls by age and gender is presented in Table 1. Cases
188 and controls were similar with regard to language spoken at home, highest level of education
189 and indigenous status. Cases and controls mainly originated from urban areas in SWS as
190 opposed to rural. More than half of case patients (40 or 58.8%), compared with 27 (40.3%)
191 neighbourhood controls and 15 (41.7%) Pertussis cases were males. The age distributions
192 varied between cases and controls with the median age being eight (± 19.4) years for giardiasis
193 cases, 58 (± 20.8) years for neighbourhood controls, and for Pertussis cases, eight (± 17.9) years
194 (see Table 1).

195 In comparison to the cases there were significantly fewer neighbourhood controls aged 0-4
196 years (36.8% vs 2.9%). Conversely, significantly more Pertussis cases were aged 0-4 years
197 (28.6% vs 41.7%). There were also significantly more older females as neighbourhood
198 controls in comparison to the Pertussis cases which had significantly more children aged less
199 than five years.

200 Univariate analyses revealed that males were significantly more likely to be cases when
201 compared with neighbourhood controls, hence sex was controlled as a potential confounder
202 in the multivariable analysis. When controlling for sex in the multivariable analysis, cases
203 aged under five years had a seven times greater risk of *Giardia* infection (aOR = 7.08; 95%
204 CI 1.02 – 49.36) when compared with neighbourhood controls. There was no difference
205 between the ages and genders of giardiasis cases and Pertussis cases.

206 *Risk factors for giardiasis*

207 Univariate analysis of the comparison between neighbourhood controls and cases revealed
208 that cases who, (a) were males aged under five years, (b) visited their / parent's country of
209 birth, (c) had a child that attends childcare, (d) had a household member diagnosed with
210 gastrointestinal illness, (e) were individuals who swim in pools, (f) had contact with domestic
211 animals, wildlife or livestock, and (g) were individuals who visited a farm, zoo or wildlife
212 park, were at increased risk for giardiasis ($P < 0.05$) (Table 2). Those who temporarily stored
213 their water in jars, bottles or cisterns at home and for those who consumed green salad or
214 lettuce on a daily basis were at a decreased risk ($P < 0.05$) (Table 2). When age and location
215 were controlled in the multivariable analysis, all variables lost their significance except for
216 having a member of household diagnosed with gastrointestinal illness and having contact
217 with farm, domestic or wild animals. Those who reported swimming in pools had an
218 elevated risk, but this was not significant ($P = 0.06$) (Table 2).

219 The univariate analysis matching cases with the second group of controls (i.e. Pertussis cases)
220 found that giardiasis cases were more likely to have travelled overseas and had a household
221 member diagnosed with gastrointestinal illness. Notably, there was a negative association
222 found between giardiasis cases and living in close proximity to wildlife. All three variables
223 except travelling overseas and outside Australia lost their significance in the multivariable
224 analysis (Table 2).

225 **DISCUSSION**

226 This matched case-control study represents the value of continuing to monitor giardiasis in
227 south-western Sydney and other parts of NSW and recommends further studies to examine
228 the genotypes in circulation and their potential for zoonotic transmission. The results from
229 this study indicate that some common risk factors of *Giardia* infection seen in other
230 developed countries were not found to be significant risk factors in south-western Sydney.

231 Notably, the multivariable analyses among cases and neighbourhood controls, and cases and
232 Pertussis cases found no significant association between giardiasis and those using water
233 sourced from alternative supplies such as roof-harvested rainwater (RHRW), tank water or
234 bore wells. An overall low number of individuals reporting drinking non-municipal water
235 long-term may lead to this lack of association [14]. However, the result is in keeping with
236 other Australian studies that could not identify untreated RHRW tanks as sources of infection
237 for giardiasis, which is likely due to the fact that RHRW tanks are likely to be mainly used
238 for potable replacement for flushing toilets, washing clothes, or watering gardens [14, 15].

239 Furthermore, while initial univariate analyses between cases and neighbourhood controls
240 found a significant association between giardiasis and those who reported swimming in pools
241 (chlorinated, salt-water or non-chlorinated) three months prior to illness onset, this
242 significance was lost in the multivariable model that controlled for age and sex. This
243 suggests there may be a relationship between age, sex and swimming that is confounding
244 their association with giardiasis infection in this setting. On the other hand, there are multiple
245 studies that have established the association between swimming in pools and giardiasis
246 infection [16, 17, 18].

247

248 Giardiasis cases were also more likely to have a household member diagnosed with
249 gastrointestinal illness, when compared with neighbourhood controls. A similar risk found in

250 the univariate analysis with Pertussis cases and may be due to a low response rate.
251 Notwithstanding, studies in Turkey and other countries have reported an increased risk of
252 infection amongst household members infected with giardiasis [19, 20]. This indicates a
253 potential for person-to-person transmission of infection occurring within households in SWS
254 with infected family members (or household members) serving as sources of infection. There
255 is also the prospect of transmission through food or water prepared by the infected individual.
256 This study emphasises the importance of screening all household members for giardiasis once
257 a case has been diagnosed.

258

259 In this study, multivariable analysis found a seven times greater risk of infection for those
260 aged under five years. However, when compared with Pertussis cases the risk was
261 insignificant. While other case-control studies have observed no significant risk associated
262 with age, it is more likely that this result is due to the small participant numbers in the
263 Pertussis cases group. Individuals of all age groups can be infected by *G. duodenalis* although
264 the majority of literature maintains that giardiasis is most prevalent in school-age and
265 younger children [21, 22]. Children tend to have a higher exposure to contaminated faeces
266 particularly in close-contact facilities such as childcare centres putting them at greater risk of
267 infection [23, 24, 16].

268

269 While univariate analyses among cases and neighbourhood controls observed that males were
270 at an increased risk of giardiasis, this association lost its' significance in the multivariable
271 analysis after being controlled for sex and age and was likely due to the fact that there were
272 overwhelmingly more females among neighbourhood controls [25, 26].

273 Cases coming in contact with domestic animals, farm animals and even wildlife were at
274 increased risk of infection when compared with neighbourhood controls, but not when

275 compared with Pertussis cases. The lack of significance when compared with the Pertussis
276 cases may be due to a lack of difference in exposure between the two groups, hence diluting
277 the risk. The possible role of animals as a source of *G. duodenalis* infection to humans is still
278 unclear, although most studies agree that animals can play an indirect role in transmission [6,
279 27]. Molecular investigations on *G. duodenalis* and the potential for zoonotic transmission
280 observed that humans can only be infected with human-specific assemblages (A or B) and not
281 from animal-adapted genotypes (C-H) [28]. A possible explanation for the present results is
282 that animals are carriers of assemblages A or B and act as vehicles for mechanical
283 transmission to humans who come in contact with animal's faeces at parks or wildlife settings
284 where hand-washing facilities may not be available [29], or other environmental exposures
285 to cysts attached to the fur of domestic animals [30].

286 Interestingly, the vast majority (80.9%) of *G. duodenalis* cases did not report travelling
287 overseas within the 3 months prior to illness onset suggesting that most of the giardiasis cases
288 were locally acquired. This is the first case control study to examine travel history amongst
289 giardiasis cases in this setting and is consistent with other case-control studies conducted in
290 other developed countries [23, 16, 31]. However, multivariable analyses found that when
291 compared with Pertussis cases, giardiasis cases were 20 times more likely to have been
292 travelling overseas. The most popular countries visited were in South & South-East Asia,
293 West Central Asia/North Africa and Oceania. Overseas travel to endemic regions is widely
294 believed to be the principal risk factor for giardiasis in developed countries. However, due to
295 detection bias associated with physicians testing for giardiasis more commonly among
296 returning travellers, overseas acquired infection rate is likely to be overestimated; and
297 consequently underestimating locally acquired giardiasis [32].

298

299 There are some limitations to this study. Although care was taken to recruit controls
300 representative of the source population of cases, some selection bias may exist among
301 controls. There was a larger response rate for among older females residents in urban areas in
302 SWS, indicating that women were more likely to respond to the neighbourhood control
303 questionnaire. There was also an underrepresentation of children seen in the neighbourhood
304 controls when compared with Pertussis cases. This selection bias emphasised the sex and age
305 differences between cases and neighbourhood controls and could explain why some
306 exposures were also present among the control group, thus diluting the exposure rates
307 amongst cases. A matched analysis was done to reduce selection bias and improve internal
308 validity, by controlling for the sex, age, and region of residence differences between cases
309 and neighbourhood controls. The matched design reduced the risk of error from the
310 confounding effect of age, sex and location but due to the resulting close matching on these
311 variables, their effects on giardiasis risk could not be assessed. However, controlling for these
312 well-known confounders was valuable as it allowed the assessment of other risk factors
313 without their confounding influences. Admission risk bias is a potential problem with
314 Pertussis cases, which were selected based on being a group of patients available through
315 NCIMS, did not have a gastrointestinal symptoms or diagnosed with giardiasis, and hence
316 they may have a different exposure profile to the general population. Since giardiasis cases
317 matched to pertussis cases were quite similar in sex distribution, there was no association and
318 hence no further need for controlling this variable. Like most studies that utilises surveillance
319 data as a sampling frame, only symptomatic *G. duodenalis* cases that sought medical
320 attention and had a positive laboratory test were included in the study. This means that this
321 study represents only a proportion of the overall burden of the disease in the community.
322 Cases with undiagnosed and asymptomatic giardiasis would not have been considered.

323 Therefore, this study cannot be generalised to all of Australia and must be interpreted in the
324 context of these limitations.

325 **CONCLUSION**

326 The study showed an increased risk of giardiasis in children aged under five years, amongst
327 individuals who have a household member diagnosed with gastrointestinal illness and have
328 contact with domestic animals, wildlife or livestock. The study also found that cases who
329 travelled overseas were at a greater risk of infection. There is a need to educate residents
330 living in urban areas in SWS on the potential of person-to-person transmission of giardiasis;
331 particularly if a household member is ill with gastroenteritis. Targeted intervention and health
332 messages are needed for the parents/ carers of younger children especially during high-risk
333 seasons such as warmer months, with emphasis on potential risks and appropriate hygiene
334 practices when visiting farms and wildlife parks or where contact with animals is to be
335 expected. Likewise, people travelling overseas to endemic countries should be appropriately
336 informed of the risks and possible control strategies that can be implemented. This study
337 illustrates the value of continuing to monitor giardiasis in south-western Sydney and other
338 parts of NSW and recommends further studies to examine the genotypes in circulation and
339 their potential for zoonotic transmission.

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341

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347

348 **DECLARATION OF INTEREST**

349 None.

350 **ETHICAL STANDARDS**

351 Ethical approval for this study was received from the South-Western Sydney Local Health
352 District Human Research Ethics Committee which is accredited by the NSW Ministry of
353 Health (HREC approval number: HE16/079 LNR).

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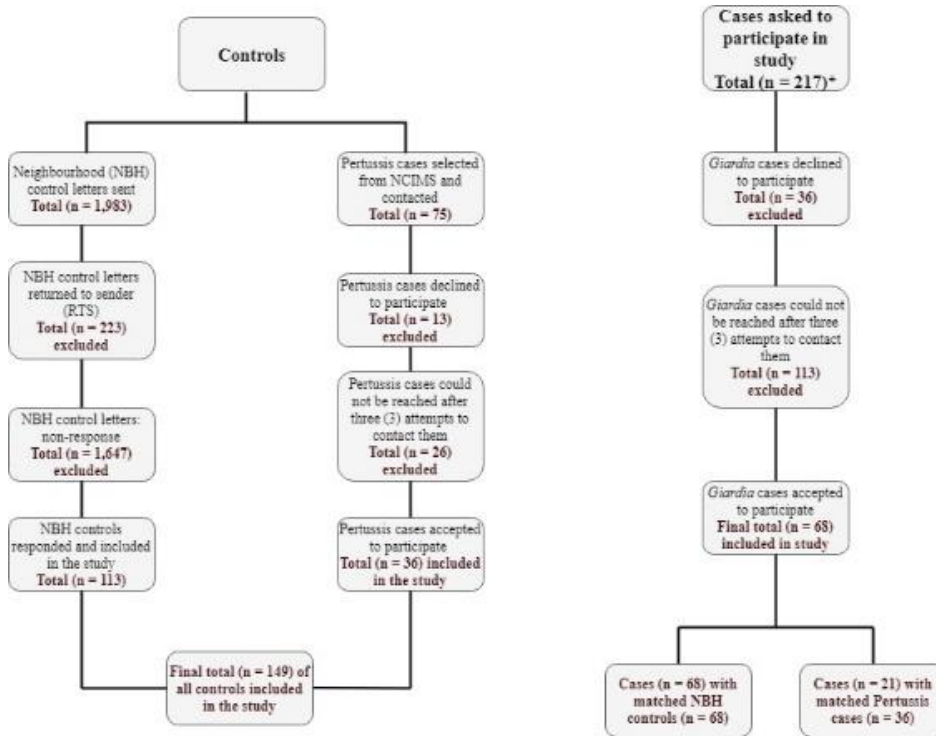
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371 **LEGENDS FOR ILLUSTRATIONS**

372 **Fig. 1. A flowchart summary of the two different control types (neighbourhood control**
373 **and Pertussis case) and the number of cases used in the study.**



374

*including notified *Giardia* cases from January 2016 to January 2017

375

376 Table 1. Demographics of cases and controls.

377

Demographics	Cases % (N = 68)	Neighbourhood Controls % (N = 113)	P-value	Cases % (N = 21)	Pertussis Cases (N = 36)	P-value
Median age in years (range)	8.0 (Range 0-83; Mean 18.3 (13.6-23.0) ±19.36)	57.5 (Range 0-84; Mean 51.0 (46.0-56.1) ±20.8)	NR ^a	7.0 (Range 1-70; Mean 19.4 (10.0-28.9) ±20.8)	8.0 (Range 1-65; Mean 16.3 (10.2-22.3) ±17.9)	NR
<i>0-4yrs</i>	36.8% (25)	2.9% (2)	0.001*	28.6% (6)	41.7% (15)	0.242
<i>5yrs or older</i>	63.2% (43)	97.1% (66)		71.4% (15)	58.3% (21)	
Gender						
<i>Male</i>	58.8% (40)	40.3% (27)	0.027*	47.6% (10)	41.7% (15)	0.662
<i>Female</i>	41.2% (28)	60.3% (41)		52.4% (11)	58.3% (21)	
Residence						
<i>Urban</i>	86.8% (59)	86.8% (59)	1.000	76.2% (16)	75.0% (27)	0.920
<i>Rural</i>	13.2% (9)	13.2% (9)		23.8% (5)	25.0% (9)	
Language spoken at home						
<i>English</i>	85.3% (58)	14.7% (10)	NR	76.2% (16)	100.0% (4)	NR
<i>Arabic</i>	2.9% (2)	0.0% (0)		0.0% (0)	0.0% (0)	
<i>Hindi</i>	3.0% (2)	0.0% (0)		9.5% (2)	0.0% (0)	
<i>Other languages</i>	7.4% (5)	0.0% (0)		14.3% (3)	0.0% (0)	
Aboriginality						
<i>Aboriginal but not Torres Strait Islander</i>	0.0% (0)	1.5% (1)	NR	0.0% (0)	2.8% (1)	NR
<i>Not Aboriginal and Torres Strait Islander</i>	98.5% (67)	83.8% (57)		95.2% (20)	97.2% (35)	

<i>Both Aboriginal and Torres Strait Islander</i>	1.5% (1)	0.0% (0)		4.8% (1)	0.0% (0)	
Highest Level of Education^c						
<i>No formal education</i>	4.4% (3)	2.9% (2)	NR	4.8% (1)	0.0% (0)	NR
<i>Primary or elementary school (Year K-6 or equivalent)</i>	1.5% (1)	4.4% (3)		4.8% (1)	2.8% (1)	
<i>Secondary school (Year 7-12 or equivalent)</i>	22.1% (15)	22.1% (15)		9.5% (2)	41.7% (15)	
<i>Vocational (e.g. TAFE or skills training)</i>	27.9% (19)	33.8% (23)		28.6% (6)	25.0% (9)	
<i>University</i>	36.8% (25)	35.3% (24)		42.9% (9)	30.6% (11)	
<i>Other form of education</i>	7.4% (5)	1.5% (1)		9.5% (2)	0.0% (0)	

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379 ^a NR not reported and / or calculated.

380 ^b Other languages spoken by 1 person each: Bengali, Cantonese, Macedonian, Mandarin, and Spanish.

381 ^c If the case was under 12 years of age, the educational level was provided by the parent /head of household answering the survey.

383 * Statistically significant ($P < 0.05$).

384

385 Table 2. Univariate and multivariable analysis of risk factors for *G. duodenalis* infection.

386

Risk Factors	Cases % (N = 68)	Neighbourhood Controls % (N = 68)	Unadjusted OR ^a (95% CI)	Adjusted OR ^b (95% CI)	Cases % (N = 21)	Pertussis Cases % (N = 36)	Unadjusted OR (95% CI)	Adjusted OR ^c (95% CI)
Gender								
<i>Male</i>	58.8 % (40)	40.3% (27)	2.17 (1.09 – 4.30)*	1.31 (0.47 – 3.67)	47.6 % (10)	41.7% (15)	1.27 (0.43 – 3.76)	1.13 (0.32 – 3.97)
<i>Female</i>	41.2 % (28)	60.3% (41)			52.4 % (11)	58.3% (21)		
Age Category								
<i>0-4yrs</i>	36.8 % (25)	2.9% (2)	19.19 (4.32 – 85.18)*	7.08 (1.02 – 49.36)*	28.6 % (6)	41.7% (15)	0.56 (0.18 – 1.78)	
<i>5yrs or older</i>	63.2 % (43)	97.1% (66)			71.4 % (15)	58.3% (21)		
Travel within Australia								
<i>Yes</i>	8.8% (6)	10.8% (7)	0.80 (0.25 – 2.53)	NR ^d	9.5% (2)	8.3% (3)	1.16 (0.18 – 7.56)	NR
Travel overseas								
<i>Yes</i>	19.1 % (13)	11.9% (8)	1.74 (0.67 - 4.53)	NR	23.8 % (5)	2.8% (1)	10.94 (1.18 – 101.41)*	0.13 (0.01 – 1.50)
Visit country of birth or parent's country of birth								
<i>Yes</i>	76.9 % (10)	18.8% (3)	14.44 (2.39 – 87.40) *	NR	80.0 % (4)	0.0% (0)	NR	NR
Countries visited overseas								
<i>South & Southeast Asia</i>	38.5 % (5)	37.5% (3)	NR	NR	20.0 % (1)	100.0% (1)	NR	NR

<i>West Central</i>	7.7%	12.5% (1)			20.0	0.0% (0)		
<i>Asia/North</i>	(1)				% (1)			
<i>Africa</i>								
<i>Oceania</i>	30.8	25.0% (2)			40.0	0.0% (0)		
	% (4)				% (2)			
<i>Europe</i>	0.0%	12.5% (1)			0.0%	0.0% (0)		
	(0)				(0)			
<i>Latin America</i>	7.7%	12.5% (1)			0.0%	0.0% (0)		
<i>& Caribbean</i>	(1)				(0)			
<i>Multiple</i>	15.4	0.0% (0)			20.0	0.0% (0)		
<i>Regions</i>	% (2)				% (1)			
Camp or caravan								
<i>Yes</i>	9.0%	10.4% (7)	0.84 (0.27 -	NR	9.5%	0.0% (0)	NR	NR
	(6)		2.66)		(2)			
Children at home attending childcare								
<i>Yes</i>	50.0	7.7% (5)	12.00 (4.29	2.46	42.9	30.6%	1.71 (0.56 -	
	%		- 33.57) *	(0.63 -	% (9)	(11)	5.21)	NR
	(34)			9.70)				
Member of household diagnosed with gastrointestinal illness								
<i>Yes</i>	24.2	1.5% (1)	21.44 (2.75	15.89	21.1	5.6% (2)	4.53 (0.75 -	0.35
	%		- 167.08)*	(1.53 -	% (4)		27.50)*	(0.05 -
	(16)			164.60)*				2.62)
Unfiltered or non-boiled tap water								
<i>Yes</i>	65.2	73.5% (50)	NR	NR	65.0	58.3%	1.33 (0.43 -	NR
	%				% (13)	(21)	4.12)	
	(43)							
Filtered tap water								
<i>Yes</i>	45.5	33.8% (22)	NR	NR	40.0	38.9%	1.05 (0.34 -	NR
	%				% (8)	(14)	3.20)	
	(30)							

Sydney water connected to home									
<i>Yes</i>	91.7 % (55)	86.8% (59)	1.68 (0.53 - 5.32)	NR	82.4 % (14)	91.2% (31)	0.45 (0.08 – 2.52)	NR	
Roof-harvested rain water to home									
<i>Yes</i>	5.9% (4)	13.2% (9)	0.41 (0.12 - 1.40)	NR	9.5% (2)	2.8% (1)	3.68 (0.31 – 43.32)	NR	
Bore water or shallow well water used in home									
<i>Yes</i>	0.0% (0)	1.5% (1)	NR	NR	0.0% (0)	0.0% (0)	NR	NR	
Tank water used in home									
<i>Yes</i>	22.1 % (15)	10.3% (7)	2.47 (0.94 - 6.50)	NR	19.0 % (4)	27.8% (10)	0.61 (0.17 – 2.27)	NR	
Temporary storage of water in jars, bottles, cisterns at home									
<i>Yes</i>	1.6% (1)	32.4% (22)	0.03 (0.00 - 0.26) *	NR	0.0% (0)	2.8% (1)	NR	NR	
Swimming in pool									
<i>Yes</i>	57.6 % (38)	28.4% (19)	3.43 (1.67 - 7.05) *	2.63 (0.95 – 7.27)	52.4 % (11)	52.8% (19)	0.98 (0.34 – 2.89)	NR	
Swimming in river, lake, lagoon, pond or similar setting									
<i>Yes</i>	13.2 % (9)	4.4% (3)	3.31 (0.85 - 12.79)	NR	9.5% (2)	13.9% (5)	0.65 (0.12 – 3.71)	NR	
Swimming in the ocean									

<i>Yes</i>	10.3 % (7)	16.2% (11)	0.60 (0.22 – 1.64)	NR	14.3 % (3)	16.7% (6)	0.83 (0.19 – 3.75)	NR
Always wash hands before eating								
<i>Yes</i>	60.3 % (41)	67.2% (45)	0.74 (0.37 – 1.50)	NR	61.9 % (13)	69.4% (25)	0.72 (0.23 – 2.22)	NR
Always wash hands after playing with animals								
<i>Yes</i>	80.3 % (49)	76.5% (52)	1.26 (0.54 – 2.92)	NR	76.5 % (13)	74.3% (26)	1.13 (0.29 – 4.35)	NR
Changing nappies of child/children								
<i>Yes</i>	13.8 % (9)	20.9% (14)	0.61 (0.24 – 1.52)	NR	14.3 % (3)	8.3% (3)	1.83 (0.34 – 10.04)	NR
Engaging in sexual activity with contact with faeces								
<i>Yes</i>	1.8% (1)	1.5% (1)	1.20 (0.07 – 19.57)	NR	0.0% (0)	0.0% (0)	NR	NR
Onsite septic system at home								
<i>Yes</i>	12.1 % (8)	14.9% (10)	0.79 (0.29 – 2.14)	NR	15.8 % (3)	22.9% (8)	0.63 (0.15 – 2.74)	NR
Contact with farm/ domestic animal/ wildlife								
<i>Yes</i>	61.8 % (42)	32.4% (22)	3.38 (1.67 – 6.84)*	3.03 (1.08 – 8.54)*	71.4 % (15)	72.2% (26)	0.96 (0.29 – 3.18)	NR
Visited a farm, zoo, wildlife park								
<i>Yes</i>	28.4 % (19)	9.1% (6)	3.96 (1.47 – 10.69)*		19.0 % (4)	38.9% (14)	0.37 (0.10 – 1.33)	NR

Wildlife in close proximity to house									
<i>Yes</i>	26.5 % (18)	17.9% (12)	1.65 (0.72 – 3.76)	NR	14.3 % (3)	41.7% (15)	0.23 (0.06 – 0.94)*	3.68 (0.82 – 16.51)	
Consumes green salad/lettuce daily									
<i>Yes</i>	17.9 % (12)	38.8% (26)	0.34 (0.16 – 0.76) *	0.48 (0.15 – 1.52)	14.3 % (3)	22.2% (8)	0.58 (0.14 – 2.49)	NR	

387

388 ^aUnadjusted odds ratio.

389 ^bOdds ratio from multivariable model adjusted for sex and age and all exposures that have been previously reported to be
390 associated with giardiasis and showed a significant association ($p < 0.05$) in the univariate model.

391 ^cOdds ratio from multivariable model adjusted for sex and all exposures that have been previously reported to be associated
392 with giardiasis and showed a significant association ($p < 0.05$) in the univariate model.

393 ^dNR not reported and / or calculated.

394