Article

Epidemiology and geographical distribution of enteric protozoan infections in Sydney, Australia

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Significance for public health

This research is significant since it provides the most recent epidemiological update on the common enteric protozoa affecting Australians. It reveals that enteric protozoa cause considerable disease burden in high risk city dwellers, and provides the evidence base for development of targeted interventions for their prevention and control in high risk populations. The prevalence of enteric protozoa in this metropolitan setting underscores that microorganisms do not respect borders and that a collaborative approach is needed to contain the global spread of infectious diseases. Incorporating spatial analysis is valuable in providing a compelling picture of the geographical distribution of these often neglected diseases. Local and State Public Health departments can use this information to support further investigations into the risk factors for giardiasis in regional/rural areas is needed.

Abstract

Background. Enteric protozoa are associated with diarrhoeal illnesses in humans; however there are no recent studies on their epidemiology and geographical distribution in Australia. This study describes the epidemiology of enteric protozoa in the state of New South Wales and incorporates spatial analysis to describe their distribution.

Design and methods. Laboratory and clinical records from four public hospitals in Sydney for 910 patients, who tested positive for enteric protozoa over the period January 2007 - December 2010, were identified, examined and analysed. We selected 580 cases which had residence post code data available, enabling us to examine the geographic distribution of patients, and reviewed the clinical data of 252 patients to examine possible links between protozoa, demographic and clinical features.

Results. Frequently detected protozoa were *Blastocystis* spp. (57%), *Giardia intestinalis* (27%) and *Dientamoeba fragilis* (12%). The age distribution showed that the prevalence of protozoa decreased with age up to 24 years but increasing with age from 25 years onwards. The geographic provenance of the patients indicates that the majority of cases of *Blastocystis* (53.1%) are clustered in and around the Sydney City Business District, while pockets of giardiasis were identified in regional/rural areas. The distribution of cases suggests higher risk of protozoan infection may exist for some communities.

Conclusions. These findings provide useful information for policy makers to design and tailor interventions to target high risk communities. Follow-up investigation into the risk factors for giardiasis in regional/rural areas is needed.

Introduction

Several species of enteric protozoa are associated with diarrhoeal illnesses in humans. Some cause severe debilitating conditions in immunosuppressed and immune-competent populations.[1] Protozoan related morbidity and mortality in humans worldwide is well documented. However, little attention has been paid to human infections in developed countries, where the risk of transmission is presumed to be low.[2] Focus is usually placed on opportunistic infections with protozoa such as *Cryptosporidium* spp., *Cyclospora cayetanensis* and *Cystoisospora beli*, implicated in prolonged diarrhoea in immunosuppressed patients, such as those with HIV/AIDS, compensated idiopathic hepatic cirrhosis, protein energy malnutrition, and Hodgkin's lymphoma.[3,4] However, in addition to others such as *Giardia intestinalis* (also known as *G. duodenalis*), *Dientamoeba fragilis* and *Entamoeba histolytica* these protozoa contribute to a variety of acute, persistent and chronic gastrointestinal illnesses in immune-competent persons.[3,5,6] Protozoan related morbidity and mortality in humans worldwide is well documented. However, little attention has been paid to human infections in developed countries, where the risk of transmission is assumed to be low.[2,7] Several species of enteric protozoa exist in Australia, with some being endemic. In (the State of) New South Wales, Australia, infectious disease surveillance captures only two protozoan infections: giardiasis and cryptosporidiosis. Once a diagnosis has been confirmed, public health units must be notified to take preventative actions.[8] Pathogenic protozoa such as *Cyclospora* and *Entamoeba histolytica* are less prevalent, being usually associated with travel to developing regions.[5,9]

Blastocystis spp. is the most common protozoan diagnosed in developed countries, although its role in eliciting gastrointestinal pathology and symptoms remain uncertain and controversial.[10,11] Clinical features of illness which have been attributed to *Blastocystis* spp. include nausea, anorexia, abdominal pain, flatulence and acute or chronic diarrhoea.[7,10,11] It is often associated with chronic gastrointestinal illness of unknown aetiology and with irritable bowel syndrome (IBS)-like symptoms.[7,12] Some clinicians consider the identification of *Blastocystis* from patient stools only as a potential marker of exposure

to faecal contamination and not necessarily the cause of specific pathology.[13]

Cryptosporidium spp., accounts for about 20% of diarrhoeal episodes in children in developing countries, but <9% in developed settings.[14] Infections are usually characterised by self-limiting diarrhoea associated with severe abdominal pain in immunosuppressed and immunocompetent persons, especially those HIV-infected and children worldwide.[3,6] In developed countries transmission occurs from person-to-person, especially in day care settings and between men who have sex with men (MSM), as well as through water borne and zoonotic infections.[6,15]

Cyclospora cayetanensis has emerged as an important cause of endemic or epidemic diarrhoeal illness in children and adults worldwide. Clinical illness is characterised by persistent diarrhoea, bloating, flatulence, abdominal cramps, constipation, and fatigue.[6] Cycloisosporiasis is a common cause of illness amongst returned international travellers,[5,9] although non-travel and water-borne related cases and food-borne outbreaks have been reported in developed countries.[16,17] The pathogenicity of *Dientamoeba fragilis* has been widely debated.[3,18] Infection can be acute or chronic, and symptomatic patients exhibit abdominal pain, persistent diarrhoea, loss of appetite, weight loss and flatulence, as well as IBS like symptoms.[18] Studies have found *D. fragilis* to be of similar or greater prevalence when compared with *Giardia.*[7]

Giardiasis, caused by the protozoon *Giardia intestinalis* (*aka G. lamblia*), has been reported in both animals and humans, being particularly common in infants, young children and young adults. Symptoms include diarrhoea, stomach cramps, bloating, nausea, fatigue, and if chronic, weight loss.[19] The faecal-oral route is the most important mode of infection, and various studies have found evidence of zoonotic transmission.[20] In Australia giardiasis is frequently associated with waterborne infections, day care centre disease outbreaks, and travel-associated diarrhoea.[21]

Protozoa are often found as co-infections with enteric bacteria and other parasites, particularly in developing settings and in outbreaks.[22,23] Co-infection with multiple pathogens is usually an indication of exposure to sources contaminated with animal and or human excreta through a variety of routes.[24,25]

Geographical information systems (GIS) have emerged as important tools to improve quantification and understanding of spatial variation in disease risk and spread.[26] Disease maps are useful tools for understanding the distribution of the disease incidence, identification of underlying geographical risk factors, and enabling rapid decision making and response for the containment, management and eradication of infectious diseases.[27] However, very few studies have incorporated spatial tools for enteric protozoa, with little or no evidence of this from Australia. Geographical, environmental and other socio-cultural differences among the populations across Sydney, may impact on the distribution of cases of enteric protozoan infections. However no scientific assessment or epidemiological studies of the prevalence of enteric protozoa and their geographical distribution has been done in Australia.

The aim of this study was to provide a detailed description of i) the epidemiology and ii) geographical distribution of enteric protozoa infections in the Greater Sydney Metropolitan Area. Laboratory and clinical data were used to describe the demographic and clinical features and to map the geographical distribution of enteric protozoa detected in patients seen at four major public hospitals in Sydney: Liverpool Hospital (Hospital A); Children's Hospital at Westmead (Hospital B); St. Vincent's Hospital, Sydney (Hospital C); Prince of Wales Hospital (Hospital D). Symptomatic patients seen in these hospitals come from across the Sydney Region, including persons within the Sydney metropolitan area as well as cases referred to these hospitals from all over the State of New South Wales (NSW). The NSW Public Health Services, divided into eight rural and metropolitan Area Health Services, have responsibility for hospitals, clinics, community health centres and support programs in their respective area. Four hospitals located in Metropolitan Sydney, including two of thirteen principal referral hospitals for adults, one major public hospital and the largest stand-alone paediatrics hospital State-wide, were included in this study. Hospitals A and D captured both adults and children; Hospital B captured paediatric cases state-wide and only adult cases (≥15 years) were included from Hospital C. Clinical laboratories within all four Hospitals provide laboratory services for smaller hospitals within their respective Area Health Service, and for some rural health services in the Newcastle, Illawarra and Hunter regions and therefore captures a wide cross section of the NSW State population. Hence the data linked to patent's illness histories should reflect an unbiased picture of the distribution of cases across NSW State. In asymptomatic protozoan infections, the likelihood of patients reporting to hospitals is low, and reporting to hospital for a microbiological test would be strongly influenced by the location of the hospitals, and whether or not testing facilities are conveniently located in relation to their daily activities. Obtaining clinical information from comparable asymptomatic cases and or a control group proved difficult in this setting, and hence only symptomatic cases were analysed and discussed in this study. The data represents cases seen over the period 2007-2010.

Design and methods

Ethical approval for this study was received from the Human Research Ethics Committees (HREC) for each of the four Hospitals, and the University of Technology, Sydney (UTS) and was guided by the Australian National Statement on Ethical Conduct of Research involving humans. The data presented represent a subset of data from a larger study investigating the prevalence of gastrointestinal pathogens in patients seeking care in Sydney. Laboratory and clinical records were identified for all persons who had a stool specimen positive for enteric protozoa over the period January 2007 – December 2010. Of the 25,914 stool specimen tested for enteric protozoa, there were 910 individual cases positive for one or more enteric protozoa across the four centres. Hospital ethical guidelines prohibited the collection of personal identifiers, excepting for postal code of the patients' residences. The postal code of residence for each patient was sourced for 580 cases, and was

Table 1. Distribution of specimen tested and proportion of cases positive reviewed in the four study sites.

Hospital laboratory	Stool specimen tested	Positive for protozoa % (n)	Medical records reviewed % (n)
Hospital A	2138	8.8 (187)	46 (85)
Hospital B	10,123	1.2 (118)	67 (81)
Hospital C	7575	6.9 (525)	13 (70)
Hospital D	6078	1.3 (80)	20 (16)
Total	25,914	3.5 (910)	27.7 (252)

used as the spatial location identifier for cases.

The patients were classified based on gender, age group and species of protozoa. To provide an epidemiological picture of protozoan infections in Sydney, we reviewed the clinical data for a subset of 252 cases (28% of 910 positive cases) who presented with watery, liquid or loose stool specimens. These include 85 (34%) from Hospital A; 81 (32%) cases from Hospital B; 70 (28%) from Hospital C; and 16 (6%) from Hospital D as presented in Table 1.

Microbiological methods

Laboratory diagnoses were performed using standard quality controlled procedures in the National Association of Testing Authorities, Australia (NATA) accredited laboratories at the four Sydney Hospitals. Each laboratory tested on average one stool sample per patient using microbiological procedures previously described.[28,29]

All hospitals routinely tested for both viral and bacteriological pathogens when patients present with gastrointestinal symptoms. Bacteriology and virology studies were done on stool specimen using standard methods.

Parasitology

All hospitals processed stools by a wet preparation in saline, and examined for white blood cells, red blood cells and cysts, ova and parasites (COP).[30,31] Direct microscopy was routinely performed on all stool specimens for the detection of COP, and concentration techniques performed on request at Hospitals A, B and D, and routinely at Hospital C. In order to detect COP, an aliquot of faeces was emulsified in sodium acetate acetic acid formalin (SAF) fixative and processed for permanent staining by modified iron haematoxylin staining technique (to identify cysts and trophozoites)[28] and formal ethyl acetate concentration (for the identification of helminths and ova).[32]

Specifically, when a COP test was requested and if any parasites were seen in the wet preparation, a sample of stool was placed into SAF fixative (Oxoid, Australia) using a 1:5 ratio and processed for faecal concentration and stained using the Faecal Parasite Concentrator (FPC) (Evergreen Scientific, LA, CA, USA) which uses centrifugation at 500 g ×10 minutes and examined for COP using oil immersion (Hospital B). If the specimen was not received in SAF (Hospitals A and D only), then an enzyme immunoassay (EIA) screen was performed for the detection of *Giardia/Cryptosporidium* (ProSpecT™ *Giardia/Cryptosporidium* Microplate Assay) and *Entamoeba histolytica/dispar* (ProSpecT™ *Entamoeba histolytica*, Remel). A 10% suspension of stool was prepared in 10% formalin (for *G. intestinalis* and *Cryptosporidium*) and the EIA was performed in accordance with the manufacturer's instructions and without modification. All positive EIA findings were confirmed by microscopy (*i.e.* iodine preparation and acid fast stain). *Cryptosporidium* smear was alternatively done using a Modified Kinyoun's Acid-Fast Stain (Cold) at Hospital B. In some cases, the fixed smear was prepared for permanent staining by iron haematoxylin staining (IHS) with modified acid fast stain (Hospitals A and C). Additionally at Hospital C, the wet preparation was examined under a low power objective (10×) and then scanned under the high dry (40×) objective. All stool specimens were emulsified in SAF fixative (Oxoid Australia) using a 1:3 ratio, then was centrifuged at 500 g for 10 minutes. Samples were then processed for permanent staining by a modified iron haematoxylin staining (mIHS) technique incorporating a carbol fuschin step to stain for acid fast organisms (*Isospora*, *Cryptosporidium* and *Cyclospora*).[28] Polymerase chain reaction (PCR) was employed where stool samples underwent direct DNA extraction using a QIAamP DNA stool minikit (Qiagen, Hilden, Germany) using a portion of fresh stools sample for the identification of *Entamoeba* spp. These methods have been previously described by Stark and colleagues.[31,33,34]

Data description and analysis

While some of the hospitals employed different testing algorithms, all hospitals used accredited standard, quality controlled procedures for the detection of enteric protozoa. We thus considered that merging of the data from the four hospitals into a single database was reasonably justified in order to explore the geographic distribution and epidemiological history of the patients. Going forward therefore, the data are analysed and discussed without further distinction in regards to the hospitals in which they were generated.

The results of the microbiology tests were de-identified and entered in a database including: post-code of residence of patient; age; gender; species of protozoa diagnosed.

Frequencies and means of basic demographic, clinical and laboratory findings for cases were described. Pearson's chi-squared (χ^2) analysis and Spearman correlation (r) were used for correlation analysis test associations between non-parametric relationships. The associations between protozoa detected and gender, age, symptoms and post code of residence were examined by binary logistic regression analysis to calculate odds ratios and 95% confidence intervals. *Blastocystis* was selected as the reference protozoan as it was the most common protozoan detected and not routinely considered as pathogenic. Hospital C was selected as the reference facility because it tested for all protozoan species. The 0-5 age group was selected as the reference age group for convenience and Sydney region was selected as the reference region, as the majority of cases came from there. All post codes were categorized based on districts in New South Wales (NSW) (http://www.homehelp4u.net/postcode_tool/postcode_list_NSW.php), and the districts were listed in ascending order based on the grouping of post codes. The IBM SPSS Statistic version 19 (SPSS Inc. Chicago, IL, USA) was used for data analysis.

To display and analyse the geographic distribution of the patients as a base map of the Australian postal areas (POA) 2006 Digital Boundaries (ESRI shape file) from the Australian Bureau of Statistics archive (http://www.abs.gov.au/) was used. The commercial software ArcGIS Desktop (version 9.3) was used to extract the postcodes for the state of NSW, and joined by the postcode fields to the hospital data to generate a table of attributes for the extracted layer. We queried this layer to produce derivative layers of the distribution of individual parasites across the state by Postcode. The derivative layers were then used to construct the maps shown in Figures 1 and 2.

Results

Clinical and epidemiological profile

The demographic and clinical features of cases are presented in Table 2. There appears to be no identifiable distinction between genders in the incidence of pathogens. However in the 25-75 years age groups there were more males than females [χ^2(df) 11.5(4); P=0.04] (Figure 3). A bimodal age distribution was observed with the prevalence of protozoa decreasing with age in the 24 years and under age group, and increasing with age in the over 25 years age groups. Watery diarrhoea (82%), abdominal pain (48%) and vomiting (45%) were the most frequent symptoms reported by patients presenting at each hospital (Table 2).

The age specific distribution of the cases based on clinical symptoms and protozoa detected is presented in Table 3. Vomiting [χ^2=15.6(4); P=0.004] and fever [χ^2=14.2(4); P=0.007] were more common in younger patients, compared with nausea in older persons [χ^2=32.9(4); P=0.001].

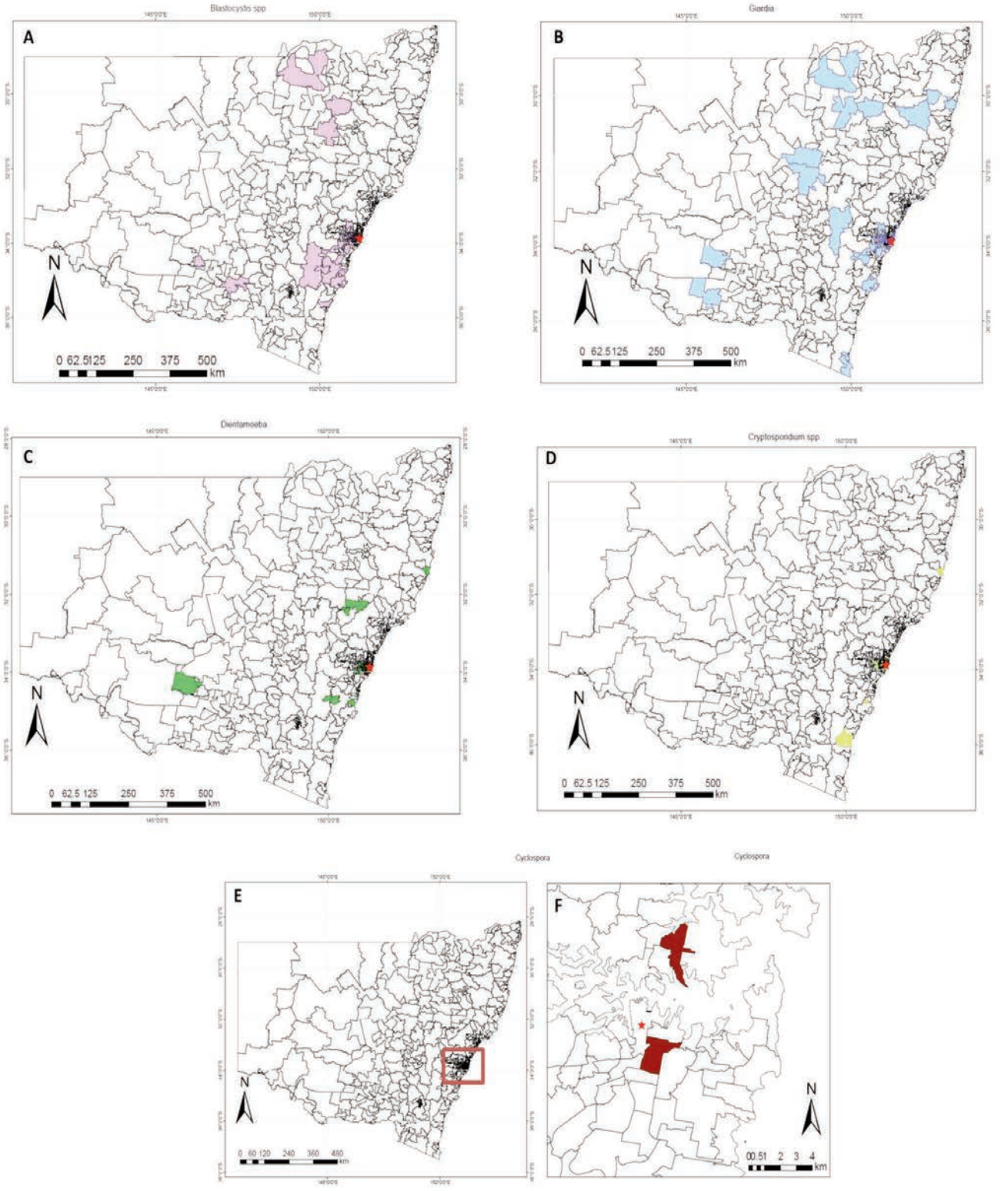

Figure 1. Map of New South Wales showing distribution of cases affected by the five enteric protozoa *Blastocystis* (A), *Giardia* (B), *Cryptosporidium* (C), *Dientamoeba* (D), and *Cyclospora* (E). Note the change of scale in (F) zooming in on *Cyclospora* cases in Sydney City Business District.

Enteric protozoa were identified in an average of 4% (95%CI 1.1-11.2%) of 980 specimens which tested positive from all hospitals. Overall, the most common enteric protozoa detected were *Blastocystis* spp., in 5% (95%CI 5-6%) of cases, followed by *G. intestinalis* (1%; 95%CI 1-1.2%), *D. fragilis* (1%, 95%CI 0.7-1%) and *E. histolytica/dispar* in 0.5% (95%CI 0.4-0.6%). On average, between 1% and 9% of cases from each hospital were infected with enteric protozoa (Table 1). Of the 580 cases included in the review, a co-infecting organism was reported in 23% (132); including infectious bacteria in 30% (39), pathogenic protozoa (including *Blastocystis* spp.) in 39% (52), non-pathogenic protozoa in 24% (31) and enteric viruses in 7% (9) (data not shown). Amongst cases with gastrointestinal symptoms *Blastocystis* spp. (61%),

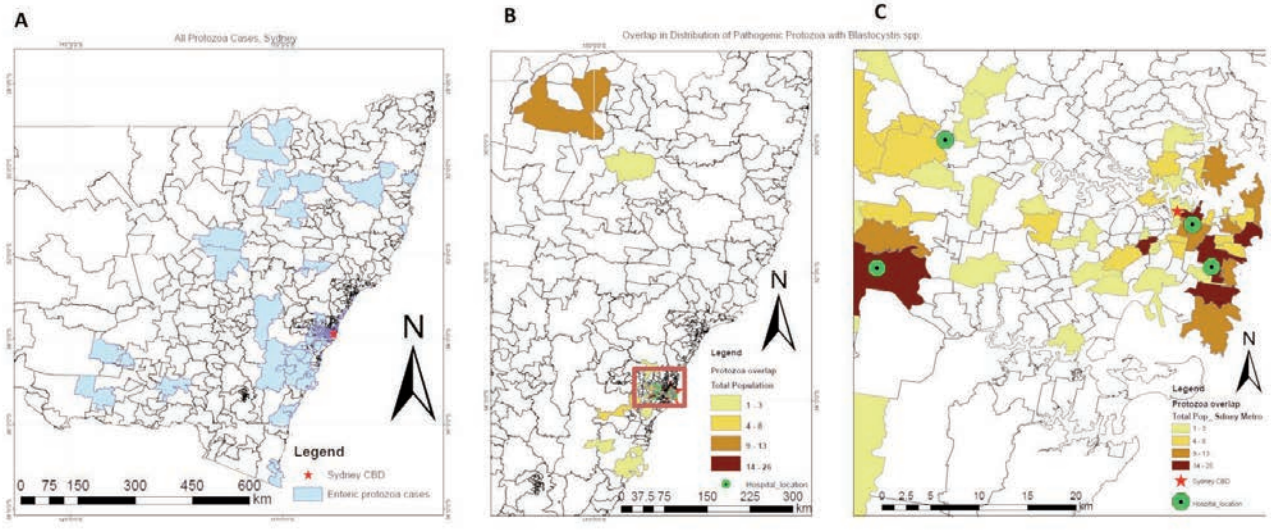

Figure 2. Distribution of cases of protozoa, and location of co-infections with *Blastocystis* spp and infectious protozoa, based on New South Wales Post Codes. The map illustrates (A) the total protozoa detected across the State from four hospitals; (B) where pathogenic protozoa cases overlap with cases of *Blastocystis* infection. Note change of scale in (C) showing a zooming in on dense cases in the Sydney City Business District.

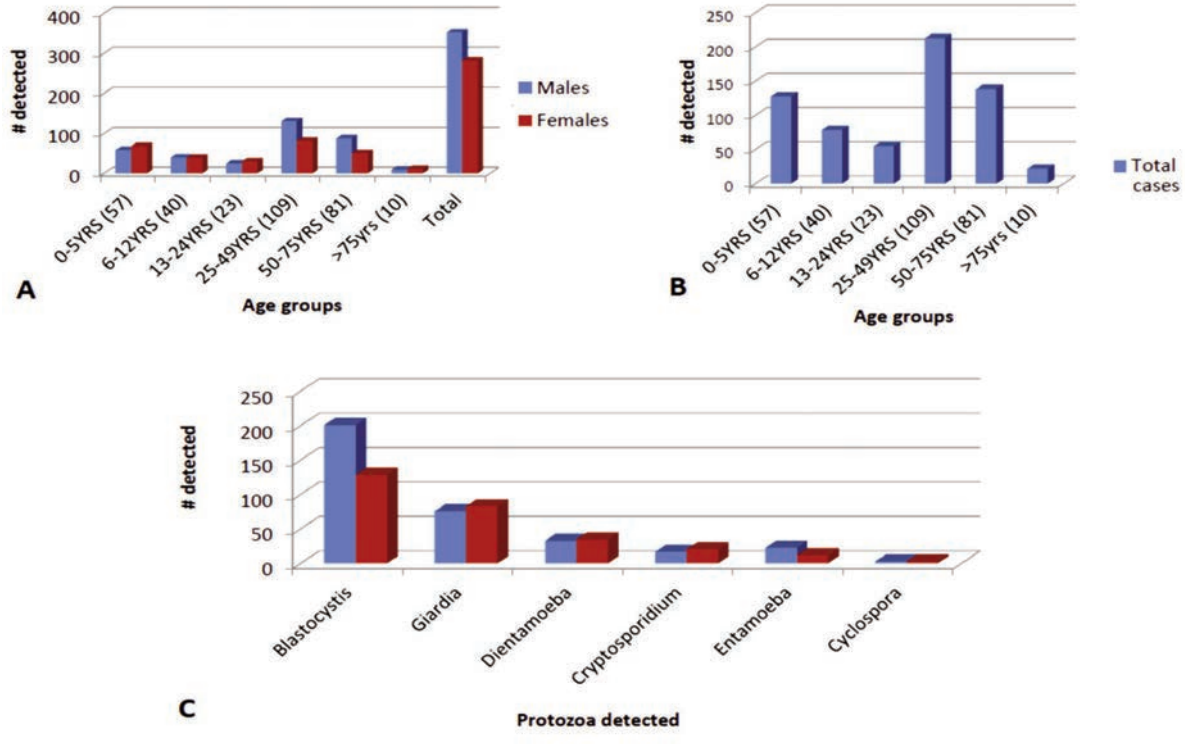

Figure 3. Distribution of protozoa cases based on age and gender (A), age groups (B), and gender and protozoa detected (C). Numbers in brackets represent the number of cases in each category. There were co-infections with two or more protozoa in some cases.

Giardia intestinalis (22%), *Dientamoeba fragilis* (13%), *Cryptosporidium* spp. (7%), and *Entamoeba* spp. (4%) were frequently detected. The incidence of *Blastocystis* spp., increased with age, with persons over 50 years of age having the highest incidence, compared with higher incidence of *Cryptosporidium* spp. [χ^2=11.2(4); P=0.025] and *Giardia* in the under 13 years age groups [χ^2=45.1(4); P=0.001]. The incidence of *Dientamoeba fragilis* also increased with age, and was mainly evident in the under-25 age groups [χ^2=19.5(4); P=0.001].

Diarrhoea was reported by the majority of persons infected with *D. fragilis* (91%), *Cryptosporidium* spp. (88%), *Giardia* (82%) and *Blastocystis* spp. (81%). Vomiting was significantly associated with *Giardia intestinalis* infection [58%; χ^2=4.5 (1); P=0.046]. Several of the cases (30/250) reported overseas travel in the past 6 weeks, with 50% being to Asia (mainly the Indian sub-continent), 23% to South Pacific Islands, 13% to Africa and two each to Europe and North America (data not shown).

Table 2. Demographic and clinical characteristics of diarrhoeal cases by Hospitals.

Characteristic	Hospital A, % (n)	Hospital B, % (n)	Hospital C, % (n)	Hospital D, % (n)	Total % (n)
Age group, years					
0-5	3.5 (3)	43.2 (35)	0 (0)	56.3 (9)	18.7 (47)
6-12	5.9 (5)	38.3 (31)	0 (0)	12.5 (2)	15.1 (38)
13-24	3.5 (3)	18.5 (15)	8.6 (6)	0 (0)	9.5 (24)
25-49	34.1 (29)	0 (0)	48.6 (34)	25.0 (4)	26.6 (67)
50-75	52.9 (45)	0 (0)	42.9 (30)	6.25 (1)	30.2 (76)
Gender					
Male	52.9 (45)	50.6 (41)	71.4 (50)	56.3 (9)	57.5 (145)
Female	47.1 (40)	49.4 (40)	28.6 (20)	43.8 (7)	42.5 (107)
Symptoms					
Diarrhoea	73.8 (62)	82.7 (67)	95.7 (67)	62.5 (10)	82.1 (206)
Vomiting	44.0 (37)	61.7 (50)	28.6 (20)	50.0 (8)	45.8 (115)
Nausea	35.7 (30)	9.9 (8)	44.3 (31)	12.5 (2)	28.3 (71)
Abdominal pain	57.1 (48)	44.4 (36)	44.3 (31)	31.3 (5)	47.8 (120)
Fever	29.8 (25)	48.1 (39)	25.7 (18)	3.5 (6)	35.1 (88)
Dehydration	15.5 (13)	13.6 (11)	8.6 (6)	31.3 (5)	13.9 (35)
Anorexia/loss of appetite	15.3 (13)	24.7 (20)	7.1 (5)	6.3 (1)	15.5 (39)
Lethargy	14.1 (12)	28.4 (23)	11.4 (8)	18.8 (3)	18.3 (46)
Respiratory symptoms	3.6 (3)	17.3 (14)	1.4 (1)	12.5 (2)	8.0 (20)
Total per hospitals	33.7 (85)	32.1 (81)	27.8 (70)	6.4 (16)	100.0 (252)

Variables coded as dichotomous variable with: diarrhoea, vomiting, nausea, abdominal pain, fever, and dehydration- each coded as No=0, Yes=1; Anorexia, lethargy, and respiratory symptoms each coded as No=1, Yes=2; *Blastocystis*: No=1, Yes=2; *Cryptosporidium*: No=1, Yes=2; *Dientamoeba fragilis*: No=1, Yes=2; *Entamoeba* species: No=1, Yes=2; *Giardia*: No=1, Yes=2.

Table 3. Age specific distribution of cases based on demographic, clinical signs and protozoa detected.

Responses	0-5 yrs	6-12 yrs	13-24 yrs	25-49 yrs	50-75 yrs	% total cases (n)	χ^2 (df); P value
Female	10.3 (26)	7.5 (19)	5.2 (13)	34.3 (23)	10.3 (26)	42.5 (107)	
Male	8.3 (21)	7.5 (19)	4.4 (11)	17.5 (44)	19.8 (50)	57.5 (145)	9.34 (4); 0.053
Diarrhoea	80.9 (38)	76.3 (29)	91.7 (22)	77.6 (52)	86.7 (65)	82.1 (206)	4.39 (4); 0.356
Vomiting	66.0 (31)	50.0 (19)	58.3 (14)	32.8 (22)	38.7 (29)	45.8 (115)	15.56 (4); 0.004
Fever	51.1 (24)	47.4 (18)	33.3 (8)	32.8 (22)	21.3 (16)	35.1 (88)	14.20 (4); 0.007
Abdominal pain	14.9 (7)	71.1 (27)	66.7 (16)	62.7 (42)	37.3 (28)	47.8 (120)	41.30 (4); 0.001
Dehydration	14.9 (7)	7.9 (3)	20.8 (5)	13.4 (9)	14.7 (11)	13.9 (35)	2.19 (4); 0.701
Lethargy	34.0 (16)	18.4 (7)	20.8 (5)	16.4 (11)	9.2 (7)	18.3 (46)	12.28 (4); 0.015
Anorexia	23.4 (11)	23.7 (9)	25.0 (6)	7.5 (5)	10.5 (8)	15.5 (39)	10.59 (4); 0.032
Respiratory symptoms	21.3 (10)	13.2 (5)	4.2 (1)	1.5 (1)	4.0 (3)	8.0 (20)	18.66 (4); 0.001
Nausea	2.1 (1)	10.5 (4)	41.7 (10)	41.8 (28)	37.3 (28)	28.3 (71)	32.93 (4); 0.001
Blastocystis spp.	5.9 (9)	9.8 (15)	11.1 (17)	31.4 (48)	41.8 (64)	61.0 (153)	62.46 4; 0.001
Giardia	45.5 (25)	23.6 (13)	1.8 (1)	16.4 (9)	12.7 (7)	21.9 (55)	45.05 (4); 0.001
Dientamoeba fragilis	18.8 (6)	21.9 (7)	28.1 (9)	9.4 (3)	21.9 (7)	12.7 (32)	19.46 (4); 0.001
Cryptosporidium spp.	44.4 (8)	16.7 (3)	5.6 (1)	27.8 (5)	5.6 (1)	7.2 (18)	11.15 (4); 0.025
Entamoeba spp.	11.1 (1)	0 (0)	0 (0)	66.7 (6)	22.2 (2)	3.6 (9)	8.35 (4); 0.080

Pearson's Chi squared test: diarrhoea vomiting, nausea, abdominal pain, fever, and dehydration, each coded as dichotomous variable with No=0, Yes=1; Anorexia, lethargy, and respiratory symptoms each coded as No=1, Yes=2; *Blastocystis*: No=1, Yes=2; *Cryptosporidium*: No=1, Yes=2; *Dientamoeba fragilis*: No=1, Yes=2; *Entamoeba* species: No=1, Yes=2; *Giardia*: No=1, Yes=2.

Spatial distribution

The post-code distribution of patients is shown in Figure 1. Some interesting patterns emerge. Infection with *Blastocystis* spp., the most commonly detected protozoa, was focused in the Metropolitan Area, with additional clusters in the Southern Coast and Hunter New England Area. The distribution of *G. intestinalis* had some overlaps with *Blastocystis* spp., except that additional cases were also identified in the Central Tablelands, North West and Murray regions. *Dientamoeba fragilis, Cryptosporidium* spp., and *Entamoeba* spp., had a less wide distribution.

Based on its high prevalence in the population and controversy surrounding the pathogenicity of *Blastocystis* spp., the distribution of the other known pathogenic protozoa in relation to the distribution of *Blastocystis* spp. was further examined. Figure 2 illustrates the distribution of four known pathogenic protozoa: *G. intestinalis, Cryptosporidium* spp., *D. fragilis,* and *Cyclospora,* in areas where infections coincided with infections with *Blastocystis* spp. The figure shows that overlaps in the distribution of cases were mainly distributed around the Sydney Metropolitan Area, with other clusters identified in the Hunter New England Health Area [Post Code 2400 (4 *Giardia* and 7 *Blastocystis* from 11 cases); and Post Code 2347 (2 *Giardia* and 1 *Blastocystis* from 2 cases)] and the Southern Coast: [two cases identified in PC 2540 (*Blastocystis* and *G. intestinalis*), two in 2535 (*Blastocystis* and *D. fragilis*) and two in PC 2579 (*Blastocystis* and *D. fragilis*)]. There was less overlap in the distribution of protozoa in the Western, Southern and Northern areas of the State, and a few cases scattered in hinterland areas.

An examination of the relationships between different protozoa revealed that there was a weak positive correlation between region of residence and infection with *Giardia intestinalis* (0.148; P=0.001) and a weak negative relationship with *Blastocystis* (−0.130; P=0.002). While not statistically unequivocal, the evidence suggests that future studies should set out to test the possibility that infection with *Giardia intestinalis* may be more common in persons living further away from the Sydney City Business District (CBD), compared with *Blastocystis* infections having a stronger association with living close to or within the CBD. A closer look at the relationship between these two protozoa reinforces this interpretation, because there is a strong negative relationship between the distribution of *G. intestinalis* and *Blastocystis* (Spearman's R: −0.545; P=0.0001). This suggests that there were significantly more cases of *Blastocystis* infections in areas where *Giardia intestinalis* infections were absent. No other significant relationships were observed in the distribution of the other enteric protozoa.

Discussion

This multi-centre study presents the first detailed description of i) the epidemiology and ii) geographical distribution analysis of patients with gastrointestinal symptoms and infected with enteric protozoa in New South Wales, Australia. The study found that enteric protozoa prevalence is age related and there is a possible association between the prevalence of individual protozoan species and geographic distribution of cases.

The age distribution of enteric protozoa reveals that in this population, persons under 13 years old had a higher incidence of infection with *Cryptosporidium* spp. and *Giardia*. These infections in younger children are consistent with current knowledge, especially since those in the under-five age group have poorly developed hygiene habits and are more susceptible to enteric infections [35]

Both *Cryptosporidium* spp. and *Giardia* are considered to be neglected tropical diseases (NTDs), infections commonly affecting the world's poorest people, including the *bottom billion*, (approximately 1.4 billion people living on no money), who are affected by one or more NTDs.[36] These diseases result in high levels of global disability and destabilization of communities and tend to trap people in poverty through their adverse effects such as the impairment of child development, pregnancy outcomes, reduced agricultural productivity and food security.[36] Infections with NTDs in Australia can disproportionately affect indigenous populations,[37] however infections such as giardiasis and cryptosporidiosis have been reported amongst non-Aboriginal urban dwellers in outbreak and non-outbreak settings.[15,37]

Nearly one in every five protozoa detected had a co-infecting organism, with the majority of co-infections being with either pathogenic protozoa (39%) or infectious enteric bacteria (30%). Co-infection with multiple parasites is not uncommon and has been widely found and is likely an indication of transmission via the faecal oral route through contaminated food or water or other unhygienic practices.[7,22,23]

A specific trend was observed for the 25-49 years age group around the Sydney Central Business District (CBD) (R=0.09; P=0.025). In this location, more men were infected with protozoa than women, against a general state-wide statistics in which no observable large scale difference between genders was observed. We suggest that this may be due to the presence of a high risk population of MSM residing in and seeking care in the CBD. Data on MSM status was unavailable for some hospitals, although previous studies showed 52% of stool specimens from MSM were positive for protozoa compared with 13% from non-MSM.[15]

The relatively high prevalence of *Blastocystis* spp. is not surprising considering its ubiquitous nature and usual high prevalence.[7,15] It is interesting that all clinical laboratories involved in this study routinely test for this protozoan, and despite controversies about its role as a pathogen, it is usually used as a marker/indicator of exposure to faecal contamination, and can raise enough suspicion to look for other recognized pathogens. More than 10% of cases reported overseas travel – mainly to Asia, which is a major risk factor, and important indicator for protozoan infection monitoring in developed settings.[5,9,38]

Sufficient data was unavailable to explain the incidence of *Giardia* infections in the Southern Coast and Hunter New England areas. It is possible that these are linked to environmental sources of infection (water, sewage), but further investigations in these areas is warranted to establish conclusively the causes of infection and to suggest possible control strategies. However, that the data reveals an increase in the cases of *Giardia* over the March to May period (Southern hemisphere autumn) during the four year period of 2008-2012, which was consistent with State infectious disease surveillance data for this period.[39] In addition, the NSW State Bureau of Meteorology reported that in 2010, NSW experienced the wettest autumn since 2000, and, north-western NSW rivers were awash with floodwaters, following torrential rain in Queensland, during early March 2010. Furthermore, an East Coast Low brought heavy rainfall to most southern regions at the end of May 2010.[40] These phenomena could have resulted in contamination of drinking water supplies in the affected areas. The affected areas would include the Southern Coast and Hunter New England areas where the clustering of cases outside of the CBD has been observed. A Brazilian study however, found no associations between Giardiasis and seasonality or rainfall.[41] In the absence of a clear explanation for the cases of giardiasis reported from these areas, there is a need for further studies to understand the relationships observed.

A 2004 report indicated that Giardiasis is a major public health problem in the New England Area, and may be associated with drinking water from rain-water tanks.[42] The report indicated that these infections occurred in an area in close proximity to Aboriginal (indigenous) settlements. Giardiasis infections among Aboriginal communities are considered to be common especially in children under five years of age.[43] However, the Aboriginal status of these cases could not be ascer-

tained. If communities in these regional areas of Sydney are faced with increased risk of giardiasis infections, there is therefore need for rural hospitals to have the diagnostic capacity for the timely diagnosis of enteric protozoa. In addition, water authorities should ensure frequent monitoring of water quality for enteric protozoa.

The study also revealed that infections with *G. intestinalis* were more likely to be detected in persons who were not infected with *Blastocystis.* This could be an indication that *Blastocystis* spp. were in fact the actual cause of gastrointestinal symptoms or that patients were infected with enteric pathogens that were not detected by microbiological tests. Wet preparation and concentrates are routinely used in majority of hospitals to detect *Blastocystis* spp., but have limited sensitivity compared with microscopy of fixed, permanently stained (modified iron-haematoxylin, trichrome staining) smears for the detection of some protozoa.[28] Antigen detection by immunoassays[44,45] and PCR have demonstrated higher sensitivities when compared with microscopy and permanent staining, but are more costly and not widely used in this setting.[12,28,31] The submission of (up to three) repeat stool specimen at daily intervals has been widely considered standard for improved detection of enteric protozoa, due to intermittent shedding of trophozoites in stool,[28,46] but compliance with the submission of repeats specimen is not consistently done in Sydney and is often not realistic owing to the distances people need to travel to health facilities for diagnostic tests. Hence the use of retrospective data to identify possible geographic trends is valuable for the development of public health preventative measures that are likely to have more long term impact in managing protozoan infection risk, than the testing procedures for sick patients.

Whilst this study did not set out to investigate antimicrobial sensitivity patterns for common enteric protozoa, clinical and in vitro studies conducted by other members of this research cluster have revealed increasing ineffectiveness of commonly recommended antiprotozoal compounds against *Dientamoeba fragilis*, including Iodoquinol, Paromomycin, and Tetracycline.[34,47] Treatment failure and resistance to Metronidazole and Iodoquinol has been increasingly being reported amongst *Blastocystis* spp.[48,49] Newer antiprotozoal compounds, such as 5-nitroimidazoles, ornidazole and ronidazole which are believed to have fewer adverse effects on the patient, have proven to be more effective in treating *D. fragilis* in vitro and should be considered as suitable alternatives to current drugs. Further studies are needed to determine the level of antiprotozoal resistance amongst common infectious enteric protozoa at the community level.

Conclusions

There are no current national or state-wide estimates which describe the epidemiology of enteric protozoa in Australia. This is the first study to incorporate geographic analysis to define the epidemiology of enteric protozoa in New South Wales State, since the last 20 years. The findings suggest a widespread distribution of several infectious protozoa and indicate the need for further studies to estimate the prevalence and burden of enteric protozoa infections at a national level. Understanding the geographical distribution of patients diagnosed with enteric protozoan infections is beneficial for identifying areas with unusually high rates, early detection of outbreaks, and informing planning and implementation of public health interventions. This information is also useful to inform the public of where potential risks may exist so they can take the necessary precautions, such as attending to hand and personal hygiene and the boiling of rainwater to be used for drinking. This study has identified several enteric protozoa species with substantial prevalence in Sydney, with the very young and aging persons being more susceptible to infection. This highlights the need for public health interventions such as hand and personal hygiene messages to be reiterated especially amongst school age children and aged care settings. Spatial distribution suggests that underlying behavioural and geographical risk factors may be driving the prevalence in some areas. Further investigation needed to determine these risk factors. The findings of this study can provide useful information for policy makers to design and where possible, tailor interventions to target high risk communities.

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