# GASTROINTESTINAL ILLNESSES CAUSED BY MICROBES IN SYDNEY, AUSTRALIA

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# CERTIFICATE OF ORIGINAL AUTHORSHIP

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

…….  …….

Signature of Student  Date

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3. **Fletcher SM**, Stark D, Ellis J, Harkness J. 2010. Etiology of Gastrointestinal Illness in patients in Sydney. 74th Annual Educational Conference (AEC) & Exhibition 2010 June 6-9, 2010; 2010 June 6-9, 2010; Albuquerque, New Mexico, USA.: National Environmental Health Association, 720 S Colorado Blvd., Suite 1000-N, Denver, CO 80246-1926. (Oral)
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9. **Fletcher SM**, Stark D, Li Y, McLaws M-L, Ellis J. 2011. Prevalence of Gastrointestinal Pathogens in Developed and Developing Countries: a Meta-Analysis 7th World Congress of the World Society for Pediatric Infectious Diseases – WSPID 2011; 16-19 November, 2011; Melbourne, Australia: World Society for Pediatric Infectious Diseases. (Poster)

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# Executive Summary

Gastrointestinal illness (GIT) is a leading cause of preventable illness and death worldwide. Only a few studies provide estimates of the prevalence of pathogens associated with GIT illnesses in developed countries. In Australia, there are an estimated 17.2 million cases of GIT occur annually, however, the disease surveillance system only captures a few GIT illnesses, and the majority of pathogens causing illness are unknown. This study therefore seeks to fill this gap in knowledge by providing an overview of the epidemiology and prevalence of specific pathogens, clinical characteristics and risk factor for disease among hospitalised patients in Sydney, Australia.

Chapter One is a general overview which seeks to provide background and context for this study. The chapter sets the study in the Australian context in terms of the health system, infectious disease surveillance, health seeking behaviour and related determinants and also provides some perspective on why a hospital based study was conducted. An overview of the study sites is also provided.

In order to set the broad context for the topic, a systematic review of the literature is presented in Chapter Two. It provides age- and region-specific random-effect estimates of the detection rates of diarrhoeal pathogens in developed and developing settings. The review outlines that developing regions have significantly more pathogens detected than the OECD countries, and individual pathogen prevalence differs between developed and developing settings.

Chapter Three outlines a retrospective cross-sectional survey of laboratory and clinical records for patients seen at four major public hospitals in Sydney, from January 2007 to December 2010. The aim was to describe the clinical and epidemiological characteristics of GIT illnesses, in symptomatic patients presenting to hospital in Sydney. The chapter describes and discusses the prevalence of several viral, bacterial and protozoan pathogens that cause GIT illnesses in older children/adults and children, and associated risk factors.

In Chapter Four, a similar approach is used to describe the distribution of gastrointestinal pathogens in symptomatic children 0-5 years old in Sydney. It presents the clinical features and prevalence of pathogens associated with gastrointestinal illnesses for children 0-5 years old, presenting to two major public hospitals in Sydney with diarrhoea, for the period January 2007-December 2010.

An in-depth analysis of enteric protozoa was undertaken in Chapters Five and Six since enteric protozoa were frequently implicated in diarrhoeal illness and there is limited information about their epidemiology in Australia. Chapter Five evaluates the prevalence of enteric protozoa based on testing algorithms used to diagnose enteric protozoan infections in four hospitals, and suggest that a gold standard approach is needed. Chapter Six then incorporates spatial analysis to describe the epidemiology and geographical distribution of enteric protozoa in the state of New South Wales (NSW). Chapter Seven concludes with a discussion of the implications of the main findings and proposes recommendations to address them.

# 1 General Introduction

## Chapter Overview

Gastrointestinal illness (GIT) is a leading cause of preventable illness and death worldwide. Only a few studies provide estimates of the prevalence of pathogens associated with GIT illnesses in developed countries. Population based estimates indicate that approximately 17.2 million cases of GIT occur annually in Australia. However, the majority of pathogens causing illness are still unknown, and the disease surveillance system captures only a few pathogens. This hospital based study therefore seeks to address some of the gaps in knowledge by providing an overview of the prevalence of specific pathogens, clinical characteristics and associated risk factor for GIT amongst hospitalised patients in Sydney, Australia. This general overview chapter seeks to provide background and context for this study. The chapter sets the study in the Australian context in terms of the health system, infectious disease surveillance, health seeking behaviour and relate determinants and also provides some perspective on why a hospital based study was conducted. An overview of the study sites is provided.

## *1.1 Overview of the Health Status and Health Care System in Australia*

Australia is a federation of 6 states (New South Wales, Queensland, South Australia, Tasmania, Victoria and Western Australia) and 2 territories (the Australian Capital Territory and the Northern Territory). The Australian Bureau of Statistics estimated that at the end of September 2012, the Australian population was approximately 22.79 million, with 7.3 million living in the State of New South Wales, where this study was conducted (Australian Bureau of Statistics 2013). According to the 2011 census, approximately 4.61 million (64%) of the State's population resided in Greater Sydney (Australian Bureau of Statistics 2012).

The Australian health services is delivered through a network of primary care services and different types and levels of hospitals, classified by the Australian Institute of Health and Welfare (AIHW) as government delivered, mixed private and public services, and private sector services (Steering Committee for the Review of Government Service Provision 2010). In 2011–12, there were 1,354 hospitals in Australia, including 753 public acute hospitals accounting for 68% of hospital beds (58,420) and the remaining beds provided by 592 private hospitals (AIHW 2013b). Public hospitals dealt with under 8 million presentations to emergency departments in 2011–12, with an increase of about 8% and 6% of Emergency patients (clinical care is required within 10 minutes) and Urgent patients (clinical care is required within 30 minutes) seen over the 2007–08 period (AIHW 2013a, 2013b).

Australians enjoy a good quality of life as evidenced by Life expectancy among the highest in the world for quite some time. Life expectancy at birth in 2007 was ~ 84 years for females and 79 for males. Death rates vary across different population groups, with an estimated 13% higher death rate amongst those with the lowest socio-economic status (SES) compared with a 17% lower rate amongst the most advantaged quintile in comparison with the national rate (*A Picture of a Nation* 2006). Indigenous Australians experience nearly twice the death rate for Australia as a whole, as well as substantially lower life expectancy at birth (LEB) (59.4 for males and 64.8 for females in the 1996-2001 period) compared with all Australians (76.6 males and 82.0 females for the 1998-2000 period) (Australia's health 2010 2010).

Both infant (under one year) and child (age 1-14) mortality rates for have continued to fall (Blakeman et al. 2001). However, Australia's infant mortality is ranked amongst the lowest third for OECD countries, although most other health indicators rank among the top three (*Australia's health in 2010 in brief* 2010). The majority of child mortality occurs in the first year of life, with the neonatal mortality rate being approximately 3.6 per 1000 live births (in the first four weeks of life) and 1.4 per 1,000 in the remainder of the first year (Keleher et al. 2007). Differences in the distribution of the social determinants of health (Goold &

Usher 2006), have largely resulted in a disproportionate burden of ill health among Australian Aboriginal and Torres Strait Islander peoples, who are estimated to suffer 2.5 times more than other Australians (*Health at a glance: OECD Indicators 2005* 2005; *The Health and Welfare of Australia's Aboriginal and Torres Strait Islander Peoples* 2008).

An ageing population has resulted in increased demand for health services mainly due to chronic conditions, which are projected to increase over time (Commonwealth of Australia 2009). Attempts have been made to shift the health care service delivery focus from the acute (hospital) to the primary health care (community) sector, to reduce hospitalisation cost (Council of Australian Governments (COAG) Reform Council 2010) and manage patients expectations (Warren, Holloway & Smith 2000).

## *1.2 Health seeking behaviour in Australia*

High rates of hospitalisation are common for some potentially preventable illnesses. The Australian Institute for Health and Welfare categorises potentially preventable hospitalisations (PPHs) broadly as vaccine preventable acute and chronic (AIHW 2013a). *Vaccine-preventable diseases* such as influenza, bacterial pneumonia, tetanus, measles, mumps, rubella, pertussis and polio, are considered to be preventable by proper vaccination. *Acute conditions* including dehydration and gastroenteritis, pyelonephritis, cellulitis, ear, nose and throat infections and dental conditions are not necessarily preventable, but with adequate and timely care should not result in hospitalisation. *Chronic conditions* such as diabetes complications, asthma, angina, hypertension, congestive heart failure are considered preventable through the modification of behaviour and lifestyle and with timely care and management can effectively prevent complications requiring hospitalisation (AIHW 2013a). More than 672,000 hospital separations were classified as PPHs in 2011–12, accounting for 7.3% of overall and 9.4% of public hospital separations; with the majority (77%)being reported by public hospitals. A 5% increase in the number of hospitalisations for acute conditions lead to an overall increase in PPHs over the period. Approximately 68,271 separations for dehydration and

gastroenteritis were reported nationally, with 29% being reported from New South Wales, accounting for the second highest number of reported cases behind Victoria with 20,482 cases (AIHW 2013a).

There is evidence that persons from lower socioeconomic backgrounds and some Indigenous Australians have more problems to access and utilise primary health services and hence higher rates of hospitalisation for illnesses that could be easily managed with primary care (Katterl 2011; Katterl et al. 2012). The AIHW 2011-12 statistics found that overall rates of hospital separations for PPHs was highest for residents of Remote and Very remote areas and lowest for residents of Major cities. Although the rates for acute conditions followed a similar pattern, acute conditions accounted for the most common cause of separation in both major cities and regional areas and amongst those with higher socioeconomic status of area of residence (AIHW 2013a). High rates of hospitalisation for PPHs is due to a multiplicity of factors including: poor access to primary care, improved detection of illnesses by primary care services, socioeconomic disadvantage, rural dwelling, comorbidities and demographics factors (Katterl et al. 2012; Weinberger, Oddone & Henderson 1996). The foregoing factors could result in notable differences in health seeking behaviour and potential differences between data from hospital-based and community-based studies.

## *1.3 Gastrointestinal Illnesses Surveillance in New South Wales State*

State and territory health departments collect notifications of communicable diseases under their respective public health legislation (NNDSS Annual Report Writing Group 2012). A variety of disease surveillance methods with different notification requirements are utilised by medical practitioners, laboratories and hospitals across Australian states and territories; and as a result some diseases are only notifiable in some of the 8 jurisdictions to the National Notifiable Diseases Surveillance System (NNDSS) (NNDSS Annual Report Writing Group 2012). Only a fraction of symptomatic cases seek medical

care and are clinically diagnosed, become notified to the health authority by the Laboratory/Clinician, which in turn are captured by the NNDSS (NNDSS Annual Report Writing Group 2012). The NNDDSS system is complemented by other surveillance systems, which provide information on various diseases. Gastroenteritis, defined as three or more loose stools, and/or 2 vomits in a 24 hour period excluding cases who have a known cause, (such as bowel disease, alcohol, or pregnancy) are captured by two surveillance networks: the OzFoodNet and the Australian Sentinel Practices Research Network (ASPREN). The OzFoodNet conducts enhanced foodborne disease surveillance, investigates and reports on outbreaks and clusters of foodborne disease nationally and burden of illness studies (Australian Government Department of Health and Ageing 2012). A national network of general practitioners (ASPREN) provide weekly reports of defined medical conditions, as an indicator of the burden of disease in the community (Australian Government Department of Health and Ageing 2012; Kirk et al. 2008).

In 2010, gastrointestinal diseases were amongst 65 diseases and conditions nationally notifiable in Australia. In Australia, *Campylobacter* (excluding NSW), cholera, cryptosporidiosis, giardiasis, haemolytic uraemic syndrome (HUS), *Hepatitis* A and E, listeriosis, salmonellosis, Shiga toxin- and verocytotoxin-producing *Escherichia coli* (STEC/VTEC), shigellosis, and typhoid fever are the gastrointestinal illnesses captured by disease surveillance systems (National Notifiable Diseases Surveillance System 2013). Once a diagnosis has been confirmed, public health units must be notified to take preventative actions (NSW Department of Health 2012). In 2010, gastrointestinal diseases accounted for 15.1% (31,548 of 209,079 notifications) of communicable diseases notifications from States and territories reported to the National Notifiable Diseases Surveillance System (NNDSS Annual Report Writing Group 2012); indicating a decrease from down from 20% in 2006 (Begg et al. 2008).

## *1.4 Estimating Disease Burden*

Australian hospital data is used to estimate the incidence of diarrhoea resulting in hospitalization (AIHW 2003). Diarrhoeal diseases are recorded based on ICD-10-AM codes and are mainly included in the categories Infectious and parasitic diseases (A00–B99) (National Centre for Classification in Health 2010) Box 1 shows the national hospital separations in the infectious and parasitic diseases categories (A00–B99) for the 2008-2009 and 2009-2010 periods (AIHW 2013c). State data for NSW was not publicly available to provide a comparison.

**Table 1-1: Australia National hospital separations for ICD-10-AM categories (A00–B99), 2008/09 and 2009/10**

| Disease and injury categories and ICD-10-AM codes¶ | 2008–09 | 2009–10 |
|---|---|---|
| | N | N |
| Intestinal infectious diseases (A00−A09) | 60,487 | 69,316 |
| Certain zoonotic bacterial diseases (A20−A28) | 302 | 358 |
| Other viral diseases (B25−B34) | 19,045 | 17,588 |
| Protozoan diseases (B50−B64) | 819 | 870 |
| Helminthiases (B65−B83) | 245 | 235 |
| Pediculosis, acariasis & other infestations (B85−B89) | 397 | 487 |
| Sequelae of infectious & parasitic diseases (B90−B94) | 2 | 1 |
| Bacterial, viral and other infectious agents (B95−B97) | 30 | 46 |
| Other infectious diseases (B99) | 277 | 282 |

¶National data publicly available on the AIHW website. State data is only available at cost.

Previous studies have used The National Hospital Morbidity Database (AIHW 2003) to estimate the total number of hospitalizations for gastroenteritis). However limitations were found with the use of ICD-09 data which did not include coding for some pathogens such as *Aeromonas* and had limited codes for *E. coli* pathotypes. A review combining both

hospital clinical and laboratory databases was considered an alternative to determine actual organisms detected in association with GIT illness.

## *1.5 Overview of the study*

### 1.5.1 Why hospitalised patients?

Hospital data was used for this study. Access to community based data is very difficult. Attempts were made to enlist private laboratories that handle the bulk of specimen from General Practice (community/primary health), unfortunately, these organisations all declined to participate in the research. Additionally, there are strict ethical guidelines to govern research involving direct contact with patients, and research requiring linking patient records to individuals. Based on the foregoing, it was practical and less onerous to conduct this study in hospital settings. Hospitals capture a significant amount of patients and have clear guidelines, systematic approaches to guide the use of patient data. Hospital data is routinely collected and fairly reliable. Hospital data was therefore used to provide insights into what is happening in the community.

Several studies have been done to elucidate the number, epidemiology and cause of the enteric disease outbreaks. However outbreak data represents a very small proportion of the cases and causes of infectious diarrhoeal illness. Since much emphasis has been placed on community based studies and outbreaks, this study sought to look at hospital based patients, as there was very little data for this group. Hospital data was used for this study and looked at data that is not routinely reported to surveillance. The majority of the pathogens detected by hospital laboratories are not included in State or National disease surveillance. For example, *Campylobacter* and *C. difficile* are not routinely reported to surveillance in NSW State. It is true that mainly symptomatic patients will seek medical care and the most severe cases are more likely to be referred to hospital. However there is merit in using hospital based data as it is consistent and it provides the opportunity to link laboratory and clinical records to paint a complete picture of the patient's illness and subsequent diagnosis and management. Sydney (NSW) has mixed population, with

amenities and services relatively homogenous throughout hence is fairly representative of NSW population. The four hospitals selected had a wide-enough area of coverage to ensure that patients were fairly representative of the NSW population demographics.

### 1.5.2 Study sites

Four major public hospitals all located in different geographic areas across Sydney were included in the study. These facilities were included based on the population served, and represent a cross section of different socio-economic and cultural influences across the Sydney metropolitan region. Liverpool Hospital (Hospital A) is a tertiary referral hospital for south western Sydney, providing medical, surgical, emergency medicine, intensive care, oncology, mental health, women's health and newborn care services, and a major trauma centre for NSW. The Children's Hospital at Westmead (Hospital B) is a stand-alone service dedicated to paediatrics. The Hospital provides community medical care, paediatric and tertiary level paediatric services to patients from all over NSW and interstate. St. Vincent's Hospital, Sydney (Hospital C) is a major public and a principal referral hospital attracting referrals on a State-wide and national basis. It specializes in heart/lung transplantation; bone marrow transplantation; cardiology; cancer; HIV medicine; respiratory medicine; mental health; and drug and alcohol services. The Prince of Wales Hospital (Hospital D) is a major teaching hospital and one of thirteen principal referral hospitals for adults based in eastern Sydney and also serves all of New South Wales. The Hospital provides a broad range of medical and surgical services as well a variety of allied health services, as well as hosting the South East Area Laboratory Services (SEALS) providing laboratory services to the Prince of Wales, Sydney Children's Hospitals and the Royal Hospital for Women, all located on adjacent premises, as well as referral services to other hospitals in the Area Health Service. All four hospital hosts a fully accredited laboratory service, providing comprehensive biomedical laboratory services based on standard quality controlled procedures, accredited by the National Association of Testing Authorities, Australia (NATA).

Patients seen in these hospitals come from across the Sydney Region, including persons within the Sydney metropolitan area as well as case 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. 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.

### 1.5.3 Research Ethics

Ethical approval for this study was received from the Human Research Ethics Committees (HREC) at Sydney South West Area Health Service, Western Zone, Children Hospital at Westmead and St. Vincent's Hospital, Sydney, and the University of Technology, Sydney (UTS) and was guided by the Australian NHMRC National Statement on Ethical Conduct in Human Research (2007). The Authors are unsure whether written consent was given to each hospital by the patients including the next of kin for minors/children participants for their information to be stored in the hospital database and used for research. However, each hospital's HREC committee waived the need for individual consent from patients to view records in keeping with the Australian NHMRC National Statement on Ethical Conduct in Human Research sections 3.2.9, 2.3.5 and 2.3.6; since the researched was approved on the basis of carrying no more than low/negligible risk (see also paragraphs 2.1.6 and 2.1.7) to participants. All data were analysed anonymously.

## *1.6 Outline of the Thesis*

The main objective of this thesis was to provide an overview of the prevalence of specific pathogens, clinical characteristics and associated risk factor for infectious GIT illness amongst hospitalised patients in Sydney, gathered through laboratory and clinical databases with the following specific objectives:

1. To describe the clinical and epidemiological characteristics of infectious gastrointestinal (GIT) illnesses, in patients seeking care in Sydney;
2. To describe the clinical features and pathogens associated with gastrointestinal illnesses for children;
3. To estimate the prevalence of enteric protozoa, and evaluates the outcomes of testing algorithms used to diagnose enteric protozoan infections; and
4. To provide a detailed description of (i) the epidemiology and (ii) geographical distribution of enteric protozoa infections in the Greater Sydney Metropolitan Area

In order to set the broad context for this thesis, a systematic review of the literature is presented in Chapter one. It provides age- and region-specific random-effect estimates of the prevalence of diarrhoeal pathogens in developed and developing settings. This is followed by four other stand-alone chapters prepared as journal articles for publication, based on the four objectives and a summary and conclusion chapter. Citations are provided where articles have been accepted, in-press or published in the peer-reviewed literature.

# 2 Systematic Review of the Prevalence of Gastrointestinal Pathogens Worldwide

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## Chapter Overview

In order to set the broad context for this thesis, a systematic review of the literature is presented in this chapter. It provides an overview of gastrointestinal illnesses, various definitions and disease transmission and risk factor information. It then utilizes a systematic approach to provide age- and region-specific random-effect estimates of the rates of diarrhoeal pathogens detected in developed and developing settings worldwide. The review discusses that developing regions have significantly more pathogens detected than the OECD countries, and individual pathogen prevalence differs between developed and developing settings, and provides recommendations for how this information could be useful to the public health community.

## *2.1 Abstract*

**Background:** Diarrhoeal illness is responsible for an estimated one in five deaths in children worldwide. There are no precise or current estimates of the prevalence of pathogens associated with diarrhoeal illnesses in developed and developing countries.

**Methods:** This systematic review assessed data from 60 studies (67 data sets) published in the English language from five developing regions and developed countries worldwide to provide region specific prevalence of major enteric pathogens affecting older children/adults and young children. The studies were published from 1983 to 2009, and were conducted before 1978 and 2007. Studies were either cross-sectional, prospective cohorts or case-control studies conducted in community or hospital settings. Of the 67 data sets assessed, 60 reported data for children while 7 reported data for older children and older children/adults. The random-effect method was used to establish the weighted average prevalence of pathogens in older children/adults and young children for each region.

**Results**: Significantly more pathogens were reported by studies from developing regions compared with OECD countries ($P<0.016$). The identification rates of pathogens from

community based and hospital based studies were similar (58.5% and 58.1% respectively, P<0.619). The overall detection of enteric pathogens in developing countries was higher in older children/adults (74.8%; 95%CI 63.1%-83.8%) compared with children (56.7%; 95%CI 53.0%-60.4%) (P<0.001). Rotavirus was the most frequently detected pathogen in all regions with the highest rate, 24.8% (95%CI 18.0%-33.1%), detected in the developed countries.

**Conclusions**: This systematic review is the first to provide an estimate of the prevalence of enteric pathogens associated with diarrhoeal illnesses in older children/adults and children in developed and developing settings. Although the burden of pathogens is greater in developing regions the consistently high prevalence of rotavirus infection underscores the need for urgent access to rotavirus vaccines in both the developed and developing settings. With increasing tourism to and travel from developing regions, resource rich countries have a vested interest in ensuring that adequate funds are available to assist developing nations with the implementation of prevention and control programs for gastrointestinal infections.

*Key words:* older children/adults, bacteria; children; developed countries; developing country; diarrhoea; enteric pathogens; viruses; parasites; systematic review,

## *2.2 Introduction*

Gastrointestinal (GIT) Illnesses contribute significantly to the burden of illness from infectious diseases worldwide. Diarrhoea is the second leading cause of preventable illness in children under age five (Black et al. 2010; Kosek, Bern & Guerrant 2003; Wardlaw et al. 2010). Despite the strong association between gastrointestinal illnesses and factors such as poor sanitation, inadequate access to safe drinking water and other risk factors, both resource-rich and less developed countries alike are impacted by gastrointestinal illness (UNICEF/WHO 2009; Wardlaw et al. 2010). The risk factors however appear to be distributed differently between developed and developing countries and as a result, the incidence of specific pathogens may differ between each setting (Keusch et al. 2006).

Several studies have described the pathogens, associated risk factors and the costs and burden of illness on health care (Frenzen 2005; Jones et al. 2007; Kuusi et al. 2003; Majowicz et al. 2006; O'Brien et al. 2010; Sargeant, Majowicz & Snelgrove 2008). However, there are few studies that estimate the prevalence of pathogens affecting populations in different regions worldwide (Fischer Walker, Sack & Black 2010; Kotloff et al. 2012; Levine et al. 2012). Developing countries world often experience similar sanitation and poverty related risk factors, which predisposes their population to diarrhoeal illnesses. However, the incidence of illness in developed countries tend to be less generic and more related to seasonality, travel and food-borne transmission (UNICEF/WHO 2009).

Several enteric micro-organisms are responsible for GIT illnesses and are bacterial, viral or parasitic in nature. (Schmidt et al. 2003) A review of the literature world-wide indicates that a causative organism is identified in about 50% of symptomatic cases (Abba et al. 2009; Boga et al. 2004; Dutta et al. 1991; Georges et al. 1984) in resource limited settings. Under-reporting is common in all settings, with the data biased towards certain pathogens and relate mainly to specific age groups (Boschi-Pinto, Velebit & Shibuya 2008; Green, Small & Casman 2009; Kosek, Bern & Guerrant 2003).

## *2.3 Overview of Gastrointestinal Illness*

Gastrointestinal illnesses can be either acute self-limiting infections or chronic idiopathies (Lamps 2007). Acute infections are usually caused by pathogenic bacteria, viruses and parasitic organisms. Idiopathic diseases arising from internal dysfunctions of gastrointestinal tract include diseases such as Idiopathic bowel disease, Crohn's disease and ulcerative colitis (Lamps 2007; Pintér & Kolesárová 2004). While the majority of gastrointestinal illnesses are self-limited (Lamps 2007), certain risk factors such as malnutrition, immunosuppression, and young age predisposes to the development of persistent diarrhoea (Heymann 2008). Patients with Immune deficiencies (congenital, iatrogenic or acquired) are unusually more susceptible to infections and are at an increased risk for malignancy (Ferrell 1984).

## *2.4 Definition of 'gastrointestinal illness'*

The term 'gastrointestinal illness' is used to refer to several conditions affecting the gastrointestinal system, that exhibits with watery or unformed stools and are usually caused by infections or intoxications with a biological agent (Gilbert 2008). Because of its various causes, variable symptomology, and numerous different terms used to describe disease of the gastrointestinal tract, no standard definition of 'gastrointestinal diseases' has been presented in the medical literature (Roy, Scallan & Beach 2006).

**Box 1: Definition of 'gastrointestinal illness'**

For the purposes of this study, Gastrointestinal illness refers to any illness of the gastrointestinal tract caused by a microbe which involving chronic or acute diarrhoea, whether or not accompanied by nausea, or vomiting, combined with abdominal pain, or systemic symptoms such as fever.

- A suspected case is any case having met the case definition by a clinical diagnosis.
- A confirmed case is any suspect case that has a laboratory confirmation of an enteric disease pathogen isolated from a clinical specimen.

Diarrhoeal illness is usually divided into three main categories based on its clinical presentation (Heymann 2008):

1. **Acute watery diarrhoea** associated with several pathogens such as *Vibrio cholera* and rotavirus, usually last for several days, and poses a high risk for dehydration which can be fatal especially in young children, the elderly and immuo-compromised persons (Heymann 2008).

2. **Acute bloody diarrhoea** or dysentery is usually evidence in infection with *Campylobacter, Salmonella, Shigella,* enterohaemorrhagic *E. coli* pathotypes*, Entamoeba histolytica* or other organisms, and can lead to dehydration. The major risks include intestinal damage, sepsis and malnutrition (Heymann 2008).

3. **Persistent diarrhoea** is diarrhoea lasting for 14 or more days is usually associated with parasitic aetiology such as *Giardia intestinalis, Blastocystis,* and *Entamoeba*

*histolytica*, but can also be due to non-infectious causes such as inflammatory bowel disease. Due to the prolonged duration of diarrhoea, there is risk for nutrient deficiency, extra intestinal infection and increased risk of dehydration especially in children, the elderly and immuno-compromised (Heymann 2008).

## *2.5 Disease transmission and risk factors*

Infectious gastrointestinal illnesses are transmitted through a variety of routes including contaminated food or water-borne, the faecal oral route, and person to person (Fletcher, Stark & Ellis 2011; Gilbert 2008). Several attempts have been made to estimate the burden of disease from foodborne illnesses (Flint et al. 2005). Reports from the USA, England, Europe and Australia have estimated that a significant proportion of gastrointestinal illnesses are attributable to food-borne transmission (Flint et al. 2005)(Mead et al. 1999). An older USA report found that among all illnesses attributable to food-borne transmission, about 30% were caused by bacteria, 3% by parasites, and 67% by viruses (Mead et al. 1999). A more recent USA report indicated that non-typhoidal *Salmonella* spp. (35%), norovirus (26%), *Campylobacter* spp. (15%), and *Toxoplasma gondii* (8%) were the leading causes of hospitalisation in 2000–2008 (Scallan et al. 2011). *Salmonella*, *Listeria*, *Toxoplasma*, and norovirus are responsible for more than 75% of deaths related to known causes of food-borne illness each year (Mead et al. 1999; Scallan et al. 2011; Schmidt et al. 2003). In Australia, about 32% of all gastroenteritis are food-borne, and *Campylobacter,* non-typhoidal *Salmonella,* pathogenic *E. coli* and norovirus are responsible for over 80% of foodborne illness from 'known' pathogens (Hall et al. 2005b; Kirk et al. 2008). In Australia the highest rates of gastroenteritis in the general population has been reported among young children and adult carers (Kirk & Hall 2005).

The majority of gastrointestinal illnesses can be transmitted through the faecal-oral route. Infections with pathogenic *E. coli* strains are usually considered an indication of poor hygiene. Up to 63% of children with persistent diarrhoea in low and middle income countries, have tested positive for *E. coli* strains (Abba et al. 2009). Travel associated

diarrhoea has been described and travel to a developing region is a risk factor often considered among patients presenting with diarrhoea in developed settings (Leung, Robson & Davies 2006; Marcos & DuPont 2007). Studies have found that travelers to low and middle income countries are between 9 and 151 times more likely to develop diarrhoeal illness (Greenwood et al. 2008; Swaminathan et al. 2009), with the highest risk from travel to areas in South America, Africa, and South Asia (Greenwood et al. 2008; Jiang et al. 2002; Steffen et al. 2004).

In developed settings, enteric protozoa are often ignored as a cause of diarrhoea due to better hygiene conditions. The evidence suggest that while some enteric protozoa such as *Entamoeba spp*., *Cryptosporidium* and *Giardia* were frequently isolated from diarrhoeal cases in developing regions, like Asia and sub-Saharan Africa (Fletcher, Stark & Ellis 2011; Nair et al. 2010; Shah, DuPont & Ramsey 2009); others like *Blastocystis* spp., and *Dientamoeba fragilis* are mainly isolated in the developed countries (Stark et al. 2009b). Zoonotic (animal to human) transmission associated with the handling of livestock and domestic pets has been reported and more recently the prominence of open farms and petting zoos have featured in several zoonotic outbreaks (Chalmers et al. 2011; Collier et al. 2011; Giangaspero, Berrilli & Brandonisio 2007). Infections are associated with recent travel to developing regions (Stark et al. 2007a); amongst immigrants and refugees (Gualdieri et al. 2010; Mody et al. 2007); and domestic transmission (Stark et al. 2009a). In many developed countries however, parasitic protozoa are rarely included in operational surveillance systems, and where this occurs, they are indicators of outbreaks of foodborne and waterborne diseases (Lake et al. 2009a; Waldron et al. 2011; Yoder, Harral & Beach 2010a, 2010b). Asymptomatic carriage of protozoan parasites is also common in developed countries as several types have been detected in non-diarrhoeal cases (Dogruman-Al et al. 2009; Olesen et al. 2005 ).

There are few estimates of the prevalence and distribution of pathogens that cause GIT illnesses, which indicate there may be significant differences in the prevalence of certain pathogens in circulation in developing and developed settings (Fletcher et al. 2012;

Greenwood et al. 2008; Savarino & Bourgeois 1993). Determining the prevalence of pathogens on a regional basis will assist in the development of appropriately targeted prevention and control strategies, identify gaps in surveillance and provide support for the strengthening of laboratory diagnostic capacity at the regional level. It can also assist in the management of the health needs of travelers to different regions. The aim of this systematic review is to provide region-specific prevalence of pathogens associated with GIT illness cases in developed countries and developing regions.

## *2.6 Methods*

### 2.6.1 Search strategy

In order to identify studies reporting the aetiology of diarrhoeal illness, a literature search of databases including Science Direct, PubMed, PubMed Central, and Google scholar, was conducted for articles published from 1980 and 2010. The search was not restricted to publications in the English Language, but only studies that had adequate information in English were included. The key subject terms included one or combinations of the following: *"infectious intestinal pathogen AND humans", "diarrhea (or diarrhoea) and pathogen", aetiology (etiology) of acute gastroenteritis OR diarrhoea", enteric infectious pathogens.* Boolean operators (*not*, *and*, *or*) were also used in succession to narrow and widen the searches. Other articles were identified by using the PubMed option of "related articles" and checked the reference lists of the original and review articles.

### 2.6.2 Eligibility and study selection criteria

Eligibility for inclusion includes the following criteria:

(i) detailed results of microbiological analysis of stool samples and the number of samples tested must be reported;

(ii) The study must define whether the subjects tested were clinically symptomatic or asymptomatic;

(iii) The number of study subjects and positive results for both cases /controls must be reported.

(iv) Symptomatic subjects (case) defined as persons who had diarrhoea (defined as three or more loose/watery stools in a 24 hour period)(WHO 2010), or loose stools associated with gastrointestinal symptoms including: vomiting, abdominal pain or cramps, and blood or mucus in stool.

(v) Age-specific detection rates for older children/adults and young children available.

**Excluded studies**

Studies that presented data without adequate information for the detection rate to be calculated were excluded. In addition, studies that focused on a single pathogen, presented number of diarrhoeal episodes instead of samples tested and mixed age groups were also excluded.

### 2.6.3 Data abstraction and analysis

The selected studies were summarized in tabular format to include data on study design, study population, source of sample and sample size, methods to detect enteric pathogens, and detection rates. Where studies included combined age-groups that provided adequate data for children and older children/adults separately, the data for children and older children/adults were recorded separately. Study countries were categorized into developing regions categories, based on the World Bank List of Economies published in July 2009 (The World Bank Group 2009), including: East Asia Pacific (EAP), Latin America and Caribbean (LAC), Middle East and Northern Africa (MENA), Sub-Saharan Africa (SSA), and South Asia and the Pacific (SAP). Developed countries were categorized based on the Organisation for Economic Co-operation and Development (OECD) category of countries (International Monetary Fund 2009; The World Bank Group 2009). Two non-OECD developed countries (Republic of [South] Korea and Turkey) were also included in this category (Table 9.1). Of the studies reviewed, one presented multi-country data (two in EAP, one in LAC, two in SAP) which was tabulated and analysed under individual country/region. Three studies had sufficient data to allow the tabulation and analysis of findings for children and older children/adults separately. These brought the total number of studies reviewed to 67.

### 2.6.4 Analysis and synthesis of results

Estimates of the prevalence of diarrhoeal pathogens were prepared for each region. In order to estimate the pooled prevalence of pathogens, the DerSimonian-Laird random-effect (RE) method (Jackson, Bowden & Baker 2010) was used , since studies were

conducted in different regions using different methods and approaches. The pooled or weighted RE estimate of pathogen prevalence was calculated with the assistance of the Comprehensive Meta- analysis (CMA) programme (Fletcher et al. 2012). The estimated mean detection rate was reported with 95% Confidence Intervals (95% CI).

## *2.7 Estimating the prevalence of gastrointestinal pathogens*

### 2.7.1 Description of studies

The search produced 10288 papers and the abstracts of 121 were critically reviewed. Data from 60 studies (from 47 developing and 13 developed countries) meeting the criteria, were included in the analysis (Al-Gallas et al. 2007; Albert et al. 1999; Anyanwu 1997; Barnes et al. 1998; Barreto et al. 2006; Bodhidatta et al. 2002; Brink et al. 2002; Cajetan et al. 2010; Colomba et al. 2006; De Mol et al. 1983; Djeneba et al. 2007; Dutta et al. 1991; El-Mohamady et al. 2006; El-Sheikh & El-Assouli 2001; Elamreen, Abed & Sharif 2007; Flores-Abuxapqui et al. 1994; Georges et al. 1984; Germani et al. 1998; Ghosh et al. 1991; Haque et al. 2003; Henry et al. 1995; Hien et al. 2007; Hoge et al. 1995; Huilan S et al. 1991; Isenbarger et al. 2001; Jansen et al. 2008; Kain et al. 1991; Kelly et al. 1996; Klein et al. 2006; Kyung-Hee et al. 1989; Levidiotou et al. 2009; Liu et al. 2005; Lu et al. 2006; McIver et al. 2001; Montgomery et al. 2006; Musiime et al. 2009; Nakano et al. 1998; Nguyen et al. 2006; O'Neill et al. 2002; Ochoa et al. 2009; Ogbu et al. 2008; Ogunsanya, Rotimi & Adenuga 1994; Olesen et al. 2005 ; Olowe et al. 2003; Olsen et al. 1995; Paniagua et al. 2007; Pazzaglia et al. 1991; Podkolzin et al. 2009; Rautelin et al. 1989; Reither et al. 2007; Seigel et al. 1996; Soenarto et al. 1983; Tin et al. 1989; Torres et al. 2001 ; Uysal et al. 1997; van Eijk et al. 2010; Vargas et al. 2004; Yamashiro et al. 1998 ; Yongsi 2008; Youssef et al. 2000; Yu et al. 2008). The studies included are summarised in Table 9.1. Of the studies reviewed, one presented multi-country data (two in EAP, one in LAC, two in SAP) (Huilan S et al. 1991), which was recorded under individual country/region. Three studies had sufficient data to allow the recording of findings for children and older children/adults separately (Al-Gallas et al. 2007; O'Neill et al. 2002; Podkolzin et al. 2009). These brought the total number of studies reviewed to 67. The studies were published from 1983 to 2009, and were conducted before 1978 and 2007. Studies were either cross-sectional, prospective cohorts or case-control studies. Most studies (73%, 44/60) recruited subjects from Hospital settings and the remainder were

recruited from community or mixed settings. Studies were classified into six groups: five developing regions and OECD (developed) countries.

The summary of the laboratory procedures employed in each study is presented in Table 9.2. Of the 67 studies, 63 conducted testes for bacteria, 55 for viruses and 45 for parasites. For the detection of bacteria, standard culture methods often coupled with serological tests, DNA hybridization, enzyme immunoassays (EIA) and polymerase chain reaction (PCR) were employed for the detection of different pathotypes of pathogenic *E. coli.* In some cases antibiotic susceptibility testing was performed by the disk diffusion or plate dilution methods. The majority of viral studies focused on rotavirus, employing mainly enzyme linked immunosorbent assay (ELISA), EIA, and less frequently, latex slide agglutination test and PCRs. Microscopic examination of permanently stained films have been generally used for the detection of ova cyst and parasites, along with a modified Ziehl-Neilsen stain for coccidian parasites.

### 2.7.2 Overall detection of pathogens

**Source of cases:** Overall, there was no significant difference in the detection of pathogens between community and Hospital settings (P>0.05). In 13 community based studies from four regions, enteric pathogens were detected in an average of 57.8 % (95%CI 49.2%-65.9 %) of cases, ranging from 52.4% in the EAP region to 62.6% in the SSA region. There was a significant difference in detection rates from 44 Hospital based studies across the six regions, (P=0.001). Enteric pathogens were detected in an average of 58.1% (95% CI 54.0%-62.0%) of cases, ranging from 36.9% (95% CI 31.7%-56.2%) in the MENA region to 66.7% (95% CI 58.9%-72.4%) in the SAP region (Table 2.1).

**Age groups:** In children, a pathogen was detected in an average of 56.7% (95% CI 53.0%-60.4%), and the lowest rate of 43.6% (95% CI 31.7%-56.2%) detected amongst children in the MENA region, and the highest detection rate of 64.4% (95% CI 57.6%-70.7%) observed in the SAP region. Older children/adults on the other hand had a pathogen detected in an

average of 74.8% (95% CI 63.1%-83.8%). The highest rate amongst older children/adults was detected in the MENA region (90.4 %; 95% CI 81.2%-95.4%), and the lowest rates detected in OECD countries (32.5%; 95%CI 14.7%-57.3%) (Table 2.1).

**Table 2-1: Age specific and source related regional estimate of overall pathogen detection**

| Overall detection rate | Children | | Older children/adults | | All cases | | Hospital | Community |
|---|---|---|---|---|---|---|---|---|
| **Regions** | # of studies | Detection rate % (95% CI) | #. of studies | Detection rate % (95% CI) | # of studies | Detection rate % (95% CI) | Detection rate % (95% CI) | Detection rate % (95% CI) |
| **SAP** | 7 | 64.4 (57.6-70.7) | 0 | N/R | 13 | 54.1 (43.9-63.9) | 66.7 (58.3-74.1) | 58.7 (55.6-61.7) |
| **LAC** | 8 | 61.0 (51.2-70.1) | 0 | N/R | 8 | 61.0 (51.2-70.1) | 65.0 (56.3-72.8) | 52.6 (35.6-69.1) |
| **SSA** | 16 | 59.4 (48.5-69.3) | 2 | 69.7 (39.6-89.0) | 6 | 54.5 (40.2-68.1) | 52.8 (39.9-65.4) | 62.6 (43.3-78.6) |
| **EAP** | 13 | 54.1 (43.9-63.9) | 0 | N/R | 7 | 64.4 (57.6-70.7) | 59.4 (49.7-68.5) | 52.5 (27.7-76.1) |
| **MENA** | 5 | 43.6 (31.7-56.2) | 1 | 90.4 (81.2-95.4) | 18 | 60.5 (50.6-69.7) | 36.9 (22.0-54.9) | N/R |
| **OECD** | 11 | 50.1 (42.5-57.7) | 4 | 32.5 (14.7-57.3) | 15 | 45.2 (37.2-53.5) | 50.4 (42.5-58.3) | N/R |
| **Total** | 60 | 56.7 (53.0-60.4) | 7 | 74.8 (63.1-83.8) | 67 | 57.2 (53.4-60.9) | 58.1 (54.0-62.0) | 58.5 (55.6-61.4) |

**Random-Effect estimate** (Q (df) P-value) for difference between hospital and community based studies = 0.25 (1) 0.619; Older children/ adults = (20.7 (2) 0.001 and children = 13.2 (5) 0.022; regions = 13.9 (5) 0.016. The weighted mean detection rate is calculated by dividing number of positive stool tests for individual pathogens by the total number of specimen tested. DerSimonian-Laird random-effect (RE) methods was calculated on the basis of the Cochran's Q-test with alpha set at the 5% level. Regions**:** EAP=East Asia & the Pacific; MENA = Middle East and North Africa; SAP= South Asia; LAC= Latin America and the Caribbean; SSA= Sub-Saharan Africa; OECD= Developed Countries including non-OECD developed countries. N/R= not reported.

### 2.7.3 Regional Distribution of Enteric Pathogens

**Children**

The weighted average detection rate for enteric organisms in children for each region is presented in Table 2.2. Rotavirus was the most frequently detected pathogen in children ranging from a low of 4.8% (95% CI 2.6%-8.7%) in SSA region to a high of 24.8% (95% CI 18.0%-33.1%) in OECD countries. The detection of rotavirus from diarrhoeal cases was up to five (5) times more in OECD countries than in developing regions. Adenovirus 4.5% (95% CI 3.3%-6.1%) and norovirus 3.3% (95% CI 1.9%-5.7%) were also most frequently detected in OECD countries when compared with developing regions. On average, *E. coli* pathotypes (Enterotoxigenic *E*. *coli* (ETEC), Enteropathogenic *E. coli* (EPEC)) and other pathogenic *E. coli* spp. were quite prevalent across all developing regions. The highest rates of EPEC were detected in the SAP region (8.5%; 95%CI 5.4%-13.1%) followed by the LAC region (5.6%; 95%CI 2.3%-12.6%); while ETEC was mostly prevalent in the SAP region, and other *E. coli* pathotypes common in the MENA region (10.6%; 95%CI 4.2%-24.3%). *Campylobacter* spp, was another frequently detected pathogen in developing regions with the highest rate of detection in the SAP (6.6%; 95% CI 3.9%-10.9%) and LAC (5.5%; 95%CI 2.8%-10.4%) regions. *Salmonella* spp and *Shigella* spp were also frequently detected amongst children in developing regions. *Salmonella* (4.1%; 95%CI 2.8%-5.9%) and *Campylobacter* spp (3.4%; 95%CI 2.3%-4.9%) were the most frequently detected bacterial pathogens in children in developed countries.

**Table 2-2: Weighted average prevalence of enteric pathogens from children 0-12 years in developing regions and OECD countries**

| | EAP | LAC | MENA | OECD | SAP | SSA |
|---|---|---|---|---|---|---|
| **NO. OF STUDIES (60)** | 13 | 8 | 5 | 11 | 7 | 16 |
| **Pathogens** | **Detection rate % (95% CI)** | **Detection rate % (95% CI)** | **Detection rate % (95% CI)** | **Detection rate % (95% CI)** | **Detection rate % (95% CI)** | **Detection rate % (95% CI)** |
| | | | **Bacteria** | | | |
| ***Aeromonas*** | 0.1 (0.0-0.6) | 0.3 (0.00-6.9) | 0.7 (0.3-1.9) | 0.1 (0.04-0.2) | 2.9 (1.4-6.1) | 0.3 (0.2-0.6) |
| ***Campylobacter jejuni*** | 2.1 (1.0-4.2) | 5.5 (2.8-10.4) | 2.4 (1.1-5.5) | 3.4 (2.3-4.9) | 6.6 (3.9-10.9) | 2.7 (1.5-4.8) |
| **EPEC** | 2.9 (1.7-4.8) | 5.6 (2.3-12.6) | 1.4 (0.3-6.6) | 0.2 (0.1-0.9) | 8.5 (5.4-13.1) | 3.2 (2.0-5.2) |
| **ETEC** | 4.1 (2.2-7.5) | 5.9 (3.1-11.2) | 5.4 (1.7-15.6) | 0.1 (0.01-1.6) | 12.7 (8.6-18.3) | 1.0 (0.5-2.1) |
| **Other diarrh *E. coli* pathotypes** | 1.7 (0.8-3.4) | 1.9 (0.5-6.8) | 10.6 (4.2-24.3) | 0.4 (0.1-1.2) | 1.8 (0.5-5.6) | 4.3 (2.1-8.6) |
| ***Salmonella* spp.** | 2.8 (1.5-5.4) | 1.7 (0.3-9.2) | 3.2 (1.6-6.5) | 4.1 (2.8-5.9) | 2.5 (1.6-3.8) | 3.6 (2.5-5.0) |
| ***Shigella* spp.** | 4.4 (2.8-7.0) | 2.9 (1.4-6.1) | 3.5 (2.4-5.3) | 0.5 (0.1-2.1) | 5.6 (3.0-10.1) | 4.3 (2.6-7.0) |
| ***Vibrio cholerae*** | 0.2 (0.1-0.4) | 0.2 (0.1-0.5) | 0.2 (0.1-0.7) | 0.1 (0.04-0.3) | 2.1 (1.0-4.8) | 0.4 (0.1-0.9) |
| | | | Viruses | | | |
| **Astrovirus** | 0.1 (0.0-0.6) | 0.4 (0.2-0.8) | 0.2 (0.1-0.7) | 1.3 (0.7-2.7) | 0.1 (0.04-0.3) | 0.2 (0.1-0.7) |

| | EAP | LAC | MENA | OECD | SAP | SSA |
|---|---|---|---|---|---|---|
| **NO. OF STUDIES (60)** | 13 | 8 | 5 | 11 | 7 | 16 |
| **Adenovirus type 40/41** | 0.4 (0.2-0.9) | 0.5 (0.2-1.6) | 0.2 (0.1-0.7) | 4.5 (3.3-6.1) | 1.5 (0.6-3.4) | 0.5 (0.2-1.5) |
| **Norovirus** | 0.2 (0.1-0.7) | 0.3 (0.1-1.6) | 0.2 (0.1-0.7) | 3.3 (1.9-5.7) | 0.7 (0.3-1.8) | 0.2 (0.1-0.9) |
| **Rotavirus** | 12.1 (7.4-19.3) | 12.0 (7.4-19.0) | 14.4 (7.8-25.0) | 24.8 (18.0-33.1) | 7.9 (4.7-12.8) | 4.8 (2.6-8.7) |
| | | | **Parasites** | | | |
| ***Ascaris*** | 0.2 (0.0-0.7) | 0.3 (0.1-1.0) | 0.5 (0.2-1.1) | 0.1 (0.04-0.2) | 0.1 (0.04-0.3) | 0.3 (0.1-1.1) |
| ***Blastocystis* spp.** | 0.1 (0.0-0.2) | 0.1 (0.1-0.4) | 0.2 (0.1-0.7) | 0.1 (0.04-0.5) | 0.1 (0.04-0.3) | 0.2 (0.1-0.3) |
| ***Cryptosporidium* spp** | 0.1 (0.1-0.2) | 0.2 (0.1-2.0) | 1.0 (0.2-4.9) | 0.3 (0.1-0.5) | 1.7 (0.8-3.1) | 0.3 (0.1-0.9) |
| ***Dientamoeba fragilis*** | 0.1 (0.1-0.2) | 0.1 (0.1-0.4) | 0.2 (0.1-0.7) | 0.1 (0.03-0.2) | 0.1 (0.1-0.3) | 0.2 (0.1-0.3) |
| *Entamoeba* spp. | 0.1 (0.1-0.9) | 0.6 (0.1-6.5) | 1.5 (0.6-4.2) | 0.1 (0.03-0.4) | 0.6 (0.1-2.5) | 1.5 (0.9-2.5) |
| *Giardia intestinalis* | 0.1 (0.1-0.3) | 1.9 (0.6-6.5) | 1.2 (0.5-3.2) | 0.3 (0.1-0.8) | 3.0 (1.5-5.9) | 2.7 (1.8-4.3) |

DerSimonian-Laird random-effect (RE) method was calculated on the basis of the Cochran's Q-test with alpha set at the 5% level. EAP=East Asia & the Pacific; MENA = Middle East and North Africa; SAP= South Asia; LAC= Latin America and the Caribbean; SSA= Sub-Saharan Africa; OECD= Developed Countries including non-OECD developed countries. EPEC- Enteropathogenic *E. coli*; ETEC- Enterotoxigenic *E. coli*

Parasites were less frequently detected; however *Giardia intestinalis* was the most frequently detected protozoa in developing regions, with the highest prevalence found in SAP (3.0%; 95%CI 1.5%-5.9%) and SSA (2.7%; 95%CI 1.8%-4.3%) regions. *Entamoeba* spp were frequently detected in MENA (1.5%; 95%CI 0.6%-4.2%) and SSA (1.5%; 95%CI 0.9%-2.5%) regions; while *Cryptosporidium* was more prevalent in in the MENA (1.0%; 95%CI 0.2%-4.9%) and SAP (1.7; 95%CI 0.8%-3.1%) regions. Other protozoa such as *Dientamoeba fragilis* were found in <1% of cases in each region.

**Older children/adults**:

Adult patients were assessed for only three regions: MENA, SSA and the OECD countries (Table 2.3). Amongst adult patients *Cryptosporidium* sp. (9.4%; 95%CI 1.0-50.2%), *Salmonella* spp (4.0%; 95%CI 0.2%-43.5%), other pathogenic *E. coli* (3.6%; 95%CI 0.3%-36.2%), and *Shigella* sp. (2.2%; 95%CI 1.1%-4.5%) were quite common in the SSA region. Older children/adults in OECD countries had more norovirus (10.5%; 95%CI 7.5%-14.7%), rotavirus (3.6%; 95%CI 1.4%-9.2%) and *Campylobacter* (3.3%; 95%CI 0.9%-12.0%) detected from their stools.

**Table 2-3: Weighted average prevalence of enteric pathogens from older children/adults >12 years in developing regions and OECD countries.**

| REGIONS | MENA | OECD | SSA |
|---|---|---|---|
| **No. of Studies (7)** | **1** | **4** | **2** |
| Pathogens | **Detection rate % (95% CI)** | **Detection rate % (95% CI)** | **Detection rate % (95% CI)** |
| | **Bacteria** | | |
| *Aeromonas* sp. | 0.7 (0.01-9.9) | 0.2 (0.01-0.6) | 0.3 (0.01-2.3) |
| *Campylobacter jejuni* | 1.4 (0.2-9.1) | 3.3 (0.9-12.0) | 0.3 (0.01-2.3) |
| EPEC | N/R | 0.1 (0.01-0.5) | 0.3 (0.01-2.3) |
| ETEC | N/R | 0.1 (0.01-0.5) | 1.0 (0.3-2.7) |
| Other diarrh *E. coli* pathotypes | 37 (26.7-48.6) | 0.1 (0.01-0.5) | 3.6 (0.3-36.2) |
| *Salmonella* spp. | 0.7 (0.01-9.9) | 1.9 (0.4-7.7) | 4.0 (0.2-43.5) |
| *Shigella* spp. | 4.1 (1.3-12.0) | 0.2 (0.01-1.0) | 2.2 (1.1-4.5) |
| *Vibrio cholerae* | 0.7 (0.01-9.9) | 0.1 (0.01-0.5) | N/R |
| | **Viruses** | | |
| Astrovirus | 0.7 (0.01-9.9) | 0.4 (0.01-2.9) | 0.3 (0.01-2.3) |
| Adenovirus type 40/41 | 6.8 (2.9-15.4) | 1.0 (0.4-2.4) | 0.3 (0.01-2.3) |
| Norovirus | 0.7 (0.01-9.9) | 10.5 (7.5-14.7) | 0.3 (0.01-2.3) |

| REGIONS | MENA | OECD | SSA |
|---|---|---|---|
| Rotavirus | 1.4 (0.2-9.1) | 3.6 (1.4-9.2) | 0.3 (0.01-2.3) |
| | **Parasites** | | |
| *Ascaris* | 0.7 (0.01-9.9) | 0.1 (0.01-0.5) | 0.3 (0.01-2.3) |
| *Blastocystis* spp. | 0.7 (0.01-9.9) | 0.2 (0.01-1.5) | 4.0 (0.5-24.7) |
| *Cryptosporidium* spp | 0.7 (0.01-9.9) | 0.3 (0.01-3.1) | 9.4 (1.0-50.2) |
| *Dientamoeba fragilis* | 0.7 (0.01-9.9) | 0.1 (0.01-0.5) | 0.3 (0.01-2.3) |
| *Entamoeba* spp. | 1.4 (0.2-9.1) | 0.2 (0.01-0.6) | 2.5 (1.2-5.1) |
| *Giardia intestinalis* | 0.7 (0.01-9.9) | 0.3 (0.01-2.3) | 1.4 (0.6-3.2) |

DerSimonian-Laird random-effect (RE) method was calculated on the basis of the Cochran's Q-test with alpha set at the 5% level. EAP=East Asia & the Pacific; MENA = Middle East and North Africa; SAP= South Asia; LAC= Latin America and the Caribbean; SSA= Sub-Saharan Africa; OECD= Developed Countries including non-OECD developed countries. EPEC- Enteropathogenic *E. coli*; ETEC- Enterotoxigenic *E. coli*

## *2.8 Discussion*

The paucity of information concerning the prevalence of diarrhoeal pathogens in different world regions has resulted in the conduct of this systematic review. This is the first study that has attempted to provide an estimate of the prevalence of enteric pathogens in both developed and developing settings. Several fundamental conclusions can be drawn from these findings. This review found (i) developing regions have significantly more pathogens isolated than the OECD countries, (ii) the identification of pathogens from community based studies was similar to those in hospital based studies, (iii) the overall detection of enteric pathogens is higher amongst older children/adults than in children in developing settings, (iv) enteric viruses (rotavirus, adenovirus and norovirus) were more frequently detected in paediatric cases in developed countries than in developing countries, and (v) bacterial pathogens are frequently detected amongst children and older children/adults in developing regions.

This review found that developing regions had significantly more pathogens isolated than the OECD countries. A WHO report indicated that in 2008 the SSA and SAP regions accounted for more than three quarters of the deaths in children from diarrhoeal disease (Boschi-Pinto, Velebit & Shibuya 2008). Several travel based studies suggest that travel to South America, Africa, and South Asia poses the greatest risk (Greenwood et al. 2008; Jiang et al. 2002; Steffen et al. 2004). South Asia continues to be affected by a disproportionately higher incidence of diarrhoeal illness in countries such as India and Bangladesh. Support for the development of targeted interventions such as food safety programs that can successfully reduce the rates of travelers' diarrhoea (Ashley et al. 2004; Fletcher, Maharaj & James 2009; Steffen et al. 1999), and provide evidence for vaccine development (Sanchez-Padilla et al. 2009) in developing regions should be a priority for developed countries. While the source of difference in pathogen prevalence between both settings cannot be determined from the information provided in the studies, we suggest that it is likely to be related, in part, to factors such as access to socioeconomic

status, potable water and sanitation solutions. These factors were not explicitly described in many studies but are known important predictors of diarrhoea incidence in developing settings (Genser et al. 2008).

The identification of pathogens from community based studies was similar to those in hospital based studies; however regional differences may exist between the settings. Even though there were no significant differences in the overall detection of pathogens between community and hospital based studies, differences were observed within hospital based studies between regions. One explanation for this may be hospital setting cases have severe clinical symptoms and were more likely to be tested compared with cases from the community setting.

The overall detection of enteric pathogens was higher amongst older children/adults than in children in developing settings. The contrary is true for developed settings. Whilst this may be due to underlying sample sizes and testing methodologies employed, there is some evidence to suggest that several new and emerging pathogens are not routinely detected in clinical laboratories. Considering the majority of the studies involving children were from developing regions, limited laboratory capacity may have precluded the identification of organisms that require very sensitive diagnostic techniques, such as viral pathogens (Richardson et al. 1998; Robins-Browne & Levine 2012) and some enteric protozoa (Bruijnesteijn van Coppenraet et al. 2009; Stark et al. 2008a). There is a need for the development of inexpensive sensitive and specific diagnostic methods to improve pathogen detection in clinical laboratories (Fletcher et al. 2012; Fotedar et al. 2007; Stark et al. 2011; Stark et al. 2010a; Stark et al. 2010b). Some exposures increase the risk for infections in older children and older children/adults, that could influence the types of pathogens that infect them including: poor hygiene practices, foodborne infections, and different environmental risk factors (Putignani & Menichella 2010).

Enteric viruses were more frequently detected in paediatric cases in developed countries than in developing countries. This finding is not unusual since rotavirus is considered to be the most common cause of childhood diarrhoea in both developed and developing

countries (Thapar & Sanderson 2004). Infections with enteric viruses have a strong relationship with seasons and may be the reason for the higher incidence in OECD countries. This relationship is particularly evident in OECD countries with a temperate climate where rotavirus and norovirus infections often peak in the cooler months (Fletcher et al. 2013; Glass R. 2009). There is a less obvious seasonal distribution in tropical countries (Cook et al. 1990). The burden of rotavirus diarrhoea worldwide has resulted in the World Health Organization (WHO) placing priority on the development and distribution of rotavirus vaccines globally (Wardlaw et al. 2010). More recently, a gradually decrease in the number of hospitalisations from severe dehydrating diarrhoea in children has been observed since the introduction of the rotavirus vaccine in the USA in 2006 (Curns et al. 2010) and in Australia in 2007 (Chiu et al. 2010; Hull et al. 2010; NCIRS et al. 2007). Efficacy trials conducted in Africa (Madhi et al. 2010) and a post-marketing study conducted in Mexico support the use of rotavirus vaccines in the developing countries (Santosham 2010).

Norovirus infection in developed settings was prevalent in older children/adults and nearly three times more common compared with children. Norovirus is recognized as a leading cause of epidemic gastroenteritis affecting all age groups, with sporadic cases occurring all year round with increased outbreaks in colder months (Glass R. 2009). In contrast to rotavirus, norovirus is the principal cause of healthcare associated viral diarrhoea (Svraka et al. 2007). Enteric adenoviruses types 40 and 41 and astrovirus are less frequently implicated but are also important causes of acute diarrhoeal illnesses in sporadic and outbreak settings (Fletcher et al. 2013; Svraka et al. 2007).

*Campylobacter* spp, *E. coli* pathotypes and *Shigella* spp are frequently detected in children from developing regions while older children/adults are predominantly affected by *Cryptosporidium* spp, *Salmonella* and pathogenic *E. coli*. Bacterial diarrhoea can be spread through various routes including contaminated food, water and the faecal-oral route; providing multiple sources of infections among the exposed (Kirk & Hall 2005; Schmidt et al. 2003). The relatively high prevalence of different strains of pathogenic *E. coli* found in

both older children/adults and children in developing settings has been previously described (Abba et al. 2009). One recent estimate of multiple diarrhoea pathogens in older children and older children/adults in outpatient and inpatient settings, found ETEC and *Vibrio cholera* as the leading causes of hospitalisation and *Salmonella* spp, *Shigella* spp, and *E. histolytica* as the leading causes in out-patients (Fischer Walker, Sack & Black 2010). The prevalence of pathogenic *E. coli* in developed countries is usually sporadic (Thapar & Sanderson 2004) and more closely associated with travel to a developing regions (Leung, Robson & Davies 2006; Marcos & DuPont 2007). *Campylobacter* spp and *Salmonell*a spp were the most common bacteria isolated in patients from OECD countries. Evidence from the USA, New Zealand and Australia suggest that *Salmonella* infections are a major cause of hospitalisations and deaths annually, and are frequently associated with foodborne illnesses in industrialized countries (Bambrick et al. 2008; Rabsch, Tschäpe & Bäumler 2001; Voetsch et al. 2004).

Enteric protozoa, mainly *Giardia intestinalis* and *Entamoeba* spp were common in children from developing regions compared with children from developed settings. A recent meta-analysis found that *Giardia intestinalis* was not associated with acute paediatric diarrhoea but was associated with persistent diarrhoea in developing countries (Muhsen & Levine 2012). Older children/adults, predominantly those in the SSA region, were mainly affected by *Cryptosporidium* spp and *Blastocystis* spp., which has been previously described in the SSA region (Fletcher, Stark & Ellis 2011). The prevalence of parasitic infections is consistent with findings from other developing settings, where sanitation and access to clean water is compromised (Mor & Tzipori 2008; O'Ryan, Prado & Pickering 2005; Raso et al. 2004). There are conflicting interpretations about the role of *Blastocystis* spp as a pathogen, and as a result some laboratories may not place priority on looking for or reporting the presence of this parasite (Boorom et al. 2008; Jones et al. 2009; Stark et al. 2009b). However, several reports have established its association with abdominal pain, persistent diarrhoea and irritable bowel syndrome (IBS) (Dogruman-Al et al. 2009; Jimenez-Gonzalez et al. 2011; Stark et al. 2007c), and other reports hypothesize that pathogenicity may be sub-type dependent (Roberts et al. 2012).

**Limitations**

In systematically reviewing the literature, it became apparent that only some studies utilised rigorous study design and analytical techniques that could identify a broad range of enteric pathogens. Accordingly, we limited our analysis to include only those studies that utilised a rigorous methodology and clearly defined the patient group (diarrhoea cases) and the enteric pathogens detected. Additionally, OECD countries are likely to have better testing regimes than many developing countries, and as such they are more likely to identify pathogens from infected persons. As such, care should be taken in making comparisons across developed and developing regions.

### 2.8.1 Conclusions and Policy implications

This systematic review of the literature has attempted to provide an estimation of the types and prevalence of gastrointestinal pathogens associated with diarrhoeal illness in both developing and developed settings. Similar studies to provide a comparison were not found as this was the first study to employ a broad definition of diarrhoeal illness, for older children/adults and children. The current study addresses the gap in the literature by providing region specific estimates of pathogen prevalence for older children/adults and children worldwide. It concludes that pathogens are consistently isolated from persons seeking medical attention for diarrhoeal illnesses in secondary or primary care settings. Appropriate estimates of the pathogens associated with gastrointestinal illnesses assist in advancing the global burden of disease initiative and help in setting research priorities for diarrhoeal illnesses. The review found that while similar enteric pathogens are in circulation in developed and developing settings, they occur with different frequency in each setting. In developed settings, enteric viruses such as rotavirus, norovirus and adenovirus are the major causes of diarrhoeal illness in older children/adults and children. Public health measures in developed settings should therefore include the development of vaccines against viral infections.

The consistently high prevalence of rotavirus infection in developing countries underscores the need for access to rotavirus vaccines in developing settings. Rotavirus vaccines have proven to be efficacious in reducing the burden of rotavirus and all cause gastroenteritis in settings where they have been introduced, and there is evidence that current vaccines will be efficacious in developing settings. The global public health community should support efforts to make rotavirus vaccines available at affordable costs to the poorest people of the world, where disease burden and mortality is usually highest. Developed countries have a vested interest in ensuring that adequate funds are available to developing regions to assist in the implementation of programs for the prevention and control of gastrointestinal infections, since increase in transmission of infectious pathogens between developed and developing regions has become possible, with increasing travel to developing areas.

To our knowledge this systematic review is the largest and first to provide an estimate of the region specific prevalence of enteric pathogens associated with diarrhoeal illnesses, in developed and developing countries. It highlights the need for development of inexpensive sensitive and specific diagnostic methods to improve pathogen detection in clinical laboratories, and for continued research and development activities for cost effective interventions to prevent and control diarrhoeal illnesses worldwide.

# 3 Epidemiology and Clinical Profile of Gastrointestinal Illnesses in Sydney

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## Chapter Overview

There is a paucity of information about the actual causes and relative prevalence of GIT pathogens resulting in hospitalisation in the Australian setting. It is believed that gastrointestinal illnesses contribute a significant burden to the health system, therefore knowing the actual causes and their prevalence is important, to inform prompt diagnosis and treatment, to prevent complications and reduce the burden on the health care system. The systematic review in the preceding chapter sets the worldwide global context for the detection of enteric pathogens in both developed and developing contexts. This chapter now looks specifically at Australia, and provides an overview of the clinical and epidemiological characteristics of GIT illnesses, caused by infectious pathogens amongst hospitalised patients in Sydney.

## *3.2 Abstract*

**Objective:** To describe the clinical and epidemiological characteristics of infectious gastrointestinal (GIT) illnesses, in patients seeking care in Sydney.

**Design and Setting:** A retrospective cross-sectional survey of laboratory and clinical records for patients seen at four major public hospitals in Sydney, from January 2007 to December 2010.

**Patients:** Patients presenting to Hospital with GIT symptoms, and had an enteric organism detected in their stool.

**Main Outcome Measure(s):** Age-specific pathogen detection rates for infected cases.

**Results**: Gastrointestinal illnesses are caused by several pathogens, which varies between older children/adults and children. Children 0-5 years old were mainly affected by rotavirus (22.4%), norovirus (19.6%), and adenovirus (17.5%). *Campylobacter* (57.5%) and *Salmonella* spp, (51.9%) were frequently diagnosed in persons age 6-59 years old (P<0.001), while *Clostridium difficile* (57.3%), was more frequently diagnosed in persons 60 years and older. Infections with *Blastocystis* spp increased with increasing age

(P<0.001), and the incidence of *G. intestinalis* was highest in persons under 13 years (P=0.018). Nearly all protozoan infections were associated with diarrhoea (P<0.001). Other symptoms associated with protozoan infection include abdominal pain and vomiting (P<0.05). The risk of infection with *C. difficile* was more likely in persons who were on prolonged antibiotic therapy (OR 6.3; 95%CI 3.2-12.2; P<0.001), had a recent surgery (OR 2.2; 95%CI 1.1-4.6; p=0.030), and a chronic GIT illness (OR 2.4; 95%CI 1.1-5.3; 0.035); however infection was 60% less likely in females compared with men (OR 0.4; 95%CI 0.2-0.9; P=0.031). Persons infected with *Shigella* spp were five times more likely to be men who have sex with men (MSM) (OR 5.0; 95%CI 1.6-16.0; P=0.007) and three times more likely to be HIV infected (OR 3.3; 95%CI 1.0-10.9; P=0.055). Young older children/adults in the 13-24 years and 25-49 years were more likely to be diagnosed with *Campylobacter* (OR 2.5; 95%CI 0.8-7.4; P=0.101 and OR 1.8 95%CI 0.7-4.4; P=0.206) and non-typhoidal *Salmonella* spp (OR 14.4; 95%CI 2.4-85.8; P=0.003 and OR 3.6 95%CI 0.8-16.9; P=0.102) respectively.

**Conclusion**: This study is the largest hospital based study to incorporate data from multiple sites to describe the epidemiology of infectious GIT illnesses in NSW State in the last 20 years. This study has revealed that GIT illness is a major issue for healthcare in Sydney, with implications for resource management and disease surveillance and control. It emphasises the importance of laboratory diagnosis of enteric infections and highlights the need for better data collection in hospital settings to better understand and manage disease risk factors in the community.

## *3.3 Introduction*

It is estimated that annually, there are approximately 17.2 million cases of gastrointestinal (GIT) illnesses in Australia (Cretikos, Telfer & McAnulty 2008a; Gilbert 2008; Hall et al. 2006). As a result, approximately one third of working older children/adults miss on average one day of work each year when they have gastroenteritis (Hall et al. 2005b). About 32% of GIT illnesses are foodborne (Hall et al. 2005a), contributing to a significant burden on health care from cost of medication, paid work time lost, hospitalisation costs and death. In Australia, it has been estimated that GIT illness costs $1249 million annually (Cretikos, Telfer & McAnulty 2008a; Gilbert 2008).

Gastrointestinal (GIT) illnesses are caused by several species of microorganisms, including viruses, bacteria and parasites, which are transmitted mainly via the faecal oral route, contaminated food and from person to person. In Australia, the disease surveillance system only captures a few GIT infections, and reports indicate that a significant proportion of illnesses are not reported, and the majority of pathogens causing illness is unknown (Hall et al. 2006; Hall et al. 2008). Hospital clinical and laboratory databases may be able to fill this gap.

Reports suggest that about 75% of the causes of GIT illnesses are unknown (Gilbert 2008). The fact that a minority of cases have a known pathogen identified creates a large gap in the knowledge of the causes of GIT illness in the Australian population. Several studies conducted by the NSW Department of Health coupled with surveillance data reveal that enteric viruses, mainly norovirus and rotavirus, are the most common causes of non-food GIT illness, accounting for approximately 15-18% of all GE cases (Cretikos, Telfer & McAnulty 2008b; Neville & McAnulty 2004). The OzFoodNet Working Group reported that approximately 32% of all gastroenteritis are foodborne and that pathogenic *Escherichia coli*, *norovirus*, *Campylobacter* and non-typhoidal *Salmonella* were responsible for over 80% of foodborne illness from 'known' pathogens (Hall et al. 2005b; Kirk et al. 2008).

Population based studies estimated that between 20% (USA) (Scallan et al. 2006) and 31% (Australia and New Zealand) (Hall & the OzFoodNet Working Group 2004b; Lake, Adlam &

Perera 2009; Lake et al. 2009b). of persons with acute diarrhoea will seek medical attention for GIT illness and between 7-50% of these persons will have a stool specimen requested for laboratory confirmation of their illness, based on duration of illness (Hall & the OzFoodNet Working Group 2004b; Scallan et al. 2006). Since only a selected few pathogens are reportable to the infectious disease surveillance system (Hall & the OzFoodNet Working Group 2004a; National Notifiable Diseases Surveillance System 2010), several emerging and re-emerging enteric pathogens are not routinely captured. This has resulted in a paucity of information about the actual causes and relative prevalence of GIT pathogens in the Australian population. It is believed that gastrointestinal illnesses contribute a significant burden to the health system, therefore knowing the actual causes and their prevalence is important, to inform prompt diagnosis and treatment, to prevent complications and reduce the burden on the health care system.

The aim of this study is to describe the clinical and epidemiological characteristics of GIT illnesses, caused by infectious pathogens amongst hospitalised patients in Sydney. It is believed that a retrospective analysis of the clinical and laboratory databases will provide information on a broad spectrum of known enteric pathogens associated with intestinal illnesses amongst hospitalised patients. This paper provides insights into the common infectious organisms associated with GIT illnesses, which is important to clinicians to inform diagnosis and treatment, and inform public health policy for their prevention and control.

## *3.4 Methods*

### 3.4.1 Study sites

Four hospitals in different geographic areas across Sydney were included in the study. These facilities were included based on the population served, and in order to have a representative cross section of different socio-economic and cultural influences across the Sydney metropolitan region. Liverpool Hospital (Hospital A) is the tertiary referral hospital for south western Sydney, providing medical, surgical, emergency medicine, intensive care, oncology, mental health, women's health and newborn care services. The Children's Hospital at Westmead (Hospital B) is a stand-alone service dedicated to paediatrics. The hospital provides community medical care, paediatric and tertiary level paediatric services to patients from all over NSW and interstate. The St. Vincent's Hospital, Sydney (Hospital C) is a major public and a principal referral hospital, specializing in heart/lung transplantation; bone marrow transplantation; cardiology; cancer; HIV medicine; respiratory medicine; mental health; and drug and alcohol services. The Prince of Wales Hospital (including cases from Sydney Children's Hospitals and the Royal Hospital for Women) (Hospital D) is a major teaching hospital, based in Sydney's eastern suburb, and one of thirteen principal referral hospitals for older children/adults that serves all of New South Wales. The hospital provides a broad range of medical, surgical services and allied health services. All hospitals attract referrals on a State-wide basis. The cases reviewed include inpatients and outpatients seen at each hospital's emergency department and/or its affiliated clinics.

Ethical approval for this study was received from the Human Research Ethics Committees (HREC) at Sydney South West Area Health Service, Western Zone, Children Hospital at Westmead and St. Vincent's Hospital, Sydney, and the University of Technology, Sydney (UTS).

### 3.4.2 Laboratory methods (to add Methods from Revised chapter 2 and 4)

The laboratory methods for the diagnosis of enteric organisms have been previously described (Fletcher et al. 2013; Stark et al. 2010a). However briefly, all laboratories conducted testing for adenovirus and rotavirus routinely in all children ≤5 years of age unless otherwise indicated or requested by the clinician. However, Hospital A tested for norovirus on request or where outbreaks were suspected. Hospital A tested for rotavirus, adenovirus serotypes 40 and 41 and norovirus using the enzyme immunoassay (EIA) method for each species respectively. Hospitals B and C used the RIDA Quick Rotavirus/Adenovirus Combi immunochromatographic test (ICT) and the RIDASCREEN norovirus test (EIA). All tests were conducted following the manufacturers recommendations. The adenovirus test used at Hospital B detects all adenovirus serotypes, and not just the enteric serotypes 40 and 41.

The identification of bacteria was routinely performed in all four laboratories using standard culture methods. In summary, selective media (Xylose, Lysine Deoxycholate agar (XLD) were inoculated for the detection of *Salmonella*, *Shigella*,*Yersinia; Aeromonas, Plesiomonas* and *Vibrio* spp on Horse Blood Agar (HBA), *Campylobacter* sp, (*Campylobacter* agar) and *Clostridium difficile* (*C. difficile* agar (CD) Oxoid Australia). *Clostridium difficile* was detected using the EIA method for hospitalisations greater than three days or otherwise indicated (history of antibiotic use, chemotherapy or immuno-suppressed).

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). 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) and formal ethyl acetate concentration (for the identification of helminths and ova). Additionally Hospital C

utilized a modified iron haematoxylin staining incorporating a carbol fuchsin step to enhance the detection of acid-fast *Isospora*, *Cryptosporidium* and *Cyclospora*, and direct DNA extraction using a QIAamP DNA stool minikit (Qiagen,Hilden, Germany) for the identification of *Entamoeba* sp., These methods have been previously described by Stark and colleagues (Stark et al. 2005b; Stark et al. 2007b; Stark et al. 2010c). An enzyme immunoassay (EIA) was performed routinely at Hospitals A and D as a screening test for the detection of *Giardia intestinalis* and Cryptosporidium parvum (ProSpecT™ *Giardia/Cryptos*poridium Microplate Assay) and the detection of *Entamoeba histolytica*/*dispar* (ProSpecT™ *Entamoeba histolytica*, Remel). All positive findings from the EIAs were confirmed by microscopy (i.e. iodine preparation and acid fast stain).

### 3.4.3 Patient data collection and analysis

A retrospective cross-sectional survey of patients who presented to hospital with GIT symptoms, and had an enteric organism detected in their stool, was conducted at each of the four hospitals. Laboratory results for all stool specimens that tested positive for an enteric organism were collected for the January 2007 to December 2010 period, for all hospitals except for Hospital C that included data for 2008-2010. The total specimen tested and individual cases were ascertained from each laboratory, from de-identified laboratory records over the study period.

Patients with diarrhoea were identified from the laboratory records provided by the four hospitals. Cases were matched by medical record number (MRN) and accession numbers/laboratory reference number (and date of birth where available). The data was then sorted to ensure that multiple specimen/episodes were merged into one record. Only one record per person was kept in the sampling frame in order to avoid the possibility of duplicate records being included in the study. Based on the volume of specimen tested at each hospital, a sample of cases was selected for review. Sample size was determined as follows. The level of confidence was set at 95%, with 80% power and a 2% margin of error based on these assumptions: that each laboratory receives

approximately 10,000 specimen; an estimated prevalence of microbes in 5% of diarrhoea cases. A sample size of 436 was required for each site. Oversampling was performed for Hospitals A, B and C in order to make up for any shortfalls in missing medical records, resulting in the following numbers reviewed: Hospital A- 494/971 cases; Hospital B- 576/932 cases; Hospital C- 500/1954 cases and Hospital D- 399/1932 cases. Lower numbers than expected were achieved at Hospital D, due to factors such as the unavailability of medical records, cost and time constraints. In the case of Hospital C, all medical records for cases seen over the January 2008-December 2009 period were reviewed. Table 3.1 summarises the sample selected for each hospital.

The cleaned list for each hospital was stratified by date of service into two main yearly seasons: cool season (Autumn- March to May / Winter-June to August]) and warm season (Spring- September to November / Summer -December to February). Cases were further stratified by three age groups (children 0-12 yrs.; young older children/adults 13-49 yrs.; and older children/adults 50-70+ years. A random number generator was used to select the sample. Clinical summaries were reviewed for signs and symptoms, risk factors, diagnosis and treatment data. The clinical data was then matched with the de-identified laboratory results.

A standardised questionnaire (See appendix 9.1) was used to collect data on demographic details, clinical data and risk factors from the patients' medical records. The data was inputted into a database created with the commercial software PASW Statistics Release version 18.0 (SPSS Inc. 2011).

### 3.4.4 Statistical analysis:

Analysis included descriptive statistics such as the median, mean and standard deviation (SD), for distribution of demographic characteristics, clinical symptoms, proportion of pathogens isolated amongst cases. The association between demographic characteristics, clinical symptoms, proportion of pathogens isolated and risk factors was examined using

the Pearson's Chi-square ($\chi^2$) test. The associations between independent variables and selected pathogens were placed into a binary logistic regression model where the dependent variable was each organism (*Blastocystis* spp, *D. fragilis*, *Campylobacter*, *C. difficile*, non-typhoidal *Salmonella*, and *Shigella* coded as 1=No, 2= Yes), and the independent variables being age group (five categories), surgery, transplant, HIV/AIDS, cancer, chronic GIT illness, antibiotic use, travel history, consumption of suspect food, and MSM status (all coded as No = 1; Yes = 2). A backward stepwise elimination process was employed, using a likelihood ratio test, to produce the most parsimonious model (Hotez 2011). In response to the small sample size and potential confounders, all variables with a bivariate P-value of <0.25 was considered to be statistically significant and placed into the model. Odds Ratios (OR) and 95% confidence intervals (95%CI) for the association were reported. Statistical analyses were performed using PASW Statistics Release version 18.0 (SPSS Inc. 2011).

**Table 3-1: Distribution of diarrhoeal cases based on specimen tested and sample reviewed¶ in the four study sites**

| Hospital laboratory | Stool specimen tested[a] | Individual cases[b] | Positive for pathogen (%) | Medical records reviewed n (% of positives) | Cases included in this study n (% of reviewed) |
|---|---|---|---|---|---|
| **Hospital A** | 7115 | 4252 | 971 (14%) | 494 (51%) | 464 (94%) |
| **Hospital B** | 10123 | 5229 | 932 (9%) | 576 (62%) | 550 (95%) |
| **Hospital C** | 8613 | 6237 | 1954 (23%) | 500 (22%) | 421 (88%) |
| **Hospital D** | 6078 | 3772 | 1932 (32%) | 399 (21%) | 287 (72%) |
| **Total** | **40508** | **19490** | **5789 (14%)** | **1890 (33%)** | **1722 (91%)** |

[a]Includes individuals who submitted multiple stool specimen

[b]Represent individual cases (regardless of number of stool specimen). Hospital C- medical records reviewed for 500 cases seen in 2008-2009 only; complete data available for 421 cases, Hospital D- medical records unavailable for a number of patients, only 399 reviewed and 112 cases with formed stools removed from the analysis. ¶Sample assumptions: baseline prevalence 5%; 95%CI; 2% margin of error;

## *3.5 Results*

### 3.5.1 Demographic and clinical characteristics.

The selection of cases from the four sites is presented in Table 3.1. A total of 1722 cases including 464 from Hospital A, 550 from Hospital B, 421 from Hospital C and 287 from Hospital D were included in this study. The patients aged 0-99 years (mean ± SD= 28.3± 29.5 years), all presented with loose stools or diarrhoea. The distribution of the cases from each hospital by age and gender is presented in Table 3.2. Hospitals A and C had more adult cases with 42% and 48% respectively, being 50 years or older. Conversely, Hospitals B and D had predominantly children 0-5 years accounting for 76% and 44% respectively. There were a few more males (overall 55%) than females across all sites. The age specific distribution of the cases based on gender, clinical profile and diagnosis is presented in Table 3.3. There were more males than females in all age groups except for the >75 years age group that had 1.5:1.0 females to male ratio Females were slightly older with an average age of 45 years (mean ± SD= 44.6 ± 29.7) compared with males (mean ± SD= 36.2 ± 24.7 years).

On average, children presented with elevated body temperature (37.8 ± 1.1°C), but older persons were more likely to have normal body temperature. The overall length of stay in hospital was 8.9± 21.4 days; and persons 60 years and older stayed for an average of 15 days compared with younger persons spending 5 days on average. Most persons paid at least one visit to hospital for a diarrhoea related illness, with illness in children lasting for an average of 7 days compared with older persons, being ill for an average of 11 days. The reporting of vomiting, fever, abdominal pain, and respiratory symptoms decreased with increasing age. Younger persons were more likely to have a principal diagnosis of a GIT infection compared with older children/adults.

**Table 3-2: Distribution of cases from the four hospitals based on age and gender**

| ***Characteristics*** | | ***Hospital*** | | | | |
|---|---|---|---|---|---|---|
| | | Hospital A % (n) | Hospital B % (n) | Hospital D % (n) | Hospital C % (n) | Total % (n) |
| **Age Group (N=1718)** | 0-5 yrs. | 27.8 (129) | 76.3 (422) | 44.3 (127) | 0.0 (0) | 39.5 (678) |
| | 6-12 yrs. | 5.2 (24) | 15.5 (85) | 6.6 (19) | 0.0 (0) | 7.5 (128) |
| | 13-24yrs | 7.3 (34) | 7.8 (43) | 9.1 (26) | 14.6 (61) | 9.6 (164) |
| | 25-49yrs | 17.7 (82) | 0.0 (0) | 17.1 (49) | 37.4 (156) | 16.7 (287) |
| | 50-75yrs | 26.5 (123) | 0.0 (0) | 9.8 (28) | 28.3 (118) | 15.7 (269) |
| | >75yrs | 15.5 (72) | 0.0 (0) | 13.2 (38) | 19.7 (82) | 11.2 (192) |
| **Gender (N=1715)** | Male | 52.2 (241) | 56.9 (313) | 50.5 (143) | 61.7 (259) | 55.7 (956) |
| | Female | 47.8 (221) | 43.1 (237) | 49.5 (140) | 38.3 (161) | 44.3 (759) |
| | Total per Hospital | 27.0 (464) | 31.9 (549) | 16.7 (287) | 24.5 (421) | 100.0 (1721) |

**Table 3-3: Age distribution of diarrhoea cases based on gender, clinical profile and pathogens detected**

| *Characteristics* | *0-5 yrs % (n)* | *6-12 yrs % (n)* | *13-24yrs % (n)* | *25-49yrs % (n)* | *50-75yrs % (n)* | *>75yrs % (n)* | *Total % (n)* | *$X^2$(df) P-value* |
|---|---|---|---|---|---|---|---|---|
| **Gender** | | | | | | | | |
| Male | 56.3 (379) | 56.3 (72) | 53.0 (87) | 60.8 (174) | 61.7 (166) | 39.1 (75) | 55.7 (953) | 29.12(5) 0.001 |
| Female | 43.7 (294) | 43.7 (56) | 47.0 (77) | 39.2 (112) | 38.3 (103) | 60.9 (117) | 44.3 (759) | |
| **Symptoms** | | | | | | | | |
| Diarrhoea | 97.9 (663) | 99.2 (127) | 98.8 (162) | 98.6 (283) | 98.5 (265) | 99.5 (191) | 98.5 (1691) | 3.25(5) 0.662 |
| Vomiting | 66.5 (450) | 54.7 (70) | 55.5 (91) | 41.8 (120) | 33.8 (91) | 32.8 (63) | 51.5 (885) | 133.57(5) <0.001 |
| Nausea | 3.4 (23) | 14.8 (19) | 36.6 (60) | 43.6 (125) | 33.8 (91) | 23.4 (45) | 21.1 (363) | 267.41(5) <0.001 |
| Abdominal pain | 19.8 (134) | 75.0 (96) | 75.6 (124) | 69.0 (198) | 36.8 (99) | 27.1 (52) | 40.9 (703) | 378.68(5) <0.001 |
| Fever | 57.2 (387) | 60.2 (77) | 50.0 (82) | 54.7 (157) | 29.7 (80) | 20.8 (40) | 47.9 (823) | 128.50(5) <0.001 |
| Dehydration | 29.5 (200) | 22.7 (29) | 23.8 (39) | 20.6 (59) | 19.7 (53) | 21.4 (41) | 24.5 (421) | 16.36(5) 0.006] |
| Anorexia/Loss of appetite | 43.7 (212) | 39.7 (31) | 32.7 (36) | 23.0 (41) | 29.5 (44) | 33.7 (35) | 36.1 (399) | 29.39(5) <0.001 |
| Lethargy | 56.5 (257) | 35.7 (25) | 19.2 (20) | 14.1 (23) | 26.7 (36) | 32.3 (30) | 38.3 (391) | 129.35(5) <0.001 |
| Respiratory symptoms | 32.3 (147) | 18.6 (13) | 3.8 (4) | 4.3 (7) | 3.7 (5) | 12.9 (12) | 18.4 (188) | 116.33(5) <0.001 |
| **Diagnosis** | | | | | | | | |

| *Characteristics* | *0-5 yrs % (n)* | *6-12 yrs % (n)* | *13-24yrs % (n)* | *25-49yrs % (n)* | *50-75yrs % (n)* | *>75yrs % (n)* | *Total % (n)* | *$X^2$(df) P-value* |
|---|---|---|---|---|---|---|---|---|
| **ICD-10-AM A00-A09** | 72.6 (486) | 76.8 (96) | 71.2 (116) | 64.6 (181) | 29.2 (78) | 25.9 (49) | 59.4 (1006) | |
| Non-infectious GE | 1.6 (11) | 4.8 (6) | 7.4 (12) | 8.2 (23) | 4.9 (13) | 2.6 (5) | 4.1 (70) | |
| **Co-morbidities** | | | | | | | | |
| Surgery | 3.8 (26) | 3.9 (5) | 8.0 (13) | 7.4 (21) | 29.2 (78) | 24.7 (47) | 11.1 (190) | 172.6(5) <0.001 |
| Transplant (organ, bone marrow) | 1.3 (9) | 0.8 (1) | 5.5 (9) | 5.3 (15) | 8.6 (23) | 0.5 (1) | 3.4 (58) | 43.5(5) <0.001 |
| HIV/AIDS | 0 (0) | 0 (0) | 0 (0) | 12.0 (34) | 4.5 (12) | 1.1 (2) | 2.8 (48) | 120.3(5) <0.001 |
| Cancer | 2.5 (17) | 3.9 (5) | 4.9 (8) | 6.0 (17) | 23.5 (63) | 8.4 (16) | 7.4 (126) | 130.4(5) <0.001 |
| Neonatal illness | 2.8 (19) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 29.3(%) <0.001 |
| **Pathogens detected** | | | | | | | | |
| Adenovirus | 17.5 (118) | 3.9 (5) | 2.4 (4) | 0.0 (0) | 0.4 (1) | 1.0 (2) | 7.6 (130) | |
| Rotavirus | 22.4 (151) | 4.7 (6) | 0.6 (1) | 0.0 (0) | 1.1 (3) | 0.0 (0) | 9.4 (161) | |
| Norovirus | 19.6 (132) | 6.3 (8) | 3.7 (6) | 1.4 (4) | 3.7 (10) | 12.0 (23) | 10.7 (183) | |
| *Aeromonas* | 0.0 (0) | 0.0 (0) | 1.8 (3) | 0.3 (1) | 2.2 (6) | 2.6 (5) | 0.9 (15) | |
| *C. difficile* | 4.4 (30) | 5.5 (7) | 10.4 (17) | 17.1 (49) | 46.1 (124) | 54.2 (104) | 19.3 (331) | *394.78(5) <0.001* |
| *Campylobacter* | 15.9 (107) | 30.5 (39) | 42.7 (70) | 31.0 (89) | 16.4 (44) | 14.6 (28) | 22.0 (377) | *85.64(5) <0.001* |
| *Shigella* spp | 0.9 (6) | 1.6 (2) | 2.4 (4) | 9.8 (28) | 1.5 (4) | 0.5 (1) | 2.6 (45) | 69.81(5) |

| ***Characteristics*** | ***0-5 yrs % (n)*** | ***6-12 yrs % (n)*** | ***13-24yrs % (n)*** | ***25-49yrs % (n)*** | ***50-75yrs % (n)*** | ***>75yrs % (n)*** | ***Total % (n)*** | ***$X^2$(df) P-value*** |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | <0.001 |
| *Salmonella* spp (Non typhoidal). | 13.1 (88) | 24.2 (31) | 22.0 (36) | 16.4 (47) | 9.7 (26) | 5.7 (11) | 13.9 (238) | |
| *Salmonella typhi* A | 0.3 (2) | 3.1 (4) | 0.6 (1) | 1.4 (4) | 0.4 (1) | 0.0 (0) | 0.7 (12) | 43.57(5) <0.001 |
| Enteric protozoa | 6.2 (42) | 20.3 (26) | 12.8 (21) | 19.5 (56) | 19.0 (51) | 8.3 (6) | 12.3 (212) | 58.6(5) <0.001 |
| | | | | | | | | |
| Other bacteria (MRSA, NORSA, *Plesiomonas*) | 0.1 (1) | 0.0 (0) | 0.6 (1) | 2.4 (7) | 1.5 (4) | 1.0 (2) | 0.9 (15) | |
| **Total Age-groups** | **39.4 (675)** | **7.5 (128)** | **9.6 (164)** | **16.7 (287)** | **15.7 (269)** | **11.2 (192)** | **100.0 (1715)** | |

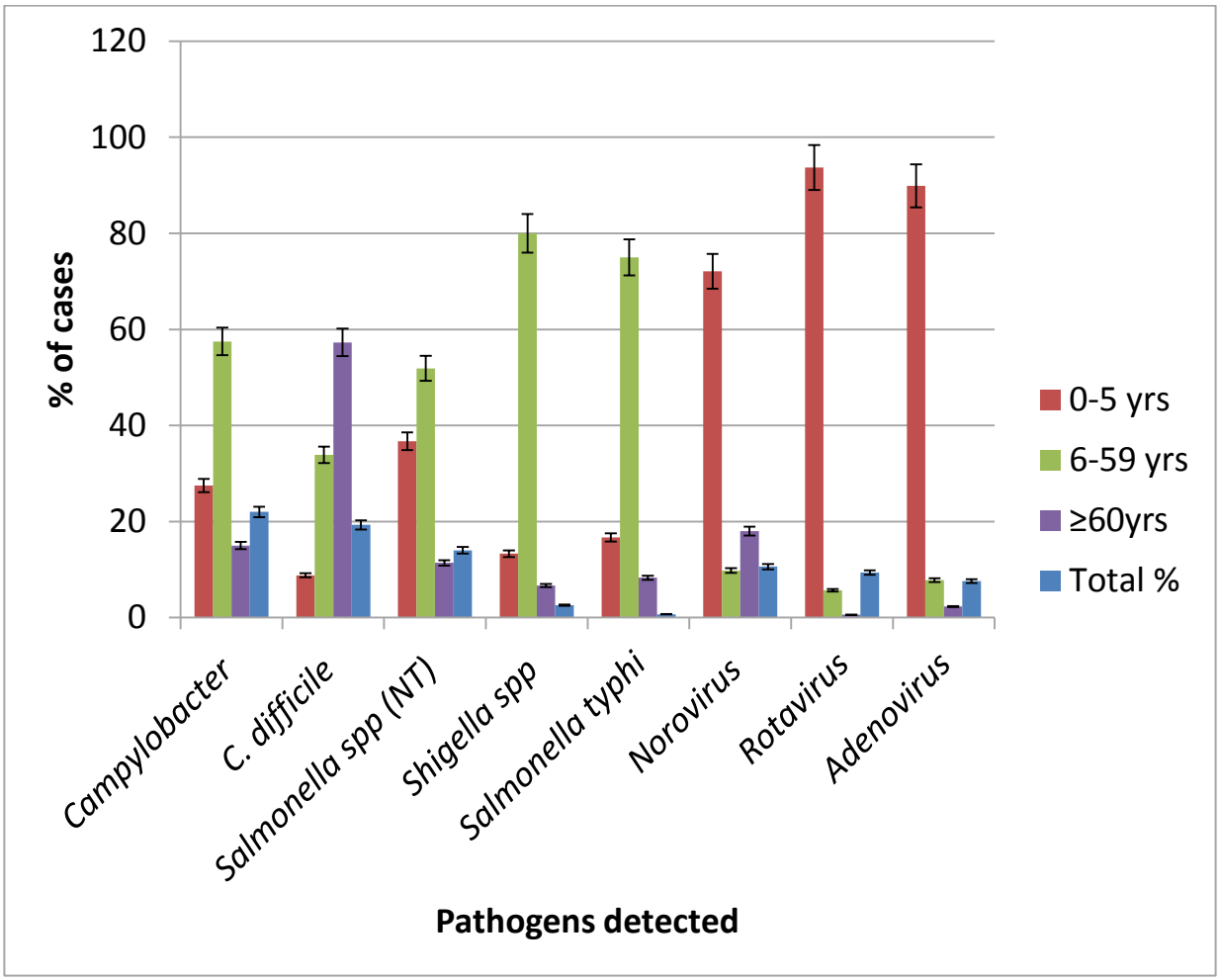
### 3.5.2 Pathogens associated with diarrhoea

An enteric pathogen was detected in between 9%-32% of stool specimen in each hospital. Patients were diagnosed with a variety of bacterial, viral and protozoan infections. The distribution of pathogens and associated clinical factors are presented in Table 3.4. *Campylobacter* spp, was most frequently detected (22.0%) followed by *C. difficile* (19.3%), non-typhoidal *Salmonella* (14.0%), norovirus (10.6%), rotavirus (9.4%) and adenovirus (7.6%). *Blastocystis* spp (7.2%), *Giardia intestinalis* (2.9%) and *Dientamoeba fragilis* (1.8%) were the most common enteric protozoa detected. Other bacterial infections diagnosed included *Shigella* spp (2.6%), *Salmonella typhi* (0.7%), and *Aeromonas, Plesiomonas, Yersinia enterocolitica*, multi-resistant *Staphylococcus aureus* (MRSA) in <1% of cases respectively. Approximately one third of those infected with *Campylobacter* were 0-5 years old. Persons with *Campylobacter* infection mainly presented with watery diarrhoea (49.1%), blood or mucous in stools (33.8%), abdominal pain (63.1%) and fever (56.5%) (*P*<0.001). *Clostridium difficile* infections increased with age, with 68% of those affected were 50 years or older (P<0.001) and mainly presented with watery diarrhoea (62.8%). More than one third (37%) of those infected with non-typhoidal *Salmonella* spp were 0-5 years old. Non-typhoidal Salmonella infections were associated with vomiting (60.8%; P<0.001), abdominal pain (59.2%; P<0.001), and fever (66.3%; P<0.001).

The age distribution of enteric pathogens is presented in Figure 3.1. The majority of cases in the 0-5 age group were infected with rotavirus (22.4%), norovirus (19.6%), and adenovirus (17.5%), and incidence of each virus decreased with increasing age (P=0.001). The majority of persons with norovirus infection reported vomiting (63.4%; P<0.001) and lethargy (51.4%: P<0.002). Rotavirus infection was associated with vomiting (85.7%; P<0.001), fever (68.3%; P<0.001), dehydration (50.9%; P<0.001) and lethargy (66.4%: P<0.001). Adenovirus infections were also associated with vomiting (61.5%; P=0.011), lethargy (54.2%: P<0.002) and upper respiratory symptoms (41.0%; P=0.001). Infections with *Blastocystis* spp increased with increasing age (P<0.001), and the incidence of *G. intestinalis* was highest in persons under 13 years (0.018). Nearly all protozoan infections

were associated with diarrhoea ($P<0.001$). Other symptoms associated with protozoan infection include abdominal pain and vomiting ($P<0.05$).

**Figure 3-1: Age-distribution of patients with enteric bacteria and viruses diagnosed in diarrhoeal cases in Sydney**




Bars show prevalence with 5% error bars.

**Table 3-4: Distribution of bacterial and viral pathogens based on clinical and risk factors in diarrhoea cases in Sydney, 2007-2010**

| Patient Characteristics | *Campylobacter* % (n) | *C. difficile* % (n) | *Salmonella* spp (N-T) % (n) | *Shigella spp* % (n) | *Salmonella typhi* A % (n) | Noroviruses % (n) | Rotaviruses % (n) | Adenoviruses % (n) |
|---|---|---|---|---|---|---|---|---|
| ***Total Prevalence (all age)*** | ***22 (379)*** | **19.3 (331)** | ***14.0 (240)*** | ***2.6 (45)*** | ***0.7 (12)*** | **10.6 (183)** | **9.4 (161)** | **7.6 (130)** |
| 0-5 yrs | 27.5 (103) | 8.8 (29) | 36.7 (87) | 13.3 (6) | 16.7 (2) | 72.1 (132) | 93.7 (149) | 89.9 (116) |
| 6-59 yrs | 57.5 (215) | 33.9 (112) | 51.9 (123) | 80.0 (36) | 75.0 (9) | 9.8 (18) | 5.7 (9) | 7.8 (10) |
| ≥60yrs | 15.0 (56) | 57.3 (189) | 11.4 (27) | 6.7 (3) | 8.3 (1) | 18.0 (33) | 0.6 (1) | 2.3 (3) |
| **Signs and symptoms** | | | | | | | | |
| Diarrhoea | 99.7 (378) | 98.2 (325) | 100.0 (240) | 100.0 (45) | 91.7 (11) | 97.8 (179) | 98.8 (159) | 97.7 (127) |
| Vomiting | 44.1 (167) | 29.9 (99) | 60.8 (146) | 53.3 (24) | 58.3 (7) | 63.4 (116) | 85.7 (138) | 61.5 (80) |
| Abdominal pain | 63.1 (239) | 28.4 (94) | 59.2 (142) | 75.6 (34) | 58.3 (7) | 17.5 (32) | 16.8 (27) | 12.3 (16) |
| Fever | 56.5 (214) | 27.8 (92) | 66.3 (159) | 68.9 (31) | 83.3 (10) | 41.5 (76) | 68.3 (110) | 44.6 (58) |
| Dehydration | 23.7 (90) | 13.6 (45) | 31.3 (75) | 44.4 (20) | 25.0 (3) | 19.1 (35) | 50.9 (82) | 30.0 (39) |
| Anorexia/Loss of appetite | 34.2 (81) | 27.4 (45) | 45.1 (73) | 16.7 (6) | 54.5 (6) | 31.9 (37) | 48.6 (67) | 43.7 (38) |
| Lethargy | 29.0 (62) | 25.0 (37) | 38.5 (57) | 15.6 (5) | 60.0 (6) | 51.4 (56) | 66.4 (89) | 54.2 (45) |
| Respiratory symptoms | 14.5 (31) | 10.7 (16) | 11.5 (17) | 0 (0) | 60.0 (6) | 33.0 (36) | 24.6 (33) | 41 (34) |
| **Stool description** | | | | | | | | |
| Severe-explosive | 10.3 (39) | 1.5 (5) | 12.9 (31) | 26.7 (12) | 25.0 (3) | 6.6 (12) | 9.9 (16) | 6.9 (9) |
| Watery | 49.1 (186) | 62.8 (208) | 49.2 (118) | 44.4 (20) | 16.7 (2) | 71.6 (131) | 73.3 (118) | 73.8 (96) |
| Loose-unformed | 2.9 (11) | 13.9 (46) | 2.9 (7) | 0 (0) | 33.3 (4) | 11.5 (21) | 5.6 (9) | 10.8 (14) |

| Patient Characteristics | *Campylobacter* % (n) | *C. difficile* % (n) | *Salmonella* spp (N-T) % (n) | *Shigella spp* % (n) | *Salmonella typhi* A % (n) | Noroviruses % (n) | Rotaviruses % (n) | Adenoviruses % (n) |
|---|---|---|---|---|---|---|---|---|
| Bloody/mucous | 33.8 (128) | 9.4 (31) | 30.8 (74) | 28.9 (13) | 8.3 (1) | 4.4 (8) | 6.2 (10) | 6.9 (9) |
| Persistent >14days | 2.9 (11) | 8.8 (29) | 4.2 (10) | 0 (0) | 16.7 (2) | 4.9 (9) | 4.3 (7) | 1.5 (2) |
| **Seasonal distribution** | | | | | | | | |
| Autumn | 27.2 (102) | 21.1 (68) | 34.3 (80) | 26.7 (12) | 33.3 (4) | 12.6 (21) | 16.9 (26) | 30.9 (38) |
| Winter | 22.9 (86) | 26.0 (84) | 15.5 (36) | 24.4 (11) | 16.7 (2) | 33.5 (56) | 31.8 (49) | 26.0 (32) |
| Spring | 20.3 (76) | 26.3 (85) | 15.5 (36) | 17.8 (8) | 16.7 (2) | 27.5 (46) | 33.8 (52) | 19.5 (24) |
| Summer | 29.6 (111) | 26.6 (86) | 34.8 (81) | 31.1 (14) | 33.3 (4) | 26.3 (44) | 17.5 (27) | 23.6 (29) |
| **Co-morbidities** | | | | | | | | |
| Surgery | 4.0 (15) | 34.0 (112) | 2.9 (7) | 2.2 (1) | 0 (0) | 8.2 (15) | 2.5 (4) | 6.2 (8) |
| Transplant (organ, bone marrow) | 1.9 (7) | 9.4 (31) | 0.8 (2) | 0 (0) | 0 (0) | 2.7 (5) | 0.6 (1) | 3.1 (4) |
| HIV/AIDS | 0.8 (3) | 2.4 (8) | 0.8 (2) | 37.8 (17) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Cancer | 5.0 (19) | 15.5 (51) | 3.8 (9) | 2.2 (1) | 8.3 (1) | 7.1 (13) | 0.6 (1) | 3.8 (5) |
| **Other risk factors** | | | | | | | | |
| Antibiotic/chemotherapy | 7.4 (28) | 65.9 (216) | 14.2 (34) | 25.0 (11) | 16.7 (2) | 22.0 (40) | 11.9 (19) | 15.4 (20) |
| Chronic Gastrointestinal illness | 6.4 (24) | 17.3 (57) | 4.6 (11) | 6.8 (3) | 0 (0) | 4.9 (9) | 3.1 (5) | 2.3 (3) |
| Consumption of suspect food | 14.4 (54) | 0.6 (2) | 15.5 (37) | 20.5 (9) | 1 (0) | 1.1 (2) | 2.5 (4) | 0.8 (1) |
| Involved in FBI outbreak | 0.8 (3) | 0.3 (1) | 2.9 (7) | 0 (0) | 0 (0) | *0.5 (1)* | *0.6 (1)* | *1.5 (2)* |
| MSM status[¶] | 4.3 (3) | 3.6 (4) | 4.0 (1) | 58.1 (18) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Travel history<6wks | 6.9 (26) | 2.4 (8) | 13.0 (31) | 27.3 (12) | 75.0 (9) | 3.8 (7) | 9.3 (15) | 0.8 (1) |

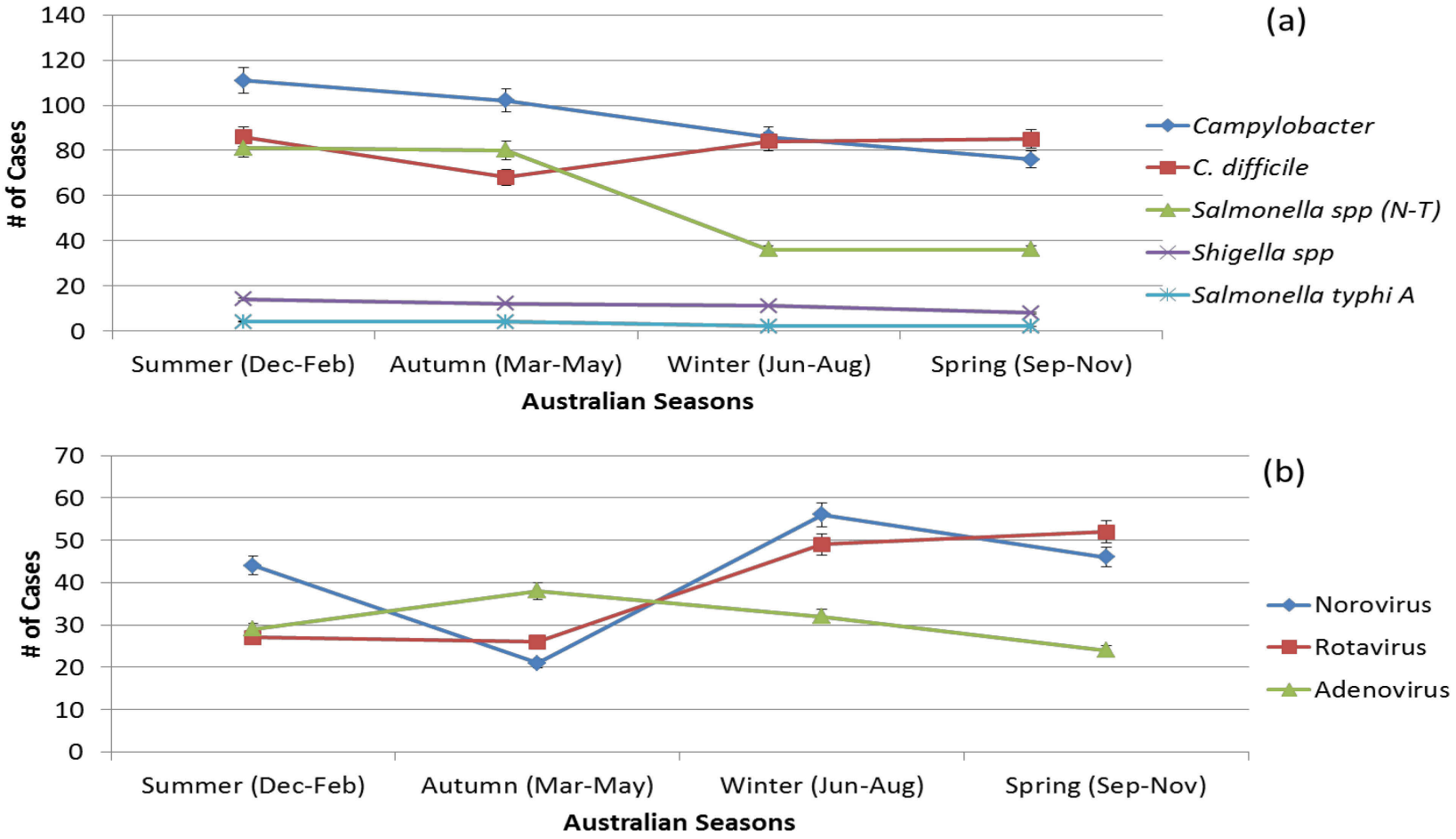
[¶]Information available for cases from Hospital C only (N=313).

### 3.5.3 Relationship between pathogens and disease risk factors

The seasonal distribution of bacterial infections was not very pronounced (Figure 3A), except that infections with *Campylobacter* and N-T *Salmonella* spp, were slightly more common in the summer and decreased gradually over the cooler months, and the incidence of all *Salmonella* species doubled in both autumn and summer. However there were clear seasonal patterns with viral infections (Figure 3B). Rotavirus presented a distinct seasonal pattern with the lowest rates occurring in the warm months and peaking in the cooler months ($P<0.001$). Adenovirus showed a less consistent monthly trend, and norovirus detection increased in the cooler months.

Bivariate analysis revealed significant associations ($P <0.05$) between various risk factors for GIT infection and the common pathogens. A suspected foodborne illness was closely associated with infection with *Campylobacter* spp ($X^2$(df) = 40.1(1)) and non-typhoidal *Salmonella* spp ($X^2$(df) =30.5(1)). *Clostridium difficile* infections were associated with prolonged antibiotic/chemotherapy use ($X^2$(df) = 386.1(2)); surgery ($X^2$(df) = 216.9(1)); chronic GIT illness ($X^2$(df) =42.20(1)); organ or blood marrow transplant ($X^2$(df) =46.9(1); and diagnosis of any form of cancer ($X^2$(df) = 39.5(1)). *Salmonella typhi* infection was associated with overseas travel ($X^2$(df) = 76.9(1)). Infection with *Shigella* spp, was associated with several risk factors including recent overseas travel ($X^2$(df) = 24.1(1)); consumption of suspicious food ($X^2$(df) =12.41(1)); HIV/AIDS sero-status ($X^2$(df) =206.9(1)); and MSM status ($X^2$(df) = 61.1(2)). A total of 237 patients had diarrhoea onset >72 hours after hospitalisation (suspected nosocomial infection). Among these 56% (132) were diagnosed with *C. difficile* infection; 78% (103/132) of whom reported prolonged antibiotic therapy. Bivariate logistic regression analysis revealed that the risk of *C. difficile* infection was significantly increased among persons who had a recent surgical intervention (OR 2.1; 95%CI 1.1-4.0), were on prolonged antibiotic treatment (OR 8.1; 95%CI 4.2-15.6) and reported a Chronic GIT illness (OR 3.9; 95%CI 1.1-14.7).

**Figure 3-2: Seasonal distribution of (A) Bacterial pathogens and (B) Viral pathogens associated with diarrhoea**




A. Bacterial pathogens associated with diarrhoea based on season of diagnosis B: Viral pathogens associated with diarrhoea based on season of diagnosis. Graph shows prevalence with 5% error bars.

The majority of these associations with the pathogens lost their significance when further examined by logistic regression (Table 3.5). Some associations remained significant (OR; 95%CI; P-value). Persons aged 25-49 years (OR 3.0; P<0.070), 50-75 years (OR 3.1; P<0.050); and MSM (OR 2.3; P<0.070) were more likely to be infected with *Blastocystis*. Men who have sex with men were thirteen times (OR 12.8; P=0.031) more likely to be infected with *D. fragilis*. Infection with *C. difficile* was more likely in persons who were on prolonged antibiotic therapy (OR 6.3; P<0.001), had a recent surgery (OR 2.2; P=0.030), and a chronic GIT illness (OR 2.4; P=0.035). However infection was 60% less likely in females compared with men (OR 0.4; P=0.031), and in those who were HIV infected (OR 0.4; P=0.070). Infection with *Campylobacter* spp was 90% less likely in persons on prolonged antibiotic therapy (OR 0.1; P<0.001), 80% less likely in MSM (OR 0.2; P<0.011), and 70% less likely in those 50-75 years old (OR 0.3; P<0.038),; but was more than seven times more likely in those who had a recent transplant (OR 7.0; P<0.010), and those with a diagnosis of cancer (OR 7.5; P<0.002). Persons infected with *Shigella* spp were five times more likely to be MSM (OR 5.0; P=0.007) and three times more likely to be HIV infected (OR 3.3; P=0.055). Young older children/adults in the 13-24 years and 25-49 years were more likely to be diagnosed with *Campylobacter* (OR 2.5; P=0.101 and OR 1.8 P=0.206) and non-typhoidal (N-T) *Salmonella* spp (OR 14.4; P=0.003 and OR 3.6 P=0.102) respectively.

**Table 3-5: Association between selected pathogens, demographic, clinical and other risk factors**

| Co-variates | Odds Ratio | 95% CI | P-value |
|---|---|---|---|
| **Blastocystis spp.** | | | |
| MSM Status (Ref= No) | 2.3 | 1.0-5.4 | 0.055 |
| Comorbidity surgery (Ref= No) | 2.1 | 0.9-4.7 | 0.068 |
| Age group (Ref >75 years) | | | 0.087 |
| 13yrs-24yrs | 0.5 | 0.1-5.1 | 0.582 |
| 25yrs-49yrs | 3.0 | 0.9-9.9 | 0.070 |
| 50yrs-75yrs | 3.1 | 1.0-9.8 | 0.050 |
| **Dientamoeba fragilis** | | | |
| MSM Status (Ref= No) | 12.8 | 1.3-130.7 | 0.031 |
| Comorbidity transplant (Ref= No) | 10.1 | 0.7-146.2 | 0.089 |
| Comorbidity cancer (Ref= No) | 13.8 | 1.6-122.1 | 0.018 |
| **Clostridium difficile** | | | |
| Age group (Ref >75 years) | | | **0.000** |
| 13yrs-24yrs | 0.1 | 0.0-0.3 | 0.001 |
| 25yrs-49yrs | 0.3 | 0.1-0.6 | 0.003 |
| 50yrs-75yrs | 0.8 | 0.4-1.8 | 0.611 |
| Gender (Ref= Male) | 0.4 | 0.2-0.9 | 0.031 |
| Comorbidity surgery (Ref= No) | 2.2 | 1.1-4.6 | 0.030 |
| Comorbidity HIV (Ref= No) | 0.4 | 0.1-1.1 | 0.070 |
| Comorbidity cancer (Ref= No) | 0.3 | 0.1-0.9 | 0.030 |
| MSM Status (Ref= No) | 0.3 | 0.1-1.0 | 0.051 |
| Antibiotic use (Ref= No) | 6.3 | 3.2-12.2 | 0.000 |
| Chronic GE (Ref= No) | 2.4 | 1.1-5.3 | 0.035 |
| Suspect food (Ref= No) | 0.2 | 0.0-1.5 | 0.113 |
| **Campylobacter spp.** | | | |
| MSM Status (Ref= No) | 0.2 | 0.0-0.7 | 0.011 |
| Comorbidity transplant (Ref= No) | 7.0 | 1.6-30.6 | 0.010 |

| Co-variates | Odds Ratio | 95% CI | P-value |
|---|---|---|---|
| **Comorbidity cancer (Ref= No)** | 7.5 | 2.1-26.6 | 0.002 |
| **Antibiotic use (Ref= No)** | 0.1 | 0.0-0.2 | 0.000 |
| **Age group (Ref >75 years)** | | | 0.001 |
| **13yrs-24yrs** | 2.5 | 0.8-7.4 | 0.101 |
| **25yrs-49yrs** | 1.8 | 0.7-4.4 | 0.206 |
| **50yrs-75yrs** | 0.3 | 0.1-0.9 | 0.038 |
| **Non-Typhoidal Salmonella spp.** | | | |
| **MSM Status (Ref= No)** | 0.2 | 0.0-1.8 | 0.154 |
| **Comorbidity surgery (Ref= No)** | 0.2 | 0.0-1.4 | 0.094 |
| **Age group (Ref >75 years)** | | | 0.003 |
| **13yrs-24yrs** | 14.4 | 2.4-85.8 | 0.003 |
| **25yrs-49yrs** | 3.6 | 0.8-16.9 | 0.102 |
| **50yrs-75yrs** | 0.7 | 0.1-4.6 | 0.693 |
| **Gender (Ref= Male)** | 7.4 | 2.2-24.8 | 0.001 |
| **Shigella spp.** | | | |
| **MSM Status (Ref= No)** | 5.0 | 1.6-16.0 | 0.007 |
| **Comorbidity HIV (Ref= No)** | 3.3 | 1.0-10.9 | 0.055 |
| **Suspect food (Ref= No)** | 3.5 | 1.1-11.2 | 0.037 |

## *3.6 Discussion*

This study is the largest hospital based study to incorporate data from multiple sites to describe the epidemiology of infectious GIT illnesses in NSW State in the last 20 years. We have provided an overview of the epidemiological profile of GIT illnesses associated with enteric pathogens, amongst persons seeking care in Sydney across four major public hospitals.

The study found that GIT illnesses affect both adult and children; however the severity of clinical symptoms and the prevalence of enteric pathogens are different for each age group. Slightly more males than females report to Hospital with GIT illness, however amongst the elderly, women are more likely to be seen in Hospital. This is not in keeping with Australian national data which suggests an overall higher rate of GIT illness in women especially in the 20-40 age group (Hall et al. 2006). The reason for these differences in age group is not clear but it may be related to the differences in exposure between the genders of the two groups, at different spectrum of the lifespan. A USA study also found that more men than women will seek medical attention for severe GIT symptoms (Scallan et al. 2006).

Persons 60 years old had a longer length of stay (LOS) in hospital (average of 15 days) compared with younger persons (average 5 days). The extended LOS for older patients is may be due to dysfunction of the immune system with aging, that leads to an increased incidence of infectious disease, and elderly patients are likely to have co-morbidities that also require medical attention (McGlauchlen & Vogel 2003; Nikolich- & Zcaron 2008). Similar lengths of stay was found in other studies of adult patients in Brazil (Borges et al. 2008). Additionally, infection in older persons could be associated with underlying immuno-suppression and prolonged antibiotic therapy. Other studies have established that the presence of co-morbidities in older age patients is a significant predictor of a prolonged length of stay in hospital for GIT illness (Dubberke et al. 2008; Jansen et al. 2008). Infectious diarrhoea in children is usually self-limiting and is managed conservatively in developed settings like Australia, where adequate facilities are available

for the management and care of children. Gastrointestinal symptoms in children is more likely to result in a diagnosis of infectious GIT illness, since children have less developed immune systems rendering them more susceptible to some infections (Thompson 2000). Children are not necessarily clean and often have poor hygiene practices, that can lead to spread of infection from person to person (Curtis, Cairncross & Yonli 2001; Feachem 1984). The medical literature suggests that in cases with immuno-suppressive status and co-morbidities such as recent surgeries, recurrent antibiotic or chemotherapy use, and chronic GIT illness, diarrhoeal illness may be considered as side effects or due to non-infectious aetiologies (Fine, Seidel & Do 2000; Fiore & Cutsem 2009).

Diarrhoeal illness in children and the elderly, in the Australian setting has economic significance since the illness is not only costly to the health care system but to the parents/carers who miss many days of work as a result of caring for sick children and elderly parents. The impact of the GIT illness is far reaching in terms of the economic costs of diagnosis, treatment, man-hours loss due to frequency of illness, and cost of medication (Frenzen 2005; Majowicz et al. 2005). In resource constrained settings, household economic costs of diarrhoea episodes can have both an economic impact, by reduced household resources for other activities and a health impact, by influencing whether households seek care and how much care they seek. Households are repeatedly balancing health risks and associated economic costs (Andersen & Davidson 2001; Rheingans et al. 2012a; Rheingans et al. 2012b).

Several enteric pathogens were detected from between 9%-32% of patients with diarrhoea, and the types and frequency of organisms detected varied based on the age of patients. This study found that elderly patients were more likely to be diagnosed with infections with *C. difficile*. Increased risk of *C. difficile* infections and mortality has been described in elderly patients in Australia (Thomas et al. 2002) and other developed settings (Freeman et al. 2010; Loo et al. 2005). Other Australian based studies looking at GIT illness in the elderly have found that infections with *Campylobacte*r, toxigenic *Escherichia coli* infections, and shigellosis were higher in community-based residents, compared with higher rates of *Salmonella* infection in residents of long-term care facilities

(LTCFs) (Kirk et al. 2012). Young older children/adults and older children on the other hand were prone to infection with food-borne pathogens such as *Campylobacte*r and *Salmonella* spp, travel related illnesses such as *Shigella* spp, and enteric protozoa (for e.g. *Blastocystis* spp). Children were significantly more likely to be infected with the enteric viruses: rotavirus, norovirus and adenovirus; as well as the enteric protozoa *G. intestinalis*. These findings may indicate that differences in health seeking behaviour (community versus hospital) may be associated with not only the severity of illness but also with specific pathogens.

*Clostridium difficile* and *Campylobacter* were the most common bacterial pathogens found in this study. The increased risk of *C. difficile* infection associated with prolonged antibiotic use and particularly amongst persons with extended length of stay in Hospital indicate a need for good antibiotic stewardship, and that existing protocols and practices for the control of *C. difficile* should be carefully reviewed and modified where necessary (Vonberg et al. 2008). Similar rates were found in adult cases in Sweden (Svenungsson, Lagergren & Lundberg 2001) and nosocomial infections in Costa Rica, (Zumbado-Salas et al. 2008). *Clostridium difficile* has been linked to between 20–50% of cases of antibiotic-associated diarrhoea and almost 100% of pseudomembranous colitis cases (Svenungsson, Lagergren & Lundberg 2001). However the high incidence in this population may be an indication of an increase in community acquired *C. difficile* infections in otherwise healthy patients, which has been observed in other developed countries (Abrahamian et al. 2006). Infection control programmes in Hospitals and aged-care settings should ensure attention is given to the monitoring of *C. difficile* infections.

Campylobacteriosis is the most common enteric infection reported to surveillance systems across Australian States, excepting for NSW (National Notifiable Diseases Surveillance System 2010) where it is not notifiable. This study has found that in keeping with data from other Australian states, Campylobacteriosis is significantly more common than Salmonellosis and hence consideration should be given to including it in routine surveillance. A significant association was made between infection with *Shigella* spp, HIV/AIDs patients and MSM, which warrants further investigation. *Shigella* spp are easily

transmitted via faecal-oral sexual contact (Stark et al. 2006b). Outbreaks of shigellosis linked to unsafe sexual practices have been described in this setting among MSM (O'Sullivan et al. 2002). The incidence of HIV/AIDS is particularly high amongst MSM in Australia (Van de Ven et al. 2000). The co-infection with enteric pathogens amongst these individuals suggests unsafe practices among this high risk group. Studies have shown that sexual risk behaviour has increased among HIV-infected MSM since the introduction of highly active antiretroviral therapy (HAART) in developed countries (Stark et al. 2007b; Stolte et al. 2004; Van de Ven et al. 2000). Public health interventions to control GIT illnesses such as health education and promotion should be developed to target these groups.

Infection with enteric viruses was most common among children, and is expected in this setting. Rotavirus, adenovirus and norovirus predominantly affected the 0-5 age group. The low rate of viral infections amongst older children/adults is expected, due to the acuity and self-limiting nature of viral GE, and lower susceptibility of older age groups (Johnson & Ericsson 1990; Middleton 1996). Viral infections follow seasonal patterns. Rotavirus presented a distinct seasonal pattern with the lowest rates occurring in the warm months and peaking in the cooler months ($P<0.001$). Adenovirus showed a less consistent monthly trend, and norovirus detection increased in the cooler months. These seasonal trends have been previously described in this population (Fletcher et al. 2013) and in similar settings (Grimwood et al. 2006; Mounts et al. 2000).

*Blastocystis* spp. was the most common parasite detected in this study. This is quite unusual as *Giardia* and *Cryptosporidium* are considered the main intestinal parasites associated with enteric infections in Australia (Kirk & Hall 2005; Sinclair et al. 2005). In this study *Giardia* and *Cryptosporidium* were detected in 3% and 1% of cases respectively. Studies have reported *Blastocystis* spp as the most commonly detected enteric protozoa in developed settings (Fletcher et al. 2012). Despite much controversy about the pathogenicity of *Blastocystis* spp*,* several reports have established its association with abdominal pain, persistent diarrhoea and irritable bowel syndrome (IBS)-like symptoms, (Dogruman-Al et al. 2009; Jimenez-Gonzalez et al. 2011; Stark et al. 2007c) and other

reports postulate that pathogenicity may be sub-type dependent (Roberts et al. 2012). Similar rates were detected by other Australian studies (Roberts et al. 2011; Sawangjaroen, Luke & Prociv 1993; Stark et al. 2010b) and is comparable to developing settings (Fletcher, Stark & Ellis 2011). *Dientamoeba fragilis,* an emerging protozoan pathogen was found in 3% of cases. Advanced molecular diagnostics has led to the increased detection of *D. fragilis* in Australia, and several studies suggest it is as common as *Giardia* in developed settings (Fletcher et al. 2012; Stark et al. 2010b; Stark et al. 2007c).

In addition to other risk factors already discussed, infection with *Campylobacter* and non-typhoidal *Salmonella* spp were associated with reportedly eating a suspicious food item; both occurring more frequently in warmer months. However, although these associations lost their significance in the logistic regression model, both *Campylobacter* and non-typhoidal *Salmonella* are considered important causes of foodborne illnesses in Australia (Hall et al. 2005b; Kirk et al. 2008) and in other developed countries (Scallan et al. 2011). Seasonal relationships in keeping with our findings have been previously described for non-typhoidal *Salmonella* (Ekdahl et al. 2005; Zhang, Bi & Hiller 2008) and *Campylobacter* spp (Kovats et al. 2005). Additionally, more than a quarter of persons diagnosed with *Shigella* spp, and 75% of those infected with *Salmonella typhi* reported recent overseas travel, mainly to Asia. Travellers to some low and middle income countries have been found to be between 9 and 151 times more likely to develop diarrhoeal illness (Fletcher, Maharaj & James 2009; Greenwood et al. 2008; Swaminathan et al. 2009), with increased risked from travel to areas in South Asia and Africa (Greenwood et al. 2008; Jiang et al. 2002; Steffen et al. 2004). Because of the increased travel to developing areas and the globalization of the food supply, clinicians in developed countries should be on the alert for sporadic cases and outbreaks of diarrhoea caused by unusual pathogens/subtypes, such as enteric protozoa, *Shigella* and *Salmonella typhi* (Fletcher et al. 2012; Wilson 2005).

This study has potential limitations. There were inconsistencies in the data collected in different hospitals across Sydney and as a result, information on some disease risk factors (for example MSM status, HIV/AIDS diagnosis, and exposure to suspicious food) was more

detailed at some hospitals than others. The association between these risk factors and specific pathogens could therefore be underestimated in this study, due to lack of information.

### 3.6.1 Conclusion

This study has revealed that GIT illness is a major issue for healthcare in Sydney, Australia, with implications for resource management and disease surveillance and control. The extended duration of GIT in older patients, requiring longer periods of hospitalisation for symptoms, has implications for the planning of health care resources in terms of the clinical management of diarrhoeal illnesses, Hospital bed capacity and infection control programs.

The study has identified various risk factors that can be addressed by public health interventions. Increased incidence of paediatric diarrhoea in cooler months and foodborne illnesses in warmer months implies that health promotional messages should be developed to target the respective high risk groups in each season. The relatively high prevalence of antibiotic associated *C. difficile* infections, suggest that existing protocols and practices for the control of *C. difficile* should be carefully reviewed and modified where necessary. Information on disease risk factors is essential to inform knowledge and practice in the control of infectious diarrhoea, and should be routinely collected in a systematic way across Hospitals.

These findings have implications for healthcare and policy. Clinical and laboratory records can provide useful insights into the prevalence, and associated risk factors for GIT illnesses in Australia. There is therefore the need for a systematic approach to the collection of clinical and risk factor information across NSW hospitals, and clear policies to guide this process. The consistent use of well organised electronic medical records is recommended as an alternative. Priority should also be given to the development of a gold standard approach for diagnosis of pathogens, especially enteric protozoa, including the incorporation of molecular diagnostic methods to provide consistency and reliability across the State.

# 4 Gastrointestinal pathogen distribution in symptomatic children in Sydney, Australia

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## Chapter Overview

There is a paucity of information about the actual causes and relative prevalence of GIT pathogens in the Australian setting. It is believed that gastrointestinal illnesses contribute a significant burden to the health system, therefore knowing the actual causes and their prevalence is important, to inform prompt diagnosis and treatment, to prevent complications and reduce the burden on the health care system. This chapter follows from the big picture view of the clinical and epidemiological characteristics of GIT illnesses, caused by infectious pathogens amongst hospitalised patients in Sydney. This chapter looks specifically at the 0-5 age group and describes the clinical features and pathogens associated with gastrointestinal illnesses for children presenting to two major public Hospitals in Sydney with diarrhoea, for the period January 2007-December 2010. It interprets the findings in light of rotaviral vaccination coverage in Australia.

## *4.1 Abstract*

**Background and Methods**: There is limited information on the causes of paediatric diarrhoea in Sydney. This cross sectional study used clinical and microbiological data to describe the clinical features and pathogens associated with gastrointestinal illnesses for children presenting to two major public Hospitals in Sydney with diarrhoea, for the period January 2007-December 2010.

**Results:** Of 825 children who tested positive for an enteric pathogen, 430 medical records were reviewed. Adenovirus, norovirus and rotavirus were identified in 20.8%, 20.3% and 21.6% of reviewed cases respectively. Younger children were more likely to have adenovirus and norovirus compared with rotavirus (P= 0.001). More viruses were detected in winter than in the other three seasons (P= 0.001). Rotavirus presented a distinct seasonal pattern with the lowest rates occurring in the warm months and peaking in the cooler months. Adenovirus showed a less consistent monthly trend, and norovirus detection increased in the cooler months (P=0.008). A decline in the number of rotavirus cases was observed after mid-2008.

**Conclusion:** The majority of childhood diarrhoeal illnesses leading to Hospital presentations in Sydney are caused by enteric viruses with most infections following clear seasonal patterns. However, a sustained decrease in the incidence of rotavirus infections has been observed over the period.

Keywords: children, diarrhoea, adenovirus, norovirus, rotavirus, Australia

## *4.2 Introduction*

Acute diarrhoeal illnesses continue to be an important cause of morbidity in children, affecting both developed and developing countries (Widdowson et al. 2009). Enteric viruses, especially rotavirus have been recognized as the leading cause of childhood diarrhoea worldwide (Parashar et al. 2003; Widdowson et al. 2009). Several studies reveal that rotavirus, norovirus, adenovirus and astrovirus are the main viral causes of acute childhood diarrhoea (Carraturo, Catalani & Tega 2008). Reports from the United Kingdom have shown viral agents responsible for up to 50% of all community acquired and health-care associated gastroenteritis (Cunliffe et al. 2010). Australian data for all ages reveal that enteric viruses, mainly norovirus and rotavirus, are common causes of gastroenteritis, accounting for about 15-18% of all gastroenteritis cases (Cretikos, Telfer & McAnulty 2008a).

Nearly all children worldwide become infected with rotavirus by their fifth birthday; with those aged between 6 months and 2 years more likely to be diagnosed with severe disease resulting in hospitalisations (Chiu et al. 2010). The burden of rotaviral diarrhoea worldwide has resulted in the World Health Organization (WHO) placing priority on the development and distribution of rotavirus vaccines globally (UNICEF/WHO 2009). Norovirus infections on the other hand, is recognized as a leading cause of epidemic gastroenteritis affecting all age groups, with sporadic cases occurring all year round with increased incidence observed in colder months (Glass R. 2009). In contrast to rotavirus, norovirus is the principal cause of healthcare associated viral diarrhoea (Svraka et al. 2007). Enteric adenoviruses types 40 and 41 and astrovirus are less frequently implicated but are also important causes of acute diarrhoeal illnesses in sporadic and outbreak settings (Svraka et al. 2007).

Bacterial GIT illnesses are also common in children under five years old, including those in institutional settings are at increased risk for GIT Illness (Heymann 2008; Kirk & Hall 2005; Lamps 2007; O'Ryan, Prado & Pickering 2005; Scallan 2005). Infants and young children,

especially those with low nutritional status, severe poverty and poor sanitation, are susceptible to *Salmonella* infections (Georges et al. 1984; Lamps 2007) and co-infection with non-typhoidal *Salmonella* spp and enteric parasites is not unusual (Gordon 2008; Hamer, Gorbach & Kris 2008; Haque et al. 2003; Paniagua et al. 2007). There is also an association between persistent and recurrent *Salmonella* bacteraemia and Schistosomiasis especially in African children, and person to person transmission amongst susceptible children is common (Gordon 2008).

*Escherichia coli* pathotypes are the most common causes of persistent diarrhoea in children in low and middle income countries (Abba et al. 2009; Fischer Walker, Sack & Black 2010). However, in developed countries the EHEC group especially the E. coli O157:H7 are usually implicated (Podewils et al. 2004). Infections with E. coli O157:H7 (also known as Shiga toxin producing E. coli or STEC) is associated with the complication of Hemolytic Uremic Syndrome (HUS) which occurs in 2-8% of cases (Heymann 2008; Kuntz & Kuntz 1999; Podewils et al. 2004) and up to 15% in children (Heymann 2008).

Infections with *Campylobacter* in children are not uncommon and are usually associated with handling of domestic animals and birds, faecal-oral, and consumption of contaminated water or food (Bahrani-Mougeot et al. 2009; Heymann 2008). Person to person transmission is mainly between infected infants and carers (McIver 2005).

Over the last fifteen years, great progress has been made towards the development and introduction of rotavirus vaccines, despite the withdrawal of an early vaccine due to safety concerns (Parashar, Alexander & Glass 2006). Vaccination programmes are estimated to prevent approximately 85–100% of hospitalisations due to rotavirus at least one year following vaccination (Snelling et al. 2007). The introduction of the rotavirus vaccine in the USA in 2006 and in Australia in 2007 has led to a dramatic reduction in the incidence and hospitalisations for acute gastroenteritis (Buttery et al. 2011; Curns et al. 2010). A USA report projected that the administration of the rotavirus vaccine at ages 2, 4, and 6 months would result in an estimated 255,000 fewer physician visits, 137,000 fewer ED

visits, 44,000 fewer hospitalisations, and 13 fewer deaths per year in children aged <5 years (Parashar, Alexander & Glass 2006).

The rotavirus vaccination programme was implemented in the Australian National Immunisation program in the year 2007 (Chiu et al. 2010). Immunisation against rotavirus using Rotarix® at 2 and 4 months of age started in the Northern Territory from October 2006, while universally funded immunisation against rotavirus at 2 and 4 months of age (Rotarix®) or at 2, 4 and 6 months of age (Rotateq®) began from July 2007 in other States. (Chiu et al. 2010) Immunisation or catch-up programmes for older children and older children/adults is not recommended in Australia (NCIRS 2009).

Little is known about the risk factors of paediatric gastrointestinal illnesses for children presenting to Hospital with diarrhoea in Sydney. Knowledge of the actual causes and their prevalence is important to inform prompt diagnosis and treatment and evaluate the impact of the rotavirus vaccination program. This retrospective study utilizes the review of laboratory and Hospital databases to describe the prevalence of diarrhoeal pathogens and associated clinical features in children presenting to Hospital in urban Sydney up to 3 years after widespread vaccine uptake.

## *4.3 Methods*

### 4.3.1 Study setting

Two large Hospitals serving the paediatric population of Sydney were included in the study; a major general public Hospital in South Western Sydney (Hospital A) and a tertiary/quaternary paediatric centre in the Sydney children's Hospital network (Hospital B). Ethical approval was granted by the Human Research Ethics Committees of both hospitals and the University of Technology, Sydney.

### 4.3.2 Microbiology methods

Both laboratories routinely test for enteric pathogens in patients presenting with gastrointestinal symptoms. Both laboratories use the standard methods for the identification and isolation of enteric pathogens as described below.

#### 4.3.2.1 *Virology*

Both laboratories conducted testing for adenovirus and rotavirus routinely in all children ≤5 years of age unless otherwise indicated or requested by the clinician. However, Hospital A tested for norovirus on request or where outbreaks were suspected. Hospital A tested for rotavirus, adenovirus serotypes 40 and 41 and norovirus using the enzyme immunoassay (EIA) method for each species respectively. Hospital B used the RIDA Quick Rotavirus/Adenovirus Combi immunochromatographic test (ICT) and the RIDASCREEN Norovirus test (EIA). All tests were conducted following the manufacturers recommendations. The adenovirus test used at Hospital B detects all adenovirus serotypes, and not just the enteric serotypes 40 and 41.

#### 4.3.2.2 *Bacteriology*

Bacterial identification was done routinely in all laboratories using standard culture methods. Selective media (Xylose, Lysine Deoxycholate agar (XLD), *Salmonella* selective broth, *Campylobacter* selective agar, and *Yersinia* selective agar were inoculated for the detection of *Salmonella* spp, *Shigella* spp, Campylobacter spp and *Yersinia enterocolitica*. Detection of *Aeromonas, Plesiomonas* and *Vibrio* spp was attempted only on special request, or where relevant clinical notes such as overseas travel or seafood consumption were provided. *Clostridium difficile* testing was performed EIA for diarrhoea greater than three days after Hospital admission, on special request, or where relevant clinical notes were provided (e.g. history of antibiotic use, chemotherapy or immuno-suppressed). In Hospital A, *C. difficile* toxin testing was performed on one semi-formed/loose sample if requested.

#### 4.3.2.3 *Parasitology*

At both sites, direct microscopy was routinely performed on all stool specimens for the detection of ova, cyst, and parasites. However, concentration techniques were performed only on special request or when indicated by certain circumstances; for e.g. history of overseas travel or prolonged diarrhoeal illness. At Hospital A, stool specimen are routinely collected in sodium acetate acetic acid formalin (SAF) fixative (Oxoid Australia), and direct wet preparation microscopy was routinely performed on all stool specimens. In the instances where no clinical information was received and the patient was an older children/adults or age ≤10 years old, or the specimen was not received in SAF, then a *Giardia/ Cryptosporidium* screen enzyme immunoassay (EIA) (ProSpecT™ Giardia/Cryptosporidium Microplate Assay) was performed. A 10% suspension of stool samples was prepared in 10% formalin (for *Giardia intestinalis* and *Cryptosporidium*) and the EIA was performed in accordance with the manufacturer's instructions and without modification. A full COP test was done on all positive microscopy and EIA results using an Iron haematoxylin stain with modified Acid fast stain. A similar procedure was employed for all stool specimens received at Hospital B, and samples positive by direct microscopy are placed into SAF fixative followed by confirmation by Iron haematoxylin staining.

### 4.3.3 Medical record review

#### 4.3.3.1 *Selection criteria*

The primary selection criteria was all children aged 0-5 years seen in each hospital and/or its affiliated clinics that had gastrointestinal symptoms and had a stool specimen testing positive for an enteric organism. Patients presenting with gastrointestinal symptoms including diarrhoea- (defined as the passing of three or more unformed [loose, liquid, watery] stools within a 24-hour period), with or without fever, abdominal/colicky pain, vomiting and nausea were included in the sample.

#### 4.3.3.2 *Sampling*

Paediatric cases are a sub-group of a larger study involving older children/adults and adolescents/children. Children with diarrhoea were identified from the microbiology results based on date of birth and or age 5 years or younger at the date when the sample was tested. Laboratory data was then stratified based on two seasons (Spring/Summer and Autumn/Winter) in each year. Attempts were made to review 100% (154/154) of medical records at Hospital A and 50% (335/671) of records at Hospital B, owing to larger numbers of children being seen. These proportions were chosen based on cost and time constraints. Samples were randomly selected using a random number generator.[vi] The medical record charts were obtained for each case using their unique medical record number (MRN), and matched by date of visit/service date. Clinical summaries were reviewed for signs and symptoms, risk factors, diagnosis and treatment data.

### 4.3.4 Statistical methods

Analysis included the median, mean and standard deviation (SD), for distribution of demographic characteristics, clinical symptoms, proportion of pathogens isolated amongst all positive cases, association between clinical symptom, season and viral pathogens using Pearson's Chi-square test. Odds Ratios (OR) and 95% confidence intervals (95%CI) for the association between age and viral agents detected were calculated using the Binary logistic regression model where the dependent variable was each virus (rotavirus, adenovirus, norovirus) coded as 1=No, 2= Yes, and age groups being the independent variable, using the Enter method. Statistical analyses were performed using PASW Statistics Release version 18.0 (SPSS Inc. 2011).

[vi] Available at http://stattrek.com/Tables/Random.aspx

**Table 4-1: Total cases tested for enteric pathogen in 0-5 years old children in two hospitals 2007-2010, Sydney.**

| Sampling details | Hospital A | Hospital B |
|---|---|---|
| Total specimen tested[a] | 15694 | 9239 |
| Individual cases[b] | 5020 | 5229 |
| Total positives | 971 | 932 |
| Total negative | 4049 | 4297 |
| Children 0-5 yrs (% of total) | 868 (17.3%) | 3910 (74.8%) |
| Total positives | 154 (19.5%) | 671 (17.2%) |
| Total negative | 714 | 3239 |
| Reviewed N (%) | 132 (78.1%) | 298 (44.4%) |

[a]Includes individuals who submitted multiple stool specimen
[b]These are only individual cases (regardless of number of stool specimen) counted to get this number. Hospital A had 5020 individual cases, 868 of which were children 0-5years. Hospital B had 5229 individual cases, 3910 of which were children 0-5 years.

## *4.4 Results*

### 4.4.1 Demographics

A total of 825 children aged 0-5 years (154 at Hospital A and 671 at Hospital B) who presented to the Hospitals had a stool specimen testing positive for an enteric organism over the period January 2007-December 2010. From Hospital A, only 78% (132/154) of cases were reviewed because the remaining medical records were either not available, or the age of the subject could not be determined. Of the 335 (50%) of cases selected from Hospital B, only 89% (298/335) were reviewed due to either unavailability of records or legal/ethical reasons. The medical records for a total of 430 children were reviewed from the two hospitals (see Table 4.1). The median (LQ, UQ) age of children was 1.4 (0.8, 2.0) years [mean 1.6 yrs, SD 1.2]. There was slightly more males (56%) than females.

### 4.4.2 Clinical profile

Of the children reviewed, 89% (382/430) had symptoms prior to admission for 1 to 4 days with a median of 3 days and requiring admission for a median of 1 day. A total of 28% (120/430) of children required admission to the emergency department for 2 or more nights. Just over half (58%) of all cases presented with elevated body temperature [mean ± SD: 37. 8 °C ± 1.2°C], ranging from 35.0°C -41.0°C. Clinical symptoms differ significantly between the two hospitals. The majority of children, 68% (264/430), presented with explosive or watery stools, 21% (90/430) had blood/mucous in their stools, and 3% (11/430) experienced persistent diarrhoea lasting for ≥14 days. Vomiting was frequently experienced (68%, 293/430), followed by dehydration (31%, 132/430) and abdominal cramping/pain (19%, 80/430). Other major signs and symptoms included: anorexia (31%, 134/430), lethargy 38% (165/430), and respiratory symptoms (25%, 106/430) (Table 4.2).

**Table 4-2: Summary of clinical findings associated with diarrhoea in 0-5 years old children.**

| Clinical symptoms (N=430)[c] | Hospital A % (n) (N=132) | Hospital B % (n) (N=298) | Total % (n) | P, Pearson's $\chi^2$(df) |
|---|---|---|---|---|
| Vomiting | 78 (103) | 64 (190) | 68 (293) | **0.003,** 8.58(1) |
| Abdominal pain | 22 (29) | 51 (17) | 19 (80) | 0.149, 1.39(1) |
| Fever | 73 (96) | 51 (152) | 58 (248) | **0.001,** 17.68(1) |
| Dehydration | 39 (52) | 27 (80) | 31 (132) | **0.007,** 6.77(1) |
| Anorexia | 58 (76) | 20 (59) | 32 (135) | **0.001,** 60.26(1) |
| Lethargy | 39 (52) | 38 (113) | 38 (165) | 0.772, 0.084(1) |
| Upper respiratory symptoms | 14 (18) | 30 (88) | 25 (106) | **0.001**, 12.44(1) |

[c]Reviewed 430 cases with one or more symptoms.

According to discharge summaries, 79% (338/430) of children were classified on presentation with an infectious gastrointestinal illness, 76% (323/430) of cases had a principal diagnosis and 70% (301/430) had an additional diagnosis of infectious gastrointestinal illness. Co-morbidities were noted in few cases including recent surgery 2% (10/429), complications related to neonatal period 4% (16/430) and cancer/lymphomas 3% (11/430). About 20% (82/430) of cases had a family member or close contact with gastrointestinal symptoms around the same time of their illness that included up to a week before or after onset. Prolonged antibiotic-therapy or chemotherapy was reported by 12% (53/429) but neither *C. difficile* antigens nor toxins were detected in any of these cases. Only 6% (27/430) of cases developed diarrhoea 48-hours or more after hospitalisation, and significantly more were infected with norovirus (56%, 15/27) compared to rotavirus (33%, 9/27) and adenovirus (11%, 3/27) (P= 0.022).

### 4.4.3 Pathogen distribution

There was near equal distribution of each viral agent isolated as a percentage of the total pathogens isolated (Table 4.3). Overall, rotavirus was identified as a single pathogen in 22%, adenovirus in 21% and norovirus in 20% of cases reviewed. However, Table 4.4a shows that when laboratory results were considered there was slightly more rotavirus (33.8% and 17.1%) isolated from cases at both hospitals than adenovirus and norovirus. *Campylobacter* spp. (11.0% and 27.1%) and non-typhoidal *Salmonella* spp. (14.9% and 22.2%) were the most common bacteria isolated in both hospitals A and B respectively. *Giardia intestinalis* was the most common protozoa found in 3.2% and 3.7% of cases in each hospital respectively. Non-typhoidal *Salmonella* spp. and adenovirus were frequently found as second pathogen in a few cases (Table.4b).

**Table 4-3: Comparison of rate of enteric pathogens isolated from 0-5 years old children in two hospitals**

| | Hospital A %(n) | Hospital B %(n) | Total (within pathogen) %(n) |
|---|---|---|---|
| **Rotavirus** | 36 (47) | 17 (51) | **23 (98)** |
| **Adenovirus** | 23 (30) | 25 74 () | **24 (104)** |
| **Norovirus** | 1 (1) | 32 (97) | **23 (98)** |
| **Other: Bacteria**[d] | 52 (39) | 24 (71) | **28 (121)** |
| **Enteric protozoa** | 2 (2) | 1 (2) | **1 (4)** |
| **Adenovirus + rotavirus** | 2 (2) | 1 (3) | **1 (5)** |
| **Rotavirus +norovirus** | 0 (0) | 0.3 (1) | **0.2 (1)** |
| % of Total **(within Hospital)** | **31 (132)** | **69 (297)** | **100 (429)** |

[d]Others:-Bacteria: *Campylobacter* sp (61), *Salmonella* spp (55) *Shigella* sp (3), *Clostridium difficile* (3) and *Yersinia enterocolitica* (4); Enteric protozoa: *Blastocystis* sp (1), *Giardia intestinalis* (2), and *Dientamoeba fragilis* (1)

**Table 4-4: Prevalence of (A) single enteric pathogens and (B) multiple enteric pathogens in children 0-5 years in Sydney 2007-2010.**

**A. Single enteric pathogens in children 0-5 years**

| Organisms | | Hospital A | | | Hospital B | |
|---|---|---|---|---|---|---|
| **Single pathogen** | **n/868** | **% of positive cases (154)[e]** | **% of specimen tested (868)[f]** | **n/3910** | **% of positive cases (671)[e]** | **% of specimen tested (3910)[f]** |
| **Bacteria** | | | | | | |
| *Aeromonas* spp. | 0 | 0 | 0 | 3 | .4 | 0.1 |
| *Campylobacter* | 17 | 11.0 | 2.0 | 182 | 27.1 | 4.7 |
| *C. difficile* | 5 | 3.2 | 0.6 | *N/T* | N/T | N/T |
| *Salmonella enteric* spp. | 23 | 14.9 | 2.6 | 149 | 22.2 | 3.8 |
| *Salmonella typhi* | *0* | 0 | *0* | 3 | 0.4 | 0.1 |
| *Shigella* | 2 | 1.3 | 0.2 | 1 | 0.1 | 0.0 |
| *Yersinia enterocolitica* | 1 | 0.6 | 0.1 | 9 | 1.3 | 0.2 |
| **Viruses** | | | | | | |
| Adenovirus | 40 | 26.0 | 4.6 | 81 | 12.1 | 2.1 |
| Norovirus | 5 | 3.2 | 0.6 | 79 | 11.8 | 2.0 |
| Rotavirus | 52 | 33.8 | 6.0 | 115 | 17.1 | 2.9 |
| **Parasites** | | | | | | |
| *Blastocystis* spp. | 2 | 1.3 | 0.2 | 8 | 1.2 | 0.2 |
| *Cryptosporidium* | 1 | 0.6 | 0.1 | 7 | 1.0 | 0.2 |
| *Dientamoeba fragilis* | *N/T* | N/T | N/T | 9 | 1.3 | 0.2 |
| *Giardia intestinalis* | 5 | 3.2 | 0.6 | 25 | 3.7 | 0.6 |
| *Schistosoma* | 1 | 0.6 | 0.1 | *N/T* | N/T | N/T |

| B. Multiple enteric pathogens in children 0-5 years | | | | | | |
|---|---|---|---|---|---|---|
| **Organisms** | | **Hospital A** | | | **Hospital B** | |
| **Co-infections Pathogen** | **n** | **% of positive cases (154)** [g] | **% of specimen tested (868)** [h] | **n** | **% of positive cases (671)** [g] | **% of specimen tested (3910)** [h] |
| *Campylobacter* spp. | 3 | 1.9 | 0.3 | 20 | 3.0 | 0.5 |
| *Clostridium difficile* | 2 | 1.3 | 0.2 | 0.0 | 0.0 | 0.0 |
| *Salmonella enterica spp.* | 6 | 3.9 | 0.7 | 36 | 5.4 | 0.9 |
| *Salmonella typhi* | 0 | 0 | 0 | 1 | 0.1 | 0.0 |
| Adenovirus | 4 | 2.6 | 0.5 | 9 | 1.3 | 0.2 |
| Norovirus | 0 | 0 | 0 | 4 | 0.6 | 0.1 |
| Rotavirus | 0 | 0 | 0 | 26 | 3.9 | 0.7 |
| *Blastocystis* spp. | 1 | 0.6 | 0.1 | 2 | 0.3 | 0.1 |
| *Cryptosporidium* | 1 | 0.6 | 0.1 | 1 | 0.1 | 0.0 |
| *Dientamoeba fragilis* | 0 | 0 | 0 | 1 | 0.1 | 0.0 |
| *Giardia intestinalis* | 3 | 2.6 | 0.5 | 6 | 0.9 | 0.2 |

[e] The number of specimen positive for each pathogen was divided by the number of positive cases expressed as '% of positive cases'. [f] The number of specimen positive for each pathogen was divided by the number of specimen tested expressed as '% of specimen tested'. N/T: Tests were not conducted or organism not detected.

[g] The number of specimen positive for each pathogen was divided by the number of positive cases expressed as '% of positive cases'.

[h] The number of specimen positive for each pathogen was divided by the number of specimen tested expressed as '% of specimen tested'.

Infection with adenovirus and norovirus decreased with increasing age (P=0.001), but the opposite was true for rotavirus (Figure 4.1). The lowest rate of rotavirus (12%) was observed in children under 1 year old, and approximately half of the adenoviruses (52%) and norovirus (46%) cases were in children under one year old (P=0.001). The relationship between age and the three viral agents was examined using a logistic regression model and adjusted for seasonal variations. Children in the under 1 year old age group were five to seven times more likely to have adenovirus and norovirus, than rotavirus, *Campylobacter* and *Salmonella* spp, isolated from their stools. Children in the 1-2 years age group had increased risk of infection with norovirus (OR 3.2, 95%CI= 1.5-6.8), P=0.003 (Table 4.5). Younger children were less likely to have *Campylobacter* (OR range 0.3 [95%CI= 0.1-0.6] to 0.6 [95%CI=0.3-1.2]) detected in their stools and this risk decreased further with decreasing age (P<0.05). The risk of infection with *Salmonella* spp was significantly less and consistent in children under 3 years old when compared with those 3-5years old (OR range 0.4 [95%CI= 0.2-0.9; P<0.05]. Amongst the children infected with the three viral agents, those with rotavirus were significantly more likely to exhibit anorexia (P= 0.001) and lethargy (P= 0.005), whilst those with adenovirus were more likely to exhibit respiratory symptoms; however, this relationship was not significant (P= 0.603). There was no variation in symptom profile between infection with *Salmonella* and *Campylobacter* (Table 4.6).

**Figure 4-1: Age distribution of viral pathogens associated with childhood diarrhoea in Sydney, 2007-2010.**

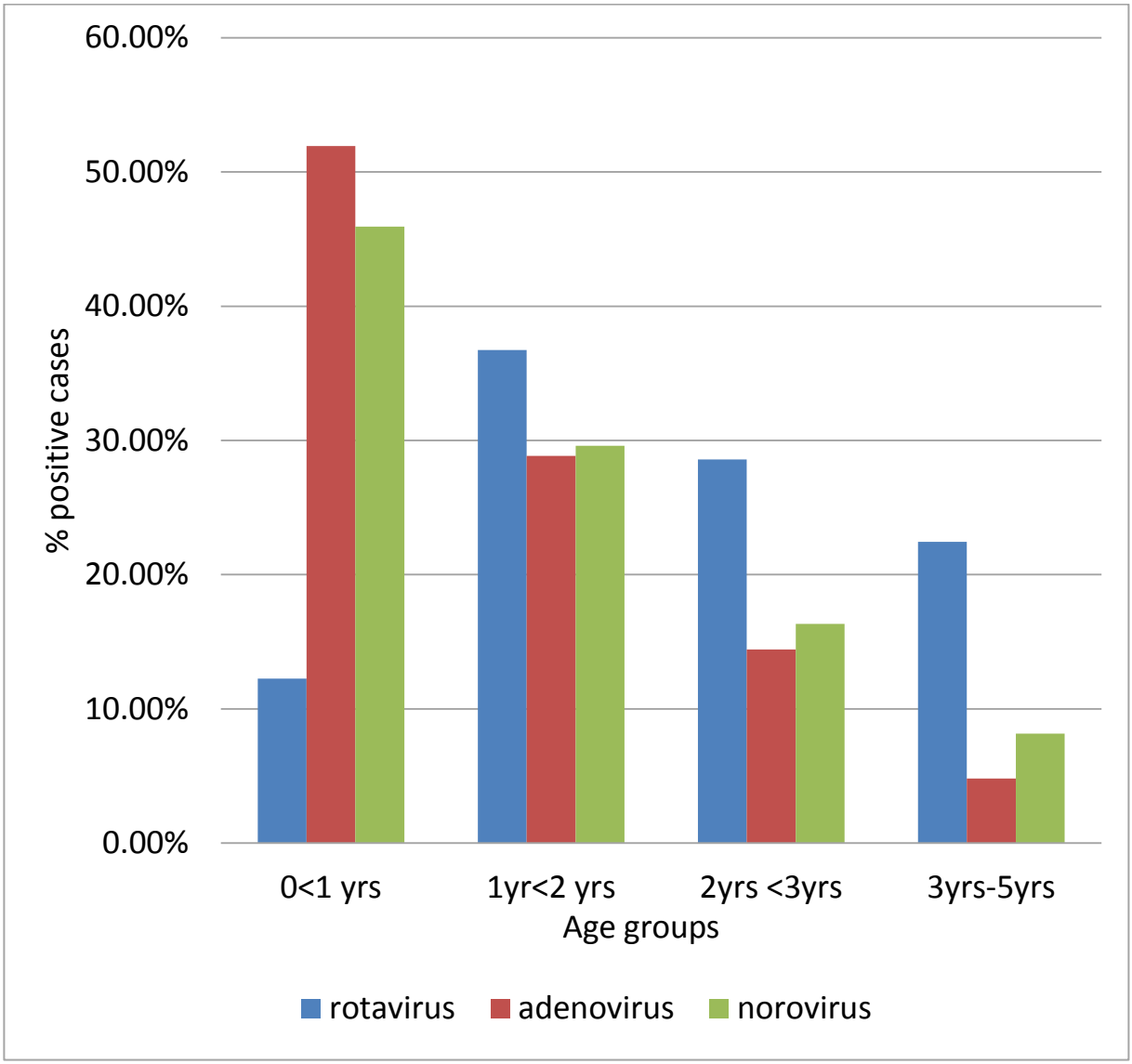

**Table 4-5: Adjusted Odds Ratio of the association between enteric pathogen and age group in children**

| Age groups | N (%) | Odds Ratio (95% Confidence Interval) P- value | | | | |
|---|---|---|---|---|---|---|
| | | **Rotavirus** | **Adenovirus** | **Norovirus** | **Campylobacter spp.** | **Salmonella spp.** |
| 0<1 yrs | 143 (37%) | 0.2 (0.1-0.4), 0.001 | 6.5 (1.8-22.5) 0.003 | 5.4 (2.5-11.3), 0.001 | 0.3 (0.1-0.6) 0.002 | 0.4 (0.2-0.9) 0.031 |
| 1yr<2 yrs | 122 (32%) | 0.9 (0.5-1.7), 0.680 | 3.3 (0.9-12.1) 0.068 | 3.2 (1.5-6.8), 0.003 | 0.4 (0.2-0.9) 0.021 | 0.4 (0.2-0.8) 0.015 |
| 2yrs <3yrs | 87 (19.7%) | 1.1 (0.5-2.1), 0.890 | 1.8 (0.43-7.9) 0.445 | 2.3 (1.0-5.1), 0.049 | 0.6 (0.3-1.2) 0.151 | 0.4 (0.2-0.99) 0.047 |
| 3yrs-5yrs (constant) | 67 (11.7%) | 0.3, 0.005 | 0.01, <0.001 | .24, 0.001 | 0.5, 0.129 | 0.7, 0.402 |
| **Overall prediction success rate** | | 77%. | 88%. | 66%. | 86% | 87% |

[i]Adjusted by season.

**Table 4-6: Relationship between enteric pathogen, clinical signs and symptoms in hospitalised children, 2007-2010**

| Clinical symptoms (n/300 or 115) | Rotavirus % (n) | Adenovirus % (n) | Norovirus % (n) | P, Pearson's $\chi^2$(df) | Campylobacter spp % (n) | Salmonella spp. % (n) |
|---|---|---|---|---|---|---|
| **Diarrhoea ( 286)** | 32 (93) | 35 (100) | 33 (93) | 0.901, 0.21(2) | 52 (59) | 48 (55) |
| **Vomiting (211)** | 41( 86) | 31 (65) | 28 (60) | 0.001, 21.21(2) | 49 (34) | 51 (35) |
| **Abdominal pain (36)** | 44 (16) | 25 (9) | 31 (11) | 0.244, 2.82(2) | 53 (21) | 47 (19) |
| **Fever (160)** | 44 (70) | 32 (51) | 24 (39) | 0.001, 20.88(2) | 48 (38) | 52 (42) |
| **Dehydration (102)** | 51 (52) | 29 (30) | 20 (20) | 0.001, 25.17(2) | 46 (12) | 54 (14) |
| **Anorexia (92)** | 47 (43) | 37 (34) | 16 (15) | 0.001, 18.75(2) | 49 (17) | 51 (18) |
| **Lethargy (128)** | 42 (54) | 33 (42) | 25 (32) | 0.005, 10.43(2) | 47 (14) | 53 (16) |
| **Upper respiratory symptoms (84)** | 29 (24) | 38 (32) | 34 (28) | 0.603, 1.01(2) | 48 (10) | 52 (11) |

Persons with a single virus (rotavirus, adenovirus, norovirus) detected= 300; Campylobacter and Salmonella spp = 115.

### Seasonal distribution

Seasonal distribution of viral infections was assessed. Overall, more viruses were isolated in winter than in the other three seasons ($\chi^2$ = 37.0(9); P= 0.001). Significantly more norovirus (41 %) was found in the winter followed by rotavirus (32%; P= 0.001). In autumn, significantly more adenovirus (49%) was isolated, whilst in the spring, rotavirus (48%) dominated. Rotavirus accounted for the smallest proportion of cases (25%) in the summer. The distribution of the three viruses by month of detection is presented in Figure 4.2. Rotavirus presented a distinct pattern, with the lowest numbers detected in the warmer months and gradually increasing and peaking in the cooler months. Adenovirus on the other hand showed a less consistent monthly trend, however a gradual increase in laboratory confirmed cases was observed in the cooler months of 2010. An increase in the number of norovirus detected was also observed in the cooler months of 2008-2010.

## 4.4.4 Management and Treatment of Diarrhoea

Generally speaking, children are managed conservatively with oral rehydration however in cases where there was prolonged vomiting, dehydration, lethargy and anorexia, children received intravenous or nasogastric rehydration therapy. It was observed that IVF was given only in cases of dehydration or when children did not tolerate oral intake or nasogastric tube (NG) feeds

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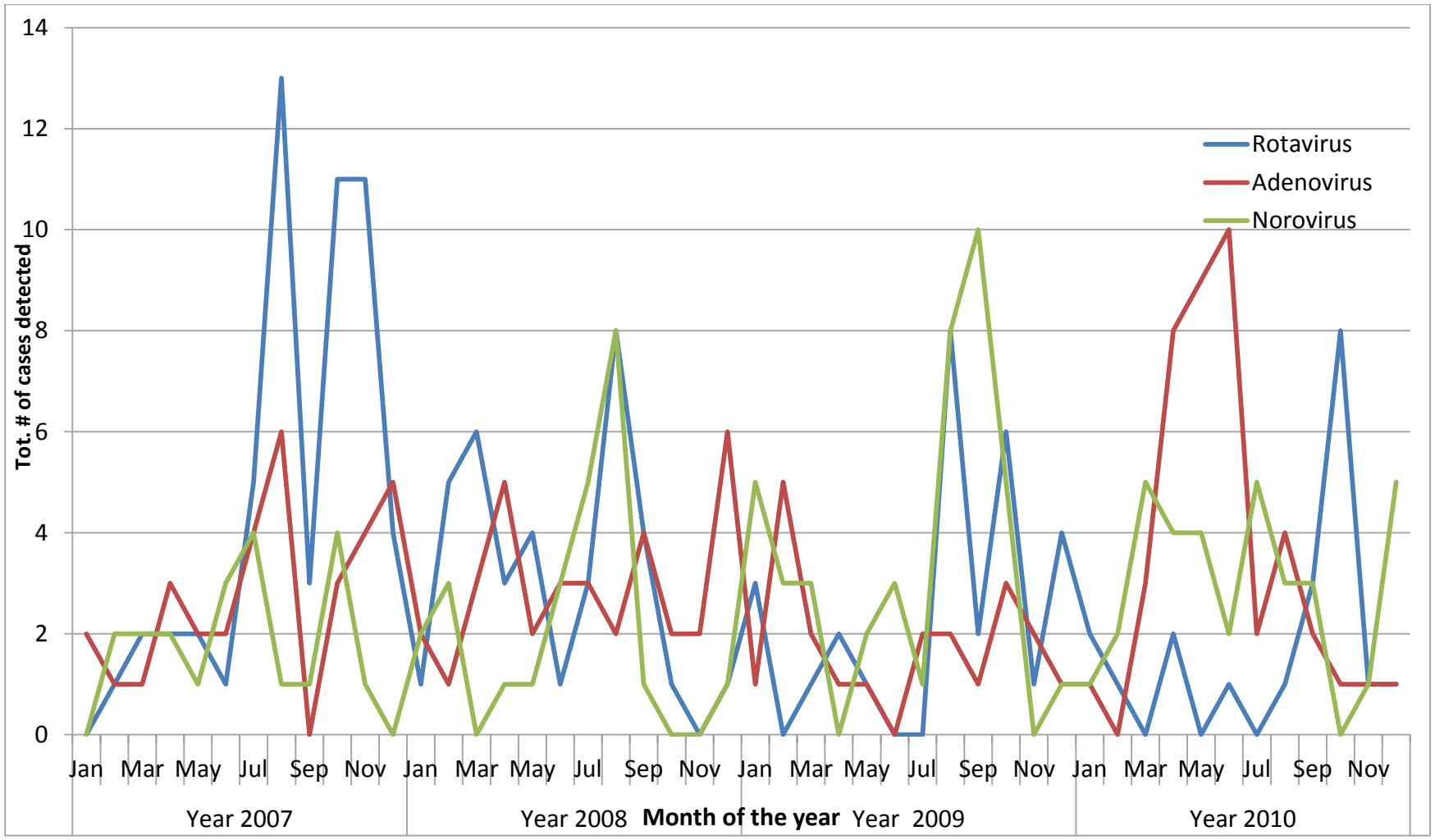
**Figure 4-2: Monthly distribution of viral pathogens associated with childhood diarrhoea in Sydney, 2007-2010**



## *4.5 Discussion*

This four year multi-centre retrospective study used clinical and microbiological data to describe the clinical features and pathogens associated with gastrointestinal illnesses in children presenting to two major public Hospitals in Sydney, for the period January 2007-December 2010. The study found that viral pathogens are the major causes of childhood diarrhoea in Sydney, accounting for nearly two thirds of cases who have a pathogen detected. *Campylobacter* spp. and non-typhoidal *Salmonella* spp., two common food-borne pathogens, were other common causes of diarrhoea in children.

A review of clinical and laboratory records showed nearly equal distribution of rotavirus (21.6% and 2.9%), adenovirus (20.6% and 2.1) and norovirus (20.3% and 2.0%) isolated from children, which is rather unusual. In childhood diarrhoea, rotavirus usually dominates in sporadic cases, with norovirus being more prominent in outbreak settings. (WHO 2011) Australian data reveal that enteric viruses, mainly *norovirus* and *rotavirus*, are the most common causes of non-food gastroenteritis, accounting for about 15-18% of all gastroenteritis cases. (Cretikos, Telfer & McAnulty 2008a) The WHO reported that in 2009, rotavirus was detected in a median of 36% (range 12%–68%) of children aged <5 years hospitalised for diarrhoea and were tested for rotavirus. (WHO 2011) Previous reports from Australia found norovirus as the most common cause of gastroenteritis in the community. (Kirkwood et al. 2005; Sinclair et al. 2005) The testing for norovirus only in outbreak settings in one hospital may have resulted in a testing bias. The incidence of adenoviruses in this population (21%) is also quite surprising, since its prevalence is usually significantly less than rotavirus. The reason for the high incidence detected in this study is not particularly clear. Other reports have found adenovirus rates ranging from 1.5% to 15%; (Sdiri-Loulizi et al. 2009; Wanke 2008) although an older report from Sydney found similar rates. (McIver et al. 2001) The fact that reports from Hospital B including non-diarrhoeal adenovirus serotypes, may account for the overall high prevalence, as has

been observed in other studies.(Basu et al. 2004) However, slightly more were isolated in Hospital A (24% vs. 20%) that used the EIA method to detect the enteric serotypes 40/41.

A major finding was that significantly more adenovirus and norovirus were isolated from children under 1 year old, compared with rotavirus. The most likely explanation for this is the impact of the new rotavirus vaccine implemented in 2007. This vaccine would have covered mainly children under one year old; hence this age group would have benefitted from the largest reduction in new cases. (Chiu et al. 2010) In the Northern Territory, the median age for non-indigenous Australian children infected with rotavirus was 16 months old. (Schultz 2006) The introduction of the rotavirus vaccine for infants has resulted in an upward shift in the age of children being infected with rotavirus. Modelling and analysis of post-vaccination rotavirus rates predicts an increase in the age of first infection, which results in later onset, in fewer cases and less severe symptoms and subsequently less hospitalisations. (Pitzer et al. 2009) However, some models have suggested that the incidence of severe rotaviral infections could increase in older individuals following vaccine introduction. (Pitzer et al. 2012) A Finnish study also found norovirus to be of similar prevalence to rotavirus, with infections peaking in the 0-18 months age group post rotavirus vaccination. (Puustinen et al. 2011) Bacterial infection was less common (28.7% of cases), and the rate of enteric protozoa was quite low, emphasizing that viral pathogens are the major causes of childhood diarrhoea in Sydney. Only a few cases of nosocomial diarrhoeal illness were observed; the majority of whom were infected with norovirus. A previous report from Hospital B found nearly 15%-19% of rotavirus infections were hospital acquired. (Snelling et al. 2007) Other reports have found norovirus as the second most common cause of nosocomial diarrhoeal infections. (Cunliffe et al. 2010)

There was a clear seasonal distribution of viral childhood diarrhoea, with winter peaks observed in this study and has been confirmed by other studies in Australia and other parts of the world. (Koopmans et al. 2000) A 20 year old report described higher rates of rotavirus in NSW occurring in August and September (Ferson 1996) and more recently from June to November Australia wide.(Chiu et al. 2010; NCIRS et al. 2007) In temperate

climates, rotavirus infections peak in the winter and spring, (Glass R. 2009)with less obvious seasonal distribution in tropical countries. (Cook et al. 1990) While the seasonal distribution for norovirus was less obvious, the incidence was highest in winter and is consistent with the wintertime seasonality described in temperate climates for norovirus. (Glass R. 2009) Significantly more adenovirus was isolated in autumn. An older report found that peaks in diarrhoeal illness in the late summer and early autumn in Sydney, were due to adenovirus infection, (McIver et al. 2001) although a report from Melbourne found no consistent seasonal distribution. (Barnes et al. 1998)

A gradually decrease in the number of cases of rotavirus infection was observed after the autumn of 2008. This dramatic and sustained decline in the number of cases is most likely attributed to the introduction of the rotavirus vaccines in July 2007. (Hull et al. 2010)The rotavirus vaccination programme was universally implemented in Australia in the year 2007. (Chiu et al. 2010; NCIRS et al. 2007) Immunisation against rotavirus using Rotarix® at 2 and 4 months of age started in the Northern Territory from October 2006, while universally funded immunisation against rotavirus at 2 and 4 months of age (Rotarix®) or at 2, 4 and 6 months of age (Rotateq®) began from July 2007. (Chiu et al. 2010) The effect of the Rotavirus vaccination in NSW was evident from the obvious decline in cases from 2008 and 2010. (NNDSS Annual Report Writing Group 2011) A reduction of 83% in South Australia, (Clarke et al. 2011) 68% in Queensland notifications, (Lambert et al. 2011) and 70.8% Australia wide hospitalisations, (Dey et al. 2011) was observed in rotavirus AGE and between 33.9% to 48% in all cause AGE (Dey et al. 2011) following the introduction of the rotavirus vaccination. An unexplained increase in the number of cases noted in the summer of 2010 has also been observed in Queensland in all age groups as well. (Lambert et al. 2011) Careful attention should therefore be placed at ensuring that all eligible children are vaccinated, to provide herd protection for older and younger children not immunized for various reasons and hence susceptible. (Hull et al. 2010)

Genotyping is not routinely conducted on for enteric viruses in NSW hospital laboratories, however a fraction of rotavirus positive specimen (including some from Hospital B) are

submitted to the Australian Rotavirus Surveillance Program in Melbourne, for serotyping and has been documented. Over the mid-2005-mid-2008 period, serotype G1 was the most the dominant strain identified nationally and in NSW, followed by serotype G9 (2005-2007), (Kirkwood et al. 2006; Kirkwood et al. 2007) and serotype G2 (2007 2008). (Kirkwood et al. 2008) Genotype analysis from 2008 revealed that genotype G1P[8] was the most common nationally and in NSW over the 2008-2011 period, followed G3P[8] and G2P[4] in mid-2008-2009; (Kirkwood et al. 2009) and genotype G2P[4] in 2009-2010.(Kirkwood et al. 2010) In 2010-2011, there was a shift to G2P[4] strain being the most common genotype identified nationally and in NSW, followed by G3P[8]. (Kirkwood et al. 2011) Prior to 1995, rotavirus genotypes G1P[8], G2P[4], G3P[8] and G4P[8] were the most common serotype in circulation worldwide. (Santos & Hoshino 2005; Zeller et al. 2010) Since then, genotype G9 has increased dramatically and is now considered the fifth globally important rotavirus genotype.(Matthijnssens et al. 2008; Zeller et al. 2010) Over the study period, G1 and G1P[8] remained the dominant serotype in the study area, but a shift to G2P[4] has been observed at the state and national levels since 2010.

### 4.5.1 Limitations

This study like most retrospective studies has potential limitations. The difference between the proportions of pathogens isolated between each hospital is likely due to the different stool testing protocols, mainly for the identification of protozoa. It is also important to note that pathogens differ in terms of their level of severity; therefore the hospitalization rate may be higher for some pathogens. In this study the rate of adenovirus infection was similar to that of rotavirus and norovirus. It must be noted that Hospital B used the Immunochromatographic test that detects all adenovirus serotypes and not just the enteric serotypes 40 and 41; hence a positive result does not necessarily mean the serotype found was the cause of diarrhoeal illness. Adenoviruses can cause a broad spectrum of clinical diseases, most of which are self-limiting; (Mandelboim et al. 2011) and the rate of isolation in both hospitals was quite similar (24% vs. 20%). In addition, testing for norovirus at Hospital A was mainly when outbreaks were suspected,

which may have resulted in a testing bias. Finally, current clinical guidelines for the management of acute gastroenteritis in children do not recommend the routine collection and testing of stools for an aetiological agent, (NSW Department of Health 2002) hence these cases are likely not representative of the full spectrum of paediatric community gastroenteritis. These results represent only a sub-section of cases with diarrhoeal illness that presented to hospital. However, one of these Hospitals is the largest children's hospital in the Sydney Children's Hospital network that treats large numbers of children with acute diarrhoea from across the State whilst the other represents a culturally diverse area in Sydney.

### 4.5.2 Conclusion

This study has found that the vast majority of children seeking medical attention for diarrhoeal illnesses, who have a pathogen detected, are infected with an enteric virus. It highlights the need for careful monitoring and the rapid assessment and treatment of young children with gastrointestinal symptoms especially in the cooler months. The implementation of a rotavirus vaccine has proven effective in reducing the incidence of rotavirus infection in young children in Sydney; hence attention should be paid to ensuring that all eligible children are vaccinated.

# 5 Comparison of algorithms used to diagnose enteric protozoan infections: towards determination of a gold standard.

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## Chapter Overview

The previous two chapters indicated that enteric protozoa accounted for a substantial proportion of known enteric pathogens implicated in gastrointestinal illnesses in Sydney, affecting both adults and children. The aim of this chapter is to look closer at the testing protocols for the detection of enteric protozoa. This chapter provides overall estimate of the detection of enteric protozoa amongst hospitalised patients, and discusses the outcomes and differences between testing algorithms used to diagnose enteric protozoan infections, in four large, public clinical laboratories in Sydney.

## *5.1 Abstract*

**Aim and Methods**: This research seeks to estimate the rate of detection of enteric protozoa, and evaluates the outcomes of testing algorithms used to diagnose enteric protozoan infections. This multi-centre retrospective study assesses microbiological data from four large, public clinical laboratories in Sydney.

**Results**: Across the four hospitals, the most common enteric protozoa detected was *Blastocystis* spp., identified in an average of 5.4% (95%CI 5.0%-5.7%) of cases, followed by *Giardia intestinalis* (1.1%; 95%CI 1.0%-1.2%) and *Dientamoeba fragilis* (0.8%; 95%CI 0.7%-1.0%). Detection rates varied between hospitals and the prevalence of *G. intestinalis* was higher in the 0-5 age group compared to *Blastocystis* being more frequently detected in persons over 5 years old ($P<0.05$).

When similar testing algorithms were used, microscopy of wet preparations detected *Blastocystis* spp. in an average of 2.9% (95%CI 2.5-3.4) in two hospitals. The use of permanent staining with iron haematoxylin staining (IHS) and modified IHS (mIHS) incorporating a carbol fuschin step detected *Dientamoeba fragilis* in an average of 1.1% of cases (95%CI 0.9-1.4) in two hospitals. Microscopy combined with screening enzyme immunoassays (EIA) detected *G. intestinalis* in an average of 0.9% of cases (95%CI 0.7-1.1) and *Cryptosporidium* spp., in an average of 0.4% (95%CI 0.3-0.5) in two hospitals.

Detection rates for the individual species using different testing algorithms were significantly different between hospitals ($P<0.05$). The mIHS method consistently detected higher rates of *Blastocystis* spp, and *G. intestinalis* in comparison with light microscopy of wet preparations ($P<0.001$), as well as higher rates of *G. intestinalis* and *Cryptosporidium* when compared with screening EIA ($P<0.0002$).

**Conclusion**s: Hospitals across Sydney employ different testing protocols for the detection of enteric protozoa. In this study, the modified IHS consistently detects more parasites than light microscopy of wet preparation and the EIAs. The variability in the detection rates between the tests done at different hospitals, indicate the need for the development of a gold standard approach for diagnosis of enteric protozoa. Incorporation of molecular diagnostic methods such as PCR would provide consistency across laboratories and yield more reliable estimates of the actual prevalence of enteric protozoa.

## *5.2 Introduction*

Enteric protozoa are important causes of infectious diseases affecting people in developing as well as developed countries (WHO 2008). Compared with developing countries, relatively few enteric protozoa are included in operational surveillance systems in developed countries. Where these are included, they are mainly seen as indicators of foodborne and waterborne diseases outbreaks (Cretikos, Telfer & McAnulty 2008b; Dixon et al. 2011; Kucerova et al. 2010; Sokolova et al. 2011; Stark et al. 2009b; WHO 2008). However, evidence suggests that while some enteric protozoa such as *Entamoeba spp*., *Cryptosporidium spp.,* and *Giardia intestinalis* are more frequently identified in diarrhoeal cases in developing regions, like Asia and sub-Saharan Africa (Fletcher, Stark & Ellis 2011; Nair et al. 2010); others like *Blastocystis* spp. and *Dientamoeba fragilis* appear to be more prevalent in the developed countries (Fletcher et al. 2012; Roberts et al. 2011). In developed settings however, enteric protozoa are often ignored as a cause of diarrhoea due to the often mistaken belief that better hygiene practices are occurring.

In developed settings, bacterial cultures are usually considered initially in the diagnosis for acute diarrhoeal illnesses, while parasitic infections are more likely to be considered in patients with chronic symptoms, appropriate travel histories or other risk factors (Ribes, Seabolt & Overman 2004). However, laboratory based surveillance has been used as an important tool for estimating the burden of infectious diseases in several countries worldwide (Flint et al. 2005). In Australia for example, Cryptosporidiosis and Giardiasis are the only parasitic gastrointestinal diseases included in the infectious disease surveillance (Costello et al. 2009; Cretikos, Telfer & McAnulty 2008a). Estimates of the actual prevalence of enteric protozoa in industrialized countries is often affected by (i) the lack of routine testing for these parasites and (ii) the lack of sensitive diagnostic techniques to detect them in clinical specimens, while carrier stages and sub-clinical infections are often not diagnosed (Ng et al. 2011).

The actual burden of parasitic infections affecting Australians is relatively unknown. Hall et al found that 5% and 3% of persons were infected with *Cryptosporidium* and *Giardia* respectively . Other annectdotal evidence suggests that the protozoa prevalence is relatively low, however some individuals are at increased risk of infection. Parasitic infections are considered to be common among Aboriginal communities especially in children under five years of age (Commonwealth of Australia 2000; Currie & Brewster 2001). Reports indicate that men who have sex with men are at increased risk of infection (Stark et al. 2007b; Stark et al. 2008b). However, it is assumed that the estimated prevalence of protozoan infections is relatively similar regardless of the testing protocol employed. However no scientific assessment of this has been done; nor has a gold standard approach been determined for diagnosis of disease. This information is needed for early and accurate diagnosis to aid in the optimal management of parasitic diseases. This not only allows initiation of an adequate therapy but also implementation of health and hygiene education and control measures in the patients' home and community.

Here we summarise a multi-centre study to determine the relative prevalence of enteric protozoan infections from clinical specimens examined at four public hospitals in Sydney, and the comparison of the outcome of different testing algorithms for the detection of enteric protozoa. Finally, this study suggests that molecular methods should be employed as a gold standard approach for clinical diagnosis of enteric protozoa.

## *Methods*

### 5.2.1 Setting and Study Sites

Four hospitals, all located in different geographic areas across Sydney were included in the study. These facilities were included based on the population served, and represent a cross section of different socio-economic and cultural influences across the Sydney metropolitan region. Liverpool Hospital (Hospital A) is a tertiary referral hospital for south western Sydney; The Children's Hospital at Westmead (Hospital B) is a stand-alone service dedicated to paediatrics attracting referrals on a State-wide basis; St. Vincent's Hospital, Sydney (,Hospital C) is a major public and a principal referral hospital attracting referrals on a State-wide and national basis; and Prince of Wales Hospital (Hospital D) is a major teaching hospital and one of thirteen principal referral hospitals for adults based in Sydney's eastern suburb that also serves all of New South Wales. Each hospital hosts a fully accredited laboratory service, providing comprehensive biomedical laboratory services.

Ethical approval for this study was received from the Human Research Ethics Committees (HREC) at Sydney South West Area Health Service, Western Zone; Children Hospital at Westmead, South Eastern Sydney Local Health District, the St. Vincent's Hospital, Sydney, and the University of Technology, Sydney (UTS).

### 5.2.2 Microbiology methods

All four hospitals routinely test for enteric organisms in persons who present with gastrointestinal symptoms. On average, each laboratory tested one stool sample per patient, with between 45-89% of these specimens being loose- but not taking the shape of the container. The microbiological procedures used by the laboratories were described in Chapters two and three (Fletcher et al. 2013; Stark et al. 2010a). Generally speaking each laboratory used standard methods for the identification and isolation of enteric

pathogens. Additionally, in all hospitals, stools were processed by a wet preparation in saline, and examined for white blood cells, red blood cells, cysts, ova and parasites (COP) and bacteriological pathogens were identified using standard culturing methods. Each hospital had specific criteria for the testing of viruses. In some hospitals additional tests were employed for some pathogens. A summary of the various tests done for parasitic agents is presented in Table 5.3.

### 5.2.2.1 Parasitology

**Hospital A**: stool specimen are routinely collected in sodium acetate acetic acid formalin (SAF) fixative (Oxoid Australia), and processed by direct wet preparation. Light microscopy was routinely performed on all stool specimens. In the instances where no clinical information was received and the patient was an adult or age ≤10 years old, or the specimen was not received in SAF, then a *Giardia/ Cryptosporidium* screen enzyme immunoassay (EIA) (ProSpecT™ Giardia/Cryptosporidium Microplate Assay) was performed. 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. A full COP test was done on all positive microscopy and EIA results using an iron haematoxylin staining (IHS) with modified acid fast stain.

**Hospital B:** Light microscopy of a direct saline preparation was performed on all stool specimens. Concentration techniques are performed routinely for persons with a history of overseas travel, prolonged diarrhoea illness (>7 days) , attendees at Refugee clinics and on specific requests for COP test by the clinician. When a COP test is requested and if any parasites are seen in the wet preparation, a sample of stool is 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) which uses centrifugation at 500 g X10 mins and examined for COP using oil immersion. Alternatively, the fixed smear is prepared for permanent staining by the IHS technique. Additionally,

each stool specimen has a *Cryptosporidium* smear done routinely using a Modified Kinyoun's Acid-Fast Stain (Cold)].

Hospital C: Direct wet preparation and light microscopy was performed routinely on all stool specimens. The wet preparation was examined under a low power objective (10x) and then scanned under the high dry (40x) objective. All stool specimens are emulsified in SAF fixative (Oxoid Australia) using a 1:3 ratio, then the sample was centrifuged at 500g 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). Stool samples also 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 (Banik et al. 2011; Roberts et al. 2011; Stark et al. 2010c).

**Hospital D:**

Direct wet preparation microscopy was only conducted on patients at risk to parasitic infection as indicated in the clinical history and for patients with recent overseas travel or on request by the clinicians. The wet preparation was examined by light microscopy under low power objective (10x) and then scanned under the high dry (40x) objective. A sample of stool was also placed into SAF fixative (Meridian Bioscience, Inc., Cincinnati, Ohio–) followed by faecal concentration using the Mini Parasep®SF concentration kit (DiaSys Europe LTD, Laboratory Diagnostics PTY LTD). In addition, an enzyme immunoassay (EIA) was performed routinely as a screening test for the detection of *G. intestinalis* and *Cryptosporidium* (ProSpecT™ Giardia/Cryptosporidium Microplate Assay) and the detection of *Entamoeba histolytica/dispar* (ProSpecT™ *Entamoeba histolytica*, Remel). A 10% suspension of stool is prepared in 10% formalin (for *G. intestinalis* and *Cryptosporidium*) and in specimen buffer provided in kit (for *E. histolytica*) and the EIA was performed in accordance with the manufacturer's instructions and without modification.

All positive findings from the EIAs were confirmed by microscopy (i.e. iodine preparation and acid fast stain). Samples testing positive on the *E. histolytica* EIA that could not be confirmed by direct microscopy (i.e. iodine preparation) were sent to a reference laboratory for permanent stain preparation and examination. In order to detect *Cryptosporidium* oocysts, smears were made directly from faeces and stained by the Ziehl-Neilsen (ZN) based on the procedures described elsewhere (Collins & Lyne 1984).

### 5.2.3 Data extraction and analysis

Each hospital provided a spread sheet containing de-identified microbiology test results for the period January 2007 to December 2010 (Hospital C's data was for 2008-2010). The data was then arranged by medical record number, and date of service/stool request in ascending order.

For each hospital, the testing protocols were consulted to determine the number of specimen tested for COP/intestinal parasites. The information concerning these tests were placed into an SPSS database and duplicate tests removed. Duplicates were considered to be any stool specimens from the same individual (using their medical record number as unique identifier) that was collected on the same date and had the same accession/lab request number. This was done to ensure there was no double counting of specimen. For the purposes of this analysis, each individual stool sample and results were counted. Positivity was calculated on the basis of one organism per specimen. The percentage positivity rate was calculated as the total number of stool samples positive for an enteric organism divided by the total number of specimen tested. The mean difference between tests was calculated using the Data assessed using CMA software and verified with online vassarstats (Borenstein et al. 2005; Lowry 2012); mean difference % (z-value) P-values were reported. A laboratory survey (modelled on the Caribbean EcoHealth Laboratory Survey for foodborne pathogens) was conducted to identify laboratory procedures and the nature of the information completed on laboratory request forms in the four hospitals (See appendix 9.2). The survey was administered prior to the collection of the clinical data

from each hospital. The Laboratory Manager, Senior Hospital Scientist or representative completed the survey. The survey provided information of the nature of the information completed on laboratory request forms including: date of sample collection; age and gender; and information on the actual tests and frequency with which tests were conducted for common enteric micro-organisms. Respondents were asked to answer on a scale of "rarely" to routinely.

## *5.3 Results*

A laboratory survey was conducted to identify the nature of the information completed on laboratory request forms in the four hospitals. The results of the survey found that date of sample collection; age and gender were reported routinely on all laboratory request forms in all hospitals. Differential diagnosis was reported only sometimes in two hospitals and rarely in the other two. Signs and symptoms were only reported sometimes at all sites, and date of onset of illness was rarely reported except by Hospital D, where it was routinely done. Exposure information was rarely included on sample request forms. Across all hospitals, between 1% and 10% of stool specimens received were formed while 45%-89% were unformed (loose but not taking the shape of the container). Only 10%-50% of specimens were considered liquid (taking the shape of the container). A summary of diagnostic methods employed by each hospital is presented in Supplementary Table 9.3

Tests for enteric parasites were conducted on 2138 individual specimens from 1518 persons at Hospital A; 11097 specimen from 5229 persons at Hospital B; 8613 specimen tested from 6273 persons at Hospital C; and 6078 specimen tested from 3772 persons at Hospital D.

### 5.3.1 Enteric Parasites Summary

Detection rates for different age groups varied between Hospitals. Statistical tests were conducted based on 5 year age groups but were not statistically different between hospital, hence the combination of age groups. The prevalence of *G. intestinalis* was higher in the 0-5 age group compared to *Blastocystis* being more frequently detected in

persons over 5 years old (P<0.05) (data not shown). Enteric protozoa were identified in an average of 3.6% (95%CI 1.1%-11.2%) of specimens from the four hospitals. Across the four hospitals, the most common enteric protozoa detect was *Blastocystis* spp., identified in an average of 5.4% (95%CI 5.0%-5.7%) of cases, followed by *G. intestinalis* 1.1% (95%CI 1.0%-1.2%), *D. fragilis* in 0.8% (95%CI 0.7%-1.0 %), *E. histolytica/dispar* in 0.5% (95%CI 0.4%-0.6%), *Cryptosporidium* spp. 0.3% (95%CI 0.3%-0.4%), *Cyclospora* 0.1% (95%CI 0.02%-0.1%). Non-pathogen *Entamoeba* spp., *Chilomastix mesnili*, *Endolimax nana, Enteromonas hominis and Iodamoeba butschlli* were found in less than 1% of cases respectively. At Hospital A, 29% of patients submitted multiple specimen, with an enteric protozoa found in 8%. At Hospital B, 48% of patients seen submitted multiple specimen, and an enteric protozoa detected in <1%. At Hospital C, 38% submitted multiple specimens of which 8.5% were positive for protozoa. At Hospital D, 38% of patients submitted multiple specimens and 0.5% were positive for enteric protozoa.

The results for Hospital A are summarised in Table 51. A total 9% (187/2138) of stool specimen tested for COP test and had an enteric protozoan identified. Overall, *Blastocystis* spp., (5.71% or 122/2138) was the most common protozoan identified, followed by *G. intestinalis* (1.17% or 25/2138). At Hospital B, an enteric protozoan was detected in 1% (70/ 10123) of stools that underwent a COP test (Table 5.2). Overall, *G. intestinalis* (0.5% or 48/10123) was the most common protozoa identified, followed by *Blastocystis* spp, (0.4% or 40/10123). *Giardia* was also commonly found in repeat samples. One or more protozoa were found in 12% (1003/8613) specimens at Hospital C (Table 5.3). *Blastocystis* spp was the most common protozoa found in 7% (571/8613), *Giardia* in 2% (141/8613), and *D*. *fragilis* in 1% (100/8613). At Hospital D, 1% (78/6078) of stool specimen tested positive for one or more enteric protozoa (Table 5.4). A total of 1 % (77/3772) patients had a positive result from the *Giardia*/*Cryptosporidium* coproantigen test (EIA). However, only 56% (43) of these were confirmed by microscopy of wet preparation to be *G. intestinalis* and 20 (26.0%) confirmed to be *Cryptosporidium* spp.

**Table 5-1: Overall prevalence of enteric protozoa from Cyst, Ova and Parasite test, Hospital A, 2007-2010**

| Organism identified | Total Positive -single specimen tested (n) | % | Total Positive multiple specimen tested (n) | % | Total specimen | % of Overall specimen tested |
|---|---|---|---|---|---|---|
| ***Blastocystis* spp** | 92 | 6.1 | 30 | 4.8 | 122 | 5.7 |
| ***Giardia intestinalis*** | 21 | 1.4 | 4 | 0.6 | 25 | 1.2 |
| ***Cryptosporidium* spp.** | 7 | 0.5 | 3 | 0.5 | 10 | 0.5 |
| ***Dientamoeba fragilis*** | 7 | 0.5 | 0 | 0.0 | 7 | 0.3 |
| ***Entamoeba hartmanni*** | 5 | 0.2 | 1 | 0.2 | 6 | 0.3 |
| ***Entamoeba histolytica/dispar*** | 3 | 0.1 | 1 | 0.2 | 4 | 0.2 |
| ***Chilomastix mesnili*** | 1 | 0.1 | 2 | 0.3 | 3 | 0.1 |
| ***Endolimax nana*** | 1 | 0.1 | 8 | 1.3 | 9 | 0.4 |
| ***Enteromonas hominis*** | 0 | 0.0 | 1 | 0.2 | 1 | 0.1 |
| **Subtotal Protozoa positive** | **137** | **9.2** | **50** | **8.1** | **187** | **8.8** |
| **Other pathogens** | 15 | 1.1 | 5 | 0.8 | 20 | 1.0 |
| **Positive** | 152 | 10.0 | 55 | 8.9 | 207 | 9.7 |
| **Negative** | 1366 | 90.0 | 565 | 91.1 | 1931 | 90.3 |
| Total | **1518** | **100.0** | **620** | **29.0** | **2138** | **100.0** |

**Table 5-2: Overall prevalence of enteric protozoa from Cyst, Ova and Parasite test, Hospital B 2007-2010**

| Organism identified | Total Positive -single specimen tested (n) | % | Total Positive multiple specimen tested (n) | % | Total specimen | % of Overall specimen tested |
|---|---|---|---|---|---|---|
| ***Giardia intestinalis*** | 38 | 0.7 | 10 | 0.2 | 48 | 0.47 |
| ***Blastocystis* spp.** | 29 | 0.6 | 11 | 0.2 | 40 | 0.4 |
| ***Dientamoeba fragilis*** | 19 | 0.4 | 2 | 0.0 | 21 | 0.21 |
| ***Cryptosporidium* spp.** | 8 | 0.2 | 1 | 0.0 | 9 | 0.09 |
| **Subtotal Protozoa** | **94** | **1.9** | **24** | **0.5** | **118** | **1.2** |
| **Other pathogens** | 838 | 15.9 | 101 | 2.1 | 939 | 9.3 |
| **Total positive** | 932 | 17.8 | 115 | 2.3 | 1057 | 10.5 |
| **Negative** | 4297 | 82.2 | 4769 | 97.4 | 9066 | 89.5 |
| **Total** | **5229** | **100.0** | **4894** | **100.0** | **10123** | **100.0** |

**Table 5-3: Overall prevalence of enteric protozoa from Cyst, Ova and Parasite test, Hospital C for 20008-2010**

| Organism identified | Total Positive - single specimen tested (n) | % | Total Positive multiple specimen tested (n) | % | Total specimen | % of Overall specimen tested |
|---|---|---|---|---|---|---|
| ***Blastocystis* spp.** | 429 | 5.0 | 142 | 4.3 | 571 | 6.6 |
| ***Giardia intestinalis*** | 109 | 5.7 | 32 | 1.0 | 141 | 1.6 |
| ***Dientamoeba fragilis*** | 71 | 3.7 | 29 | 0.9 | 100 | 1.2 |
| ***Endolimax nana*** | 34 | 0.4 | 30 | 0.9 | 64 | 0.7 |
| ***Cryptosporidium spp.*** | 33 | 0.4 | 4 | 0.1 | 37 | 0.4 |
| ***Entamoeba coli/hartmanni*** | 21 | 0.2 | 15 | 0.5 | 36 | 0.4 |
| ***Entamoeba histolytica/dispar*** | 47 | 0.8 | 4 | 0.1 | 51 | 0.6 |
| ***Enteromonas hominis*** | 7 | 0.1 | 9 | 0.3 | 16 | 0.2 |
| ***Cyclospora*** | 5 | 0.1 | 0 | 0.0 | 5 | 0.1 |
| ***Iodamoeba*** | 2 | 0.02 | 17 | 0.5 | 19 | 0.2 |
| ***Chilomastix*** | 1 | 0.01 | 0 | 0.0 | 1 | 0.01 |
| **Subtotal Protozoa** | **758** | **18.4** | **282** | **12.1** | **1041** | **12.1** |
| **Other Pathogens** | 1196 | 19.1 | 402 | 17.2 | 1598 | 18.5 |
| **Total positive** | 1954 | 31.2 | 684 | 29.3 | 2639 | 30.6 |
| **Total Negative** | 4319 | 68.8 | 1656 | 70.7 | 5974 | 69.4 |
| **Total specimen tested** | **6273** | | 2340 | **38.4** | **8613** | |

**Table 5-4: Overall prevalence of enteric protozoa from Cyst, Ova and Parasite test, Hospital D for 2007-2010**

| Organism identified | Total Positive - single specimen tested (n) | % | Total Positive multiple specimen tested (n) | % | Total specimen | % of Overall specimen tested |
|---|---|---|---|---|---|---|
| ***Giardia intestinalis*** | 38 | 1.0 | 5 | 0.2 | 43 | 0.7 |
| ***Cryptosporidium* spp.** | 16 | 0.4 | 4 | 0.2 | 20 | 0.3 |
| ***Blastocystis hominis*** | 5 | 0.0 | 1 | 0.04 | 6 | 0.1 |
| ***Entamoeba histolytica/dispar*** | 3 | 0.0 | 2 | 0.1 | 5 | 0.1 |
| ***Entamoeba coli/hartmanni*** | 2 | 0.1 | 1 | 0.04 | 3 | 0.1 |
| ***Endolimax nana*** | 1 | 0.0 | 0 | 0.0 | 1 | 0.02 |
| ***Sub-total Protozoa*** | 65 | 1.7 | 13 | 0.6 | 78 | 1.3 |
| **Other- organisms** | 2 | 0.1 | 0 | 0.0 | 2 | 0.03 |
| **Negative** | 3705 | 98.2 | 2293 | 99.4 | 5998 | 98.7 |
| **Total** | **3772** | | **2306** | **37.9** | **6078** | **100.0** |

### 5.3.2 Comparison of Results based on Testing Algorithms

Approximately 2.5% (95%CI 2.3%-2.7%) of protozoan infections was detected by permanent staining (IHS or mIHS), 1.1% (95%CI 1.0%-1.2%) by microscopy of wet preparations and 0.6% (95%CI 0.5%-0.7%) by EIA combined with microscopy. The detection of protozoa based on different microbiological tests done is summarised in Table 5.5.

Figure 5.1 presents a comparison of the detection rates for four protozoa based on similar testing protocol in at least two hospitals. The combination of microscopy of wet preparation and EIA detected the prevalence of *Cryptosporidium* spp., in an average of 0.4% (95%CI 0.3%-0.5%) (OR 1.4; 95 % CI 0.7- 3.0) and *G. intestinalis* in 0.9% (95%CI 0.7%-1.1%) (OR 1.7; 95 % CI 1.0 - 2.7; P = 0.05) of cases between Hospitals A and D. Microscopy of wet preparation detected *Blastocystis* spp., in an average of 2.6% (95%CI 2.2%-3.0%) of cases, with significantly higher detection rates in Hospital A compared with Hospital B (OR 14.4; 95 % CI 10.1- 20.7; P< 0.0001) and Hospital D (57.8; 25.4- 131.4; P<0.0001). Permanent staining with IHS or mIHS detected *D. fragilis* in an average of 1.1% (95%CI 0.9%-1.3 %) of cases between Hospitals A and C, with Hospital D detecting significantly higher rates by employing a mIHS (OR 3. 6; 95 % CI 1.7 - 7.7; P < 0.001).

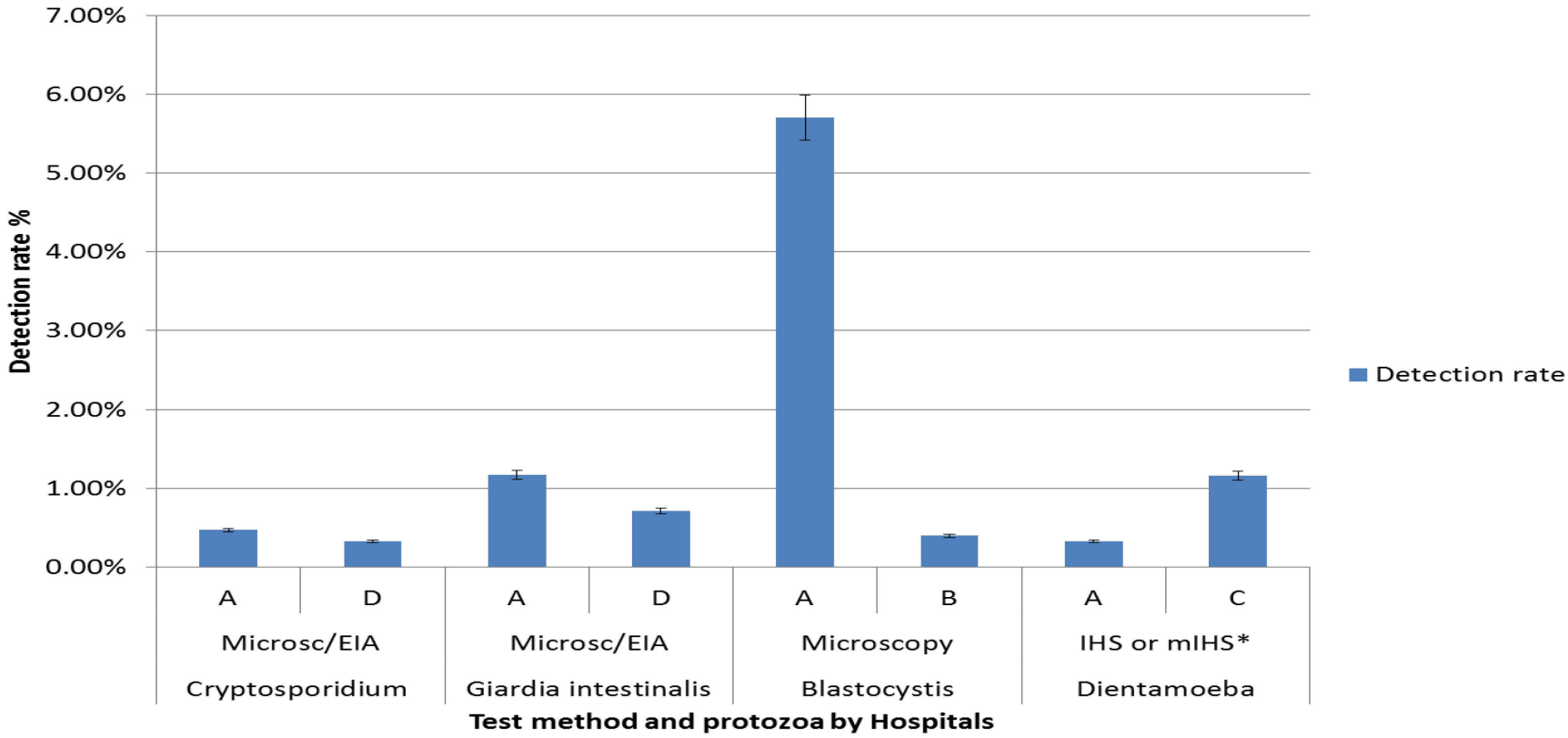
**Table 5-5: Prevalence of enteric protozoa based on microbiology test in four hospitals in Sydney**

| Organism | Hospital A % (95%CI) (N=2138) | Hospital B % (95%CI) (N=10123) | Hospital D % (95%CI) (N=6078) |
|---|---|---|---|
| | Microscopy -Wet Preparation | | |
| ***Blastocystis*** | 5.7% (4.8-6.8) | 0.4% (0.3-0.5) | 0.1% (0.04-0.2) |
| ***Giardia intestinalis*** | N/A | 0.5% (0.4-0.6) | N/A |
| ***Endolimax nana*** | 0.4% (0.2-0.8) | NT | 0.02% (0.0-0.1) |
| ***Entamoeba hartmanni/coli*** | 0.2% (0.1-0.6) | NT | 0.1% (0.02-0.2) |
| ***Dientamoeba fragilis*** | N/A | 0.2% (0.1-0.3) | NT |
| ***E. histolytica/dispar*** | 0.2% (0.1-0.5) | NT | N/A |
| ***Chilomastix mesnili*** | 0.1% (0.1-0.4) | NT | NT |
| ***Cryptosporidium*** | N/A | 0.1% (0.1-0.2) | N/A |
| ***Enteromonas hominis*** | 0.1% (0.01-0.3) | NT | NT |
| | **Permanent Staining-IHS/mIHS** | | |
| Organism | **Hospital C (N=8613)** | **Hospital A (N=2138)** | |
| ***Blastocystis*** | 6.6% (6.1-7.2) | N/A | |
| ***Giardia intestinalis*** | 1.6% (1.4-1.9) | N/A | |
| ***Dientamoeba fragilis*** | 1.2% (1.0-1.4) | 0.3% (0.2-0.7) | |
| ***Endolimax nana*** | 0.7% (0.6-1.0) | N/A | |
| ***E. histolytica/dispar**** | 0.6% (0.5-0.8) | N/A | |
| ***Cryptosporidium*** | 0.4% (0.3-0.6) | N/A | |
| ***Entamoeba hartmanni/coli*** | 0.4% (0.3-0.6) | N/A | |
| ***Iodamoeba butschlli*** | 0.2% (0.1-0.5) | N/A | |
| ***Enteromonas hominis*** | 0.2% (0.1-0.3) | N/A | |
| ***Cyclospora*** | 0.1% (0.02-0.1) | N/A | |
| ***Chilomastix mesnili*** | 0.01% (0.00-0.8) | N/A | |
| | **Enzyme Immunoassay (EIA)** | | |
| **Organism** | **Hospital A (N=2138)** | **Hospital D (N=6078)** | |

| Organism | Hospital A % (95%CI) (N=2138) | Hospital B % (95%CI) (N=10123) | Hospital D % (95%CI) (N=6078) |
|---|---|---|---|
| ***E. histolytica/dispar*** | N/A | 0.1% (1.03-1.2) | |
| ***Giardia intestinalis*** | 1.2% (0.8-1.7) | 0.7% (0.5-1.0) | |
| ***Cryptosporidium*** | 0.5% (0.3-0.9) | 0.3% (0.2-0.5) | |
| | | | |

**Key:** Micro-Wet = Microscopy of wet preparation; SAF= sodium acetate acetic acid formalin (SAF) fixative; ZN= Ziehl Neelson; IHS= iron haematoxylin stain; mIHS= modified iron haematoxylin stain with a carbol fuchsin step; EIA= enzyme immunosorbent assay; PCR= polymerase chain reaction. NT= Not tested; N/A –not applicable- another test performed. **7 seven specimen were confirmed with *E. histolytica* by PCR over the period.

**Figure 5-1: Prevalence of Cryptosporidium spp., Giardia intestinalis, Blastocystis, and Dientamoeba fragilis based on Microbiology tests conducted at Four Sydney Hospitals 2007-2010**




Odds Ratios for difference in detection: *Blastocystis* spp (14.4, 95%CI 10.1- 20.7; P< 0.0001), *Dientamoeba* (3. 6, 95%CI 1.7 - 7.7; P < 0.001), *Giardia intestinalis* (1.7, 95%CI 1.0 -2.7; P = 0.05), *Cryptosporidium* spp (1.4, 95%CI 0.7- 3.0; P > 0.05). **Key:** Microsc= Microscopy; IHS= iron haematoxylin stain / mIHS- modified iron haematoxylin staining technique incorporating a carbol fuschin step; EIA= enzyme immunosorbent assay.

The mean difference between tests conducted at each hospital is presented in Table 5.6. *Blastocystis* spp. was more frequently detected at Hospital A when compared with Hospital B and Hospital D (mean difference 5.3% and 5.6% respectively; P=0.0002), and at Hospital C when compared with Hospitals B and D (mean difference >6% each; P<0.0001). A higher rate of detection was also observed for *Giardia* at Hospital A when compared with Hospital B and Hospital D (mean difference 0.7%; P<0.0002 and 0.5%, P<0.05 respectively) and Hospital C when compared with Hospitals B and D (mean difference 1.2% and 0.93%; respectively; P<0.0001). In addition, Hospital C diagnosed significantly more *E. histolytica/dispar* when compared with Hospital D (mean difference 0.5%; P<0.0002).

**Table 5-6: Mean difference in protozoa detection rates amongst four Sydney Hospitals**

| | Hospital A/B | HOSPITAL A/C | HOSPITAL A/D | HOSPITAL B/C | HOSPITAL B/D | HOSPITAL C/D |
|---|---|---|---|---|---|---|
| Organisms detected | Mean difference % (z-value) P-value | Mean difference % (z-value) P-value | Mean difference % (z-value) P-value | Mean difference % (z-value) P-value | Mean difference % (z-value) P-value | Mean difference % (z-value) P-value |
| ***Blastocystis* spp.** | 5.3% (19.5) <0.0002 | 0.9%2 (1.6) 0.060 | 5.6% (18.0) 0.0002 | 6.2% (23.9) <0.0001 | 0.3% (3.433) 0.0006 | 6.5% (20.1) <0.0001 |
| ***Chilomastix mesnili*** | 0.1 (3) | NT | NT | NT | NT | NT |
| ***Cryptosporidium* spp.** | 0.4% (4.1) <0.0002 | 0.04% (0.2) 0.406 | 0.1% (0.9) 0.361 | 0.34% (4.7) <0.0001 | 0.2% (3.501) <0.0005 | 0.1% (1.0) <0.167 |
| ***Cyclospora*** | NT | NT | 0.03 (2) | 0.0 (0) | NT | NT |
| ***Dientamoeba fragilis*** | 0.1% (1.1) 0.291 | 0.83% (3.5) 0.0003 | 0.0 (0) | 0.95% (8.1) <0.0001 | 0.0 (0) | 0.0 (0) |
| ***Endolimax nana*** | 0.0 (0) | 0.03% (1.6) 0.052 | 0.4% (N/C) | 0.0 (0) | 0.0 (0) | 0.7% (N/C) |
| ***Entamoeba hartmanni/coli*** | 0.0 (0) | 0.14%; (0.9) 0.181 | 0.2% (N/C) | 0.0 (0) | 0.0 (0) | 0.37% (N/C) |
| ***Entamoeba histolytica/dispar* complex** | 0.0 (0) | 0.4% (N/C) | 0.1% (N/C) | 0.0 (0) | 0.0 (0) | 0.6% (4.9) <0.0002 |
| ***Enteromonas hominis*** | 0.0 (0) | 0.1% (N/C) | 0.0 (0) | 0.0 (0) | 0.0 (0) | 0.0 (0) |
| ***Giardia intestinalis*** | 0.7% (3.8) <0.0002 | 0.47% (1.6) 0.058 | 0.5% (2.0) 0.043 | 1.2% (7.9) <0.0001 | 0.2% (1.924) 0.054 | 0.9% (5.0) <0.0001 |

N/C- Not calculated. Cells contained fewer than 5 hence estimates could not be calculated. NT- Test not done. Total specimen tested Hospital A: 2138; Hospital B: 10123; Hospital C: 8163; and Hospital D: 6078.

## *5.4 Discussion*

We present the prevalence of enteric protozoa amongst persons seeking care for gastrointestinal illnesses in Sydney across four major public hospitals. The study reveals that while all four laboratories performed direct microscopy on stool specimens for the detection of cyst, ova and parasites, different approaches are used for different species and tests for some protozoa are not routinely done. The study revealed that prevalence of enteric protozoa infections varies depending on the nature of the tests conducted, and so progress towards development of a gold standard approach for diagnosis of disease is warranted.

On average only one stool specimen was tested per person, with multiple specimens being submitted by between 29%-48% of persons across hospitals, yielding similar results. This small percentage that submitted multiple samples is not ideal nor best practice, since the cysts/oocysts of some protozoa are not consistently shed in the stool, their numbers may vary from day to day (Garcia, Shimizu & Bernard 2000), and trophozoites of some protozoa could be missed (van Gool et al. 2003). Additionally, since the prevalence of enteric protozoa is relatively low in this population, the submission of single specimen is not ideal and is usually only acceptable when the prevalence among the population is about 20%, such as in developing settings (Branda et al. 2006).

The success of detection of enteric parasites between the four hospitals varied. Generally, *Blastocystis* spp., and *G. intestinalis* were the most common enteric protozoa identified in patients. However *Giardia* appeared to be more prevalent in the 0-5 age group as evidenced by higher detection rates in this age group at Hospitals A and B, compared with *Blastocystis* in the over 5 age group at the same hospitals. This age relationship was confirmed to be significant, as the odds of infection with *Giardia* decreases with age while the odds of infection with *Blastocystis* increases with age in these settings (Fletcher et al 2012, *in preparation*). The prevalence of *Giardia* among children in Sydney may indicate

that children are coming into contact with contaminated water sources or infected persons, and suggests that young children are at increased risk to infectious gastrointestinal illnesses (Fletcher et al. 2012; Yoder et al. 2012), including those in child-care settings (Plutzer, Ongerth & Karanis 2010). This high incidence of *Giardia* in children has been observed in other countries (Pardhan-Ali et al. 2012). *Blastocystis* spp., in older children/adults on the other hand suggests possible exposure through routes such as contaminated food, water and unhygienic practices (Boorom et al. 2008). In many developed countries *Blastocystis* spp. is the most common enteric protozoan identified in diarrhoeal patients (Logar, Andlovic & Poljsak-Prijatelj 1994; Özyurt et al. 2008; Svenungsson et al. 2000). However, despite being commonly detected in diarrhoeal patients, *Blastocystis* is often reported as a non-pathogenic microbe (Fletcher et al. 2012; Svenungsson et al. 2000; Tan & Suresh 2006; Vandenberg et al. 2006), however recent studies suggest that pathogenicity may be subtype dependent (Roberts et al. 2012).

The proportion of stool specimens positive for an enteric parasite varied between hospitals, ranging from a low of 1.2% at Hospital B and 1.32% at Hospital D, to a high of 11.6% at Hospital C and 8.83% of specimens at Hospital A. The difference in detection rates between hospitals is likely driven by the number of stool specimen tested, since individual hospitals had different criteria for testing for enteric protozoa, which are not necessarily based on pathogen prevalence. For example, both Hospitals A and C tested for a wider range of pathogens routinely (see Tables 4.1 and 4.3 respectively), including non-pathogenic species, which may be associated with the fact that both hospitals served high risk populations such as refugees (A), men who have sex with men, and HIV/AIDs infected persons (C). The composition of the population seen at each hospital could influence the results if risk factors are unequally distributed in the population (Mohr & Mohr 1992). Other hospitals would not routinely conduct a test if the prevalence of protozoa is relatively low, and probably likely to generate many false negatives. The testing protocols may therefore be secondary to a perceived prevalence within high risk groups in the wider population (e.g. men who have sex with men, recent immigrants and lower socio-economic groups). Both hospitals A and C had clinics that catered to high risk groups. The

differences in detection rates are also likely related to the different diagnostic techniques and handling practices between hospitals. According to Libman et al, significant variations in specimen handling and processing practices between laboratories can affect the assessment of the diagnostic processes (Libman et al. 2008).

Detection rates for the individual species using different testing algorithms were significantly different between hospitals ($P<0.05$). There were differences between hospitals that used similar methods. For example, where microscopy of wet preparation was employed, Hospital A detected significantly higher rates of *Blastocystis* spp, (5.3%; $P=0.0002$) and *G. intestinalis* (0.7%; $P<0.0002$) when compared with Hospital B using similar methodology. One difference observed between these two hospitals is the fact that Hospital B routinely examines all specimens for COP, compared with only some specimen being tested for COP at Hospital A. However, this study reveals that hospitals that employed wet preparation microscopy of fresh or fixed stool (SAF) specimen found a lower prevalence of protozoa. A disadvantage of using the microscopy method only is its low sensitivity to detect protozoa, which lead to false-negative results (Roberts et al. 2011; Stark et al. 2010a). This is particularly true for protozoa such as *D. fragilis* that requires special staining techniques to detect its nuclear structure (Garcia 1994; Stark et al. 2011; Stark et al. 2010b). It is however surprising that the detection rates obtained for *Cryptosporidium* and *Giardia* at Hospital A using EIA and wet preparation microscopy, showed no significant difference when compared to those obtained by Hospital C that employed the more sensitive methods of permanent staining with the modified IHS.

Hospital C that utilized the modified iron haematoxylin staining method, consistently detected significantly higher rates of *Blastocystis* spp, and *G. intestinalis* in comparison with Hospitals B and Hospital D that utilized microscopy of wet preparation ($P< 0.001$). However there was only borderline differences compared with Hospital A for these protozoa ($P=0.06$). The utilisation of the modified iron haematoxylin staining by Hospital C also detected significantly higher rates of *D. fragilis* when compared with the IHS-only method at Hospital A and wet preparation microscopy at Hospital B. These results suggest

that the application of permanent staining is more valuable for the detection of *Blastocystis* spp., *G. intestinalis* and *D. fragilis*. Several reports recommend molecular methods for the sensitive and specific detection of some protozoa; however these tests are not widely available even in developed settings. Microscopy remains a necessary tool for the diagnosis of multiple parasitic pathogens, even in low prevalence settings (Bruijnesteijn van Coppenraet et al. 2009). However, permanent staining is more sensitive for the detection of difficult to diagnose protozoa, and is recommended when SAF is used (Rosenblatt 2006).

Additionally, Hospital C identified *G. intestinalis* in *2 %* and *Cryptosporidium* from <1*%* of specimens using the modified IHS method, compared with Hospital D that employed screening EIA which detected slightly lower results for *Giardia intestinalis (0.7 %)* and *Cryptosporidium (0.3%)*. The weakness of the EIA used was demonstrated in that only 70% of those initially positive by EIA were confirmed as positive by microscopy (i.e. iodine preparation and acid fast stain). These findings indicate that the screening EIA method is less sensitive and specific for the detection of *Cryptosporidium* and *Giardia*. Another study has shown that a ProSpecT EIA for the detection of *Giardia*/*Cryptosporidium* antigens demonstrated 100% sensitivity and 98.4% and 98.6% specificity respectively in the detection of these protozoa in stool specimens (Katanik et al. 2001)

There was however a significant difference in the detection of *D. fragilis* (P=0.001) between the three hospitals that utilized three different methodologies. The employment of the modified IHS gave the highest detection rate of 1% at Hospital C compared with <1% by the IHS-only method at Hospital A and by microscopy of wet preparations at Hospital B respectively. Microscopy of fixed smears and permanent staining (modified iron-haematoxylin, trichrome staining) (Stark et al. 2006a; Stensvold et al. 2007), is considered to be the gold standard for diagnosis of *D. fragilis* infection. Fixation of stool is needed to prevent the degradation of morphology and to visualise characteristic nuclear structure needed for definitive diagnosis of *D. fragilis* by microscopy (Sawangjaroen, Luke & Prociv 1993; Stark et al. 2010a; Stark et al. 2005a).

Significantly higher rates of *E. histolytica/dispar* were also detected using the mIHS confirmed by PCR, compared with EIA and wet-preparation microscopy. The lower detection rates using microscopy is not surprising since microscopy is generally considered insufficient for differentiation between the pathogenic *E. histolytica*, that is morphologically identical to the non-pathogenic species, *E. dispar* and *E. moshkovskii*; of these species (Stark et al. 2010c). Although the ingested red blood cells within the cytoplasm of *E. histolytica* trophozoites can sometimes be used to differentiate it from the non-pathogenic species this phenomenon does not commonly occur and is rarely seen in clinical samples (CDC ; Fotedar et al. 2007). A number of conventional and real-time PCRs have proven to be more sensitive and specific than microscopic examination for the detection of *E. histolytica* and *E. dispar* in a single stool sample, and are now considered the gold standard for diagnosis (Verweij et al. 2004; Visser et al. 2006). In developed settings, PCR is more useful for detection of *E. histolytica* in stools rather than the antigen detection tests due to the higher sensitivities observed in PCR and the reduced chance of cross reactivity with other *Entamoeba* species (Gutiérrez-Cisneros et al. 2010; Stark et al. 2010c; Visser et al. 2006).

One potential bias is that the hospitals that looked at specimen for COP based on a specific criteria, identified higher rates of protozoa, regardless of the test used as evidenced by *Blastocystis* and *Giardia* rates at Hospital A. However, the similar approach of using specific criteria was utilized by Hospital D, which produced consistently lower results. Additionally, the use of permanent staining with fixed smears at Hospital C, produced similar rates of isolation for *Cryptosporidium* spp., *Endolimax nana*, *Entamoeba hartmanni*/*coli*, and *G. intestinalis*, when compared with microscopy at Hospital A. This variability in results with microscopy, EIA and permanent staining of fixed smears has been previously described in a similar setting (McIver et al. 2001; Roberts et al. 2011). These results are also likely to be influenced by the fact that the prevalence of some organisms is relatively low in the population, and only a proportion of persons who are infected seek medical attention and get tested. The identification of non-pathogenic species such as *Endolimax* is also important to demonstrate to the laboratory that they

can readily identify and differentiate morphologically similar parasites, as well as to serve as an indication of exposure to contaminated material, that result in further search for pathogenic organisms (Rosenblatt 2006).

The authors are mindful that that the incidence figures for each hospital should be compared with caution based on the differences between the testing protocols, and their ability to detect protozoa. However, these results can be used by local and state health authorities to guide disease surveillance activities for these organisms. This can also aid in the understanding of the burden and epidemiology of protozoa infections in Sydney, and provide the basis for setting research priorities and planning interventions to prevent the spread of disease.

The variability in the detection rates between the tests done at different hospitals, indicate the need for the development of a gold standard approach for diagnosis of enteric protozoa. In this study, the modified IHS consistently detects more parasites than light microscopy of wet preparation and the EIAs. However, IHS like the other methods also has limitations. A recent study in a similar setting found that IHS missed all *Blastocystis* subtype 2 infections, which could be attributed to the possible morphological forms and size of this subtype (Roberts et al. 2012). The prevalence of enteric protozoa is relatively low in this setting, and as such requires highly sensitive techniques to detect them in faecal specimen. This is due to several issues including: relatively low incidence of some species such as *Cryptosporidium* and *Cyclospora;* the difficulties in diagnosis of some species (e.g. *D. fragilis*), the difficulties with differentiation of *Entamoeba* species, and the low sensitivity of EIAs, to name a few. The development of a gold standard approach for diagnosis of enteric protozoa, which addresses these issues, is therefore warranted. More recently, molecular techniques have been developed to overcome some of the limitations of conventional microscopic methods.(Bruijnesteijn van Coppenraet et al. 2009; Stark et al. 2008a). Both conventional and real-time PCRs have proven to be more sensitive and specific than microscopic examination for the detection of *Cryptosporidium* spp., *D. fragilis*, *E. histolytica*, and *G. intestinalis* in a single stool sample (Stark et al. 2011; Verweij

et al. 2004; Visser et al. 2006). A single stool sample is usually sufficient for complete parasitological diagnosis, and the diagnostic yield is usually improved with the application of PCR (Bruijnesteijn van Coppenraet et al. 2009; Poirier et al. 2011; Stark et al. 2011). Molecular methods can assist in understanding the genetic diversity and the role of different genotypes amongst *Blastocystis* spp., in the causation of disease. (Roberts et al. 2011). It is therefore recommended that microscopic examination should be enhanced by molecular techniques, especially in low prevalence settings.

### 5.4.1 Conclusions

This study reveals that hospitals across Sydney employ different testing protocols for the detection of enteric protozoa. The results of microbiology tests for the same protozoan species were significantly different based on the nature of the tests performed. Factors such as the number of consecutive stool specimen tested and the inclusion of high risk populations at some hospitals may be considered as influencing the prevalence rates at individual sites. However, the evidence suggests that the use of permanent staining with fixed smears is a more sensitive methodology for the detection of some protozoa including *Blastocystis* spp., *G. intestinalis,* and *D. fragilis* and hence influencing the prevalence of protozoa reported.

While the results of microbiology tests from these hospitals can be considered reliable to inform public health practice, the consistent use of sensitive diagnostic techniques are needed to determine the true prevalence of enteric protozoa and to provide consistent data to inform policy. In this study, the mIHS consistently detects more parasites than light microscopy of wet preparation and the EIAs. The variability in the detection rates between the tests done at different hospitals, indicate the need for the development of a gold standard approach for diagnosis of enteric protozoa. Clinicians and public health practitioners must therefore be aware that depending on the protocol used, the prevalence of enteric protozoa can differ significantly, and in some cases, may underestimate their actual prevalence in the community. There is therefore need for a

more consistent approach and the application of standard testing protocols across hospitals using sensitive and specific diagnostic tools.

# 6 Geographical distribution and epidemiology of enteric protozoan infections in Sydney: A multicentre study.

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Running title: Distribution of Enteric Protozoa in Sydney

## Chapter Overview

Having examined the testing protocols used to detect enteric protozoa, the aim of this chapter is to expand on the previous chapter by providing a detailed description of the epidemiology and 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.

## *6.1 Abstract*

**Background:** Several species of enteric protozoa are associated with diarrhoeal illnesses in humans. However there is limited information about the epidemiology and geographical distribution of enteric protozoa in Australia. This study seeks to describe the epidemiology and geographical distribution of enteric protozoa in the state of New South Wales (NSW), and is the first study to incorporate spatial analysis to define the epidemiology of enteric protozoa in the Australasia Region.

Methodology and Principal Findings: Laboratory and clinical records from four public Hospitals in Sydney (NSW) 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.

Frequently detected protozoa were *Blastocystis* spp. (57%), Giardia *intestinalis* (27%), and *Dientamoeba fragilis,* (12%). The age distribution was bimodal, with the prevalence of protozoa decreasing with age up to 24 yrs., 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 (CBD),

while pockets of giardiasis were identified in regional/rural areas. The age distribution of cases suggests that schools, homes for the elderly are foci of infection.
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.

Keywords: *Blastocystis,* Epidemiology, geographical distribution, *Giardia intestinalis,* enteric protozoa, Sydney, mapping, microbiology, public health, geographical information systems.

## *6.2 Introduction*

Several species of enteric protozoa are associated with diarrhoeal illnesses in humans. Some cause severe debilitating conditions in immuno-suppressed and immune-competent populations (Sokolova et al. 2011). 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 (Escobedo et al. 2010; Kenny & Kelly 2009). Several species of enteric protozoa exist in Australia, and some are endemic. Giardiasis and cryptosporidiosis are the only protozoan infections captured by disease surveillance in NSW, for which, once a diagnosis has been confirmed, public health units must be notified to take preventative actions (NSW Department of Health 2012). Pathogenic protozoa such as *Cyclospora* and *Entamoeba histolytica* are less prevalent and are usually related to travel to developing regions (Swaminathan et al. 2009; Verweij et al. 2003).

*Blastocystis* spp. is the most common protozoan diagnosed in developed countries, although its role in eliciting gastrointestinal pathology and symptoms remain uncertain and controversial (Hotez 2000; Tan, Singh & Yap 2002). Clinical features of illness which have been attributed to *Blastocystis* spp. include nausea, anorexia, abdominal pain, flatulence and acute or chronic diarrhoea (Jones et al. 2009; Sohail & Fischer 2005). It is often associated with chronic gastrointestinal illness of unknown aetiology (Jones et al. 2009) and with irritable bowel syndrome (IBS) -like symptoms (Dogruman-Al et al. 2010; Jimenez-Gonzalez et al. 2011). Some clinicians consider the identification of *Blastocystis* from patient stools only as a potential marker of exposure to faecal contamination (Pierce, Huston & Moselio 2009).

*Cryptosporidium* spp., accounts for about 20% of diarrhoeal episodes in children in developing countries, up to 9% in developed settings (Rimseliene et al. 2011; Xiao 2009). Infections are usually characterized by self-limiting diarrhoea associated with severe abdominal pain in immuno-suppressed and immuno-competent persons, especially those

HIV-infected and children worldwide (Areeshi et al. 2008; Fayer, Morgan & Upton 2000; Stark et al. 2009b). 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 (Xiao 2009).

*Cyclospora cayetanensis* has emerged as an important cause of endemic or epidemic diarrhoeal illness in children and older children/adults worldwide (Chacín-Bonilla 2010). Clinical illness is characterized by persistent diarrhoea, bloating, flatulence, abdominal cramps, constipation, and fatigue (Ortega, Eberhard & Kris 2008). Cyclosporiasis is a common cause of illness amongst returned international travellers (Swaminathan et al. 2009; Verweij et al. 2003), although non-travel and water-borne related cases (Amin 1998) and food -borne outbreaks (Döller et al. 2002 ; Ho et al. 2002) have been reported in developed countries.

The pathogenicity of *Dientamoeba fragilis* has been widely debated (Barratt et al. 2011; Stark et al. 2009b). Infection can be acute or chronic (Barratt et al. 2011; Stark et al. 2010b), and symptomatic patients exhibit abdominal pain, persistent diarrhoea, loss of appetite, weight loss and flatulence, as well as IBS like symptoms (Banik et al. 2011; Sawangjaroen, Luke & Prociv 1993). Studies have found *D. fragilis* to be of similar or greater prevalence to *Giardia* (Fletcher et al. 2012; Stark et al. 2007b).

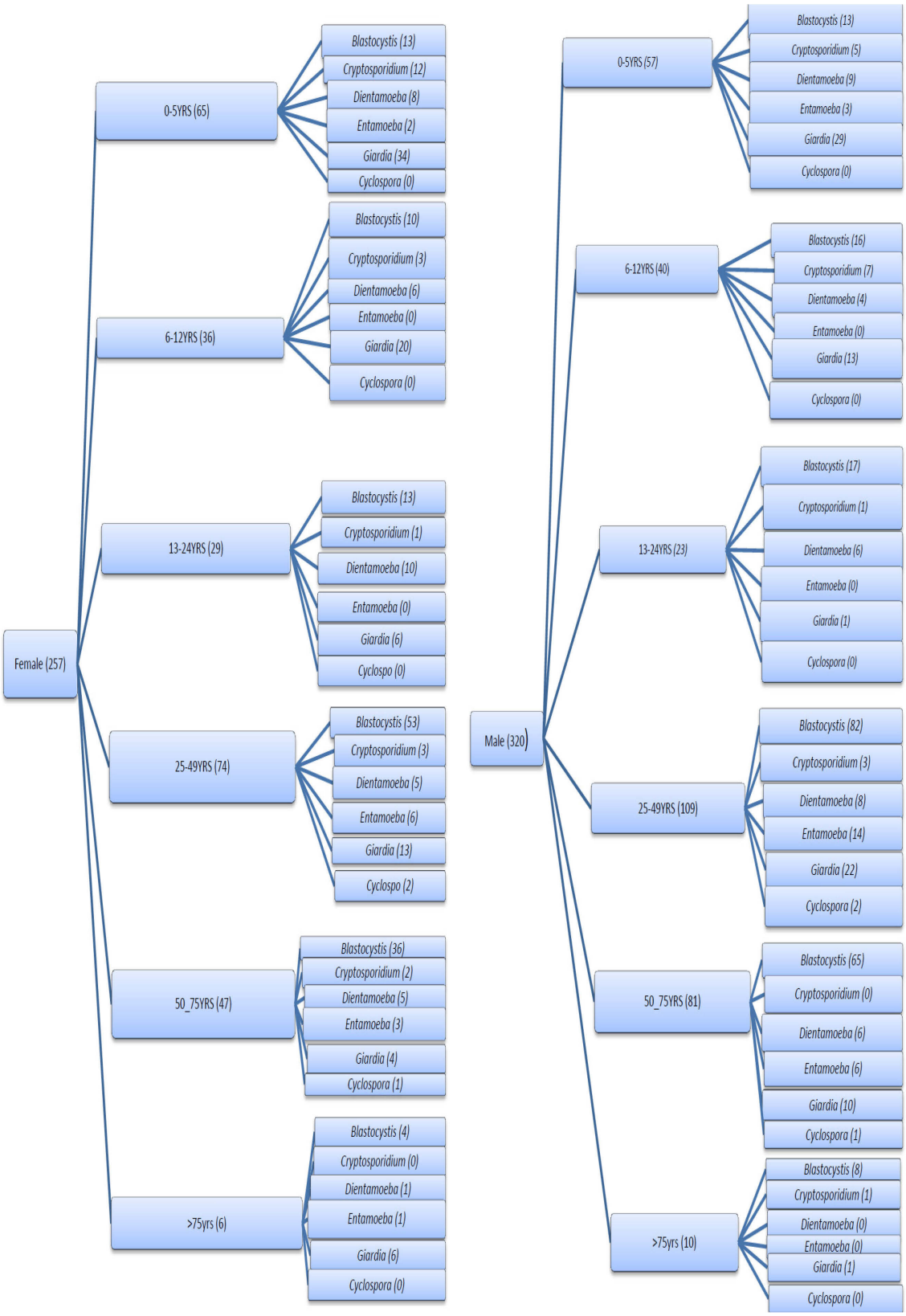
Giardiasis, caused by the protozoon *Giardia intestinalis (aka G. lamblia),* has been reported in both humans and animals, but it is particularly common in infants, young children and young older children/adults. Symptoms include diarrhoea, stomach cramps, bloating, nausea, fatigue, and if chronic, weight loss (Busatti, Santos & Gomes 2009; Cacciò & Ryan 2008). The faecal-oral route is the most important mode of infection (Boreham, Upcroft & Upcroft 1990), and various studies have found evidence of zoonotic transmission (Lebbad et al. 2011). In Australia giardiasis is frequently associated with waterborne infections, day care centre disease outbreaks, and travel-associated diarrhoea (Wilson et al. 2008).

The mapping of disease has emerged as an important epidemiological tool, and geographical information systems (GIS) have been incorporated into the analysis of health data in some countries (spatial epidemiology). Disease maps are useful tools for understanding the distribution of the disease incidence, identification of underlying geographical risk factors, and assessing potential needs for geographical variation in intervention programs (Bailey 2001; Toprak & Erdoğan 2008). However, very few studies have incorporated spatial epidemiology for enteric protozoa, and this is a first for this region. The availability of health information enables the incorporation of geographical information to describe the epidemiology of infectious diseases. Because of the geographical, economic, environmental and cultural differences among the populations across Sydney, geographical variability may exist in the distribution of cases of enteric protozoan infections.

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 was 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 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 case 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. The 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, B and C capture adult cases state-wide, while Hospital D (and to a lesser extent Hospital D) capture paediatric cases state-wide. 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 patents' illness histories should reflect an unbiased picture of the distribution of cases in whole of NSW. 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 was difficult in this setting, and hence only symptomatic cases were analysed and discussed in this study. We report on the epidemiology and geographical distribution of enteric protozoa infections in cases seen in hospital, over the period January 2007 to December 2010, from the Greater Sydney Metropolitan Area.

Figure 6-1: Flow diagram showing the distribution of cases based on gender, age and protozoa detected



## *6.3 Methods*

Ethical approval for this study was received from the Human Research Ethics Committees (HREC) at Sydney South West Area Health Service, Western Zone, Children Hospital at Westmead and St. Vincent's Hospital, Sydney, and the University of Technology, Sydney (UTS) and was guided by the Australian National Statement on Ethical Conduct in Research involving Humans. The data used in this paper represent a subset of a larger study (Fletcher et al., *in preparation*) investigating the prevalence of gastrointestinal pathogens 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. There were a total of 910 cases with a stool specimen positive for enteric protozoa from the four centres. Hospital ethical guidelines prohibited the collection of personal identifiers of the patients, but the postal code of residence for each patient was available for 580 cases, and we used it as the spatial location identifier for all these.

The patients were classified based on gender, age group and species of protozoa (Figure 6.1). We reviewed the clinical data for a subset of 252 cases (28% of 910 positive cases) having a complete clinical history, in addition to presenting with watery, liquid or loose stool specimens: 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 6.1.

**Table 6-1: Distribution of cases and specimen tested in the four study sites.**

| Hospital laboratory | Stool specimen tested | Positive for protozoa N (%) | Medical records reviewed N (%) | % reviewed |
|---|---|---|---|---|
| Hospital A | 2138 | 187 (8.8) | 85 (46) | 34% |
| Hospital B | 10123 | 118 (1.2) | 81 (67) | 32% |
| Hospital C | 7575 | 525 (6.9) | 70 (13) | 28% |
| Hospital D | 6078 | 80 (1.32) | 16 (20) | 16% |
| **Totals** | | **910** | **252** | |
| **Organisms detected** | | **Post code analysis** | **Cases reviewed** | |
| *Blastocystis* spp. | | 56.9 (330) | 61.0 (153) | |
| *Giardia intestinalis* | | 27.4 (159) | 21.9 (55) | |
| *Dientamoeba fragilis* | | 11.7 (68) | 12.7 (32) | |
| *Cryptosporidium* spp. | | 6.6 (55) | 7.2 (18) | |
| *Entamoeba* spp. | | 6.0 (35) | 3.6 (9) | |
| **Total** | | **580** | **252** | |

### 6.3.1 Microbiological methods

Laboratory diagnosis was performed using standard quality controlled procedures in the National Association of Testing Authorities, Australia (NATA) accredited laboratories of the four Sydney Hospitals. The laboratories tested on average one stool sample per patient using microbiological procedures (Fletcher et al. 2013; Stark et al. 2010a). 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.

#### 6.3.1.1 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) (McIver et al. 2001; Roberts et al. 2011). 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) (Stark et al. 2010a) and formal ethyl acetate concentration (for the identification of helminths and ova) (Garcia 2001).

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) which uses centrifugation at 500 g X10 mins 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 (10x) and then scanned under the high dry (40x) objective. All stool specimens were emulsified in SAF fixative (Oxoid Australia) using a 1:3 ratio, then was centrifuged at 500g 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*) (Stark et al. 2010a). Stool samples also 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 (Banik et al. 2011; Roberts et al. 2011; Stark et al. 2005b; Stark et al. 2007b; Stark et al. 2010c).

### 6.3.2 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 ($\chi^2$) 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. *Blastocysti*s 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. 2011) 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 the hospital data to the postcode fields 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 6.3-6.4.

**Figure 6-2: Distribution of protozoa cases based on age and gender (A), age groups (B), and gender and protozoa detected (C)**

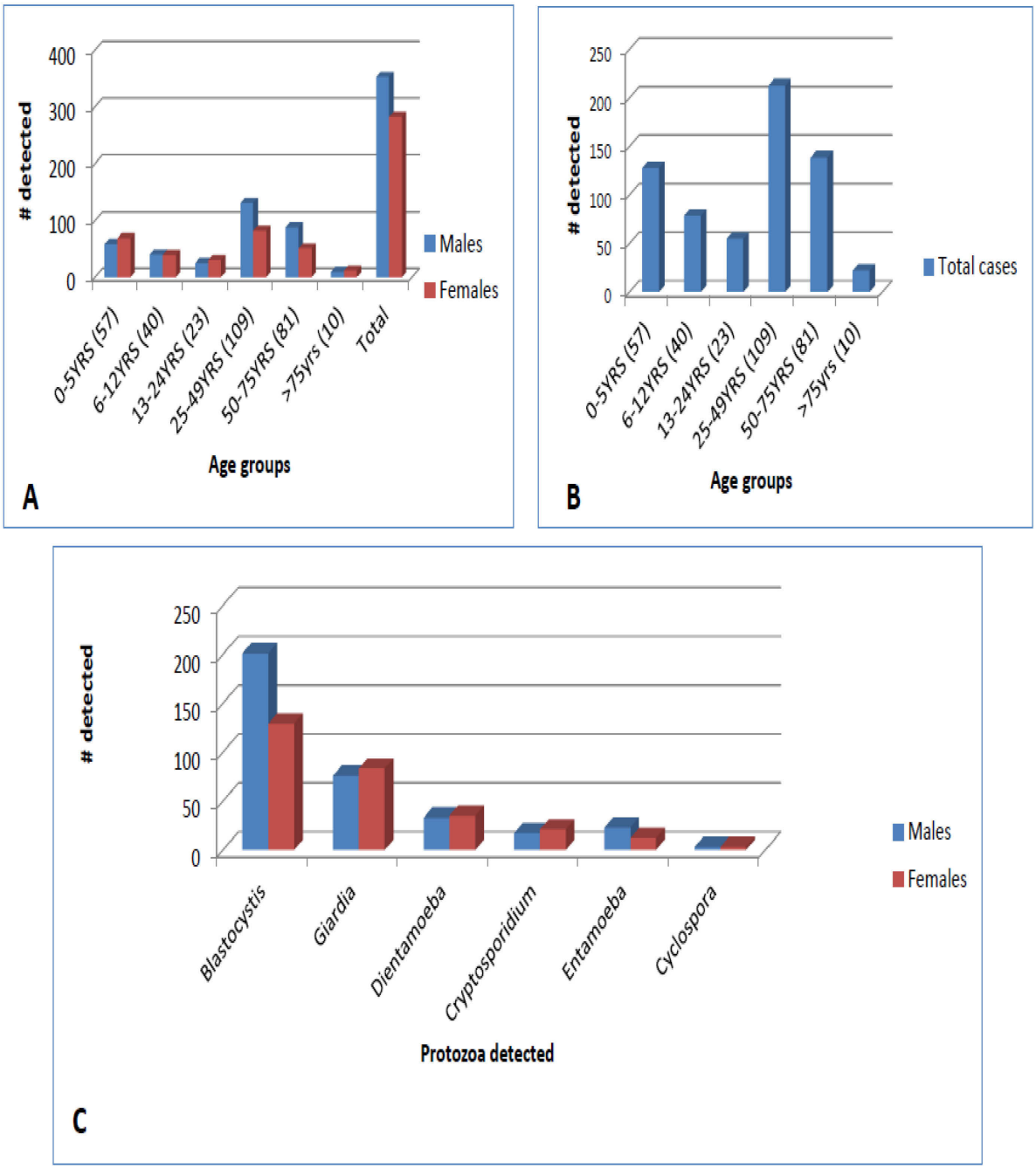


**Table 6-2: Demographic and clinical characteristics of diarrhoeal cases by Hospitals**

| Characteristics | | Hospital seen | | | | |
|---|---|---|---|---|---|---|
| | | Hospital A % (n) | Hospital B % (n) | Hospital C % (n) | Hospital D % (n) | Total % (n) |
| Age Group | 0-5 yrs. | 3.5 (3) | 43.2 (35) | 0 (0) | 56.3 (9) | 18.7 (47) |
| | 6-12 yrs. | 5.9 (5) | 38.3 (31) | 0 (0) | 12.5 (2) | 15.1 (38) |
| | 13-24yrs | 3.5 (3) | 18.5 (15) | 8.6 (6) | 0 (0) | 9.5 (24) |
| | 25-49yrs | 34.1 (29) | 0 (0) | 48.6 (34) | 25.0 (4) | 26.6 (67) |
| | 50-75yrs | 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) |

| Characteristics | | Hospital seen | | | | |
|---|---|---|---|---|---|---|
| | | **Hospital A % (n)** | **Hospital B % (n)** | **Hospital C % (n)** | **Hospital D % (n)** | **Total % (n)** |
| | Respiratory symptoms | 3.6 (3) | 17.3 (14) | 1.4 (1) | 12.5 (2) | 8.0 (20) |
| | **Total per Hospital** | **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;

## 6.4 Results

### 6.4.1 Clinical and epidemiological profile

The demographic and clinical features of cases are presented in Table 6.2. There appears to be no identifiable distinction between genders in the incidence of pathogens (Figure 6.2). However in the 25-75 years age groups there were more males than females ($\chi^2$(df) 11.5(4); P=0.04). 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. The majority or 82% (206/252) of patients in each Hospital presented with diarrhoea, abdominal pain (48%, 120/252) and vomiting (45%, 115/252), and the distribution of symptoms between the genders were quite similar (P>0.05).

The most frequently detected protozoa were *Blastocystis* spp. (57%), Giardia *intestinalis* (27%), *Dientamoeba fragilis,* (12%), *Cryptosporidium* spp, (7%), and *Entamoeba* spp (6%) (Table 6.1). The age specific distribution of the cases based on clinical symptoms and protozoa detected is presented in Table 6.3. Younger patients reported more vomiting ($\chi^2$= 15.6(4); P= 0.004), and fever ($\chi^2$= 14.2(4); P= 0.007), compared with more abdominal pain reported by the 6-49 age groups ($\chi^2$= 41.3(4); P= 0.001); and more nausea in older persons ($\chi^2$= 32.9(4); P= 0.001). Of the 580 cases included in the review, a co-infecting organism was reported in 22.8% [132]; including 29.5% (39) infectious bacteria; 39.4% (52) pathogenic protozoa (including Blastocystis spp), 23.5% (31) non-pathogenic protozoa and 6.8% (9) enteric viruses (Table 6.4). The incidence of *Blastocystis* spp, increased with age, with person over 50 years of age having the highest incidence, compared with higher incidence of *Cryptosporidium* spp ($\chi^2$= 11.2(4); P= 0.025), and *Giardia* in the under 13 years age groups ($\chi^2$=45.1(4); P= 0.001). The incidence of *Dientamoeba fragilis* also increased with age mainly evident in the under-25 age groups ($\chi^2$= 19.5(4); P= 0.001).

**Table 6-3: Age specific distribution of cases based on demographic, clinical signs and protozoa detected**

| Responses | 0-5 yrs. | 6-12 yrs. | 13-24yrs | 25-49yrs | 50-75yrs | % (n) of Total Cases | $\chi^2$ (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;

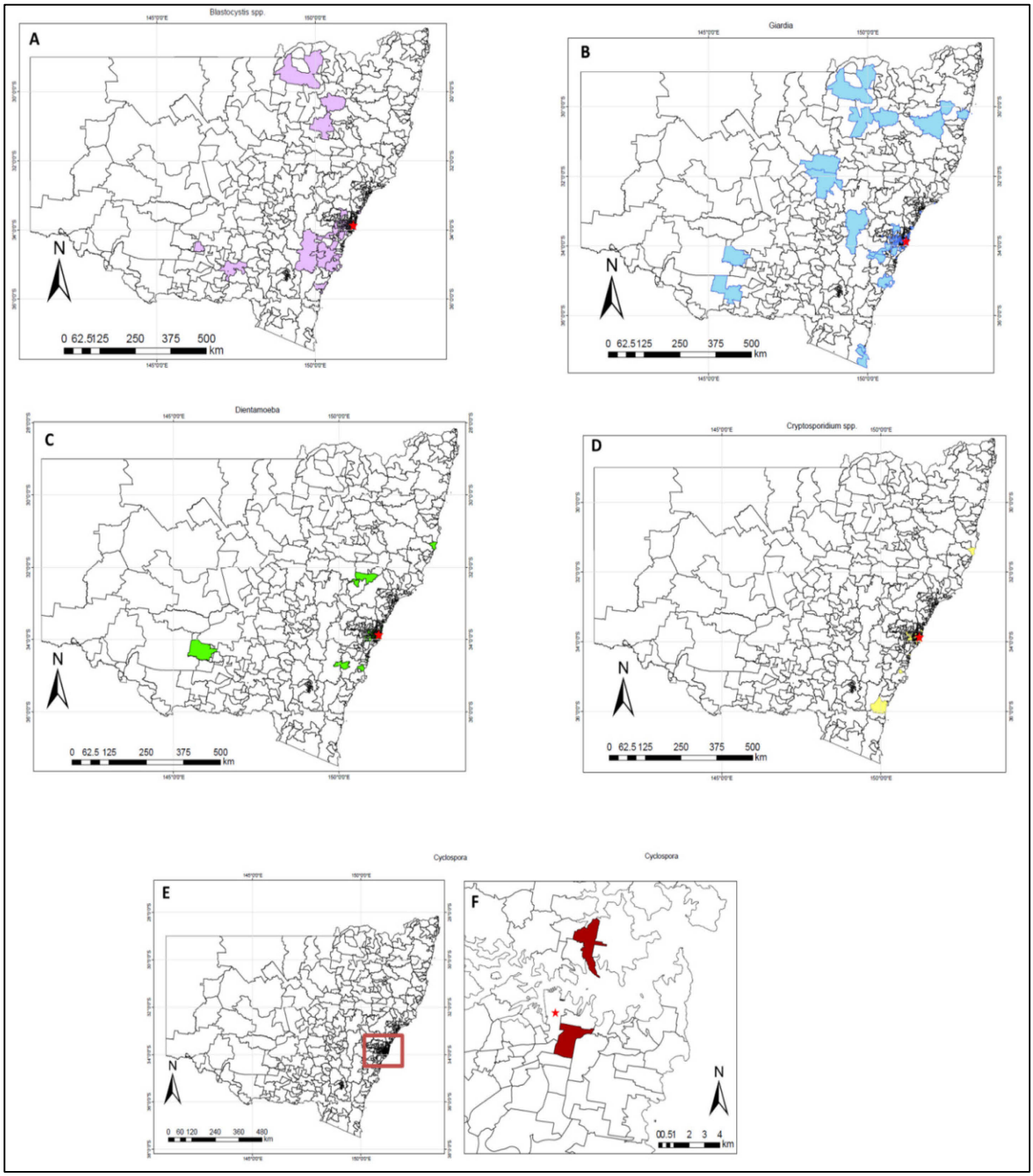
More than 80% of persons infected with *D. fragilis* (90.6% or 29/32), *Cryptosporidium* spp. (88.2% or 15/17), *Giardia* (81.8% or 45/55) and *Blastocystis* spp. (81.0% or 124/153) reported diarrhoea. However, none of these associations were significant (P>0.05). Vomiting was significantly associated with *Giardia intestinalis* infection (58.2% or 32/55; $\chi^2(1) = 4.50$; P= 0.046). Several of the cases (30/250) reported overseas travel in the past 6 weeks, with 50% (15/30) being to Asia (mainly the Indian sub-continent), 23% (7/30) to South Pacific Islands, 13% (4/30) to Africa and two each to Europe and North America.

**Table 6-4: Enteric protozoa infection and co-infecting organisms detected**

| Protozoa detected | Co-infecting parasites | Co-infecting bacteria | Co-infecting virus |
|---|---|---|---|
| ***Blastocystis* spp** | *Endolimax nana* x11; *Dientamoeba fragilis* x 9; *Entamoeba coli* x 7; *Giardia intestinalis* x 4; *Iodamoeba butschlli* x 4; *Entamoeba histolytica* x 3; *E. hartmanni* x 2; *Strongyloides stercoralis* x 1 | *Campylobacter spp x 7; Salmonella typhimurium x 2; S. typhi x 1; Shigella spp x 6; Escherichia coli x 1; C. difficile x 6; Enterobacter x 1* | Norovirus 1; Rotavirus 1 |
| ***Giardia intestinalis*** | | *C. difficile x 1* | Norovirus x 1; Adenovirus x 1 |
| ***Cryptosporidium*** | *Blastocystis* x 1 *Giardia* x 1 | *Shigella flexineri x 1* | Norovirus x 1 |
| ***Cyclospora*** | *D. fragilis* x 1 | | |
| ***D. fragilis*** | *Blastocystis* x 12; *E. histolytica* x 1; *E. nana* x 4; *Entamoeba coli* x 1; *Cryptosporidium* x 1; *Giardia* x 1 | *Campylobacter x 2;*<br>*Shigella soneii x 1;*<br>*Vibrio cholera x 1;*<br>*C. difficile x 1* | Norovirus x 1; Rotavirus x 1; Adenovirus x 1 |
| ***E. histolytica/dispar*** | *Blastocystis* x 2; *Entamoeba coli* x 1; *E. nana* x 1 | | |

| Protozoa detected | Co-infecting parasites | Co-infecting bacteria | Co-infecting virus |
|---|---|---|---|
| ***E. hartmanni*** | | *C. difficile* x 1 | |
| ***Giardia*** | *Blastocystis* x 14; *Cryptosporidium* x 1 | *Campylobacter* x 2; *Salmonella enteric* x 3; *C. difficile* x 1 | Adenovirus 1 |
| ***Entamoeba coli*** | | *Shigella* x 1 | |
| ***Total Co-infections*** | | *Campylobacter =11;*<br>*Salmonella enterica spp =5;*<br>*S. typhi = 1*<br>*C. difficile = 10*<br>*Shigella spp = 9*<br>*Vibrio cholera = 1;*<br>*Escherichia coli = 1;*<br>*Enterobacter = 1* | Norovirus = 4<br>Rotavirus = 2<br>Adenovirus = 2 |

**Figure 6-3: Map of NSW showing distribution of number 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 CBD. Colours represent number of cases*




Colours on maps represent number of cases detected from that Post Code Area

### 6.4.2 Spatial distribution

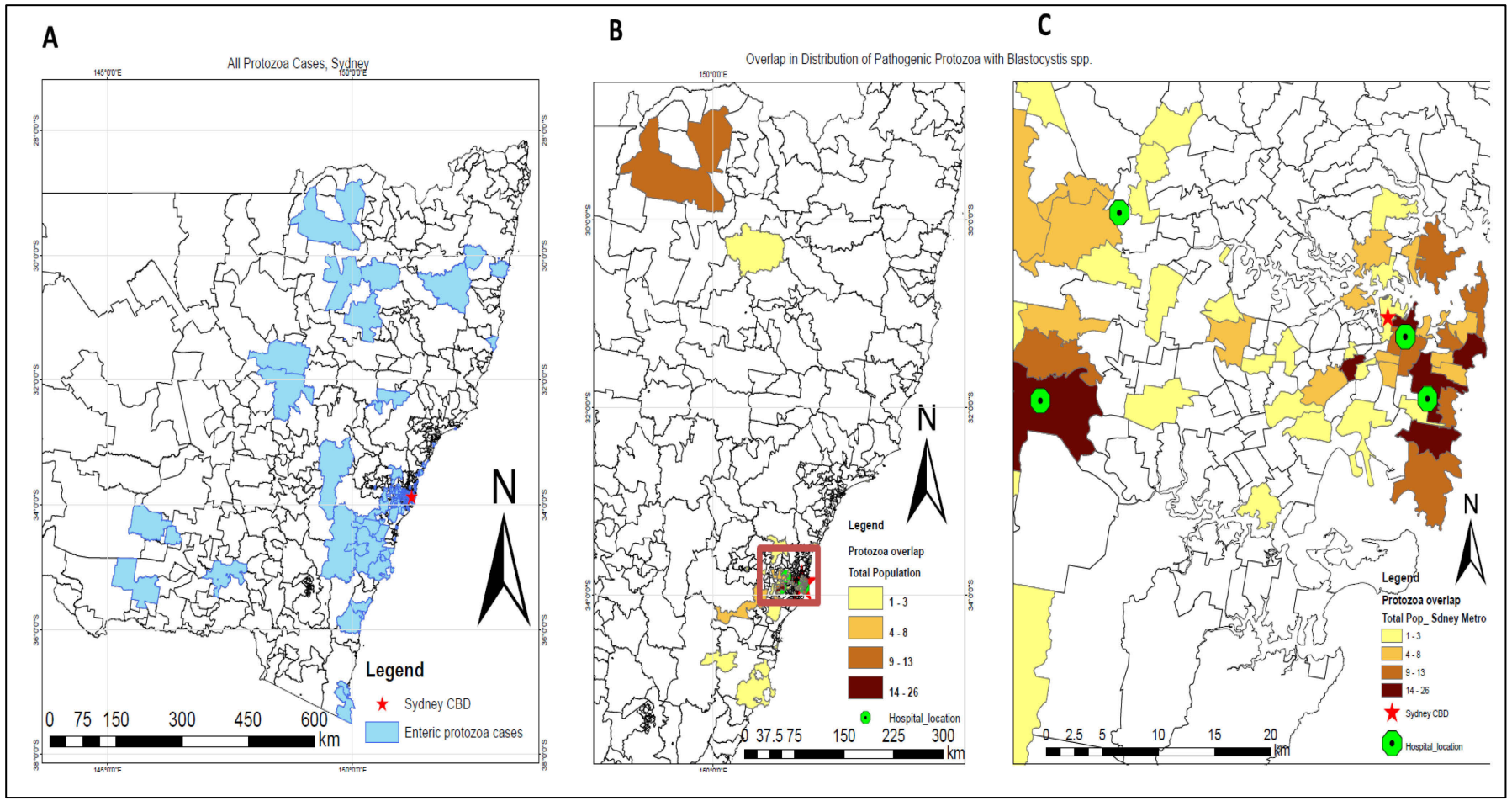
The post-code distribution of patients is shown in Figure 6.3. Some interesting patterns emerge. Infection with *Blastocystis* spp was focused in the Metropolitan Area, with additional clusters in the Southern Coast and Hunter New England Area. The distribution of *G. intestinalis* had some overlaps 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 and controversy surrounding the pathogenicity of *Blastocystis* spp., the distribution of the other pathogenic protozoa in relation to the distribution of *Blastocystis* spp was further examined. Figure 6.4 illustrates the distribution of four known pathogenic protozoa: *G. intestinalis, D. fragilis, Cryptosporidium* spp., and *Cyclospora,* in areas where infections coincided with infections by *Blastocystis* spp. The figure shows that overlaps in 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 Sydney, 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 *Giardia intestinalis* may be associated with living further away from the CBD, while *Blastocystis* infections have a stronger associated with

living close or in the CBD. A closer look at the relationship between these two protozoa reinforce this interpretation, because there is a strong negative relationship between the distribution of *G. intestinalis* and *Blastocystis (-0.545; p= 0.0001).* This further indicated that there were significantly more *Blastocystis* infections in areas where *Giardia intestinalis* infections were absent. No other significant relationships were observed in the distribution of the other enteric protozoa.

**Figure 6-4: Distribution of number of cases of protozoa, and location of co-infections with *Blastocystis* spp and infectious protozoa, based on NSW Post Codes.**




The map illustrates (A) the total numbers of protozoa detected across the State from four Hospitals; (B) where infection with pathogenic protozoa cases coincide with cases of *Blastocystis* infection. Note change of scale in (C) showing a zooming in on dense cases in the Sydney CBD.

## *6.5 Discussion.*

This multi-centre study presents the first clinical profile and geographical distribution analysis of patients with gastrointestinal symptoms and infected with enteric protozoa in NSW. We showed that enteric protozoa prevalence is age related and there is a possible association between the prevalence of individual protozoa and geographic distribution of cases.

The bimodal age distribution of enteric protozoa reveals that the epidemiology of infections is different for the younger persons compared with older persons in the population. 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 (Thompson 2000). Other studies have found similar trends (Friesema et al. 2012; Vernacchio et al. 2006).

Both *Cryptosporidium* spp and *Giardia* are considered to be neglected tropical diseases and efforts need to be made to protect the population against infection, even in this developed setting (Escobedo et al. 2010; Savioli, Smith & Thompson 2006). The neglected tropical diseases are 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 (Hotez 2011). 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 (Hotez 2011; Lobo et al. 2011). Infections with NTDs in Australia can disproportionately affect Aboriginal populations (Kline et al. 2013), however infections such as giardiasis and cryptosporidiosis have been reported amongst non-Aboriginal urban dwellers in outbreak and non-outbreak settings

(Kline et al. 2013; Sinclair et al. 2005; Stark et al. 2007b). Prevalence of D. fragilis infections was observed to be higher in older children and youth, mainly in the under 25 age groups. A similar observation was made with higher prevalence in this age group in a large study in Canada (Lagacé-Wiens, VanCaeseele & Koschik 2006 ) and is likely due to various lifestyle and poor hygienic practices resulting in faecal-oral transmission amongst this age-group.. The incidence of D. fragilis in children (Banik et al. 2011), and the higher incidence of Blastocystis in older persons have also been described (Jimenez-Gonzalez et al. 2011; Özyurt et al. 2008).

A specific trend is observed in the CBD, for 25-49 years age group (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 high incidence of MSM among the population residing in and seeking care in the CBD, who may be at higher risk of infection with enteric protozoa infections. Data on MSM status was unavailable for some Hospitals, however previous studies showed 52% of stool specimens from MSM were positive for protozoa compared with 13% from non-MSM (Stark et al. 2007b). The experience of abdominal pain was more common in older children and older children/adults.

The relatively high prevalence of *Blastocystis* spp is not surprising considering its ubiquitous nature and usual high prevalence (Özyurt et al. 2008; Stark et al. 2007b). 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 associated with enteric protozoan infections in developed countries. (Swaminathan et al. 2009; Verweij et al. 2003)

It is difficult to explain the incidence of infections with *Giardia* in the Southern Coast and Hunter New England areas: it is possible that these indicate an environmental point source of infection (water, sewage), but further data collection addressing these areas

specifically will be needed in order to establish conclusively the causes of infection and to suggest possible control strategies. In corroboration of this interpretation though, it is important to note that an increase in the cases of *Giardia* was observed over the March to May period (Southern hemisphere autumn) for the four year period of 2008-2012, and was consistent with broad NSW surveillance data for this period (NSW Health Notifiable Conditions Information Management System 2012). In addition, the NSW branch of the Bureau of Meteorology reported that in 2010, the NSW State 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 (NSW Climate Services Centre 2010). These phenomena could have resulted in an increased contamination of 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 (Newman et al. 2001). Regardless of the reason for the increased detection of *Giardia* cases, from these areas, there is a need for further studies too 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 (Nean & Pearce 2004 ). 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 (Commonwealth of Australia 2000; Currie & Brewster 2001). However, the Aboriginal status of these cases could not be ascertained. 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.

Higher rates of hospitalisation are common for some preventable illnesses (such as GIT illnesses) due to a multiplicity of factors including: poor access to primary care, improved detection of illnesses by primary care services, socioeconomic disadvantage, rurality, comorbidities and demographics factors (Katterl et al. 2012; Weinberger, Oddone & Henderson 1996). The evidence reveals that persons from lower socioeconomic backgrounds and some Indigenous Australians have more problems to access and utilise primary health services and hence higher rates of hospitalisation for illnesses that could be easily managed with primary care (Katterl 2011; Katterl et al. 2012).

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* species appearing on their own were the actual cause of gastrointestinal symptoms or that patients were exposed to other enteric pathogens that were not detected by microbiological tests. Wet preparation and concentrates are routinely used in a majority of hospitals but are not as sensitive as microscopy of fixed smears and permanent staining (modified iron-haematoxylin, trichrome staining) for the detection of some protozoa (Sawangjaroen, Luke & Prociv 1993; Stark et al. 2010a; Stark et al. 2006a). However, antigen detection by immunoassays (Garcia & Shimizu 1997; Garcia et al. 2003; Quílez et al. 1996) and polymerase chain reactions (PCR)- despite being more costly, have demonstrated higher sensitivities when compared with more commonly used methods like concentration and permanent staining (Jimenez-Gonzalez et al. 2011; Roberts et al. 2011; Sawangjaroen, Luke & Prociv 1993; Stark et al. 2010a), but are not widely used in this setting. Molecular based technologies based on PCR that can simultaneously detect several protozoa in stool are desirable and becoming more common in industrialized settings (Stark et al. 2011; ten Hove 2009). However, these are still not widely available commercially for routine application even in developed countries (Fletcher et al. 2012). The submission of repeat (up to three) stool specimen at daily intervals has been widely considered standard for improved detection of enteric protozoa, due to intermittent shedding of trophozoites in stool (Stark et al. 2010a; van Gool et al. 2003) , but compliance with the submission of repeats specimen is not consistently done in Sydney and is not

realistic owing to the distances people need to travel to health facilities with the capabilities to conduct these 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.

### 6.5.1 Conclusion

This is the first study to incorporate geographic analysis to define the epidemiology of enteric protozoa in the Australasia Region. The analysis of the geographical distribution of cases with enteric protozoan disease seeking care in Hospitals is beneficial for early detection of outbreaks, identifying areas with unusually high rates, and can inform 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 as drinking water supply (Fletcher et al. 2012; Helmreich & Horn 2009). . Several species of enteric protozoa are present with substantial prevalence in Sydney. The very young and aged persons are more susceptible to infection. Spatial distribution suggests that a majority of cases are focused around the Sydney CBD and underlying geographical risk factors may be driving the prevalence in some areas. 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* species appearing on their own were the actual cause of gastrointestinal symptoms or that patients were exposed to other enteric pathogens that were not detected by microbiological tests. Additionally, clusters of infection, especially with *G. intestinalis* have been identified in regional/rural areas that require further investigation into the underlying 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. The prevalence of enteric protozoa infection has highlighted the need for public health interventions such as hand and personal hygiene messages to be reiterated especially amongst school age children and older children/adults.

# 7 Summary and Recommendations

## Chapter Overview

This thesis set out to provide updated insight into gastrointestinal illnesses amongst hospitalised patients in Sydney, Australia. Clinical and laboratory databases have been used to provide an epidemiological study of the common enteric pathogens associated with GIT illness and to discuss implications of testing, pathogen distribution amongst other variables. The aim of this chapter is to provide a summary of the preceding chapters and to provide recommendations based on the findings. A summary of key recommendations arising from the various chapters is presented in Box 2.

Gastrointestinal illness is an important public health issue in Australia. Understanding the epidemiology of GIT illnesses is important for clinical management, disease control and prevention efforts and to inform public health policy. The distribution of enteric pathogens varies between older children/adults and children in Sydney. Children are mainly infected with enteric viruses, with the risk of infection greatest in the cooler months. Older children/adults are predominantly infected with bacterial agents, including increased risk of antibiotic associated *C. difficile* infections among elderly patients. These findings have implications for the planning of health care resources in terms of the clinical management, infection control and the planning of health care resources.

**Box 2: Summary of Key Recommendations**

| Summary of Key recommendations |
|---|
| **Public Health Interventions and Policy** |
| ❖ Increased and widened surveillance for rotavirus infections and rotavirus genotypes is warranted. |
| ❖ NSW Health should consider the feasibility of including *Campylobacter* spp and *C. difficile* intoxication in routine disease surveillance. |
| ❖ Efforts should be made to ensure that all eligible children are vaccinated against rotavirus infection. |
| ❖ Existing protocols and practices for the control of hospital acquired diarrhoea illness should be carefully reviewed and modified in hospitals. |
| ❖ There is need for good antibiotic stewardship in hospital settings. |
| ❖ Public health interventions such as health education and promotion encouraging safe hygiene practices should be developed to target high risk groups for foodborne infections. Public health messages targeting adults and school age children should emphasises the need for increased attention to food hygiene and safety practices especially in summer. |
| ❖ In addition, policies should be put in place to support the systematic collection of clinical data and risk factor information across NSW Hospitals. |
| ❖ Public health policy makers should design and tailor interventions to target communities and individuals at high risk for gastrointestinal illnesses, including hand hygiene and personal hygiene messages should be reiterated especially amongst school age children and older children/adults. |
| ❖ A user friendly well organised electronic medical records system is needed to effectively link clinical and microbiological data. |
| **Laboratory Diagnostic Capacity** |
| ❖ There is need for the development of cost effective molecular diagnostic methods that are commercially available for the sensitive and specific detection of enteric protozoa. |
| ❖ Priority should be given to the development of a gold standard approach for diagnosis of enteric protozoa, incorporating molecular diagnostic methods to provide consistency and reliability across the State. |
| **Further Research** |
| ❖ Further research is needed to determine the underlying risk factors for enteric protozoan infection in regional/rural areas. |
| ❖ Further research needed to calculate current population based estimates of gastrointestinal illnesses due to specific pathogens. |
| ❖ Further research using case controlled design is needed to ascertain the carriage of enteric pathogens in asymptomatic populations in Australia. This is useful to estimates the 'Attributable Fraction' of illness related to specific pathogens. |

## *7.1 Children*

The majority of childhood diarrhoeal illnesses leading to hospital presentation in Sydney are caused by rotavirus, norovirus and adenovirus, with most infections following clear seasonal patterns. Our systematic review found that the detection of rotavirus from diarrhoeal cases was up to five (5) times more in developed countries than in developing regions. Adenovirus and norovirus were also most frequently detected in developed countries when compared with developing regions. Viral infections in children were also more frequent in cooler months, especially rotavirus which presented a distinct seasonal pattern with the lowest rates occurring in the warm months and peaking in the cooler months. Other reports have found strong seasonal relationship with viral infections, which is particularly evident in temperate climates; a phenomena associated with many OECD countries (Fletcher et al. 2013; Glass R. 2009). This relationship is particularly evident with rotavirus and norovirus infections, with less obvious seasonal distribution in tropical countries (Cook et al. 1990).

In Chapter 3, a sustained decrease was described in the incidence of rotavirus infection observed over the study period (Fletcher et al. 2013). The likely explanation is that the introduction of the rotavirus vaccine for infants has resulted in an upward shift in the age of children being infected with rotavirus. Modelling and analysis of post-vaccination rotavirus rates predicts an increase in the age of first infection, which results in later onset, in fewer cases and less severe symptoms and subsequently less hospitalisations (Pitzer et al. 2009). Additionally, some models have predicted that the incidence of severe rotavirus infections could increase in older individuals following vaccine introduction (Pitzer et al. 2012). Serotyping for enteric viruses is not routinely done in clinical laboratories in Australia. Surveillance data for the study period 2007-2010 indicate that rotavirus serotype G1 and G1P[8] remained the dominant serotypes in the study area, but a shift to G2P[4] has been observed at the state and national levels since 2010 (Kirkwood

et al. 2010; Kirkwood et al. 2009; Kirkwood et al. 2007, 2008; Kirkwood et al. 2011). Prior to 1995, rotavirus genotypes G1P[8], G2P[4], G3P[8] and G4P[8] were the most common serotype in circulation worldwide (Santos & Hoshino 2005; Zeller et al. 2010). Since then, genotype G9 has increased dramatically and is now considered the fifth globally important rotavirus genotype (Matthijnssens et al. 2008; Zeller et al. 2010). Understanding the serotypes and genotypes in circulation is important especially for the evaluation of the effectiveness of current vaccines and to determine whether replacement with other rotavirus genotypes not covered by vaccines have taken place since the introduction of vaccines (Matthijnssens et al. 2009). The study highlights that continued surveillance for rotavirus infections and genotypes is warranted. Attention should also be paid to ensuring that all eligible children are vaccinated.

## *7.2 Older children/adults*

Diarrhoeal illness in older children/adults results in a significant burden on the health system. Other studies have established that the presence of co-morbidities in older age patients is a significant predictor of a prolonged length of stay in hospital for GIT illness (Dubberke et al. 2008; Jansen et al. 2008). Prolonged illness and extended length of stay in hospitals has cost implications. The illness is costly to both the health care system and the individual's family. These costs can be seen in terms of health care resources, the economic costs of diagnosis, treatment, cost of medication and man-hours lost due to frequency of illness (Frenzen 2005; Majowicz et al. 2005). Infectious diarrhoea in children is usually self-limiting and can be managed conservatively in developed settings like Australia, where adequate facilities are available for the rapid assessment and management of children.

This study found that *C. difficile*, *Campylobacter* and norovirus were the most common causes of infections among older patients. These findings are in keeping with a systematic review of the literature which found that older children/adults in OECD countries had

more norovirus and *Campylobacter* detected in their stools compared with their counterparts in developing regions, who were more likely to be infected with *Cryptosporidium* spp, *Salmonella* spp and pathogenic *E. coli*. The incidence of *C. difficile* in this population was associated with prolonged antibiotic therapy and nosocomial infections, which are risk factors that have been extensively discussed (Freeman et al. 2010; Pituch 2009; Svenungsson, Lagergren & Lundberg 2001; Zumbado-Salas et al. 2008). The high incidence of nosocomial diarrhoea indicate a need for good antibiotic stewardship, and that existing protocols and practices for the control of hospital acquired diarrhoea illness should be carefully reviewed and modified where necessary (Vonberg et al. 2008)

## *7.3 Pathogens and Risk factors*

This study indicated various disease risk factors were associated with diarrhoeal illness in Sydney, which is essential to inform knowledge and practice in the control of infectious diarrhoea in Australia. The findings of this study are in keeping with the estimates of the systematic review which found that *Campylobacter* spp and *Salmonell*a spp were the most common bacteria detected from diarrhoea cases in OECD countries. Evidence from the USA, New Zealand and Australia suggest that *Salmonella* infections are a major cause of hospitalisations and deaths annually, and are frequently associated with foodborne illnesses in industrialized countries (Bambrick et al. 2008; Rabsch, Tschäpe & Bäumler 2001; Voetsch et al. 2004). Infection with *Campylobacter* and N-T *Salmonella* spp were associated with eating a suspicious food item. Both *Campylobacter* and N-T *Salmonella* are considered important causes of foodborne illnesses in Australia (Hall et al. 2005b; Kirk et al. 2008) and in other developed countries (Scallan et al. 2011). Increased incidence of infection in warmer months have been previously described for N-T *Salmonella* (Ekdahl et al. 2005; Zhang, Bi & Hiller 2008) and *Campylobacter* spp (Kovats et al. 2005). Public health messages targeting older children/adults and school age children should emphasise

the need for increased attention to food hygiene and safety practices especially in summer.

The prevalence of campylobacteriosis is particularly high in NSW, but it is not routinely captured by surveillance systems. *Campylobacte*r infections in Australia have been linked to zoonotic transmission and are mainly associated with the consumption of chicken meat (Stafford et al. 2007; Stafford et al. 2008). These results justify the need for an effective surveillance system for *Campylobacter*, and continued need for health education and promotion amongst consumers and food handlers about the risks associated with the handling and cross-contamination by raw chicken (Stafford et al. 2008). A significant association was made between infection with *Shigella* spp, HIV/AIDs patients and MSM, which warrants further investigation. *Shigella* spp are easily transmitted via faecal-oral sexual contact (Stark et al. 2006b). Public health interventions such as health education and promotion encouraging safe hygiene practices should be developed to target these groups.

Traveler's diarrhoea is an important public health issue for developed countries and developing countries alike (Fletcher, Maharaj & James 2009). The association between infection with *Shigella* spp and *Salmonella typhi* was observed in cases with recent overseas travel, mainly to Asia. Because of the increased travel to developing areas and the globalization of the food supply, clinicians in developed countries should be on the alert for sporadic cases and outbreaks of diarrhoea caused by unusual pathogens/subtypes (Fletcher et al. 2012; Wilson 2005).

An in-depth analysis of enteric protozoa was undertaken in Chapters four and five since enteric protozoa were frequently implicated in diarrhoeal illness and there is limited information about their epidemiology in Australia. Enteric protozoa contribute to the burden of infectious diarrhoea illness in Sydney. In Chapter 4, it is described that hospitals across Sydney employ different testing protocols for the detection of enteric protozoa, resulting in significant variability in the detection rates between the tests done at different hospitals. The modified IHS consistently detects more parasites than light microscopy of

wet preparation and the EIAs, however microscopy of permanent stains also have limitations. Clinicians and public health practitioners must therefore be aware that depending on the protocol used, the prevalence of enteric protozoa can differ significantly, and in some cases, may underestimate their actual prevalence in the community. There is therefore the need for the development of a gold standard approach for diagnosis of enteric protozoa. Molecular diagnostic methods such as conventional and real time polymerase chain reaction (PCR) have proven to be sensitive and specific in the detection of enteric protozoa in low incidence settings like Australia. Incorporation of PCR would provide consistency across laboratories and yield more reliable estimates of the actual prevalence of enteric protozoa. There is therefore the need for the development of cost effective molecular diagnostic methods that are commercially available to address this problem.

A closer look is taken at the epidemiology and distribution of enteric protozoa in chapter five. *Blastocystis* spp., *Giardia intestinalis* and *Dientamoeba fragilis* are the most frequently detected protozoa associated with GIT illnesses in the Sydney setting. The incidence of *Blastocystis* spp is particularly high in persons over 50 years of age, compared with higher incidence of *Cryptosporidium* spp and *Giardia* in persons under 13 years old. The age distribution of cases suggests that schools, homes for the elderly are foci of infection for enteric protozoa. 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 (CBD). A strong negative relationship between the distribution of *G. intestinalis* and *Blastocystis* indicated that there were significantly more *Blastocystis* infections in areas where *Giardia intestinalis* infections were absent. Despite much controversy about the pathogenicity of *Blastocystis* spp*,* our results like other reports have found its association with gastrointestinal symptoms, (Dogruman-Al et al. 2009; Jimenez-Gonzalez et al. 2011; Stark et al. 2007c), which may be associated with the *Blastocystis* sub-type causing the infection (Roberts et al. 2012). *Dientamoeba fragilis,* an emerging protozoan pathogen was found in 3% of cases. Advanced molecular diagnostics has led to the increased detection of *D. fragilis* in Australia, and several studies suggest it is as

common as *Giardia* in developed settings (Fletcher et al. 2012; Stark et al. 2010b; Stark et al. 2007c). Further research is also needed to determine the underlying risk factors for *G. intestinalis* infection in regional/rural areas. Public health policy makers should design and tailor interventions to target communities and individuals at high risk for protozoan infection, including hand hygiene and personal hygiene messages should be reiterated especially amongst school age children and older children/adults.

## *7.4 Limitations*

This study has several limitations that must be considered when interpretation the findings as discussed in each chapter. The combination of data from several hospitals which utilised different diagnostic approaches for some pathogens is not ideal. The major issues surround the sensitivity and specificity of diagnostic tests, where some tests will detect more or less pathogens than others, resulting in variation in the detection rates reported. However, this study intends to provide estimates using available data and hence results should be interpreted accordingly. Additionally, the vast majority of stool specimens submitted were negative for a known pathogen. This may indicate that the cause of the illness was not microbial or that a majority of pathogens are either not detected by current tests or remain unknown. This indicates a need for more sensitive diagnostic tests. Additionally, we cannot clearly say that the organisms found were the actual cause of illness and associated symptom, since some organisms, especially enteric parasites have been found to occur in both symptomatic and asymptomatic individuals. This points to the need for further research using case controlled studies to ascertain the carriage of enteric pathogens in asymptomatic populations in Australia. This can then inform further estimates about the attributable fraction of illness related to specific pathogens.

## *7.5 Conclusion*

This study has revealed that GIT illness is a major issue for healthcare in Sydney, Australia, with implications for resource management and disease surveillance and control. The extended duration of GIT in older patients, requiring longer periods of hospitalisation for symptoms, has implications for the planning of health care resources in terms of the clinical management of diarrhoeal illnesses, hospital bed capacity and infection control programs.

The study has identified various risk factors that can be addressed by public health interventions. Health promotional messages should be developed to target to foodborne infections in the warm season and viral infections, especially in children in the cool season as well as hygiene messages among MSM and other high risk groups. Existing protocols and practices to support antibiotic stewardship and Hospital infection control for *C. difficile* and other hospital acquired infections should be carefully reviewed and modified where necessary. In addition, policies should be put in place to support the systematic collection of clinical data and risk factor information across NSW hospitals. The consistent use of well organised electronic medical records is recommended as an alternative. Priority should be given to the development of a gold standard approach for diagnosis of enteric protozoa, incorporating molecular diagnostic methods to provide consistency and reliability across the State.

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# 9 Appendices and Supplementary Tables

**Supplementary Table 9-1: Summary of sixty (60) studies included in the systematic review of gastrointestinal pathogens worldwide**

| Region (# studies) | Country | Author | Study period | Study design | Source of specimen | Sampling method | age groups (yrs.) | Sample size |
|---|---|---|---|---|---|---|---|---|
| **EAP (13)** | Burma | Tin A, et al. 1989 (Tin et al. 1989) | 1982-1983 | case control | Community | consecutive | 0-5 | 501 cases, 374 controls |
| | China | Huilan Sima et al. 1991 (Huilan S et al. 1991) | 1982-1985 | case control | Hospital | consecutive | 0-3 | 594 cases, 562 controls |
| | China | Kain Kevin C. et al 1991 (Kain et al. 1991) | 1989-1989 | case control | Hospital | consecutive | 0-5 | 221 cases, 108 controls |
| | Indonesia | Soenarto Yi et al 1983 (Soenarto et al. 1983) | 1978-1979 | case control | Hospital | consecutive | 0-12 | 338 cases, 69 controls |
| | Myanmar | Huilan S et al. 1991 (Huilan S et al. 1991) | 1982-1985 | case control | Hospital | consecutive | 0-3 | 813 cases, 813 controls |
| | Vietnam | Hien BTT et al. 2007 (Hien et al. 2007) | 2002-2004 | case control | Community | consecutive | 0-6 | 111 cases, 111 controls |
| | Vietnam | Isenbarger QW et al. 2001 (Isenbarger et al. 2001) | 1998-1999 | case control | Hospital/ PHC | consecutive | 0-5 | 2160 cases, 203 controls |
| | Vietnam | Nguyen TV et al. 2006 (Nguyen et al. 2006) | 2000-2002 | case control | Hospital/ Community | consecutive | 0-5 | 587 cases, 249 controls |
| | China | Yu J-M et al. 2008 (Yu et al. 2008) | 2006-2007 | cross-sectional | Hospital | consecutive | 0-5 | 1216 cases |
| | Laos PDR | Yamashiro T et al. 1998 (Yamashiro et al. 1998 ) | 1996-1997 | cross-sectional | Hospital/ PHC | consecutive | 0-15 | 880 cases |
| | Taiwan | Liu L.J et al. 2005(Liu et al. 2005) | 2000-2001 | cross-sectional | Hospital | consecutive | 0-3 | 657 cases |
| | Taiwan | Lu C-Y et al. 2006(Lu et al. 2006) | 2001-2002 | cross-sectional | Hospital | Systematic Random | 0-5 | 768 cases |
| | Thailand | Bodhidatta L et al. 2002 (Bodhidatta et al. 2002) | 1998-2000 | cross-sectional | Hospital | consecutive | 0-12 | 623 cases |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **LAC (8)** | Brazil | Seigel RR et al. 1996 (Seigel et al. 1996) | 1994-1994 | case control | Hospital | consecutive | 0-5 | 112 cases, 106 controls |
| | Mexico | Huilan S et al. 1991 (Huilan S et al. 1991) | 1982-1985 | case control | Hospital | consecutive | 0-3 | 559 cases, 559 controls |
| | Mexico | Paniagua G et al. 2007 (Paniagua et al. 2007) | 2004-2006 | case control | Hospital | consecutive | >2 to <12 | 300 cases, 80 controls |
| | Peru | Pazzaglia G et.al. 1991 (Pazzaglia et al. 1991) | 1988-1989 | case control | Hospital | consecutive | 0-2 | 391 cases, 138 controls |
| | Peru | Ochoa TJ et al 2009 (Ochoa et al. 2009) | 2006-2007 | case control | Community | consecutive | 0-1 | 936 cases, 424 controls |
| | Brazil | Barreto, Mauricio et al, 2006.(Barreto et al. 2006) | 2000-2002 | cross-sectional | Community | Random | 0-3 | 1233 cases |
| | Mexico | Flores-Abuxapqui JJ et al, 1994.(Flores-Abuxapqui et al. 1994) | 1991-1991 | cross-sectional | Community | consecutive | 0-2 | 148 cases |
| | Uruguay | Torres ME, et al, 2001.(Torres et al. 2001 ) | 1990-1994 | cross-sectional | Hospital | consecutive | 0-4 | 224 cases |
| **MENA (8)** | Tunisia | Al-Gallas N, 2007(Al-Gallas et al. 2007) | 2001-2004 | case control *(divided into 2 age gps)* | Hospital/ PHC | consecutive | (a) 0-15<br>(b) 18-80 | 115 cases, 73 controls;<br>54 cases, 29 controls |
| | Egypt | El-Mohamady H et al. 2006 (El-Mohamady et al. 2006) | 2003-2003 | cross-sectional | Hospital | consecutive | 0-5 | 356 cases |
| | Gaza, Palestine | Elamreen FHA., 2007.(Elamreen, Abed & Sharif 2007) | 2005-2005 | cross-sectional | Hospital | consecutive | 0-5 | 150 cases |
| | Jordan | Youssef M et al. 2000.(Youssef et al. 2000) | 1993-1994 | cross-sectional | Hospital | Consecutive | 0-5 | 265 cases |
| | Saudi Arabia | El-Sheikh SM et al 2001. (El-Sheikh & El-Assouli 2001) | 1995-1996 | cross-sectional | Hospital/ PHC | Consecutive | 0-5 | 576 cases |
| **nOECD (2)** | Rep. of Korea | Kyung-Hee K et al, 1989.(Kyung-Hee et | 1984-1985 | case control | Hospital | Consecutive | 0-15 | 231 cases, 104 controls |

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|---|---|---|---|---|---|---|---|---|
| | (South) | al. 1989) | | | | | | |
| | Turkey | Uysal G et al, 1997.(Uysal et al. 1997) | 1993-1994 | cross-sectional | Hospital | Consecutive | 0-14 | 400 |
| **OECD (11)** | Denmark | Olesen B et al 2005.(Olesen et al. 2005 ) | 2000-2001 | case control | Hospital | Consecutive | 0-5 | 424 cases, 866 controls |
| | Australia | Barnes GL et al,1998.(Barnes et al. 1998) | 1980-1993 | cross-sectional | Hospital | Consecutive | 0-14 | 3785 cases, |
| | Australia | McIver CJ et al 2001.(McIver et al. 2001) | 1997-1998 | cross-sectional | Hospital | Random | 0-5 | 412 cases, |
| | England | O'Neill HJ et al, 2002 **(O'Neill et al. 2002)** | 2000-2001 | cross-sectional | Hospital | Consecutive | (i) 10-90 (ii) 0-5 | 735 cases 1051 cases |
| | Russia | Podkolzin et al, 2009 (Podkolzin et al. 2009) | 2005-2007 | Prospective | Hospital | Consecutive | (i) 14-60 yrs; (ii) 0-5 | 1354 2848 |
| | Finland | Rautelin HI et al,1989.(Rautelin et al. 1989) | 1985-1986 | cross-sectional | Hospital | Consecutive | 15-60 | 253 cases, |
| | Germany | Jansen, A et al. 2008.(Jansen et al. 2008) | 2005-2007 | cross-sectional | Hospital | Consecutive | 18-60 | 132 cases, |
| | Greece | Levidiotou, S., et al., 2009.(Levidiotou et al. 2009) | 2000-2006 | cross-sectional | Hospital | Consecutive | 0-5 | 4604 cases, |
| | Italy | Colomba, C., et al., 2006.(Colomba et al. 2006) | 1999-2000 | cross-sectional | Hospital | Consecutive | 0-12 | 215 cases, |
| | New Zealand | Montgomery, David et al 2006.(Montgomery et al. 2006) | 2005-2005 | cross-sectional | Hospital | Consecutive | 0-15 | 128 |
| | USA | Klein, Eileen et al. 2006.(Klein et al. 2006) | 1998-2001 | cross-sectional | Hospital | Consecutive | 0-5 | 372 cases, |
| **SAP (7)** | Bangladesh | Albert, M John et al., 1999.(Albert et al. 1999) | 1993-1994 | case control | Hospital | Systematic Random | 0-5 | 814 cases, 814 controls |
| | India | Huilan, Sima et al., 1991.(Huilan S et al. 1991) | 1982-1985 | case control | Hospital | Consecutive | 0-3 | 916 cases, 587 controls |

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|---|---|---|---|---|---|---|---|---|
| | India | Ghosh, A.R., et al., 1991.(Ghosh et al. 1991) | 1986-1988 | case control | Hospital | Consecutive | 0-1 | 218 cases, 102 controls |
| | Nepal | Hoge, C.W., et al., 1995.(Hoge et al. 1995) | 1994-1994 | case control | PHC | Consecutive | 0-5 | 124 cases, 103 controls |
| | Pakistan | Huilan, Sima et al., 1991.(Huilan S et al. 1991) | 1982-1985 | case control | Hospital | Consecutive | 0-3 | 758 cases, 758 controls |
| | Bangladesh | Haque, Rashidul et al 2003.(Haque et al. 2003) | 1999-2002 | cross-sectional | Community | Consecutive | 0-5 | 289 cases, |
| | India | Dutta, P et al 1991.(Dutta et al. 1991) | 1990 | cross-sectional | Hospital | Consecutive | 0-5 | 383 cases, |
| **SSA (18)** | Central African Rep | Georges, MC et al 1984.(Georges et al. 1984) | 1981-1982 | case control | Hospital | Consecutive | 0-15 | 1197 cases, 748 controls |
| | Central African Rep | Germani, Y., et al. 1998.(Germani et al. 1998) | 1995-1996 | case control | Hospital | Consecutive | 18-80 | 290 cases, 140 controls |
| | Ghana | Reither, Klaus, et al. 2007.(Reither et al. 2007) | 2005-2006 | case control | PHC | Consecutive | 0-12 | 243 cases, 124 controls |
| | Nigeria | Ogunsanya, T.I. et al 1994.(Ogunsanya, Rotimi & Adenuga 1994) | 1989-1990 | case control | Hospital | Consecutive | 0-5 | 215 cases, 100 controls |
| | Zaire | De Mol, P., et al., 1983.(De Mol et al. 1983) | 1979-1979 | case control | Hospital | Consecutive | 0-5 | 355 cases, 320 controls |
| | Zaire | Henry, M.C. et al., 1995.(Henry et al. 1995) | 1990-1990 | case control | Hospital/ PHC | Cluster Random | 0-5 | 173 cases, 155 controls |
| | Burkina Faso | Djeneba, O., et al., 2007.(Djeneba et al. 2007) | 2006 | cross-sectional | GP | Consecutive | 0-5 | 66 cases, |
| | Cameroon | Yongsi, HBN, 2008.(Yongsi 2008) | 2000-2005 | cross-sectional | Community | Stratified Random | 0-5 | 437 cases, |
| | Nigeria | Olowe O.A. et al 2003. (Olowe et al. 2003) | 2001-2002 | cross-sectional | Hospital | Consecutive | 0-5 | 135 cases, |
| | Nigeria | Cajetan, I.C.I., et al.,2010.(Cajetan et | 2008-2008 | cross-sectional | Hospital | Random | 0-5 | 404 cases, |

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|---|---|---|---|---|---|---|---|---|
| | | al. 2010) | | | | | | |
| | Tanzania | Vargas, Martha et al 2004. (Vargas et al. 2004) | 1996-1997 | cross-sectional | Hospital | Consecutive | 0-5 | 451 cases, |
| | Uganda | Musiime, V., et al., 2009.(Musiime et al. 2009) | 2008-2008 | cross-sectional | Hospital | Consecutive | 0-5 | 190 cases, |
| | Zambia | Kelly, P., et al. 1996.(Kelly et al. 1996) | 1994 | cross-sectional | Hospital/ Community | Consecutive | 18-80 | 77 cases, |
| | Nigeria | Ogbu, O. et al., 2008.(Ogbu et al. 2008) | 2005-2006 | case control | PHC | Consecutive | 0-3 | 150 cases, 50 controls |
| | Uganda | Brink A-K., et al, 2002.(Brink et al. 2002) | 1995-1997 | case control | PHC | Consecutive | 0-15 | 357 cases, 127 controls |
| | Zambia | Nakano, P., et al. 1998.(Nakano et al. 1998) | 1992-1993 | cross-sectional | PHC | Consecutive | 0-5 | 639 cases, |
| | Nigeria | Anynwu, BN., 1997.(Anyanwu 1997) | 1997-1997 | case control | Hospital | Consecutive | 0-5 | 1015 cases, 401 controls |
| | Kenya | van Eijk, AM., et al, 2009.(van Eijk et al.) | 1997-2001 | prosp. cohort | Hospital | Consecutive | 0-2 | 630 cases, |

Specimens were obtained from persons seen at Hospitals, Primary Health Care Centre (PHC), General Practitioner (GP), or from the community. Sampling methods include taking consecutive (or convenience or census) specimen, systematic random, random, stratified random sampling and some studies collected samples from routine surveillance programmes.
Cas cont. = case control study; cross-sect. = cross sectional; Cohort = prospective follow-up studies.
MENA = Middle East and North Africa; EAP= East Asia & the Pacific; SAP= South Asia; LAC= Latin America and the Caribbean; SSA= Sub-Saharan Africa; OECD= Developed Countries including non-OECD. Source: World Bank Country Classification July 2009. Available at http://go.worldbank.org/D7SN0B8YU0. Accessed December 29, 2009

**Supplementary Table 9-2: Summary of laboratory methods in individual studies.**

| Country (Ref) | Study Years | Study design | Bacteriology | Virology | Parasitology |
|---|---|---|---|---|---|
| | | | **East Asia (EAP) Region** | | |
| **Burma (Tin et al. 1989)** | 1982-1983 | case control | Standard culture and serotyping methods | ELISA | N/A |
| **China (Huilan S et al. 1991)** | 1982-1985 | case control | Standard culture methods | Electron microscopy for rotavirus, Norwalk-agent-like particles, and adenovirus. immune electron microscopy Rotavirus antigen - ELISA | N/A |
| **China (Kain et al. 1991)** | 1989 | case control | Standard culture methods + DNA hybridization of stool blots AND a latex agglutination test for *E. coli* pathotypes. A standard agar dilution technique for antibiotic susceptibility testing | ELISA Rotavirus was identified with a monoclonal latex agglutination test ( | Direct microscopy +iron haemotoxylin AND modified acid-fast stain for *Cryptosporidium sp.* |
| **Indonesia (Soenarto et al. 1983)** | 1978-1979 | case control | Standard culture methods + ELISA for *E. coli* pathotypes | Electron microscopy for rotavirus, | Light microscopy |
| **Vietnam (Hien et al. 2007)** | 2002-2004 | case control | Standard culture methods + multiplex PCR and dot-blot hybridization; dot-blot-positive *E. coli* pathotypes - Serotyping and PFGE typing; Antibiotic susceptibility testing -MIC technique using dehydrated antimicrobials in microtitre wells. | Rotavirus antigen -(ELISA) | Direct microscopy of a wet mount. Samples suspected to be positive for Cyclospora were confirmed by fluorescent microscopy |
| **Vietnam (Isenbarger et al. 2001)** | 1998-1999 | case control | Standard culture methods.+ DNA hybridization of stool blots for *E. coli* pathotypes | N/A | N/A |

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|---|---|---|---|---|---|
| **Vietnam (Nguyen et al. 2006)** | 2000-2002 | case control | Standard culture methods.+ PCR for *E. coli* pathotypes | Rotavirus antigen -(ELISA) | N/A |
| **China (Yu et al. 2008)** | 2006-2007 | cross-sectional | N/A | ELISA, RT-PCR or PCR | N/A |
| **Laos PDR (Yamashiro et al. 1998 )** | 1996-1997 | cross-sectional | Standard culture methods, Antibiotic susceptibility testing -by the plate dilution technique | N/A | N/A |
| **Myanmar (Huilan S et al. 1991)** | 1982-1985 | case control | Standard culture methods | Electron microscopy for rotavirus, Norwalk-agent-like particles, and adenovirus. Immune electron microscopy using pooled sera from indigenous donors. Rotavirus antigen -(ELISA) | N/A |
| **Taiwan (Liu et al. 2005)** | 2000-2001 | cross-sectional | Standard culture methods. | Rotavirus, enteric adenovirus (types 40 and 41), and astrovirus -(ELISA) | N/A |
| **Taiwan (Lu et al. 2006)** | 2001-2002 | cross-sectional | Standard culture methods. | rotavirus, enteric adenovirus (types 40 and 41), and astrovirus -(ELISA) | N/A |
| **Thailand (Bodhidatta et al. 2002)** | 1998-2000 | cross-sectional | Standard culture methods.+ DNA hybridization of stool blots for *E. coli* pathotypes | N/A | N/A |
| | | | **Latin America and the Caribbean (LAC) Region** | | |
| **Brazil (Seigel et al. 1996)** | 1994 | case control | Standard culture methods +PCR for *E. coli* pathotypes | Rotavirus , -(ELISA) | N/A |
| **Mexico (Huilan S et al. 1991)** | 1982-1985 | case control | Standard culture methods | Electron microscopy for rotavirus, Norwalk-agent-like particles, and adenovirus. Immune electron microscopy using pooled sera from indigenous donors. Rotavirus | N/A |

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|---|---|---|---|---|---|
| | | | | antigen -(ELISA) | |
| **Mexico (Paniagua et al. 2007)** | 2004-2006 | case control | Standard culture methods +PCR | N/A | Centrifugal floatation (500 × g 2 min); light micrscopy at 40× |
| **Peru (Pazzaglia et al. 1991)** | 1988-1989 | case control | Standard culture methods+ ELISA for *E. coli* pathotypes | Rotavirus, -ELISA | Microscopically -after filtration, centrifugation, and ether extraction |
| **Peru (Ochoa et al. 2009)** | 2006-2007 | case control | Standard culture methods + Multiplex RT-PCR for *E. coli* pathotypes | ELISA | Direct observation. |
| **Brazil (Barreto et al. 2006)** | 2000-2002 | cross-sectional | Standard culture methods | Adenovirus and group A Rotavirus were detected by enzyme immunoassay and polyacrylamine gel electrophoresis | Kato—Katz technique and sedimentation. *Cryptosporidium* spp.,-formol-ether concentration method+ confirmed by the modified Ziehl—Neelsen technique |
| **Mexico (Flores-Abuxapqui et al. 1994)** | 1991-1991 | cross-sectional | Standard culture methods + ELISA for *E. coli* pathotypes. | Adenovirus and group A Rotavirus by ELISA; Rota + identification of vRNA using polyacrylamide gel electrophoresis (PAGE) | Microscopically for trophozoites and cysts, and by Faust zinc sulphate centrifugal flotation technique. |
| **Uruguay (Torres et al. 2001 )** | 1990-1994 | cross-sectional | Standard culture methods + ELISA for *E. coli* pathotypes. Antibiotic susceptibility testing -standard agar dilution technique. | Rotavirus by EIA; + identification of vRNA using polyacrylamide gel electrophoresis (PAGE) | Direct microscopic examination-with saline solution; Ritchie concentration method with a modified Ziehl-Neelsen (Kinyoun) |
| **Tunisia (Al-Gallas et al. 2007)** | 2001-2004a | case control | Standard culture methods + PCR for *E. coli* pathotypes, serotyping by slide agglutination method | Adenovirus and group A Rotavirus by ELISA; | Direct observation of stools in cosin solution |
| | | | **Middle East and Northern Africa** | | |
| **Egypt (El-Mohamady et al. 2006)** | 2003-2003 | cross-sectional | Standard culture methods | Rotavirus by ELISA; | ELISA for C. parvum |
| **Gaza, Palestine (Elamreen, Abed &** | 2005-2005 | cross-sectional | Standard culture methods + PCR. Antibiotic susceptibility by disk | N/A | N/A |

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|---|---|---|---|---|---|
| **Sharif 2007)** | | | diffusion test. | | |
| **Jordan (Youssef et al. 2000)** | 1993-1994 | cross-sectional | Standard culture methods +phase contrast microscopy; + PCR for *E. coli* pathotypes. | ELISA + Direct electron microscopy | OCP-formyl-ether technique , Crypto-Kinyoun stain and oil immersion microscope. microsporidial spores- by light microscopy, smears were stained by Weber's modified trichome method and fluorescence technique, |
| **Saudi Arabia (El-Sheikh & El-Assouli 2001)** | 1995-1996 | cross-sectional | Standard culture methods + latex slide agglutination test for *E. coli* pathotypes. | Rotavirus by ELISA; + identification of vRNA using polyacrylamide gel electrophoresis (PAGE) | Direct microscopic examination with saline solution; flotation technique with a modified Ziehl-Neelsen (Kinyoun) procedure for Crypto |
| **New Caledonia (Germani et al. 1994)** | 1990-1991 | cross-sectional | Standard culture methods + DNA hybridization for *E. coli* pathotypes. | Rotavirus -latex slide agglutination test | Direct microscopic-saline and iodine solution; formalin-ether concentrates, and stained with Merthiolate-iodine-formaldehyde solution |
| **Turkey (Uysal et al. 1997)** | 1993-1994 | cross-sectional | Standard culture methods + sensitivity of *C. jejuni* to antibiotics by the Kirby-Bauer disc diffusion method. | N/A | Direct microscopic examination |
| **South Korea (Kyung-Hee et al. 1989)** | 1984-1985 | case control | Standard culture methods; | Rotavirus antigen -(ELISA) | N/A |
| **Denmark (Olesen et al. 2005 )** | 2000-2001 | case control | Standard culture methods + *E. coli* pathotypes -colony dot blot hybridization | Rotavirus, adenovirus, and astrovirus antigens - IDEA. Norovirus and saparovirus - RT-PCR | Direct microscopic -saline/ formalin solution; a modified Ziehl-Neelsen (Kinyoun) procedure for Crypto and Cyclospora. |

| | | | | | |
|---|---|---|---|---|---|
| **Australia (Barnes et al. 1998)** | 1980-1993 | cross-sectional | Standard culture methods | Viruses, by methods such as enzyme immunoassay (EIA) and negative-stain electron microscopy (EM) + gel electrophoresis | Microscopy |
| **Australia (McIver et al. 2001)** | 1997-1998 | cross-sectional | Standard culture methods + EIA | Electron microscopy (EM), and RT-PCR for Norwalk-like viruses (NLVs) | Electron microscopy (EM), enzyme immunoassays (EIAs) for *Giardia lamblia*; a modified Ziehl-Neelsen (ZN) stain for *Cryptosporidia*. |
| **England (O'Neill et al. 2002)** | 2000-2001 | cross-sectional | N/A | One-step nested RT-PCR for the detection of norovirus; a new nested multiplex RT-PCR (nmRT-PCR) for rotavirus and group F adenovirus. | N/A |
| **Russia (Podkolzin et al. 2009)** | 2005-2007 | cross-sectional | N/A | Conventional and multiplex PCRs for group A rotavirus, norovirus genogroups I and II, astrovirus, and adenovirus. | N/A |
| **Finland (Rautelin et al. 1989)** | 1985-1986 | cross-sectional | Standard culture methods + EIA, slide agglutination test. | Electron microscopy (EM) for rotaviruses; complement fixation technique | Formalin ether concentration method and microscopically for oocysts of cryptosporidia using a modified Ziehl-Neelsen (ZN) stain. |
| **Germany (Jansen et al. 2008)** | 2005-2007 | cross-sectional | Standard culture methods + RT-PCR; EIA- *E. coli* pathotypes; + ELISA- *C. difficile*; | Norovirus, rotavirus, aichivirus, adenovirus, astrovirus, and enteroviruses were detected by RT-PCR | SAF fixation concentration technique- protozoa; direct immune fluorescence antibody test - *Giardia*; and Kinyoun method-coccidian parasites. Uvitex method- microsporidia |
| **Greece (Levidiotou et al. 2009)** | 2000-2006 | cross-sectional | Conventional laboratory methods. | Rotavirus (group A), adenovirus, astrovirus and norovirus antigens | Conventional laboratory methods. |

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|---|---|---|---|---|---|
| | | | | by EIA +Rotavirus by an 'in-house' RT-PCR, | |
| **Italy (Colomba et al. 2006)** | 1999-2000 | cross-sectional | Standard culture methods | Rotavirus (group A), adenovirus, astrovirus and norovirus antigens by EIA + RT-PCR, | Ritchie's formalin-ether sedimentation technique; after concentration |
| **New Zealand (Montgomery et al. 2006)** | 2005-2006 | cross-sectional | Standard culture methods +EIA | Rotavirus (group A), adenovirus, antigens by EIA | EIA+ confirm any positives by immunofluorescence |
| **USA (Klein et al. 2006)** | 1998-2001 | cross-sectional | Standard culture methods; EIA + PCR *for E. coli* pathotypes. | Rotavirus (group A), adenovirus, astrovirus antigens by EIA | Trichrome stains and formalin/ethyl acetate sedimentation |
| **Bangladesh (Albert et al. 1999)** | 1993-1994 | case control | Standard culture methods, EIA DNA hybridization for *E. coli* pathotypes; *Clostridium difficile* toxin by a standard tissue culture. | Rotavirus - ELISA | Direct microscopy *Giardia lamblia* and *Entamoeba histolytica*; *Cryptosporidium parvum and Cyclospora cayetanensis* by modified acid-fast stain. |
| **India (Huilan S et al. 1991)** | 1982-1985 | case control | Standard culture methods | Electron microscopy for rotavirus, Norwalk-agent-like particles, and adenovirus. Immune electron microscopy using pooled sera from indigenous donors. Rotavirus antigen -(ELISA) | N/A |
| **India (Ghosh et al. 1991)** | 1986-1988 | case control | Standard culture methods + slide agglutination | Rotavirus antigen -(ELISA) | Microscopy + cryptosporidia using a modified Ziehl-Neelsen (ZN) stain. |
| | | | **South Asia (SAP) Region** | | |
| **Pakistan (Huilan S et al. 1991)** | 1982-1985 | case control | Standard culture methods | Electron microscopy for rotavirus, Norwalk-agent-like particles, and adenovirus. Immune electron microscopy. Rotavirus antigen - | N/A |

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|---|---|---|---|---|---|
| | | | | (ELISA) | |
| **Nepal (Hoge et al. 1995)** | 1994-1994 | case control | Standard culture methods | rotavirus - EIA | Microscopy after formalin ether concentration and by modified acid-fast staining |
| **India (Nair et al. 2010)** | 2007-2009 | cross-sectional | Using conventional, immunological and molecular detection methods | Using conventional, immunological and molecular detection methods | Using conventional, immunological and molecular detection methods |
| **Nepal (Pokharel et al. 2009)** | 2007-2007 | cross-sectional | Standard culture methods; antibiotic susceptibility - Modifi ed Kirby-Bauer disc diffusion method | Rotavirus-EIA | Parasites by direct microscopy after saline and iodine preparation and by modified acid-fast staining |
| **Bangladesh** Haque et al. 2003a) | 1999-2002 | cross-sectional | Standard culture methods; hybridization technique with specific DNA probes *for E. coli* pathotypes; antibiotic susceptibility - disk diffusion method | Rotavirus, enteric adenovirus, and astrovirus-ELISA | Parasites by direct microscopy and antigen detection test |
| **India (Dutta et al. 1991)** | 1990 | cross-sectional | Standard culture methods | Tested for viral enteropathogens using standard techniques | Microscopy for ova, cysts, and parasites. |
| | | | **Sub-saharan Africa (SSA) Region** | | |
| **Central African Rep. (Georges et al. 1984)** | 1981-1982 | case control | Standard culture methods. | ELISA | Direct microscopy after saline and iodine preparation and by modified acid-fast staining |
| **Central African Rep. (Germani et al. 1998)** | 1995-1996 | case control | Standard culture methods and disc diffusion technique for antibiotic susceptibility; | Latex agglutination test. | Dark-field microscopy with staining. |
| **Ghana (Reither et al. 2007)** | 2005-2006 | case control | Standard culture methods. | RT-PCR | Microscopy |
| **Nigeria (Ogunsanya, Rotimi & Adenuga 1994)** | 1989-1990 | case control | Standard culture methods. | ELISA | Direct microscopy +iron haematoxylin staining for *Cryptosporidium sp.* |

| | | | | | |
|---|---|---|---|---|---|
| **Zaire (De Mol et al. 1983)** | 1979-1979 | case control | Standard culture methods. | ELISA | Direct microscopy. |
| **Zaire (Henry et al. 1995)** | 1990-1990 | case control | Standard culture & direct microscopy. | Latex agglutination test. | Direct observation/microscopy; |
| **Burkina Faso (Djeneba et al. 2007)** | 2006 | cross-sectional | N/A | Immunochromatographic tests. | Direct microscopy |
| **Cameroon (Yongsi 2008)** | 2000-2005 | cross-sectional | Standard culture methods. | ELISA | Microscopy |
| **Nigeria (Olowe et al. 2003)** | 2001-2002 | cross-sectional | Standard culture methods and plate dilution technique for antibiotic susceptibility testing. | N/A | N/A |
| **Nigeria (Cajetan et al. 2010)** | 2008-2008 | cross-sectional | Standard culture and slide agglutination technique; modified disc diffusion technique for antibiotic susceptibility testing; | EIA | Light microscopy & Ziehl-Neelsen (Kinyoun's) stain for *Cryptosporidium.* |
| **Tanzania (Vargas et al. 2004)** | 1996-1997 | cross-sectional | Standard culture methods & direct microscopy. | Agglutination test. | Direct observation + Hiedenhain staining, concentration by the merthiolate-iodine formalin technique and were stained with Kinyoun's carbolfuchsine |
| **Uganda (Musiime et al. 2009)** | 2008-2008 | cross-sectional | Standard culture methods and disc diffusion technique for antibiotic susceptibility. | N/A | N/A |
| **Zambia (Kelly et al. 1996)** | 1994 | cross-sectional | N/A | N/A | Light microscopy, electron microscopy and PCR+ Ziehl-Neelsen stain for Cryptosporidium. |
| Nigeria **(Ogbu et al. 2008)** | 2005-2006 | case control | Standard culture methods & direct microscopy-Antibiotic sensitivity testing by disk diffusion method | ELISA | Microscopy- formalin concentration method |

| | | | | | |
|---|---|---|---|---|---|
| Uganda **(Brink et al. 2002)** | 1995-1997 | case control | Standard culture methods & direct microscopy. | N/A | Microscopy+ modified Ziehl-Neelsen method for Crypto |
| Zambia, DTU **(Nakano et al. 1998)** | 1992-1993 | cross-sectional | Standard culture methods. | N/A | N/A |
| Nigeria **(Anyanwu 1997)** | 1997-1997 | case control | Standard culture methods. | N/A | N/A |
| Kenya **(van Eijk et al. 2010)** | 1997-2001 | prosp. cohort | Standard culture methods & bright-field microscopy. .Antibiotic sensitivity testing by the Kirby–Bauer disk diffusion method. | EIA | Microscopy- trichrome technique and Kinyoun's modified acid-fast technique and examined using bright-field microscopy+ *Giardia* and *Cryptosporidium* immunofluorescence assay (IFA). |

DNA- deoxyribonucleic acid; ELISA- enzyme linked immunosorbent assay; EIA- enzyme immunoassay; EM- electron microscopy; N/A- not applicable; OCP- ova, cysts and parasites; PAGE- polyacrylamide gel electrophoresis; PCR- polymerase chain reaction; RNA- ribonucleic acid; RT-PCR- *real-time PCR*; vRNA- viral RNA.

**Supplementary Table 9-3: Summary of Enteric protozoa, Laboratory Tests, and frequency with which tests are conducted in four Sydney Hospitals.**

| | Hospital A | | Hospital B | | Hospital C | | Hospital D | |
|---|---|---|---|---|---|---|---|---|
| **Organism** | **Microbiology Test done** | **Frequency** | **Microbiology Test done** | **Frequency** | **Microbiology Test done** | **Frequency** | **Microbiology Test done** | **Frequency** |
| ***Blastocystis* sp.** | Microscopy | S | Microscopy | S** | modifed IHS | R | Microscopy | S |
| ***Cryptosporidium*** | EIA | S | Microscopy | R | modifed IHS | R | EIA | R |
| ***Cyclospora*** | IHS stain | S | Microscopy | S** | modifed IHS | R | Microscopy | S |
| ***Cystoisospora*** | (on request) | S | Microscopy/ IHS | R | modifed IHS | R | N/A | N/A |
| ***Dientamoeba fragilis*** | IHS stain | S | Microscopy | S** | modifed IHS | R | reject | N |
| ***Entamoeba histolytica/ dispar* complex.** | Microscopy | S | Microscopy | S** | modifed HIS, PCR | R | EIA/Microscopy | S |
| ***Enteromonas*** | Microscopy | S | N/A | N/A | modifed IHS | R | Microscopy | N |
| ***Giardia* sp.** | EIA | S | Microscopy | S** | modifed IHS | R | EIA | R |

Microscopy= microscopic examination of wet preparation; EIA – enzyme immunoassay; IHS- haematoxylin staining; mIHS- modified iron haematoxylin staining technique incorporating a carbol fuschin step. S= sometimes, R= routinely, N= rarely; N/A Not Applicable.

## *9.1 Appendix 1: SURVEY QUESTIONNAIRE*

**Data Mining Survey Instrument**

This research is collaboration between ---------- **Hospital** and University of Technology, Sydney (UTS)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Name of Surveyor:** | | | **Date survey completed:** dd /mm / yyyy | | **Survey ID:** Include a code YYYY##### | | | | | |
| **Section A. Demographics** | | | | | | | | | | |
| **1. Age** In years | **2. Gender** 1. Male | 2. Female | **3. Country of birth:** | | | **4. Country of residence:** 1. Yes | | 0. No | 88. N/A | |
| **5. Aboriginal/Torres Straits Islander** | | | | | | | | | | |
| **6. Language spoken at home:** | | | **7. Translator needed:** | | | 1. Yes | | 0. No | 88. N/A | |
| **8. Postal Code** | | **9. Employment status** | 5. Employed | 4. Self empl | 3. Pension | 2. Unempld | 1. Student | | 99. Unknown | |
| **10. DRG Code** | | **11. Separation from Hospital** | 1. Discharged, 2. Transferred, 3. Dead | | **12. Vitals signs on admission:** | | | | | |
| **13. Marital status** | married/defacto | divorced | common-law | single | unknown | a). Temp:_____°C; b). Pulse Rate_____/min | | c). Systolic BP _____mmHg; d). Diastolic BP _____mmHg | | |
| **Section B: Diagnosis** | | | | | | | | | | |
| **14. Date of Onset of GE related Illness** dd /mm / yyyy | | | **15. Illness Resolution date** dd /mm / yyyy | | | **16. Admission Source** Ward, /Clinic, GP, other hospital | | | | |
| **17. Admission Date** dd /mm / yyyy | **18.Discharge Date** dd /mm / yyyy | | **19. Length of stay** (in days) | | **20. Duration of illness (GI)** (days/hr) | **21. Symptoms related to GE on Assessment** (give details in Notes if necessary) | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **22. Presenting problem(s)** use ICD-10 codes then illness | 1 Diarrhoea 1. Yes 0. No | 2 Vomiting 1. Yes 0. No | 3 Nausea 1. Yes 0. No | 4 Abd pain 1. Yes 0. No | 5 Fever 1. Yes 0. No | 6 Dehydration 1. Yes 0. No | 7 Other(s) |

| | | | |
|---|---|---|---|
| **23. Principal Diagnosis** (use ICD-10 codes then illness) | | | |
| **24. Additional Diagnosis**- include up to 6.put additional overleaf (use ICD-10 codes then illness) | 1. | 2. | 3 |
| | 4 | 5 | 6 |

**Laboratory/Microbiology:** (complete lab details from Medical Record. Note -99 if details are absent )

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **25. Type of specimen?** 25a). Stool | 1. Yes | 0. No | 25b). Urine | 1. Yes | 0. No | 25c). Blood | 1. Yes | 0. No | 25d). Other |
| **26. Is lab result for stool test available?** | 1. Yes | 0. No | 88.N/A | **27. If positive, Name organism(s) isolated** | | | | | |
| **28. Drug sensitivity/ resistance result if available** | | | 1. Yes | | | | | | |

**Section C. Treatment**

| | | | | | |
|---|---|---|---|---|---|
| **29a. Was the Patient treated before this visit? 0. No** | 1. Self | 2. GP/otr hospital | 888. Dont Know | **29b.**If yes name drugs: | |
| **30. Was the patient treated for GI?** *(If no skip to Q. 32)* | **dose** | | **route** | **31. Drug** (give names, dose, route) | |
| **32. Treatment start date** dd /mm / yyyy | **33. Treatment end date** dd /mm / yyyy | | | | |

| | | | | |
|---|---|---|---|---|
| **34. Was this a first or repeat visit?** | 1. first visit | 2. second/repeat visit (give previous date) dd /mm / yyyy | | **35. How many visits were made for GI related illnesses in the study period?** (any break in diarrhoea for >3 days counted separately) |
| **36. Was there a likely source for the GE identified?** | 2. Yes | 1. No | 88.N/A | 1 / 2 / ≥3 |

## Section D. Prognosis, outcome and complications

| | | | | | |
|---|---|---|---|---|---|
| **Outcome of hospitalisation** | | | | | |
| **37a.Treated/Discharged** (2. Yes / 1. No) | **37b**. dd /mm / yyyy | **38a. Referred** (2. Yes / 1. No) | **38b.** dd /mm / yyyy | **39a. Death date** (2. Yes / 1. No) | **39b.** dd /mm / yyyy |
| **40. If patient died-(40a)** Direct cause of death | | | | | |
| **(40b)** Antecedent cause of death | | | | | |

## Section E. Risk and Other General Factors

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **41. Was the patient seen for the following?** | Surgery (2. Yes / 1. No) | Transplant (2. Yes / 1. No) | HIV (2. Yes / 1. No) | Neonatal (2. Yes / 1. No) | Cancer/Chemo (2. Yes / 1. No) | Dialysis (2. Yes / 1. No) | Other |
| **42a. Other family members affected?** | | 2. Yes | 1. No | 99. DK | **42b**. If yes how many? | | |
| **43a. Was the GE related illness hospital acquired?** | 2. Yes | 1. No | 99. DK | **43b. If yes, state source** -if known | | | |
| **44. Method of payment:** | 1. Medicare/pensioner | 2. Private Insurance | | 3. Dept Veteran Affairs | **44b. State category** | | |

**Risk Factors-** Please record whether or not the patient was exposed to any of the following risk factors associated with GI. 99= missing; 88= N/A

| | | | |
|---|---|---|---|
| **45. History of prolonged antibiotic use** | 2. Yes | 1. No | Name antibiotic |
| **46. Travel overseas in last 6 weeks?** | 2. Yes | 1. No | Name place |
| **47. History of chronic gastrointestinal illness?** | 2. Yes | 1. No | Name illness: e.g. GORD, Crohn's, IBD, IBS etc. |
| **48. Consumption of suspect food** | 2. Yes | 1. No | Name food |
| **49. Contact with wild/ domestic** | 2. Yes | 1. No | Name animal(s) |

| | | | |
|---|---|---|---|
| **animal** | | | |
| **50. Drank contaminated water** | 2. Yes | 1. No | Give Details |
| **51. Ate Shell fish** | 2. Yes | 1. No | Name shellfish |
| **52. Patient involved in FBI outbreak?** | 2. Yes | 1. No | details |
| **53. MSM (homosexual)** | 2. Yes | 1. No | details |
| **54. Attends Day/Child Care (<5 years only)** | 2. Yes | 1. No | details |

**Medication Details** (include names of all drugs prescribed within 30 days of the date of onset of GE)

| **Drug** | **Route** | | | | **frequency** | **Date if <=30 before GI** |
|---|---|---|---|---|---|---|
| (name and dose) | *per oral* | *intravenous* | *intramuscular,* | other-state | frequency | Date if <=30 before GI |
| 53 | A | B | C | D | E | F |
| 54 | A | B | C | D | E | F |
| 55 | A | B | C | D | E | F |
| 56 | A | B | C | D | E | F |
| 57 | A | B | C | D | E | F |
| 58 | A | B | C | D | E | F |
| 59 | A | B | C | D | E | F |
| 60 | A | B | C | D | E | F |
| 61 | A | B | C | D | E | F |
| 61 | A | B | C | D | E | F |

- **Key for Medications**
- Routes: *po = per oral; im = intramuscular, iv = intravenous, sc = subcutaneous, nebs = nebulised, topical = on the skin, pr = per rectum, pv = per vagina, S/L = sublingual (under the tongue)*
- *PRN: When necessary; Stat: Given once only e.g. Flu Vax.*
- Dose: µg = micrograms, mg = milligrams, g = grams, ml = millilitres
- Frequency: daily = once per day, mane = morning, nocte= @ night, bd = twice per day, tds = three times per day, qid = 4 times per day, 6/24 (6/hrly) = every six hrs, 4/24 (4/hrly) = every 4 hrs.

NOTES

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## *9.2 Appendix 2: LABORATORY SURVEY*

**INTRODUCTION**

This survey is strictly confidential. No laboratory or individual identifiers will appear in any reports or papers arising from this study. If you have any questions, please do not hesitate to contact the co-ordinator of this study:
Stephanie Fletcher, Tel: 0405663480 Stephaniem.fletcher@student.uts.edu.au You may also contact Professor John Ellis at 02 9514-4161 or John.Ellis@uts.edu.au , in the Faculty of Science at the University of Technology, Syndey if you have any queries or concerns.

Thank you for your time in completing this form, your assistance is appreciated.

**SECTION A**

**BACKGROUND INFORMATION**

**Name of Hospital _____**

Person administering survey _____ Date: _____

Person providing information for survey: _____ Tel: _____

**Name of Laboratory ___**_____

Tel: _____ Fax_____ email:_____

Laboratory Manager/Director_____ contact #_____

**1. Which of the following best describes your laboratory?**

- ☐ Hospital based laboratory
- ☐ Private laboratory
- ☐ Regional laboratory
- ☐ Other, specify _____

**2. Which of the following descriptions characterize the population served by your laboratory?** (check all that apply)

- ☐Patients seen at a tertiary care hospital (that is, a major referral hospital for the area) and/or its affiliated clinics
- ☐Patients seen at a primary care community health centres, and/or affiliated clinics
- ☐Patients seen in general practitioners offices and/or other outpatient clinics
- ☐ Other, specify

**3. Does your laboratory provide referral services to an off-site laboratories for culturing of enteric pathogens?**

- ☐ Yes: give the approximate number of laboratories served_____
- ☐ No

□ Don't Know

## SECTION B
## GASTROENTERITIS / ACUTE DIARRHEAL SYNDROME

4. **Does your laboratory receive stool specimens from patients with acute gastroenteritis, to test for the presence of these enteric pathogens?** (tick all that apply)

□ Bacterial pathogens
□ Ova, cysts and parasites
□ Viruses
□ Microbial toxins
□ Other, specify_____

5. **Does the laboratory have a testing algorithm for the pathogens associated with acute gastroenteritis/acute diarrheal syndrome?**

□ Yes
□ No
□ Don't know

**6. On average how many specimen(s) are tested from each patient?**

□ 1
□ 2
□ 3 or more

7. **On average, approximately what percentage of stool specimens received by your laboratory each month are in the following forms?**

□ Watery or liquid: approx. % of stool specimens_____
□ Loose but doesn't take container shape: approx % of stool specimens_____
□ Solid: approx % of stool specimens_____

8. **Does your laboratory reject a stool specimen if the stool is fully formed (i.e. it is not loose, watery etc.)?**

□Yes
□ Yes, except when testing for the following specific pathogen(s) is/are requested:_____
□ No
□ No, except when testing for the following specific pathogen(s) is/are requested:_____
□ Don't know

**9. Concerning the laboratory information, how often is the laboratory request form received with the following information:**

a. Age and sex □ routinely □ sometimes □ rarely □ never
b. Clinical diagnosis □ routinely □ sometimes □ rarely □ never
c. Signs and symptoms □ routinely □ sometimes □ rarely □ never
d. Date of onset of illness □ routinely □ sometimes □ rarely □ never

e. Date of sample collection □ routinely □ sometimes □ rarely □ never
f. Exposure information □ routinely □ sometimes □ rarely □ never

10. Please complete the following tables for stools specimens tested during **Jan 1 2007-Dec 31 2010**. ***If changes were made to testing protocol, kindly note same on foot of page indicating the pathogen affected and the year the change came into effect.***

**Stools specimens tested during Jan 1 2007-Dec 31**

| Year | 2007 | | 2008 | | 2009 | | 2010 | |
|---|---|---|---|---|---|---|---|---|
| Summary | Rec'd | Tested Pos. | Rec'd | Tested Pos. | Rec'd | Tested Pos. | Rec'd | Tested Pos. |
| No. stool samples received by laboratory | | | | | | | | |
| No. (%) stool samples tested | | | | | | | | |
| No. (%) stools positive for GI pathogen | | | | | | | | |

| **Micro-organisms isolated at Lab** | | **Freq. of testing** | |
|---|---|---|---|
| **Etiology** | **Microbiology Test done** | **R=routinely, S=Sometimes, N=Never** | **Number positive** |
| | | | |
| **BACTERIA** | | | |
| ***Aeromonas*** | | | |
| ***Campylobacter jejuni*** | | | |
| ***Clostridium difficile*** | | | |
| ***Plesiomonas shigelloides*** | | | |
| **NT-*Salmonella* sp.** | | | |
| ***Salmonella* typhi** | | | |
| ***Shigella* sp** | | | |
| ***Staphylococcus areus*** | | | |
| ***Vibrio cholera*** | | | |
| ***Vibrio* sp (non cholera)** | | | |
| ***Yersinia enterocolitica*** | | | |
| **Other (name)** | | | |
| **Other(name)** | | | |
| **Other(name)** | | | |
| **VIRAL STUDY** | | | |
| **Adenovirus** | | | |
| **Astroviruses** | | | |
| **Norovirus** | | | |
| **Hepatitis A** | | | |
| **Rotavirus** | | | |

| | | | |
|---|---|---|---|
| **Other viruses** | | | |
| **PARASITES** | | | |
| ***Entamoeba* sp.** | | | |
| ***Blastocystis* sp.** | | | |
| ***Cryptosporidium*** | | | |
| ***Cyclospora*** | | | |
| ***Dientamoeba fragilis*** | | | |
| ***Enteromonas*** | | | |
| ***Giardia* sp.** | | | |
| **Other parasites** | | | |
| **Other helminths** | | | |
| **Other helminths** | | | |
| **Other non-pathogenic species** | | | |

Diagnostic test changes
New Test (pathogen): _____ Date started: