Molecular Epidemiology of Acute Infectious Diarrhoea in Paediatric Cases at the Winnipeg Children’s Hospital Emergency Room

By

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Abstract

Acute infectious diarrhea (AcID) causes a significant health burden on the population of developed countries, and even a higher burden in the developing world. The only investigation into the causes of AcID in Winnipeg was conducted in the late 1970s. That study determined that 3%-5% of Children’s Hospital Emergency Room (CHER) visits were due to AcID, and about 50% of those were due to rotavirus infections. We conducted a prospective case-control study to reveal the current spectrum of viral pathogens associated with AcID and the distribution and frequency of their occurrence among the pediatric population of Winnipeg. In this study, nucleic acid detection (NAD) and genome sequence information confirmed the presence and identity of each pathogen, and established whether an etiological shift in the distribution of pathogens, both between families and strains of specific pathogens, occurred. Stool samples were collected from pediatric cases with AcID at the ER along with asymptomatic cases for control. A panel of viral nucleic acid detection (NAD) assays was established by the Viral Gastroenteritis Study Group for human astro, calici (Noro and Sapo), entro, polio, hepA, rota and reo viruses according to the published procedures. A new assay for Aichivirus was developed, and the VGSG has established a novel rotavirus assay which is capable of detecting rotavirus from at least 4 different host species (Human, bovine, porcine and simian). Amplified viral targets were sequenced and the information submitted to GeneBank to confirm the strain of each isolate. A total of 1128 patients visited WCHER and WC during the study period and among them 242 patients were enrolled. In 104 cases viruses were identified. A total number of 114 viruses were identified either by NAD or EM assay. Out of 114 viruses, prevalence of HAdV, NoV GI/II and HRV were 44%, 23% and 23% respectively. Mixed infections were found in 4% of cases. This knowledge of pathogen distribution will facilitate design of effective methods for prevention, treatment and intervention in the spread of AcID pathogens.
Acknowledgements

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<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>AcID</td>
<td>Acute Infectious Diarrhoea</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>Reverse transcriptase polymerase chain reaction</td>
</tr>
<tr>
<td>HAdV</td>
<td>Human Adenovirus</td>
</tr>
<tr>
<td>NoV</td>
<td>Norovirus</td>
</tr>
<tr>
<td>HAsTV</td>
<td>Human Astrovirus</td>
</tr>
<tr>
<td>HRV</td>
<td>Human Rotavirus</td>
</tr>
<tr>
<td>WCHER</td>
<td>Winnipeg Children Hospital Emergency Room</td>
</tr>
<tr>
<td>WC</td>
<td>Winnipeg Clinic</td>
</tr>
<tr>
<td>NA</td>
<td>Nucleic Acid</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<tr>
<td>CDC</td>
<td>Center for Disease Control</td>
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Chapter 1: Introduction and Literature Review

1. A Introduction:

Acute infectious diarrhoea is a common health burden in humans worldwide, particularly in the paediatric population [1]. It is also one of the leading causes of child mortality worldwide, with the greatest affect in the developing world [2]. Although the mortality associated with acute infectious diarrhoea [1] has been declining, the incidence rate of diarrhoea is still high, which makes it an important concern in the context of public health. In 2000, it was estimated that the annual mortality associated with diarrhoea was 2.1 millions [3]. In 2004 annual mortality rate from diarrhoea was estimated to be 1.5 millions among children less than five years of age [4]. According to World Health Organization (WHO) report this mortality rate has declined over the past two decades from an estimated 5 million to 1.5 million [4]. In North America, improvements in lifestyle have greatly reduced cases of AcID. In the United States and Canada incidence rates of AcID vary from 0.08 episodes/person/year [5] to 1.3 episodes/person/year [6], whereas 10 or more episodes/person/year have been seen in resource-limited countries. There is a scarcity of information in the epidemiology of AcID in Winnipeg as the last study was done in 1979. So it is important to understand the frequency and distribution of enteric
pathogens in Winnipeg which will help to build up a public health policies to prevent and intervene diarrhoea.

1. B Literature Review:

Gastroenteritis is an inflammation of mucous membrane of the gastrointestinal tract (both stomach and the intestines) which is clinically manifested by vomiting and/or acute diarrhoea. The inflammation is caused most often by an infection of viruses, bacteria or by their toxins, parasites, and less commonly by reaction to food or medication. Acute infectious diarrhoea (AcID) is defined as an episode of diarrhoea which has come on over the course of 1-2 days and has lasted for less than two weeks. It may be caused by infection of the gastrointestinal system by a virus, bacteria or parasite which results in unusual bowel movements producing excessive amounts of liquid feces. It may be accompanied by fever, nausea, vomiting, and abdominal cramping and cause severe dehydration due to loss of body fluids.

There are different forms of acute infectious diarrhoea; most of which are potentially life threatening both for children and adults.

Acute watery diarrhoea is associated with considerable amount of fluid loss and dehydration. Cholera is a good example of acute watery diarrhoea. It usually lasts for several hours or days and is considered as self limiting diarrhoea. Pathogens responsible for acute watery diarrhoea include V cholerae, E coli and rotavirus.
**Bloody diarrhoea** also called dysentery, is characterized by presence of blood in the stool of the infected person. The blood is due to an invasion of bowel tissue (wall) by the invasive pathogen. It is usually a disease of the large intestine. *Shigella* is the agent which is common in the most cases of bloody diarrhoea. Bloody diarrhoea also has been found in Campylobacter food poisoning, *Salmonella* food poisoning, *Entamoeba* food poisoning and in inflammatory bowel disease.

**Persistent diarrhoea** lasts at least for 14 days. Undernourished children or immunocompromised children are the most likely to be vulnerable. Persistent diarrhoea is an important cause of death in children in the developing world.

Malnourished children are more vulnerable to diarrhoea and life threatening dehydration than healthier children [4]. This scenario is also very common in children with poor living standards than children with better living standards. Body metabolic rates are very high in young children and their kidneys are less able to store water than older children and adults. During diarrhoea there is huge loss of body fluid and that is why younger children are more susceptible to diarrhoea and dehydration.

Diarrhoeal disease is the second leading cause of death in children under five years of age, and globally it is responsible for the death of 1.5 million children every year [4]. It causes death to children more frequently than AIDS, malaria and measles together, and it’s associated mortality is close to pneumonia which is a leading cause of death among children. According to the World Health Organization (WHO), about 40% of child deaths are due to pneumonia and diarrhoea each year around the world (Figure 1.1A & B).

![Proportional distribution of cause-specific deaths among children under five years of age, 2004](image)

Over the past two decades an estimated 2.5 billion cases of diarrhoeal disease have occurred children less than five years of age and the overall incidence rate has continued stable [4]. In global perspective of childhood diarrhoea, Africa and Asia show the highest rates of diarrhoeal cases (Figure 1.2) and they also account for more than 80% of child deaths due to infectious diarrhoea (Figure 1.3). In the developed world AcID causes a considerable financial burden on the healthcare system; in the United States it is estimated that over $2 billion is spent annually for both inpatient and outpatient care of AcID [7].
Diarrhoea is also a principal cause of malnutrition among children under 5 years of age. Children with malnutrition and poor immunity are most likely vulnerable to life-threatening diarrhoea. WHO reported that in 2004, overall 6.9% of deaths in low-income countries were due to diarrhoeal disease which was the third leading cause of death at that time, 80% of the deaths were in children under two years of age [4]. In the developing world, children under 3 years of age have at least 3 episodes of diarrhoea every year [4]. These children lack necessary nutrition for growth due to each episode of diarrhoea.
Figure 1.3: Proportional distribution of deaths due to diarrhoeal diseases among children under five years of age by different region in 2004

Diarrhoea is very common in HIV infected children and adults. It is mainly due to the deterioration of the immune system during the late stage of HIV infection [4].

Massive diarrhoea in HIV infected patients is due to the common childhood infections caused by: *Campylobacter, E coli, Salmonella, Shigella* or rotavirus [8]. Persistent diarrhoea increases among HIV-infected children and is associated with an 11-fold increased mortality rate compared to uninfected children [4, 9].
1. B. 2 Transmission of Diarrhoeal Pathogens

Most of the diarrhoeal pathogens share a common means of transmission- ingestion of food/water contaminated by fecal material originating in infected individual. Water contaminated with stool is most likely the source of spreading infection among a community. More common infections have been seen where there is a scarcity of clean water for drinking, cooking and cleaning including bathing and household washing[10]. In the developing world, where there is a lack of proper personal hygiene and sanitation; rotavirus and *E coli* have been found to be the two most common pathogens. Food is another major source of diarrhoea when it is prepared using contaminated water and preserved in unhygienic conditions. During irrigation, food-items like cereals and vegetables can also be contaminated by water. In 2000 in Canada, seven people died and more than 2000 people became sick by a contaminated community water source [11]. Seafood from polluted water may also cause infectious diarrhoea; aichivirus is a good example of a virus which causes diarrhoea through contaminated sea food. Recently aichivirus has been identified in oyster samples in Europe[12].

Surfaces contaminated by diarrhoea infected individual can also cause transmission of infectious pathogens to healthy individuals [13]. Children from daycare can also be infected by rotavirus from infected fomites at the daycare settings [14]. Airborne
spread of rotavirus gastroenteritis has been identified in many recent studies [14]. It has been suggested that in hospital settings airborne spread of rotavirus may be a major route of transmission [15]. Evidence of the airborne spread of norovirus has been documented. This virus can be transmitted through fomitus [16].

Leakage from wastewater system can cause diarrhoea. Storage containers for drinking water can also be a cause of transmission of diarrhoeal pathogen. A study in South Africa, showed the growth and survival of total coliform and *E. coli* in poorly stored water causing diarrhoea [18].
1. B.3. Etiology of AcID

A wide variety of pathogens are associated with AcID including bacteria, parasites and virus (Figure 4). In developed countries, bacteria are generally responsible for 2-10% of cases of AcID with the most common bacterial pathogens being *Campylobacter, Salmonella, Shigella and entrohemorrhagic E.coli* [19]. Parasites are responsible for 1-8% of the cases of AcID, with *Giardia* and *Cryptosporidium* being the most common parasitic causes of diarrhoea in developed nations [19]. Viral
etiology accounts for a major portion of AcID in developed nations as well as in the developing world [19, 20]. Epidemiology of these pathogens varies by location, host population, and season. Viruses are the most common cause of AcID in all age groups. The figure 1.6 shows that more than half of the cases were caused by viruses irrespective of the development status of country.


Figure 1.6: Estimates of role of etiologic agents of severe diarrhoeal illnesses requiring hospitalization in developed and developing countries

Rotaviruses and calicivirus are the most frequent pathogen causing gastroenteritis but astrovirus and adenovirus also have been reported to be associated with child diarrhoea.
1. B.3.a. Viral Gastroenteritis

Viral gastroenteritis is one of the most common diseases in humans, and continues to be a significant cause of mortality and morbidity worldwide [3]. Viral gastroenteritis is an infection caused by a variety of viruses; resulting in vomiting and/or watery diarrhoea. Among the viruses rotavirus (HRV), norovirus (NoV), adenovirus (HdV) types 40 and 41 and astroviruses (HAstV) are the most important agents. Affected persons may also have low grade fever, abdominal pain and headache. Symptoms may begin within 1 to 2 days after the ingestion and last for 1 to 10 days depending upon the type of the virus. Viral gastroenteritis is contagious, infected persons can spread the virus by close contact with healthy individuals through sharing their food, drink, or by eating utensils. Contaminated food or beverage can also cause diarrhoea to individuals. Viral gastroenteritis affects people all over the world and has a seasonality effect. In the United States rotavirus and astrovirus infections are mainly observed in the winter months (October-April), whereas adenovirus infection occurs throughout the year [21]. Frequent hand-washing, disinfection of contaminated surfaces with household chlorine, bleach-based cleaners, and prompt washing of soiled articles of clothing can reduce the chance of getting infected by viral pathogen. Viral gastroenteritis can be divided into two major forms: sporadic and epidemic. The sporadic form causes vomiting, diarrhoea, abdominal discomfort, fever, or a
Sporadic form of viral gastroenteritis can be separated from the epidemic form in several aspects; it primarily affects infants and young children <2 years of age, and causes a range of responses that vary from sub-clinical infection to mild diarrhoea, to a severe and sometimes life threatening illness and dehydration [22].

The salient features of four commonly known viruses causing diarrhoea are given in Table 1.1.

Table 1.1: Salient features of the four diarrhoea causing viruses [21, 23]

<table>
<thead>
<tr>
<th>Features</th>
<th>Rotavirus</th>
<th>Calicivirus</th>
<th>Astrovirus</th>
<th>Adenovirus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genomic composition</td>
<td>dsRNA</td>
<td>ssRNA</td>
<td>ssRNA</td>
<td>dsDNA</td>
</tr>
<tr>
<td>Age group</td>
<td>Mainly 3 to 15 months old children and also under 5 years</td>
<td>People of all Ages</td>
<td>Infants, young children, and elderly persons</td>
<td>Under 2 years</td>
</tr>
<tr>
<td>Incubation Period (days)</td>
<td>1 to 2</td>
<td>1 to 3</td>
<td>1 to 3</td>
<td>7</td>
</tr>
<tr>
<td>Symptoms</td>
<td>Watery diarrhoea, vomiting, fever and abdominal pain</td>
<td>Vomiting, diarrhoea, fatigue, headache and muscle aches</td>
<td>Mild watery diarrhoea, vomiting, abdominal pain, fever and anorexia</td>
<td>Vomiting and diarrhoea</td>
</tr>
<tr>
<td>Seasonal patterns</td>
<td>November to Winter months</td>
<td>Whole year</td>
<td>October to</td>
<td></td>
</tr>
</tbody>
</table>
Rotaviruses

Rotaviruses are one of the most common enteric pathogens in humans and animals worldwide (Figure 1.8) and are the major cause of severe diarrhoea among infants and young children [24, 25]. In 2004, rotaviruses accounted for an estimated 527000 deaths among children <5 years worldwide [2].

Rotavirus was first described in 1963 by electron microscopy. It belongs to the family Reoviridae which contains eight other distinct genera. Rotavirus has a distinctive morphologic appearance by negative-stain electron microscopy (EM). It is a non-enveloped virus with icosahedral symmetry and a capsid.
The term rota was coming from a Latin word, which means wheel and this because of its sharply defined circular outline of the outer capsid gives the appearance of the rim of a wheel [26, 27].

Complete particles of rotavirus are about 70 nm in diameter and have a distinctive double layered icosahedral protein capsid that consists of an outer and an inner layer when viewed by transmission Cryo-Electron Microscopy. Inner capsid is a third layer, the core of which contains the virus genome consisting of 11 segments of double-stranded RNA. The segments size ranges from 667 (Segment 11) to 3302 base pairs (Segment 1); with the total genome containing approximately 18522 base pairs. Gene coding analysis has established that there are six structural and six non-structural proteins. Rotaviruses are classified into seven sero groups (A-G); Groups A-C are
linked with human and animal disease while groups D-G have been identified only in birds [21]. Common features of Group A, Group B and Group C rotaviruses are described in Table 1.2.

<table>
<thead>
<tr>
<th>Features</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host</td>
<td>Humans and animals</td>
<td>Mainly humans</td>
<td>Humans and animals</td>
</tr>
<tr>
<td>Incidence</td>
<td>Globally distributed</td>
<td>Geographically confined</td>
<td>Globally distributed</td>
</tr>
<tr>
<td>Age</td>
<td>Children less than 2</td>
<td>Mainly adults</td>
<td>Both adults and children</td>
</tr>
<tr>
<td></td>
<td>years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comparative severity</td>
<td>Most severe</td>
<td>Less severe</td>
<td>Less severe</td>
</tr>
<tr>
<td>of illness</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serotyping</td>
<td>15 serotype</td>
<td>Not yet established</td>
<td>Not yet established</td>
</tr>
<tr>
<td>Genotyping</td>
<td>27 genotypes</td>
<td>Not described</td>
<td>4 genotypes</td>
</tr>
<tr>
<td>Culture</td>
<td>Yes</td>
<td>Difficult</td>
<td>Difficult</td>
</tr>
</tbody>
</table>
Group A is the most common and only Group A rotaviruses cause human disease in United State, especially in young children under 2 years of age. However, this group of rotaviruses cause milder diarrhoea in older children and adults. Group B has been found in China and southeast Asia causing human infection [29]. Primarily Group C rotaviruses are identified from swine origin but has also been detected from humans in all parts of the world [30, 31]. Rotavirus gastroenteritis varies from mild to severe infection with vomiting, watery diarrhoea and low grade fever. Incubation period is approximately 2 days followed by vomiting for 3 days and watery diarrhoea for 3-8 days. Fever and abdominal pain occurs frequently. Dehydration is one of the most common manifestations of rotavirus infection because of frequent vomiting.
In United States, 3.5 million cases of rotavirus infection occur each year. A child has a 2% lifetime chance of being hospitalized for rotavirus diarrhoea [32]. Worldwide approximately 140 million cases occur each year, with almost 1 million deaths [33].

Caliciviruses are the major cause of non-bacterial acute gastroenteritis outbreaks and sporadic infections all over the world. This virus belongs to the Caliciviridae family. There are four genera in the family including Norovirus, Sapovirus, Versivirus and Lagovirus. Among these genera only two infect humans (Norovirus and Sapovirus). Norovirus (NoV) which is mainly responsible for community acquired outbreaks and it is also the major cause of non-bacterial epidemic gastroenteritis [21]. Norovirus was originally named after the outbreak of acute gastroenteritis in Norwalk, Ohio, USA in 1968. The name norovirus was accepted by the international Committee on Taxonomy of Viruses in 2002.

NoVs are highly infectious and some studies showed that a very low number of viral particles (less than 100) are enough for inducing an infection [34]. Viral transmission occurs mainly through the fecal-oral route and contaminated foods are also a source for this infection [35]. Like rotavirus, air-born transmission is possible to cause disease to a healthy individual [36]. NoVs are single stranded, positive sense, non-enveloped RNA virus with 7.5 to 7.7 kb genome size. NoVs genome consists of 7300-7800 nucleotides and a poly A tail at the 3’end. Viral genome is organized into three Open reading frames (ORF) 1, 2, 3. ORF1 encodes large non-structural proteins, 3 A-like protein, VPg, protease and RNA dependent RNA polymerase (RdRp); the major
capsid protein (VP1) is encoded by ORF2 and ORF3 encodes for a small protein which is also called minor capsid protein (VP2) [21]. Major capsid protein, VP1 is the main antigenic part of norovirus and other calicivirus. Norovirus are genetically divided into five major Genogroups: Genogroup I (GI) to Genogroup V [37]. GI, GII and GIV cause human infection whereas GIII and GV infect bovine and murine species. Human NoVs (GI, GII and GIV) are subdivided into 8, 17 and 2 genetic clusters respectively. Among the three human NoVs genogroups; GII represent 90% of the NoV isolates, and GII4 strains are the most common isolates causing diarrhoea [38]. Capsid protein mutation and recombination events among the NoVs derive the evolution of new variants. In recent years, in Europe recombinant isolates are mainly responsible for norovirus gastroenteritis [39].

During NoVs infection viral multiplication occur within the small intestine and symptoms appear within 1 to 2 days. Symptoms include nausea, vomiting, abdominal cramps and diarrhoea. This diarrhoea is self limited lasting less than 24 to 48 hr [40].

In recent years reverse transcriptase-polymerase chain reaction (RT-PCR) is the most dependable method to detect NoVs although this virus can also be detected by Electron Microscopy (EM). PCR or RT-PCR are very sensitive detection methods and can detect as low as 10 viral particles in a sample. Despite all efforts, there is yet no suitable cell culture system or human embryonic intestinal organ culture to propagate calicivirus [21]. Other tests like ELISA can also be used but due to the diverse antigenic diversity, this method lacks specificity and sensitivity [41].
Figure 1.9: Norovirus from stool sample from an individual

There is seasonality in norovirus infections and the main peak occurs between November and December [3]. According to the Centre for Disease Control (CDC), 23 million estimated cases of acute gastroenteritis worldwide are due to norovirus and 50% of food borne gastroenteritis occur due to norovirus [40]. A surveillance in Japan during 1994 to 1999 showed that the most prevalent pathogen of acute diarrhoea in the infants <3 years of age was rotavirus and norovirus was the main agent of acute diarrhoea in children >3 years of age [42]. In another study by the CDC during July 1997 to June 2000, among 232 norovirus outbreaks 57% were food borne and the settings for outbreaks was mainly restaurants and catered meals (36%), nursing home (23%), schools (13%).

*Astrovirus*
Astrovirus was identified in human stool in 1975 by electron microscopy during an outbreak of diarrhoea. This virus belongs to the Astroviridae family; both human and animal astroviruses share a star like surface structure when viewed by electron microscope (Fig. 1.10). Eight human serotypes are currently known and animal strains have also been found [3, 43].

Astroviruses are 28-35nm in diameter with plus sense, and are single stranded RNA. Astroviruses have a non-segmented positive sense RNA genome with icosahedral capsid; its genome organization can also be distinguished from other viruses of Astroviridae family [43]. The clinical importance of astrovirus has been established through multiple studies and this virus has been shown to be an important cause of gastroenteritis in young children worldwide [44]. Astroviruses are endemic worldwide and account for 3% to 5% of hospitalizations for diarrhoea. Children less than 7 years of age are mainly affected, although adults, immunocompromised patients are equally affected by astroviral diarrhoea [45]. Incubation period is 24 to 36 hours with illness lasting 1-4 days characterized by watery diarrhoea, abdominal cramps, headache, nausea vomiting, low-grade fever, etc. Person to person transmission via fecal-oral route has been described and outbreaks have been identified due to fecal contamination of sea-food or of drinking water. The infection is a self-limited infection with rare transformation into a severe form [46]. Sporadic outbreaks have been described among the military and among elderly patients. Astrovirus infection varies depending on the seasons of a particular region. In temperate regions infections are detected in the winter months whereas in tropical regions prevalence is high in the rainy season.
In 1981, Lee and Kurtz (Filed 79) isolated human astrovirus in primary cell line. This was an important step in astrovirus diagnosis. There are eight serotypes of human astroviruses and these serotypes share common epitope in the capsid protein that have been used for the diagnosis of this virus [21]. Currently there are several methods such as electron microscopy, ELISA, immunofluorescence, polymerase chain reaction used to detect astrovirus particles, antigens or viral nucleic acid from the stool of infected people [47].

**Adenovirus**

Adenovirus was first isolated in 1953. It is a double stranded DNA virus about 70 to 75 nm in diameter. It was recognized as respiratory, ocular and genitourinary virus but serotypes 40 and 41 have been found to infect the gut, contributing to 5 to 20% of hospitalization because of childhood diarrhoea in developed countries[48, 49]. There are six sub-genera of human adenoviruses (A to F). Serotypes 40 and 41 belong to serogroup F. Epidemic and serologic evidence shows that adenovirus 40 (Ad 40) and adenovirus 41 (Ad 41) can result in severe acute diarrhoea in children less than 4
years of age. These ubiquitous viruses are found throughout the year and spread by the feco-oral route. The incubation period of adenoviral gastroenteritis is 3 to 10 days; diarrhoeal illness lasting for 1 weeks or more [48, 50]. In adenoviral infection diarrhoea is a more common symptom than are vomiting or fever, and respiratory symptoms are often present. Person to person transmission is the principle mechanism of spread of the viruses. Asymptomatic shedding has been documented[51]. Outbreaks have been reported in hospitals or day care settings and children are the main target population While adults were rarely affected [52]. Ad 40 and Ad 41 have a worldwide distribution and are of comparable prevalence in the developed and developing countries and in urban and rural countries[53].

1.B. 3. b Bacterial Gastroenteritis

Bacterial gastroenteritis is an infection of the stomach and / or intestines caused by bacteria. It is a very common problem which can range from mild to severe infection. Bacterial gastroenteritis is caused by different species of bacteria including Salmonella, Shigella, Staphylococcus, Campylobacter jejuni, Clostridium, E.coli, Vibrio cholerae, Aeromonas and Yersinia. Clinical manifestations of bacterial gastroenteritis are nausea, vomiting, bloody stool, diarrhoea, fever and abdominal discomfort. Each organism involved in bacterial gastroenteritis causes slightly different symptoms but in all cases diarrhoea is common. Inflammation during bacterial gastroenteritis is often caused by the toxin or by the growth of bacteria within the intestinal wall. Diarrhoea causing bacteria can be transmitted through improperly prepared food, seafood and dairy products.
In the United States, bacterial gastroenteritis is a very common problem in children under 5 years of age [54] and 10% of hospitalization of this age group are due to bacterial gastroenteritis. Each year, 8 million doctor visits and 250,000 hospitalizations among adults worldwide are due to bacterial gastroenteritis [55, 56].

Two important pathogens related to paediatric diarrhoea have been discussed below:

**Enterohaemorrhagic Escherichia coli (EHEC)**

*Escherichia coli (E. coli)* is commonly identified in the gut of humans and warm-blooded animals. Among *E coli*, entrohaemorrhagic *E coli* (EHEC) can cause severe food-borne disease. Contaminated foods such as raw or undercooked ground meat or milk are the main source of transmission of these bacteria to humans. Significance of these bacteria as a public health problem was recognized already in 1982, following an outbreak in the United States [4]. EHEC produces a toxin, named verotoxin which is similar to the shiga toxin. *E coli 0157:H7* is the most important EHEC serotype in relation to public health; but other serotypes are also responsible for sporadic cases and outbreaks.
Symptoms of EHEC include abdominal cramps, fever, vomiting and diarrhoea; sometimes when the condition is severe bloody diarrhoea has been observed. Incubation period ranges from 3 to 8 days with a median of 3 to 4 days. Age is an important factor in the incidence of EHEC infection. The highest reported incidence was identified in children less than 15 years of age; in US the incidence is 0.7 cases per 10000 cases. Food is the main source of infection and about 63 to 85% of cases are reported to be due to contaminated food [57]. There is a huge lack of epidemiologic information on this pathogen from developing countries, as routine surveillance is not performed in many developing countries.

**Campylobacter jejuni**

*Campylobacter jejuni* is one of the most common causes of human gastroenteritis worldwide and now it is recognized as one of the main causes of bacterial food borne disease in many developed countries [58]. It is a rod-shaped, Gram negative microaerophilic bacteria, commonly found in animal feces. *C. jejuni* is mainly distributed to humans through contaminated drinking water and unpasteurized milk. Contaminated food is a major source of infection. *C. jejuni* infection is mainly characterized by abdominal pain, diarrhoea, fever and malaise. Infection persists 24 hours to a week but sometimes even longer. It is self-limited diarrhoea but sometimes it may vary from watery to bloody diarrhoea.
Salmonella

Salmonellosis is one of the most common foods born illness in children worldwide by Salmonella. It causes significant among of morbidity and mortality in children. Salmonella the causative agent of Salmonellosis are the most common bacterial pathogens found in stool cultures with gastroenteritis or severe diarrhoea[59].

Salmonella are gram-negative facultative intracellular anaerobes that cause a wide spectrum of disease including gastroenteritis, enteric fever, bacteraemia, focal infections. The type of infection depends on the serotype of Salmonella and host factors. It has a broad host range and, results in different diseases in different hosts.

In United States Salmonellosis is the most common bacterial infectious cause of food-borne disease[59]. Most of the cases Salmonella infection are food-borne but the incidence of direct contact exposure with animal carriers is also common. More than one million people in the United States are infected with nontyphoid Salmonella annually. This incidence rate is also very common in most of the industrialized nations. The worldwide estimates of nontyphoid Salmonella range from 200 million to 1.3 billion, with an estimated death of 3 million each year[60]. Presence of drug-resistant nontyphoid Salmonella also increases the likelihood of hospitalization and death[61].

Neonates are the most risk group to fecal-oral transmission of Salmonella. The incidence rates are highest during the month of May through October in temperate climates. with the incubation period is from 8-48 hours after the ingestion of contaminated food or water. Symptoms are includes fever, nausea, vomiting,
abdominal pain and diarrhoea. This diarrhoea is mainly self-limiting but usually lasting for 3 to 7 days.

1. B. 3.e Parasitic Gastroenteritis

Parasites that cause human gastroenteritis replicate in the intestine and are excreted in the feces. They can be transmitted via the fecal-oral route with wide host range from human to animal [62]. Since 1981, parasitic agents have been recognized as a leading cause of waterborne outbreaks. Surveillance by the CDC during 1991 to 1992 showed that 21% of drinking water-associated outbreaks were due to parasitic agents in the United States [62]. Several studies have shown that intestinal parasite is very common in the developing countries due to poor environmental and personal hygiene. In a recent study in Nigeria among the children less than five years of age showed in 24% cases parasite was the causative agent [63]. Giardia and Cryptosporidium are two important parasites that cause human gastroenteritis. These two agents mostly observe in children both in industrial and developing countries. Parasitic infection may sometimes cause persistent gastroenteritis but they can also stay asymptomatic as well. Cryptosporidium is mainly cause watery diarrhoea with self limiting characteristic.

1. C Background of This Study
Despite increasing knowledge of the pathogenesis of acute gastroenteritis, a major diagnostic gap remains. Although rates vary, a high percentage of cases remain without the isolation of an etiologic agent using existing diagnostic procedures. In addition, prospective epidemiological studies especially in the ambulatory setting are lacking [64, 65]. Much of the current data comes from information gathered during outbreaks, or from laboratory-based disease reporting. This data would underestimate the actual incidence rate of gastroenteritis since only 1-5% of cases seek medical attention; so a major portion of patients is missed [66, 67]. Recent international population-based studies have allowed for some estimates of the prevalence of acute diarrhoeal illness in a given community. A recent telephone-based study survey was conducted in Canada, Australia, Ireland and the United States. In the preceding 4 weeks of the study, almost 8% of the respondents reported at least 1 episode of acute diarrhoeal illness. Highest rates were seen in children <5 years of age, of whom only 1 in 5 sought medical care [68]. A prospective case control study of paediatric acute gastroenteritis is currently underway in Adelaide, Australia. Over a 3-year period more than 700 cases plus controls were recruited. Rotavirus accounted for 49.9% of cases, but 22% remained without an identified etiology (Ratcliff R., personal communication with Hazelton P., 2006).

In 2006, unpublished data from the Winnipeg Children’s Hospital Emergency Room for February 1st to July 31st, 2005 showed the following information. In the month of February 2005, 2.1% (77/3685) of the cases were associated with AcID, while in the month of July 2005, 1.2% (46/3719) of the visits to the ER were associated with AcID. Of these cases, 88% and 95% were <5 years old in February and July respectively. In 2009, a chart review for the month of January 2008 to December 2008
showed that 1.6% of visits to the ER (710/44609) were due to AcID. The last published study in Winnipeg concerning the etiology of AcID was conducted in 1979 [65]. In that study, stool culture, electron microscopy and serological tests were used to identify the pathogen. The study concluded that the most commonly identified pathogen in diarrhoea in infants was rotavirus, causing 0.03 episodes/month for those <6 months of age and increased >10-fold for those >6 months of age. However, in the majority of cases (approximately 60%) of AcID, no etiologic agent was identified. The low rate of isolation of a pathogen is reflected in more recent studies of Canadian diagnostic facilities, where pathogens have been found in 47% cases [6, 69]. Since the 1979 study new pathogens have been identified causing gastroenteritis including calicivirus, astrovirus, adenovirus and aichivirus. Among those pathogens, aichivirus is readily cultivable, whereas astovirus is cultivable only under special conditions and the calicivirus is not cultivable at all [70-72]. New diagnostic tool like Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) can be used to identify these pathogens, tools that were not available when the 1979 study was conducted.

Despite the advance in knowledge in the diagnosis and treatment of AcID, the burden of illness remains high. It is estimated that rotavirus alone is responsible for about 440,000 deaths in children <5 years of age annually worldwide, with 98% of rotavirus-associated deaths occurring in developing countries [73]. For this reason the development of a rotavirus vaccine was of particular public health interest. The first rotaviral vaccine was withdrawn in 1999 after it was found to be associated with intussusception at a rate of 1 in 10,000 [74]. Currently, there are several new candidates of rotaviral vaccines (Rotateq, Rotavirex) are in trial stage or in different stages of development [75-77]. The reports of the phase 3 trials of the two new rota-
vaccines were published in 2006 and were associated with a significant reduction of rota-associated gastroenteritis [78, 79]. An effective vaccine will evidently be the best option to reduce morbidity, mortality and hospitalization rate and to decrease the economic burden of this disease and therefore it is very important to have current information regarding the epidemiology of AcID.

Although the overall etiology of pathogens, frequency and distribution of gastroenteritis are of most importance, the molecular epidemiology, specifically of the noroviruses, can also play an important role in the diagnosis of prevention and policy making in AcID. In addition, Caliciviruses are now accepted to be the most common cause for outbreaks of nonbacterial diarrhoeal illness in developed countries [80, 81]. They have a high infection rate, low infectious dose as well as the ability to remain stable outside of a host [80]. Historically over 90% of Norovirus infections are genogroups 2 (GGII) and most others are genogroups I (GGI) [82].

In 2006-07, a one year investigation into the spectrum and prevalence of pathogens associated with AcID conducted on a remote First Nation reserve in Northern Manitoba identified norovirus in 46 of 141 cases (32%) [83]. Interestingly 21 cases were caused by GGI, 21 were GGII and 4 cases were mixed GGI and GGII infections. This distribution varies significantly from the worldwide experiences. Ongoing investigation of pathogens in AcID outbreaks in Manitoba during 2006-07 identified norovirus GGI in 11 of 34 (26% of all cases) and GGII in 28 of 34 (74% of all cases) outbreaks. These data suggest that more than one pathogen may be associated with individual out breaks.
On the other hand, emergence of new pathogens in different parts of the world like adeno, astro and aichi indicates changes of etiologic pattern associated with AcID. Astroviruses are considered predominant causes in paediatric AcID both in sporadic and outbreak cases. Aichivirus, a novel cytopathic small round virus, was isolated from sea food-associated AcID in 1989 [70] which have been isolated in Asia and some parts of Europe. Taken together, this study suggests a shift in distribution of pathogens may be occurring worldwide.

A shift of pathogens has been observed before in an unpublished study in Winnipeg. A review of specimens examined by the electron microscope unit, Department of Medical Microbiology and Infectious Disease, University of Manitoba showed that the numbers of cases where rotavirus was identified remained relatively constant at between 600-800 cases per year until the early 90s, when the number of cases slowly declined to about 200-250 cases per year by the mid 1990s. At the same time there was a significant increase in calicivirus identification, from a 20-30 cases per year in the early 1990s to 150 to 200 cases per year by the mid 1990s (Personal communication with Dr. Paul Hazelton).

This epidemiological data indicates that there has been a shift from rotavirus to noroviruses as the predominant cause of AcID in adults and paediatric cases in Manitoba. However, while noroviruses are associated with paediatric AcID [82], there has been no investigations to determine whether the distribution of specific genogroups or strains within the paediatric population parallels that of the local communities in Manitoba.
Several mechanisms may mediate a shift in pathogens. Ongoing exposure to a single pathogen of a population may cause a significant proportion of that population to develop immunity. When the proportion becomes large enough herd immunity may develop, leading to a reduction of the number of cases. A shift in pathogens may occur when a new pathogen is introduced and then emerged to take the place of the original agent to which the population became immune. Such a mechanism may explain the apparent change in relative distribution of norovirus GGI and GGII or from rotavirus to a new pathogen.

1. D Hypothesis

Our hypothesis is that the distribution and range of pathogens associated with AcID is a dynamic process which is changing over time. As herd immunity to a specific pathogen develops, the frequency at which that pathogen is isolated will decline. At the same time, new or different pathogens will be observed with an increasing frequency - as one wave ebbs, another rises – “The Wave Theory”. If the Wave Theory is correct, then we should see a change in the distribution of AcID pathogens in the paediatric population over the period of a year or more.

1. E Objective

The objectives of this study were:
1. To identify the spectrum of AcID pathogens and the distribution and frequency of their occurrence among the paediatric population in Winnipeg.

2. To define the mechanism for the shift. The knowledge of these mechanisms may help to predict the next pathogen wave to emerge and to better design treatment and methods of intervention.

Chapter 2: Methods

2. A. Objectives:

The purpose of this study was to observe the spectrum of AcID pathogens and the distribution and frequency of their occurrence among the paediatric population in Winnipeg, (described in details in section 1.E).
2. B. Methodology

2. B.1 Study Time Frame

This was a nineteen month study from July 01, 2008 to January 31, 2010.

2. B.2 Sample Collection Site

The samples were collected from the patients at the Winnipeg Children Hospital’s Emergency Room (WCHER) and also from Winnipeg Clinic (WC). The patients were residing within and out of the boundaries of the city of Winnipeg and also different parts of Manitoba.

2. B.3 Ethical Considerations

Ethical approval was obtained by the University Of Manitoba Health Research Ethics Board (HREB).

2. B.4 Questionnaire

The initial questionnaire was developed by Dr. Ethan Rubinstein, Dr. Sergio Fanella and Dr. Paul Hazelton. Modifications when needed were made by the approval of HREB the information from the questionnaires was assessed by SPSS software. See Appendix 1 for questionnaire.
2. B.5. Study Design

This study enrolled children who were visiting WCHER and Winnipeg clinic with symptoms of infectious diarrhoea from July 2008 to January 2010. Exclusion criteria for enrolling children were:

I. HIV positive status
II. Recent hospital admission
III. Chronic bowel disease
IV. History of chronic diarrhoea
V. Ongoing Diarrhoea (2 weeks or more)
VI. Chemotherapy
VII. Antibiotic treatment in the last two weeks
VIII. Treatment for constipation
IX. Unable to produce a stool sample

2. B.6. Sample Size

Sample size was calculated by “Sample size for a prevalence survey with finite population correction” method (by the help of Dr. Rasheda Rabbani, Biostatistician, Manitoba Institute of Child Health). A number of 1128 patients visited WCHER and WC during the study period. Among them 318 patients were able to produce during there stay at WCHER or WC and also fulfill the study criteria. So 318 was the total population of this study. Viral prevalence was estimated (40%) according to literature.
review and previous RT-PCR results from the lab. With the 95% confidence interval and 5 precision values the minimum sample size for the study was calculated as 109. And for 99% confidence interval the minimum sample was calculated as 215. In this study 242 samples were collected.

2. B.7 Sample Collection

Stool samples were collected from paediatric cases <18 years of age with AcID from July 2008 to January 2010 from WCHER and Winnipeg Clinic. Informed consent was obtained from the patient’s guardians when possible. Initially we aimed to recruit all patients with AcID as they came through the WCHER. However it was not possible to obtain the entire study population from WCHER. Therefore the protocol was changed and the changes were approved by Health Research Ethics Board (HREB). In the new changed process, samples were collected from Cadham Provincial Laboratory and consent form, questionnaire with a letter were sent to the guardians’ address by the Viral Gastroenteritis Study Groups (VGSG). After receiving the letter guardians were contacted by phone and the consent was taken to enrol their children. At the same time questionnaire was filled by VGSG. Time -matched outbreak samples submitted to Cadham Provincial Laboratory were also collected to investigate the cases of gastroenteritis in the community. All the samples were submitted for routine diagnostic procedures which included culture for bacterial pathogens causing diarrhoea, microscopic examination for parasites and viral detection by negative transmission electron microscopy. Additional nucleic acid detection (NAD) testing by both polymerase chain reaction (PCR) and reverse transcriptase-polymerase chain
reaction (RT-PCR) and subsequent sequence analysis of the target genomes was performed on all of the samples collected.

Figure 2.1: Study design

2. B.8 Processing of Stool Specimen

The viral detection Unit of Cadham Provincial Laboratories provided 5% stool suspension in Tris-NaCl buffer (TN Buffer) using freshly collected stool samples. The same stool suspension was used for both electron microscopy (EM) and molecular viral assay. Samples collected from Cadham laboratory were assigned a code number
and the information was entered into a password protected excel file. Samples were kept at 4°C until being purified for nucleic acid (NA).

2. B.9 Nucleic Acid Purification

Viral nucleic acid was extracted and purified using QIAGEN viral RNA extraction kit (Qiamp viral RNA kit, Qiagen, Valencia, CA) according to the manufacturer’s instruction (Vacuum manifold) with the following modification:

I. Poly Cytosine (poly C) was used as carrier RNA instead of poly Adenine (Poly A).

II. 60 μl of elution buffer was used with RNase inhibitor. Additional RNase inhibitor was added to confirm the stability of the RNA.

Previous experience from this laboratory showed that the yield of viral genome from 150 μl stool suspension using the system is sufficient to conduct 10 to 15 Nucleic Acid Detection assays (NAD). 3μl aliquots were prepared for each sample and were stored in individual tubes to avoid cross contamination and freeze thawing during individual NAD testing. These tubes were labelled and covered with mineral oil and stored at -20°C.

2. B.10 Nucleic Acid Detection (NAD) Assay

A panel of 10 different viral assays for Human Adenovirus, Aichivirus, Astrovirus, Norovirus Genogroup I and II, Enterovirus, Hepatitis A, Rotavirus (two different assays) and Reovirus was prepared to assess the pathogen distribution among the
study population. Both PCR and RT-PCR were conducted in accordance with standard laboratory protocol using the published and unpublished primers and annealing conditions which are describe in Table 2.1. To avoid the cross contamination during the RT-PCR assay, 2 steps 1 tube (RT and PCR) method were followed. All the PCR tests were performed with an assay volume of 25 μl and RT were performed with 8 μl assay volume. Superscript II Reverse Transcriptase (Invitrogen, Carlsbad, CA 92008 USA) was used for RT-PCR. Expand long template Taq (Roche, Indianapolis, IN, USA 001) was used in PCR assay except in the Enterovirus assay where Accuprime Taq DNA (Invitrogen, Carlsbad, CA 92008 USA) was used.
### Table 2.1 Primers used in this study

<table>
<thead>
<tr>
<th>Virus</th>
<th>Primer</th>
<th>Primer sequence</th>
<th>Sense</th>
<th>Location</th>
<th>Amplicon size</th>
<th>Annealing Tm (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>neHexAA1893, neHexAA1905</td>
<td>5’ GCC ACC GAG ACG TAC TTC AGC CTG 3’&lt;br&gt;5’ TGG TACGAG TAC GCG GTATCC TCG CGGTC 3’</td>
<td>-</td>
<td>18937-18960&lt;br&gt;19051-19079</td>
<td>142</td>
<td>55</td>
</tr>
<tr>
<td>Aichivirus</td>
<td>Aichi 1, Aichi 2</td>
<td>5’ CTTYCCCCGGRGTYGTGCTCT 3’&lt;br&gt;5’ TCCTTTCCGCTTCCCTGCTC 3’</td>
<td>-</td>
<td>6452-6468&lt;br&gt;6365-6383</td>
<td>120</td>
<td>51</td>
</tr>
<tr>
<td>Astrovirus</td>
<td>AC1, AC230</td>
<td>5’ ATGGCTAGCAAATGCTGACAAG 3’&lt;br&gt;5’ GGTTCAGTGGTCTTGACACC 3’</td>
<td>-</td>
<td>1450-1470&lt;br&gt;1182-1203</td>
<td>289</td>
<td>50</td>
</tr>
<tr>
<td>Entrovirus</td>
<td>neEnt 2, neEnt 1</td>
<td>5’ GAAACCCAGCACACCAAGTA 3’&lt;br&gt;5’ CCGGCCCCCTGAATGGGGCTA 3’</td>
<td>-</td>
<td>547-567&lt;br&gt;430-450</td>
<td>121</td>
<td>54</td>
</tr>
<tr>
<td>Hepatitis A</td>
<td>HAV 3, HAV 1</td>
<td>5’ CTCCTCCGAATATCATCCTCAACTTGTT 3’&lt;br&gt;5’ ACAGGTATACAAAGTCAGACATCAG 3’</td>
<td>-</td>
<td>2204-2229&lt;br&gt;2022-2048</td>
<td>208</td>
<td>54</td>
</tr>
<tr>
<td>Norovirus</td>
<td>SR 33, SR 48, SR50, SR 52</td>
<td>5’ TGACCTCACGATCTCACCACC 3’&lt;br&gt;5’ GTG AAC AGC ATA AAT CAC TGG 3’&lt;br&gt;5’ GTG AAC AGT ATA AAC CAT TGG 3’&lt;br&gt;5’ GTG AAC AGT AAA AAT CAC TGG 3’</td>
<td>-</td>
<td>4856-4876&lt;br&gt;4754-4773</td>
<td>123</td>
<td>53</td>
</tr>
<tr>
<td>Norovirus</td>
<td>SR 33, SR 46</td>
<td>5’ TGACCTCACGATCTCACCACC 3’&lt;br&gt;5’ GTG AAC AGC ATA AAT CAC TGG 3’&lt;br&gt;5’ GTG AAC AGT ATA AAC CAT TGG 3’&lt;br&gt;5’ GTG AAC AGT AAA AAT CAC TGG 3’</td>
<td>-</td>
<td>4856-4876&lt;br&gt;4754-4773</td>
<td>123</td>
<td>53</td>
</tr>
<tr>
<td>Reovirus</td>
<td>ReoL1-1, ReoL1-2</td>
<td>5’ GGCGGTCCCTGCACATCCATTGAAAA 3’&lt;br&gt;5’ ACCCGCTGCACCTGAACCCATCA 3’</td>
<td>-</td>
<td>2248-2230&lt;br&gt;1622-1643</td>
<td>653</td>
<td>60</td>
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<tr>
<td>Rotavirus</td>
<td>VP6R, VP6F</td>
<td>5’ GTCCAATTCATNCCTGTGGTG 3’&lt;br&gt;5’ GACCGVGCRCTACTACGTG 3’</td>
<td>-</td>
<td>1106-1126&lt;br&gt;747-766</td>
<td>379</td>
<td>50</td>
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<tr>
<td>Rotavirus</td>
<td>Rota S1-3, Rota s1-4</td>
<td>5’ TAGGCCCTNATTAARACIGT 3’&lt;br&gt;5’ TTCTCACCAGTAARTAYCT 3’</td>
<td>-</td>
<td>2056-2074&lt;br&gt;1832-1852</td>
<td>243</td>
<td>51</td>
</tr>
</tbody>
</table>
2. B. 11 Agarose Gel Electrophoresis and cDNA Extraction

PCR products were electrophoresed using a 2% agarose gel (Invitrogen, Carlsbad CA, USA) and stained with ethidium bromide. The gels were then examined under
ultraviolet illumination and results were recorded photographically by gel
documentation (BioRad, Hercules CA). Bands corresponding to the appropriate size
were cut and then extraction was performed by the QIAquick gel (Qiamp viral RNA
kit, Qiagen, Valencia, CA) extraction system, in accordance with the manufacturer’s
instruction.

2. B. 12 Sequence Analysis

All positive samples were confirmed by sequence analysis in both directions.
Amplified targets were sequenced using a ABI 3100 automatic sequencer at the
National Microbiology Laboratory, Winnipeg, MB, Canada. Sequencing analysis was
performed using ChromasPro Version 1.5 (Technelysium, Australia) and all sequence
pair (reverse and forward) were aligned using Mega 4, Clustal W and discrepancies
justified by examining the original sequencing chromatogram. A unique consensus
sequences was created for each sequence pair by emboss explorer
(http://emboss.imb.nrc.ca). All sequences were then submitted to the database
published in GenBank (National centre for Biotechnology Information:
(http://www.ncbi.nlm.nih.gov) to confirm each virus isolate.

2. B.13 Phylogenetic Analysis

Phylogenetic trees were created using the samples and references sequences. Using
Mega 4, a dendrogram was created for both the nucleotide and amino acid sequences
using the neighbour-joining clustering method with statistical confidence assessed by bootstrap with 1000 replicates.

2.B.14 Statistical Analysis

All the information and laboratory results were documented into SPSS 16 softwares with a password protected file. Chi square analysis was done by SPSS software to see the statistical significances between two parameters. To see the statistical difference between two proportions Z-test was done.

2. B.15 Aichivirus Assay Development

To develop a new assay for Aichivirus, VGSG collected the control virus (courtesy of S. Le Guyader) (IFREMER, Laboratoire de Microbiologie, Nantes, France). VGSG group cultured the virus in vero cell and stored the culture for further study.

Primer Design

Primer design was performed using both NCBI/Primer BLAST and Primer Select (DNA Star) based on the Aichi virus complete genome, Genbank accession number AB010145. The potential primer sets were BLAST searched using GenBank and NCBI to assess the specificity of the primers. A total of 2 primer sets were chosen from the 3CD gene region for subsequent testing; 1 primer set (Aichi 1: CTTYCCCGGRGTYGTCGTCT and Aichi 2: TGAGGGGRGCRGGTTTGT) and other primer set (Aichi 3: TCTTYTCCCGCTTCCTYCTSGTYC and Aichi 4: AGCGCCGCATGTCTTSTA)
RT-PCR

Pre-optimized conditions established in the lab were used for the RT-PCR. RT-PCR was first performed for each primer set using a temperature gradient from 51 to 61.9°C based on the predicted optimal annealing temperatures. Subsequent RT-PCRs were performed at the optimal annealing temperature shown in Table 2.1.

Sensitivity and Specificity Testing

To test and compare the sensitivity of the newly designed primer sets, 10 fold serial dilution (from $10^1$ to $10^8$) in Qiagen elution buffer (Qiagen, Valencia, CA) of the Aichi positive control were tested by RT-PCR. In order to test the specificity of the primer, it was tested against a virus panel including the following viruses: Adenovirus, Astrovirus serotype 1, 3 & 7, Calici GGI strains AX4 and BD2, Calici GII strains 5821 and 2279, Coxsackie B2, ECHO 11, Poliovirus Type 3, Reovirus serotypes T3D, Rotavirus strains 01-1 and 01-5, Cytomegalovirus (CMV), Epstein Barr virus (EMB) and Varicella-Zoster virus.

Chapter 3: Results
3.1 Laboratory Analysis of the Samples

A total of 70,473 patients visited WCHER and WC during July 2008 to January 2010. Of those 1128 patients complained of diarrhoea during the study periods. Out of these 1128, only 318 (28%) patients were able to produce stools during their visit in ER and clinic. Of these 318, we were able to collect samples from 242 (76%) children <18 years of age. Bacterial and parasitic information were obtained from the chart review of the Children Hospital. Out of the 242 children, we were able to get both epidemiological and laboratory information from 128 (52.90%) children.

A total number of 104 cases were identified as viral positive from the 242 stool samples. Of these 104 cases, 114 viruses were identified either by EM or NAD assay (Table 3.1). Of the 114 viruses, mixed infections (>one viruses) were found in 10 samples and in 94 specimens a single virus (one virus) was identified. In the 104 cases overall viral positivity was 43% (104/242*100). Out of 114 viruses, prevalence of HAdV, NoV GI/II and HRV were 44%, 23% and 23% respectively. Mixed infections were seen in 4% cases (10/242*100) (Table 3.1 & Table 3.2). Out of 242 cases, bacterial cultures were positive in 15 cases (6%) and in only 2 (0.8%) cases parasites were identified as the causative agent.

Table 3.1: EM, NAD assay, positive by both methods and total viral positive information, n=242
Total sample | EM + NAD- | NAD+ EM- assay | EM+ NAD+ | Total positive either by EM or NAD
---|---|---|---|---
242 | 15 | 76 | 23 | 114

EM= Electron microscopy, NAD= Nucleic Acid Detection assay

Table 3.2: Viral distribution in positive cases (n=114)

<table>
<thead>
<tr>
<th>Virus</th>
<th>EM+ve/ NAD –ve</th>
<th>EM-ve/ NAD+ve</th>
<th>EM+ve/NAD+ve</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adeno</td>
<td>3</td>
<td>42</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>Aichi</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Astro</td>
<td>1</td>
<td>7</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>NoV</td>
<td>9*</td>
<td>15</td>
<td>2</td>
<td>26</td>
</tr>
<tr>
<td>GI/GII</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterovirus**</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HepA</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>2</td>
<td>14</td>
<td>10</td>
<td>26</td>
</tr>
<tr>
<td>Reovirus</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>80</td>
<td>19</td>
<td>114</td>
</tr>
</tbody>
</table>

*It was not possible to differentiate NoV GI or GII by electron microscope. But after sequencing all came out NoV GII

** Enterovirus assay was done in 155 cases

Out of 242 participants, 144 male and 98 female patients were enrolled in the study (Table 3.3). Distribution of viral positivity by gender showed higher percentage of
viral prevalence in male patients (Figure 3.1). And Adenoviruses were the most prevalent in both genders (Figure 3.2).

Table 3.3: Sex distribution of participants, n=242, P=.001

<table>
<thead>
<tr>
<th>Male/Female</th>
<th>Count</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>144</td>
<td>59.5</td>
</tr>
<tr>
<td>Female</td>
<td>98</td>
<td>40.5</td>
</tr>
</tbody>
</table>

Figure 3.1 Viral positivity in different genders, n=242, p=0.002
Participants were divided into five groups based on their age. In 6 cases (out of 242) we could not find the date of birth. Among the 236 participants, the highest number of children were in the group of 6-24 month (33.5%, Table 3.4). And the highest viral prevalence (59%) was observed in group 0-5 months of age. This prevalence steadily decreased with the increase in age. (Figure 3.3).

Table 3.4 Number of participant in different age groups, n=236, p=0.16

<table>
<thead>
<tr>
<th>Age group (Month)</th>
<th>Count</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-5</td>
<td>59</td>
<td>24.3</td>
</tr>
<tr>
<td>6-24</td>
<td>81</td>
<td>33.5</td>
</tr>
<tr>
<td>25-96</td>
<td>56</td>
<td>23.1</td>
</tr>
<tr>
<td>97-120</td>
<td>23</td>
<td>9.5</td>
</tr>
<tr>
<td>121-208</td>
<td>17</td>
<td>7.0</td>
</tr>
</tbody>
</table>
Figure 3.3: Viral prevalence among the study population based on age groups, n=236, p=0.02

High prevalence of HRVs were observed in infants <5 months of age. On the other hand, HAd viruses were common in all age groups. NoVs were found more frequent in younger children <5 months of age, the prevalence gradually decreased with an increase in age (Figure 3.4).

Figure 3.4: Viral distributions in different age groups, n=114, p=0.5
Seasonal distribution of different viruses was also analyzed. Rotaviruses was mainly identified during the springtime where as most of the HAd viruses were found in winter time (Figure 3.5). Other viruses were identified round the year.

![Seasonal distribution of virus](image)

Figure 3.5: Seasonal distribution of viral positive isolates, n=236

### 3.2 Demographic Information Base on Questionnaire Data

Of the 242 participants, demographic and clinical information were obtained from 128 cases through a questionnaire filled by the VGSG from individuals participants, 46 (36%) had viral positive stool samples and 12 (9%) were identified as having a positive bacterial stool culture.

Through the questionnaire, guardians were asked about living area and 56% of the participants were residing within the perimeter highway (Hwy) of Winnipeg (Table 3.5). Participants were also asked about the source of water at home for drinking. In 72 (56%) cases municipality treated water was found as the main source of water at home. The second most common source was bottled water (20%) (Table 3.6).
Table 3.5: Living area of the participants, n=128, p=0.12

<table>
<thead>
<tr>
<th>Where live</th>
<th>Count</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inside Perimeter</td>
<td>72</td>
<td>56.2</td>
</tr>
<tr>
<td>Outside Perimeter</td>
<td>56</td>
<td>43.7</td>
</tr>
</tbody>
</table>

Table 3.6: Source of water for the participant at home, n=128, p=0.003

<table>
<thead>
<tr>
<th>Source of water</th>
<th>Count</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated Water</td>
<td>72</td>
<td>56.3</td>
</tr>
<tr>
<td>Well water</td>
<td>16</td>
<td>12.5</td>
</tr>
<tr>
<td>Bottled Water</td>
<td>26</td>
<td>20.3</td>
</tr>
<tr>
<td>Both well and bottled water</td>
<td>2</td>
<td>1.6</td>
</tr>
<tr>
<td>Both city and bottled water</td>
<td>5</td>
<td>3.9</td>
</tr>
<tr>
<td>Others</td>
<td>7</td>
<td>5.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>128</strong></td>
<td><strong>—</strong></td>
</tr>
</tbody>
</table>

### 3.3 Clinical Information Based on Questionnaire Data

Guardians were asked about their child’s overall symptoms during the diarrhoeal illness including the presence of blood in the stool. Abdominal pain, vomiting and fever were found in 66%, 61% and 49% cases respectively (Table 3.7). These three symptoms are the main characteristics of viral diarrhoea. Among the viral positive participant abdominal pain, vomiting and low grade fever was found high (Figure 3.6 & 3.7).

Table 3.7: Overall symptoms of the participants during diarrhoea, n=128
<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Count</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vomiting</td>
<td>78</td>
<td>61.0 (78/128)</td>
</tr>
<tr>
<td>Cough</td>
<td>35</td>
<td>27.3 (35/128)</td>
</tr>
<tr>
<td>Runny nose</td>
<td>31</td>
<td>24.2 (31/128)</td>
</tr>
<tr>
<td>Fever</td>
<td>63</td>
<td>49.2 (63/128)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>85</td>
<td>66.4 (85/128)</td>
</tr>
<tr>
<td>Blood</td>
<td>31</td>
<td>24.2 (31/128)</td>
</tr>
<tr>
<td>No symptoms</td>
<td>6</td>
<td>4.7 (6/128)</td>
</tr>
</tbody>
</table>

Figure 3.6: Different symptoms among the viral positive participant, n=46
Figure 3.7: Frequency of symptoms in different viral infection

Presence of blood in stool is not often seen in viral diarrhoea. In this study most of the viral positive cases (43%) blood was not found in stool and this data was statistically significant (Table 3.8). In bacterial diarrhoea bloody stool is often observed. Out of 128 participants, 12 cases were identified as bacterial diarrhoea and of 12 cases bloody stools were found in 9 (29%) cases being a statistically significant finding (Table 3.9).

Table 3.8: Presence of blood in stool in viral diarrhoea, n=128, p=.002

<table>
<thead>
<tr>
<th>Presence/Absence of blood in Stool</th>
<th>Count</th>
<th>Viral positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of Blood in Stool</td>
<td>31(24.2%)</td>
<td>4 (12.9%)</td>
</tr>
<tr>
<td>Absence of Blood in Stool</td>
<td>97 (75.8%)</td>
<td>42 (43.3%)</td>
</tr>
</tbody>
</table>
Table 3.9: Presence of blood in stool in bacterial diarrhoea, n=128, p=.001

<table>
<thead>
<tr>
<th>Presence/Absence of blood in Stool</th>
<th>Count</th>
<th>Bacterial positive culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of Blood in Stool</td>
<td>31 (24.2%)</td>
<td>9 (29%)</td>
</tr>
<tr>
<td>Absence of Blood in Stool</td>
<td>97 (75.8%)</td>
<td>3 (3%)</td>
</tr>
</tbody>
</table>

Viral diarrhoea is characterized by large number of diarrhoeal stools. This study also showed high prevalence of viruses when number of stools within last 24 hours exceeded 5 but the data was not statistically significant (Table 3.10 and 3.11).

Table 3.10: Number of stools within last 24 hours among participant, n=128, p= 0.83

<table>
<thead>
<tr>
<th>Number of stools within last 24 hours</th>
<th>Viral positive Count</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5</td>
<td>16</td>
<td>12.5</td>
</tr>
<tr>
<td>&gt;=5</td>
<td>30</td>
<td>23.4</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td></td>
</tr>
</tbody>
</table>
Table 3.11: Number of stools within last 24 hours among viral positive participants

n=46 (Positive cases), p=0.32

<table>
<thead>
<tr>
<th>Number of stools within last 24 hrs</th>
<th>Adeno</th>
<th>Astro</th>
<th>Calici GI/GII</th>
<th>Rota</th>
<th>Reo</th>
<th>Mixed infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>&gt;=5</td>
<td>10</td>
<td>1</td>
<td>6</td>
<td>10</td>
<td>0</td>
<td>4*</td>
</tr>
</tbody>
</table>

*In two cases Adeno and Calici GII, in one case Rota A and Adeno, and in one case Rota A and Astro

3.4 Environmental Data: Housing, School Going and Sanitation Information Among the Participants

To find out whether there was any external source responsible for the Child AcID; parents were asked about the number of their house members and also whether any of the household members had diarrheal experiences in the last 4 weeks. We also collected the information whether the patients attend preschool or day care (Table 3.12,3.13). In 42 (33%) cases it was found that other members of the house experienced diarrhoea before the patients, out of these 42 cases 31% of the children were found to have viral positive stools. This data was not statistically significant. In 27 cases participant went to preschool or daycare and in 10 (37%) viral infections were identified. The data was not significant (Table 3.13, p=0.38).
Table 3.12: Information of any members of the participant’s household experiencing diarrhoea within past 4 weeks before the participant and viral prevalence of the participant (n =127), p=0.38

<table>
<thead>
<tr>
<th>Other member of the house had diarrhoea before</th>
<th>Count</th>
<th>Viral Positive Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>42 (33%)</td>
<td>13 (31.0%)</td>
</tr>
<tr>
<td>No</td>
<td>85 (66.4%)</td>
<td>33 (39.0%)</td>
</tr>
</tbody>
</table>

Table 3.13: Participant attending preschool or day care, n=128; P=0.88

<table>
<thead>
<tr>
<th>Enrolled child go to preschool or day care</th>
<th>Count</th>
<th>Viral positive prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>27 (21%)</td>
<td>10 (37.0%)</td>
</tr>
<tr>
<td>No</td>
<td>101(79%)</td>
<td>35 (34.7%)</td>
</tr>
</tbody>
</table>

Parents were also asked any other children at home go to preschool or daycare. Twenty seven cases were found where other children at participant’s home go to preschool or daycare but this data did not show whether this child experienced diarrhoea or not (Table 3.14).
Table 3.14: Information of any other children at participant’s home attending preschool or daycare and viral prevalence of the participant, n=125; P=0.09

<table>
<thead>
<tr>
<th>Other children at the home go to preschool or daycare</th>
<th>Count</th>
<th>Viral Positive prevalence among the participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>27 (21%)</td>
<td>6 (22.2%)</td>
</tr>
<tr>
<td>No</td>
<td>98 (76.6%)</td>
<td>39 (39.8%)</td>
</tr>
</tbody>
</table>

To establish the overall sanitation status during the diarrheal event, parents were asked whether they wash their child after every event of diarrhoea and also were asked about their own hand washing after cleaning the child to avoid further spread. The data showed that in most of the cases proper sanitation was followed during the AcID (Table 3.15 & 3.16).

Table 3.15: The parents were asked whether they wash (Clean) their child after diarrhoea, n=128; p=0.64

<table>
<thead>
<tr>
<th>Wash child after diarrhoea</th>
<th>Count</th>
<th>Viral positive prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>124 (97%)</td>
<td>45 (36.3%)</td>
</tr>
<tr>
<td>No</td>
<td>4 (3%)</td>
<td>1 (25%)</td>
</tr>
</tbody>
</table>
Table 3.16: The parents were questioned whether they wash their own hands after cleaning the child, n=128; p=0.67

<table>
<thead>
<tr>
<th>Wash hand after cleaning the child</th>
<th>Count</th>
<th>Viral positive prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>126 (98.4%)</td>
<td>45</td>
</tr>
<tr>
<td>No</td>
<td>2 (1.6%)</td>
<td>1</td>
</tr>
</tbody>
</table>

3.5. Received Medications After Acute Gastroenteritis Before Coming to WCHER and WC

During diarrhoeal illness, parents were asked whether they visited doctors or received any medication before coming to WCHER or WC. The data showed that 46% parents came directly to WCHER or WC and did not visit any doctor or took any medication due the illness. Parents were also asked whether their children took any antibiotic within the last month. A small number of patients took antibiotics within the last month. According to our exclusion criteria, patients who took antibiotic within last two weeks before coming to WCHER or WC were excluded. Parents who visited doctor before coming to WCHER or WC mostly took medical care from envoy doctor, family doctor and general paediatrician respectively (Table 3.17, Figure 3.8).
Table 3.17: Receiving medication among the participant before WCHER or WC visit, n=128, p=0.17

<table>
<thead>
<tr>
<th>Visit doctor after child’s diarrhoea</th>
<th>Count</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>71</td>
<td>55.5</td>
</tr>
<tr>
<td>No</td>
<td>57</td>
<td>44.5</td>
</tr>
<tr>
<td>Total</td>
<td>128</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3.8: Parents were asked about the types of medical care they have used after the AcID, n=71
Parents were asked whether they will agree to receive any vaccine for AcID upon availability. Fifty four percent of parents welcomed the vaccine but 34% wanted to know more about vaccine before agreeing.

Table 3.18: Parents were asked whether they will take vaccine for AcID for their child if available, n=128, p=0.01

<table>
<thead>
<tr>
<th>Receiving vaccine upon availability</th>
<th>Count</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>69</td>
<td>54</td>
</tr>
<tr>
<td>No</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>Maybe</td>
<td>44</td>
<td>34</td>
</tr>
</tbody>
</table>

3.6. Economical Burden of AcID Based on Questionnaire Data

To calculate the economic burden of AcID, parents were asked whether they took any day off due to their child’s diarrhoea or not. This study found that 41% of parents took days off work due their child’s diarrhoea. To establish the economic burden the average time taken from work was multiplied by the average industrial wages (19.10 CAD/h) in Manitoba [108] (Table 3.19 & 3.20).

Table 3.19: Parent were asked whether they took day off due to child’s diarrhoea, n=127

<table>
<thead>
<tr>
<th>Need to take off due to</th>
<th>Count</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3.20: Parents were asked how many days off they took from the work and calculate the amount of wages loss due to diarrhoea

<table>
<thead>
<tr>
<th>Frequency of days needed to take off</th>
<th>Count</th>
<th>Average day off</th>
<th>Average amount of wages loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10</td>
<td>33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;=10</td>
<td>16</td>
<td>8.5</td>
<td>1306.4</td>
</tr>
</tbody>
</table>

3.7. Travel History of the Patients Within the Last Month

Parents were asked whether they had any travel history outside Canada within the last month. In only 7 cases travel history was found. Among those 5 (71.4%) were viral positive. This data was statistically significant. Both rota and norvirus GII were found in two cases and adenovirus was identified in one case.

Table 3.21: We asked the parents whether they travelled outside Canada within last month, n=128, p=0.04

<table>
<thead>
<tr>
<th>Travel history outside Canada</th>
<th>Count</th>
<th>Percent (%)</th>
<th>Viral positive prevalence</th>
</tr>
</thead>
</table>
### 3.8. Ethnic Background

Ethnic backgrounds of the participants were identified. According to Statistics Canada 2006 Census, 85% of the total population have non aboriginal identity (109). In this study, out of 128 participants, 66% were non aboriginal people followed by first nation (33%, Figure 3.9). Viral prevalence in different ethnic groups is shown in Figure 3.10.

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>No</td>
<td>121</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>5 (71.4%)</td>
<td>41 (33.8%)</td>
</tr>
</tbody>
</table>

![Distribution of population according to different ethnic groups](image)

Figure 3.9: Percent of population according to ethnicity
Figure 3.10: Frequency of viral positive among different ethnic background, n=46, p=0.35

3.9. Cross Analysis of Different Parameters from the Questionnaire Information

Some multi-variant analyses were done fusing the questionnaire information. Out of 128 participants viral prevalence (38%) was higher among the participants resided outside perimeter. But the data was not statistically significant (Table 3.22).

Table 3.22: Cross analysis of viral positive count with living area of the participant, n=128; p=0.74

<table>
<thead>
<tr>
<th>Living area</th>
<th>Viral positive count</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inside perimeter</td>
<td>25 out of 72</td>
<td>34.7</td>
</tr>
</tbody>
</table>
Source of water was identified among the viral positive participants. Municipality treated water was the main source among the respondents and viral prevalence was also found high among this group (Table 3.23). Cross analysis of source of water and living area of the participant also showed higher viral prevalence (27 cases) among the participant who used municipality treated water (Table 3.24). Data was not statistically significant.

Table 3.23: Cross analysis of viral positive count with source of water at participant’s house, n=128; p=0.85

<table>
<thead>
<tr>
<th>Source of Water</th>
<th>Viral positive count</th>
<th>Viral negative count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated Water</td>
<td>27 (58.7%)</td>
<td>45 (54.9%)</td>
</tr>
<tr>
<td>Well water</td>
<td>6 (13.0%)</td>
<td>10 (12.2%)</td>
</tr>
<tr>
<td>Bottle Water</td>
<td>9 (19.6%)</td>
<td>17 (20.8%)</td>
</tr>
<tr>
<td>Both well and bottle water</td>
<td>0</td>
<td>2 (2.4%)</td>
</tr>
<tr>
<td>Both City and bottle water</td>
<td>1 (2.1%)</td>
<td>4 (4.9%)</td>
</tr>
<tr>
<td>Others</td>
<td>3 (6.6%)</td>
<td>4 (4.9%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>46</strong></td>
<td><strong>82</strong></td>
</tr>
</tbody>
</table>
Table 3.24: Cross analysis of water supply in different living area among the viral positive individuals, n=46; p=0.14

<table>
<thead>
<tr>
<th>Water supply</th>
<th>Viral positive count in different Living areas</th>
<th>Total Viral Positive count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inside perimeter</td>
<td>Outside perimeter</td>
</tr>
<tr>
<td>Treated water</td>
<td>18</td>
<td>9</td>
</tr>
<tr>
<td>Well water</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Bottle water</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>City + Bottle water</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Others</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>21</td>
</tr>
</tbody>
</table>

Frequency of stools is higher in viral diarrhoea. Cross analysis of water source, number of stools in last 24 hours with viral positive individuals showed that the viral count was higher among the participants who had higher number of stools and municipality treated water was their source of drinking water the data was not statistically significant (Table 3.25).

Table 3.25 Cross analysis of source of water with number of stools last 24 hours and viral prevalence, n=128, p=0.4
<table>
<thead>
<tr>
<th>Number of stool in last 24 hours</th>
<th>Viral prevalence in different water source</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treated water</td>
<td>Well water</td>
</tr>
<tr>
<td>&lt;5</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>&gt;=5</td>
<td>17</td>
<td>6</td>
</tr>
</tbody>
</table>

**3.10. Molecular Epidemiology**

**3.10. A. Comparison of Two Different Primers for Rota virus Assay**

The Viral Gastroenteritis Study Group (VGSG) designed a novel rotavirus primer pair which is capable of detecting rotaviruses from at least 4 different host species (human, bovine, porcine and simian). To test the new primer VGSG collected rotavirus positive (by electron microscopy) isolates. Then the positive isolates were tested by the new primer and with also three other published primers [81] data were not shown. Better performance was found by the new primer and by Gomara primer [81]. Then
VGSG tested all the clinical samples by the Gomara primer and new primer. Table shows the comparison of two different assays for the AcID samples.

Table 3.26: RT-PCR result of rotavirus by VGSG primer and Gomara primer [81]

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>VGSG primer pair +/Gomara Primer pair -</th>
<th>VGSG primer pair -/ Gomara Primer pair +</th>
<th>Both positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>E4-60</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E4-64</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E4-66</td>
<td>+</td>
<td>-</td>
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<tr>
<td>E4-68</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E4-73</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E4-77</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E4-78</td>
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<td>-</td>
</tr>
<tr>
<td>E4-90</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
### 3.10. B. Molecular Epidemiology of Individual viruses

Phylogenetic trees were drawn to see the molecular epidemiology of different viruses.

Diverse population of HAdV were found in this study. Most of the isolates were genotype F but other genotypes were also seen. Most of the isolates of HRV were genogroup A, which is the most common type causing paediatric diarrhoea.

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>E4-92</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>E4-93</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>E4-94</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>E4-96</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>E4-97</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>E4-100</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>E5-03</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>E5-20</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>E5-40</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>E5-44</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>E5-45</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>E5-49</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>E5-51</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>20</td>
</tr>
</tbody>
</table>
Figure 3.10: Phylogenetic dendrogram of Human Adenovirus strains isolated from Winnipeg during the study period. The reference strains was included for the comparison along with their accession numbers. Scale indicates genetic distance.
Figure 3.11: Phylogenetic dendrogram of Human Astrovirus strains isolated from Winnipeg during the study period. The reference stains was included for the comparison along with their accession numbers. Scale indicates genetic distance.
Figure 3.12: Phylogenetic dendrogram of Human Reovirus strains isolated from Winnipeg during the study period. The reference stains was included for the comparison along with their accession numbers.
Figure 3.13: Phylogenetic dendrogram of NoV G II strains isolated from Winnipeg during the study period. The reference stains was included for the comparison along with their accession numbers.
Figure 3.14: Phylogenetic dendrogram of Rotavirus strains isolated from Winnipeg during the study period. The reference stains was included for the comparison along with their accession numbers. Scale indicates genetic distance.
Chapter 4: Discussion

Infectious diarrhoea is a significant burden particularly among children worldwide. Annually, around 2.5 billion cases of diarrhoea occur in children less than 5 years of age. Over the last two decades this incidence rate has become relatively stable [4]. Although the mortality rate of diarrhoea has decreased during last two decades from 5 million deaths to 1.5 million but it remains the second most common incidence of death in children less than 5 years globally [4]. Each year in United States, hospitalization due to diarrhoea exceeds 210,000 children less than 5 years of age with an average of 4.5 days hospitalization. The overall inpatient cost for this hospitalization is approximately $1 billion each year [84]. The occurrence of diarrhoeal diseases varies with the seasons and child’s age. According to WHO, incidence rates are high among children in the first two years of life and the rates decline among older children. In the United States, from 1973 to 1983 the average number of deaths of children less than 5 years of age due to diarrhoea was 500 children each year [85]. It also caused the loss of 25 school days/100 children each year [86]. According to the Canadian Institute for Child Health (CICH) each year about 7000 hospitalizations, 27,000 ER visits and 56,000 physician visits are attributed only due to rotaviral diarrhoea (110).
Before 1970s, there was a scarcity of diagnostic techniques for the identification of diarrhoeal pathogens. The techniques at that time were mainly limited to bacterial and protozoal identification [87]. Electron microscopy was used to identify norovirus in 1972 and rotavirus in 1978. This technique has increased the knowledge of infectious diarrhoea dramatically. Present preference in CDC’s enteric research includes [87]:

i. Improving diagnostic facilities for known diarrhoea causing pathogens and also to identify their importance in outbreaks,

ii. Identifying unknown etiologic pathogens causing diarrhoea and

iii. Determining the means of transmission and the way to prevent disease including vaccine development and characterization of natural immunity.

After the 1970s study in Winnipeg [65], this was the first application of new techniques to detect viral epidemiology of AcID within paediatric population. The initial goal of the present study was to update the local epidemiology of AcID in children, and also to characterise various enteric pathogens isolated with clinical features of AcID, including symptoms, seasonality of the pathogens and other related clinical information.

A total of 70,473 patients visited Winnipeg Children’s Hospital Emergency Room and Winnipeg Clinic during July 2008 to January 2010. A number of 1128 patients complained about diarrhoea during the study period. Out of these 1128 patients, only 318 (28%) were able to produce stools during their visit in ER and clinic. Of these
318, we were able to collect stool samples from 242 (76%) children <18 years of age with acute gastroenteritis. Two groups of patients were enrolled. In one group only stool samples were collected from 114 (47%) children who were coded as E5. A consent, questionnaire and stool samples were collected from 128 (53%) children in the other group who were coded as E4.

In 104 (43%) cases viral etiology was identified, similar to what is reported elsewhere [88-91]. A total number of 114 viruses were identified either by EM or NAD assay or by both. Of these 114 viruses, mixed infections (>one virus) were found in 10 samples and in 94 cases single infection (one virus) was identified. The most common identified pathogens were HAdV, NoV GII and HRV with 44%, 23% and 23% respectively. In 4% (10/242) cases mixed infections have been seen.

These results differ from the literature for both paediatric outpatients and inpatients as human rotavirus is the most commonly identified virus. However due to the initial sample collection limitation of the study we missed one true rota season. So, the data of this study is expected because of the seasonality of HRV. Seasonal peaks of rotaviral diarrhoea in Canada typically occur in late winter/early spring. In Winnipeg rotavirus is normally observed from late March to the end of April. This study identified the majority of the HRV infections from April to June (12 cases 57%, Figure 3.5). In the United States, rotaviral diarrhoea is mainly observed in the cooler months of the year (October-April) [87]. Rotavirus is mainly associated with mild to severe infection which is characterised by vomiting, abdominal pain and low-grade fever [92]. High prevalence of infection is observed in children under two years of age [93, 94]. In the United States, the maximum incidence of rota infection is
concentrated among the children 6 months to 2 years of age [87] In this study rotaviruses were found mainly in children <2 years of age and was characterized by vomiting, abdominal pain, fever and diarrhoea (Figure 3.5 & 3.7). Before 1982, Group A rotavirus was considered to be the only antigenic family of rotavirus that affecting human. In 1982, an epidemic of Group B rotavirus (Common diarrhoeal pathogen for swine) was identified in China and in recent years serologic evidence of Group B infection is observed among Southeast Asian people[87, 95]. Group C rotaviruses are also swine pathogens but have been identified as human pathogens in all parts of the world [21]. Phylogenetic analysis in this study showed that the most predominant serotype of rotavirus was Group A rotavirus (Figure 3.14). In this study, we tested a new primer which is capable of identifying rota virus across four different host (human, bovine, porcine and simian) species. Like influenza virus, all rotaviruses have segmented genome which capable of undertaking antigenic variation by genetic reassortment. It is hypothesized that human Group B rotavirus arose by genetic reassortment that let the swine virus to propagate in human gut[96]. The newly designed primer of this study is capable of identifying rotavirus from different host species so to examine the zoonotic effect of rotavirus this primer can be used in future study.

Human adenovirus (HAdV) is classified into seven genogroups, it causes a wide range of clinical syndromes associated with diarrhoea in infants and young children. In comparison to rotavirus, there is an enormous scarcity of information on the HAdV associated diarrhoea in paediatric population. The present study revealed high prevalence of adenovirus among the study population (44%) which is supported by another study where 37.4% HAdV were identified among children <=14 year of age.
Adenoviruses were detected in both genders but the prevalence in males was higher than in females but the data did not reach statistical significance. The rate of infection was higher in children <2 years of age (Figure 3.4). Adenovirus infection is found throughout the year with no clear seasonality [51]. In this study the highest prevalence of adenovirus infections was found in the winter months (Figure 3.5). Phylogenetic analysis showed diverse genogroups of adenovirus. In 22 cases HAd virus F was the pathogen causing diarrhoea. In 13 cases HAd virus B were identified. HAdV E was found in only one case (Figure 3.10).

This study describes the epidemiology of NoV infection and the genetic diversity of NoV strains. The study showed that the prevalence of NoV among the paediatric population with diarrhoea was 23%. Norovirus GGII was the single subtype identified by RT-PCR. This GGII predominance is consistent with other studies on both outbreaks and sporadic cases of gastroenteritis. Two recent studies; in Northern Alberta and Quebec showed that GGII 4 was predominant in outbreak cases but in sporadic cases of gastroenteritis GGII 3 were predominant [82, 98, 99]. The present study found the majority of the cases were GII 4 variants but a few other variants were also identified (Figure 3.13). Recent evidence regarding NoV-associated gastroenteritis suggests that not only GGII.4 is predominating but that the incidence of GGII.4 associated gastroenteritis is increasing [100]. The exact reasons contributing to this increase are still unknown. But it is likely that multiple factors contribute not only to this predominant nature of GII.4 but also its increased incidence in AcID. Other studies have suggested that GGII 4 may have lower infectious dose, or that GII.4 variants of the virus may be fit to survive’ or that there may be a change in the antigenic composition of the virus [82, 101].
In this study NoV infections were found in both the genders and in all age groups. However, the prevalence of infection was higher in children <2 years of age which is similar to rotavirus infection. Patients with NoV infections have been described to experience mild to moderate symptoms [102, 103] with vomiting being prominent [104] which is the main characteristic of NoV. Other studies have also found, absence of vomiting in NoV infections [105]. In this study high frequency of vomiting in NoV infections was observed (Figure 3.7). Some studies have described that NoV associated gastroenteritis occurs mainly in the winter months [42, 106], while recent studies have also demonstrated the association of NoV infections with spring or summer months [35]. This study showed the highest prevalence of NoV infections in the winter months (Oct-Dec both in 2008 to 2009 and January both in 2009 to 2010, Figure 3.5). Thus an increase number of cases in the winter time with high frequency of vomiting support the term “Winter Vomiting Disease” for NoV.

Astroviruses are a considerable cause of AcID in children. Worldwide prevalence of astrovirus is ranging from 2.7% to 8.6% [107, 108]. This study identified 9 (7.8%) cases of astrovirus in our paediatric population. In 5 cases HAst viruses were found in children between 6 to 24 months of age and in both genders. Viral prevalence was observed throughout the year (Figure 3.5). Phylogenitic dendrogram confirmed the diverse serotypes of HAstV in our study population (Figure 3.11)
Aichivirus, a novel cytopathic small round virus, was isolated from seafood-associated AcID in 1989 [109]. According to the genetic analyses, this virus should be classified in the family of Picornaviridae [110]. In our laboratory we established an assay for Aichi virus and tested all specimens. Aichi virus is considered an emerging virus causing AcID, the virus has been detected in many countries in Europe and Asia [109, 111]. To our knowledge, there is no evidence for the presence of this virus in North America.

Of the 242 participants, demographic and clinical information were obtained from 128 cases. Out of the 128 participants, 46 (36%) viral AcID were identified. The majority of the participants in this study (56%) were residing within the perimeter Highway of Winnipeg, that is within the municipality borders of the City of Winnipeg and municipality treated water was the main source of drinking water (56%, Table 3.5&3.6). Viral prevalence was similar among the participants residing within and outside the perimeter High way in Winnipeg (35% and 38% respectively) (Table 3.22). Most of the participant of the study used municipality treated water and 59% cases of viral AcID were found in this group (Table 3.23).

Abdominal pain was the major symptom of the patients with AcID (67%) followed by vomiting (61%) and fever (50%); which is the characteristic symptoms for gastroenteritis (Figure 3.7). In 31 (24%) cases blood was found in the stool and among those 31 cases viral infections were identified in 4 (13%) cases (Table 3.10). Participants were asked whether any member of the household experienced diarrhoea within the last 4 weeks. In 42 (33%) cases other members of the house had diarrhoea before the study patients (Table 3.11). Out of 42 cases, viral infection was found in 13
cases among the study patients. Further study is needed to see whether there was any transmission of viral infection between the house members and study patients. Parents were also asked whether their children attending preschool or daycare. In 128 participants, 27 (21%) children were attending preschool or daycare and 10 cases (37%) viral infections were identified (Table 3.12).

Hand washing is very important to prevent communicable disease. We asked the parents whether they washed their children and their own hands after every stool during diarrhoea. This Study found that in 97% cases parents washed their children and also washed their own hands after cleaning their child (Table 3.15 & 3.16). Parents were also asked whether they will recommend vaccine for their child if available and 54% showed interest in vaccine whereas 34% wanted to know more about the vaccine before recommending for their children (Table 3.17 & 3.18). This information enlightens the high demand of AcID vaccine in the society.

Although viral AcID is a self limiting infection but it has a great impact on economical issue by hospital expenses, wage loss etc. Out of 128 parents, 41% took an average of 8.5 days off due to their child diarrhoea which was an average of 1306.44 CAD wages loss (Table 3.19 & 3.20). According to Canadian Institute of Child Health (CICH), parents missed 1.6 days of work to take care of their children for each rotaviral episode, costing an estimated $46 million each year (110).

In this study parents were asked whether they traveled outside Canada within the last month before their child AcID. In 7 (6%) cases travel history was found and in 5 cases viral diarrhoeas were indentified (Table 3.21). Out of 5 cases, rota and NoVs
were mainly diagnosed. Some studies showed that both NoV and rotaviral diarrhoea can occur among travelers[87]. So, we can concluded that these 5 AcID cases were associated travelling.

According to Statistics Canada 2006 Census, 15% of the total population of Manitoba were of aboriginal identity (109). In this study, 33% of the patients were First Nation people and 39% prevalence of viral diarrhoea was identified among this group. This high prevalence of disease among the group may be due to their poor living standard, lack of awareness and knowledge. Further study is needed to find out the reason of this high prevalence.

In this study our hypothesis was to see whether there is any change occurring in the AcID pathogens among the paediatric population over the time of the study. But as I mentioned earlier due the sample number of sample and the short duration of the study it is very difficult to conclude whether our hypothesis was failed or not. Although in the study we found HAdV prevalent was high among the paediatric population but it was also mentioned that we missed two potential rotavial session during the study. The last study in Winnipeg was done in 1970s and since then this study has showed the current scenario of AcID pathogen among the paediatric population and this showed there were new pathogens causing diarrhoea among the paediatric world. So, a long term study is needed to make a conclusion about our Wave theory. This study lasted a year and half and not representing the whole community in Winnipeg. Therefore, we can use the information from the study to extend the sample collection area. Further study is also needed not only in viral
etiology but also in bacterial and parasitic etiology to strengthen the knowledge and understanding of the magnitude and complexity in AcID among paediatric population.

By the improvement of laboratory diagnoses of gastrointestinal infections in children, it has become clear that, apart from rotaviruses, there are other viruses like NoV, HAd, HAst viruses play a significant role in the etiopathogenesis of AcID. However, only few etiological investigations have been performed to identify the viral agents causing diarrhoea among infants worldwide. So, there is a gap remains in the knowledge of the etiology and diagnosis of AcID. The present study highlighted the molecular epidemiology of AcID among paediatric population attending Winnipeg Children Hospital’s ER and Winnipeg Children Hospital’s Clinic. This study also analyzed clinical features, age distribution, and seasonality of AcID and strain variations of viral AcID pathogens in Winnipeg. Due to the length of the study and initial sample collection limitations this study missed potential rota viral seasons and ended with a limited number of specimens. This study found a high prevalence of HAdvirus among paediatric population. By this study it is difficult to conclude whether there is any shift of pathogens causing AcID as rotavirus the most prevalent pathogen in paediatric AcID worldwide and in Canada (110). Long term studies are needed to observe if any changes are occurring in viral pathogens causing AcID and where in the molecular level these changes are taking place. Further studies not only in viral etiology but also bacterial and parasitic etiology will strengthen the knowledge and understanding of the magnitude and complexity in AcID etiology among paediatric population. Determining the incidence and spectrum of causes of paediatric diarrhoea will identify the exact proportion of cases attributable to bacterial, viral and parasitic pathogens and decrease the present around 30-35% rate
of unknown etiology. The identification of these pathogens will facilitate public health and other health-care professionals to design effective methods for diagnosis, better management, treatment and intervention in the spread of these pathogens. Cumulatively this epidemiological data will help to achieve the millennium development goals of a reduction of child mortality by two thirds between 1990 and 2015.
Chapter 5: References


40. *Norovirus: Technical Fact Sheet*, in *National Centre for Infectious Disease, CDC*.


83. Mahta-Hayward, P., An epidemiological analysis of acute infectious diarrhoea, water, sanitation and housing infrastructure in a first Nations Reserve in Northern Manitoba., in Department of Medical Microbiology. 2007, University of Manitoba: Winnipeg.


110. www.cich.ca