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Patterns and Etiologies of Diarrheal Illness Among Two Key Immunocompromised Populations: HIV-Infected and Elderly

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Patterns and Etiologies of Diarrheal Illness Among Two Key Immunocompromised Populations: HIV-Infected and Elderly

by

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PATTERNS AND ETIOLOGIES OF DIARRHEAL ILLNESS AMONG TWO KEY IMMUNOCOMPROMISED POPULATIONS: HIV-INFECTED AND ELDERLY

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Abstract

Patterns and Etiologies of Diarrheal Illness Among Two Key Immunocompromised Populations: HIV-Infected and Elderly

by

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Doctor of Philosophy in Epidemiology

University of California, Berkeley

Professor John M. Colford, Jr., Chair

Diarrhea remains a significant cause of morbidity and mortality worldwide, especially for children and persons with compromised immune status. This dissertation considers two key subpopulations that are higher risk for gastrointestinal illness due to immunocompromise, persons living with Human Immunodeficiency Virus (HIV), and older adults over 55 years of age. Three investigations with different methodologies (population survey, meta-analysis, and case-control study) on different aspects of diarrhea burden, risk patterns and etiology in these two populations are presented.

The pathogenesis of diarrhea is complex and associated with multiple etiologies including bacterial, parasitic and viral pathogens, adverse events from medications, tumors and underlying immune status. The relative contributions of these risk factors to the overall burden of diarrheal illness is unknown and may vary by geographic location, immune status and access to anti-retroviral medications. Gastrointestinal adverse events are the most frequently cited reason for discontinuation for highly active antiretroviral therapy (HAART) and significantly influence patient adherence. As treatment decisions are increasingly made with regard to tolerability, probability of adherence, and patient quality of life, the burden of diarrhea associated with various HAART regimens may be critical in determining an individual's optimal treatment course. A systematic review to estimate the rates of diarrhea among HIV-infected individuals associated with specific HAART regimens was conducted showing protease inhibitor containing regimens to confer the highest rates of diarrhea. Diarrhea presents a persistent challenge to those living with HIV under anti-retroviral treatment and varies by regimen composition. Additionally, a prospective case control study of acute, chronic and asymptomatic diarrhea was conducted among an urban HIV+ clinic cohort to evaluate the relative risk of diarrhea due to medication and infectious pathogens.

Lastly, a random digit dial serial cross-sectional survey among 2163 adults over 55 years of age was conducted in Sonoma County, California from September 2001 to September 2005 to estimate the prevalence of gastrointestinal illness. The average monthly prevalence of gastrointestinal illness (vomiting or diarrhea) was 7.34% (6.23, 8.63), corresponding to an incidence rate of 0.99 (95% CI: 0.84, 1.48) episodes per person per year. Of those reporting gastrointestinal illness, 30.0% experienced vomiting, 23.4% sought medical care, and 10.8% took antibiotics.

CHAPTER 1

INTRODUCTION

Globally, diarrhea remains an important cause of morbidity and mortality in both developed and developing nations with an estimated two billion cases annually[1]. Diarrhea is most pronounced in infants and children in developing countries where 1.5 to 2.5 million children are lost each year to this disease. It ranks as the second leading cause of death in children under five years old[1, 2]. Children under five years of age in the developing world are estimated to experience approximately 3.2 episodes of diarrhea per year[2]. Children with compromised immunity or malunutrition are most at risk for life threatening diarrheal illness. Access to clean water, sanitation and personal hygiene play key roles in conferring risk and opportunities for prevention of diarrhea. Diarrhea can be commonly caused by a variety of bacterial, parasitic and viral organisms prevalent in food, water and soil, with Rotavirus and Escherichia coli being the most prevalent etiologies in the developing world[1].

In the developed world, diarrhea is recognized more as a self-limiting illness, though with particular risk for severity and increased mortality in subpopulations with compromised immunity[3]. Estimates of the incidence of gastrointestinal infections in the in the United States range from 211 million cases per year from national surveys to 324 million cases from community based studies[4, 5]. Many of these cases may be of infectious origin due to water or food-borne sources.

Recent studies estimating the risk of waterborne gastrointestinal infections in Canada suggesting that up to one-third of cases of gastrointestinal illness are related to waterborne transmission have heightened concern about endemic gastrointestinal illness due to drinking water even in developed countries. If these estimates are correct, then between 70.3 million to 108 million cases of gastrointestinal illness may be caused by infectious organisms in drinking water[4, 6]. A broad array of data sources presents a consistent picture of widespread, low-level microbial contamination of US surface waters used as drinking water supplies[17,18]. One estimate is that the annual morbidity and mortality related to infectious waterborne disease in the United States is $5.9-24.3 billion (1991 dollars) in both direct and indirect medical costs and lost productivity and leisure[7].

This dissertation considers two key subpopulations impacted by diarrheal illness that are higher risk for gastrointestinal illness due to immunocompromise, persons living with Human Immunodeficiency Virus (HIV), and older adults over 55 years of age. Three investigations with different methodologies (population survey, meta-analysis, and case-control study) on different aspects of diarrhea burden, risk patterns and etiology in these two populations are presented. They each specify different case definitions of diarrhea or gastrointestinal illness relevant to the particular context and research question at hand, and contend with prevalence, incidence and or etiologies, infectious and non-infectious, in each specific population and its characteristics.

Diarrheal Illness in HIV-infected Persons

Diarrhea is a significant cause of morbidity among persons with HIV with cumulative incidence estimates of 30-70% in industrialized nations and up to 100% in developing countries. The pathogenesis of diarrhea is complex and associated with multiple etiologies including bacterial, parasitic and viral pathogens, adverse events from medications, tumors and underlying immune status. The relative contributions of these risk factors to the overall burden of diarrheal illness is unknown and may vary by geographic location, immune status and access to anti-retroviral medications.

HIV+ individuals represent a sensitive subpopulation at increased risk for infectious gastroenteritis and may also be at increased risk for severe diarrhea and dying of diarrhea because of their increased

susceptibility to dehydration, their waning immunity and their frequent hospitalizations[8]. HIV patients also frequently report chronic diarrhea (daily episodes of watery stool lasting 2-4 weeks or more). Chronic diarrhea has a significant impact on patient outcomes, health care utilization and quality of life.

Numerous studies on infectious diarrhea in children, hospital patients and travelers have been published, but little is known about the importance of specific viral, bacterial and protozoan agents among HIV+ individuals in a community setting in the United States[9-11].

Previous studies have reported asymptomatic fecal carriage rates for a combination of viral, bacterial and protozoan infections to be 2.6% in a community based setting and 6% in a pediatric hospital[12]. Studies in children have reported asymptomatic carriage rates of *Cryptospordium* in 6.4% of immunocompetent children and 22% in immunodeficient children[13]. Treatment of asymptomatic children in this study significantly reduced the shedding of infectious oocysts. Among HIV+ individuals in Venezuela, high rates of *Cryptosporidium* were detected in both symptomatic and asymptomatic patients. *Cryptosporidium* infection occurred in 48.4% of patients with diarrhea and in 50% of patients without diarrhea[14]. The overall prevalence of enteric viruses in these patients was 6.4%. Viruses identified included adenovirus and picobirnavirus. The detection rate in patients without diarrhea (8.3%) was higher than in those with diarrhea (2.4%) suggesting the association between enteric viruses and diarrhea in HIV+ patients needs greater clarity. The significance of differential rates of various pathogens in asymptomatic and symptomatic gastroenteritis in HIV+ persons has yet to be established.

The epidemiology of diarrhea in the immunocompromised population is very different than that in the general population and can be potentially life threatening[3]. There are a number of non-infectious causes of diarrhea such as side effects due to medications prescribed to HIV+ individuals. This association between diarrhea and medication has increased since the introduction of highly active antiretroviral therapy (HAART) in the last quarter of 1996[15]. Highly active antiretroviral therapy (HAART) is very effective in delaying AIDS onset and has led to substantial reductions in AIDS incidence and mortality[16]. In 1996, HAART became the standard of care for all HIV infected persons[16].

However, diarrhea is a known complication of Nelfinavir and other protease inhibitors in HAART regimens[15]. T he other major cause of diarrheal disease in this population is infection with infectious pathogens. Prior to the introduction of HAART, chronic diarrhea affected 50-90% of the HIV+ population, and has been attributed to viral, bacterial, and parasitic infection[17]. A more recent study suggests that though the prevalence of diarrhea has dropped, it is still notable in the HIV+ population[18, 19]. Chronic or idiopathic HIV-related diarrhea may be associated with medication or may potentially be of infectious etiology with unrecognized pathogens.

A cross-sectional study recently conducted by Colford et al found that 47% of HIV+ participants (n=226) reported diarrhea in the 7 days prior to being surveyed[20]. The aim of this study was to measure the occurrence of diarrhea among HIV+ individuals, and to examine the relationship of diarrhea to drinking water consumption patterns, risk behaviors, immune status, as well as medication use after the introduction of HAART. The data suggested that only 30% of the diarrhea reported was attributable to side effects from the HAART medication. An increase in CD4 count was protective only for those with a low risk of diarrhea associated with medication (OR = 0.6 [0.6,

0.9]). Thirty-nine percent of participants were very concerned about drinking water quality, and 77% had never heard of the CDC safe drinking water guidelines for HIV+ individuals.

As diarrhea is a common and sometimes fatal condition in HIV-infected individuals, this concern has led to guidelines for in-home water treatment from the CDC for HIV+ persons[21, 22]. The US Congress echoed this concern for individuals who may be at greater risk for adverse effects from microbial contaminants in drinking water within the mandates of the 1996 Safe Drinking Water Act Amendments, specifically directing USEPA to identify such sensitive subpopulations and evaluate their degree of risk[8].

Recently, results of a matched case control study testing the hypothesis that consumption of regular tap water was associated with the development of cryptosporidiosis among individuals with AIDS were published[23]. Cases and controls were identified through the HIV/AIDS Reporting System in San Francisco and were matched on age, gender, race, CD4 count and date of CD4 count. Forty-nine laboratory confirmed cases and 99 controls were administered a telephone survey on water consumption, sexual behavior, exposure to animals and other risk factors associated with waterborne disease. The key finding from this study was that tap water consumption inside and outside the home at the highest exposure categories was strongly associated with cryptosporidiosis (inside the home: OR=6.76,95% CI 1.37-33.50; outside the home: OR=3.16, 95% CI 1.23, 8.13)[23]. The population attributable fraction was 85%; that is, the proportion of cases of cryptosporidiosis in San Francisco AIDS patients attributable to tap water consumption may have been as high as 85% for the study time period. The authors recommended that persons with AIDS consider avoiding tap water, but this has not been accepted as an official recommendation[23].

Gastrointestinal Illness in the Elderly

Gastrointestinal (GI) illness is recognized as a significant cause of morbidity and mortality in the elderly, and their case-fatality rate is the highest compared to other age groups[24, 25]. A study reviewing deaths due to diarrhea in the United States over a 9-year period, reported that 78% of such deaths occurred in persons aged 55 years or greater[26]. A recent review of gastrointestinal illness among sensitive populations reported case fatality rates for specific enteric pathogens 10 to 100 times higher in the elderly compared to the general population[3]. Although many infectious diseases are more problematic in the elderly because of a decline in immune function and a higher incidence of pre-existing malnutrition and dehydration, it is still not known what the principal modes of transmission are and which infectious agents are most significant.

Gastrointestinal illness remains an important issue for the elderly given the severity of disease and the disproportionate case-fatality rates seen in this group[25]. The elderly also represent a sensitive subpopulation at increased risk for infectious gastroenteritis[3,5]. Severe diarrhea and deaths due to diarrhea among the elderly may be preventable through vaccines targeted at specific enteric agents and oral rehydration programs.[24]

The elderly may be particularly susceptible to gastrointestinal infections due to a decline in gastric acid output that is thought to be associated with increasing age[27]. An age-related increase in the incidence of salmonellosis and *Campylobacter* diarrhea has also been recognized[28, 29]. Furthermore, the elderly are at increased risk for severe and fatal gastrointestinal illness[3, 24]. A recent retrospective

analysis describing the disease burden and epidemiology of gastroenteritis hospitalizations in the United States found that the elderly are at highest risk of dying during a gastroenteritis-associated hospitalization, even when compared to infants[24]. Mounts *et al.* reviewed data from the National Hospital Discharge Survey for the years 1979 through 1995. Diarrhea was listed a diagnosis on an average of 452,000 hospital discharges per year, representing 1.5% of all hospitalizations among adults. Persons over 65 years of age accounted for over 75% of hospitalizations due to gastroenteritis and the case-fatality rates were highest in these older age groups; 14.4 deaths/ 1000 discharges among those 65-74 and 24.9 deaths/ 1000 discharges among those over 75 years of age. The mean length of stay increased continuously with age. The etiology of diarrheal illness was undetermined for 78% of cases. Mounts *et al.* state that until the etiology of gastrointestinal disease can be better established, specific strategies for prevention cannot be developed.

Recent Studies on Etiology of Gastrointestinal Illness

The Sensor study, a prospective population-based cohort study with a nested case-control component was conducted to estimate the incidence of gastroenteritis and the associated pathogens in the general population in the Netherlands from December 1998 to December 1999[30]. Participants (N=4860) were identified for the cohort study from all persons registered at participating sentinel general medical practices from the Netherlands Institute of Primary Health Care. Participants completed a baseline questionnaire at enrollment and a weekly card reporting the presence or absence of gastrointestinal symptoms during six months of follow-up. They estimated an age/ gender standardized incidence of gastrointestinal illness of 283 per 1,000 person-years.

All participants were instructed to contact the study coordinator by phone if they experienced diarrhea or vomiting. The study coordinator determined whether they met the case definition of gastroenteritis and invited them to participate as a case in the nested case-control study. A case was defined as three loose stools within 24 hours or vomiting three times in 24 hours, or diarrhea or vomiting with two or more additional symptoms. The additional symptoms could be diarrhea, vomiting, abdominal cramps, abdominal pain, fever, nausea, blood in the stool, or mucus in the stool. After a case episode, a 2-week symptom free period was required before a participant could become a control or a case again. For each case, a control was invited from the main cohort matched on age, degree of urbanization and region of the country. Cases and controls completed a questionnaire about risk factors and submitted a stool specimen for analysis (cases submitted four samples per episode: day 0, 7, 14 and 21, controls submitted two samples; day 0 and 7). Stool samples were sent by regular mail to the National Institute of Public Health and the Environment and were tested for pathogenic bacteria, viruses, parasites and bacterial toxins.

Of the 1052 cases identified from the cohort, 772 (73%) participated in the case-control study and 765 (90%) of 851 invited controls participated. A total of 713 cases and 684 controls submitted at least one stool sample. A pathogen was detected in 46.1% of cases and 20.7% of controls. Twenty-one percent of cases were attributed to viral pathogens (predominately Norwalk-like virus and Sapporo-like Virus), 9% to bacterial toxins, 5% to bacterial pathogens and 6% to pathogenic parasitic organisms (age/gender standardized incidence)[30]. Among controls, non-pathogenic parasitic organisms such as *Dientamoeba fragilis* (10.7%) and *Blastocystis hominis* (20.7%) were most commonly isolated, followed by bacterial toxins (6.5%), Norwalk-like Virus (5.2%) and *Giardia lamblia* (4.9%).

A comparable study was conducted in England between August 1993 and January 1996 to determine the incidence of infectious intestinal disease in England and to estimate the incidence of gastrointestinal disease in the community attributable to microbial causes[31]. The Study of Infectious Intestinal Disease in England estimated that one in five study participants had an episode of gastrointestinal illness in one year. This investigation comprised of three principal components, a population-based cohort study comprised of 9776 participants recruited from 70 general medical practices across England and followed for 6 months monitoring gastrointestinal disease, a case-control study nested within the population cohort study, and a 12 month case-control study independent of the cohort study of persons consulting their general practitioner for GI symptoms and age, sex, practice matched controls (GP component). In the nested case-control study within the population cohort 761 cases and 555 controls were identified. In the GP component, 2893 case and 2264 controls were identified. Cases and controls for both case-control components submitted stool specimens by mail to the Leeds Public Health Laboratory for microbiological testing.

Infectious organisms or toxins were detected in 54.9% of the cases in the GP component and in 36.9% of the cases in the nested case-control component. Campylobacter (12.2%), rotavirus (8.8%) and small structured round viruses (6.5%) were the principal organisms identified from stool samples in the GP component. Among cases in the GP component, 15.3% of stool tested were positive for a pathogenic *E.Coli* species (Attaching and effacing, diffusely adherent, enteroaggregative, enteropathogenic, enterotoxigenic or vero cytotoxigenic (non-O157)). Small structured round viruses (7%) and *Aeromonas* species (5.6%) were the most commonly isolated organisms in the nested case-control component. Among cases in the nested case-control component, 11.3% of stools tested were positive for a pathogenic *E.Coli* species. Multiple organisms were detected in 11.3% of cases in the GP component and in 6.4% of the nested case-control component.

The Cooperative Research Centre for Water Quality and Treatment at the Department of Epidemiology and Preventative Medicine at Monash University in Melbourne, Australia has recently published their study on the relationship between drinking water and gastrointestinal illness and their study on the prevalence of enteric pathogens among asymptomatic individuals enrolled in their drinking water intervention trial[12, 32]. The intervention trial was designed to determine whether microorganisms in a surface water supply with minimal treatment play a significant role in gastrointestinal illness in a community. Other objectives were to test whether there was a relationship between indicator microorganisms, the amount of water consumed and health outcomes, and to provide other possible indicators of water quality.

The source water was from a pristine, protected forest catchement area with no farming, human habitation or recreational activity. The water supply was disinfected with chlorine but not filtered prior to distribution. Subjects were recruited from the suburbs of Melbourne, Australia and included families that normally drink tap water as their source of drinking water, own or were purchasing their home and had at least two children between 1 and 15 years of age. Individuals suffering from immune deficiency conditions were excluded from the study sample. The study period was 16 months, and the study sample was a total of 600 families, with 300 in each treatment arm. The study was a double-blinded, randomized, controlled trial conducted between September 1997 and February 1999. Participating families were randomly allocated to two treatment groups: one group received the active water treatment device, and the other group received a placebo unit. Neither the participants nor the plumbers installing the device were aware of what type of device a particular family received. The water treatment devices were point of use devices installed underneath the

kitchen sink. The true water treatment device consisted of a 1-micron (absolute) pre-filter and an ultraviolet lamp. The placebo device was an empty filter casing and a non-UV transmitting glass sleeve.

Stool specimens were collected at baseline (not at the time of gastrointestinal illness), and blood samples were collected at the beginning, middle and end of the study. These samples were tested for various water borne pathogens and enteric viruses. Pathogens were identified in 129 (16.2%) of the 795 stool samples collected, with pathogenic strains of *Escherichia coli* being the most common organism. Other organisms detected included *Campylobacter, Salmonella*, Adenovirus, Rotavirus, *Cryptosporidium parvum and Giardia lambia.*

The major finding of this study was that there was no difference in the level of gastroenteritis over 16 months between households with active water treatment units compare to those with placebo units. There were 0.80 episodes/per person/ per year of "highly credible gastrointestinal illness" in the treatment group and 0.82 episodes/per person/per year in the placebo group, yielding a rate ratio of 0.99 (95% CI 0.85-1.15, p=0.85). The authors note that the difference in the results between the previous Canadian studies (Payment *et al*) and their study may be due to differences in source water supplies rather than differences in study methodology[6, 12, 32, 33]. The Canadian studies were conducted in communities drawing water from a heavily polluted river that was treated, chlorinated and filtered in comparison to the pristine source in Melbourne.

Specific Infectious Agents Associated with Diarrhea

Bacterial Agents. Salmonella, *Shigella* and *Campylobacter* species are the most common causes of bacterial diarrhea[34]. These three agents accounted for 1,111 cases of gastroenteritis in 6 outbreaks of water-borne disease in 1993[34]. There were 7 deaths associated with the *Salmonella* serotype *typhimurium* outbreak. *Aeromonas* spp. are aquatic bacteria and can be found in high numbers in surface waters during the warm months of the year and are frequently isolated from stool specimens during this time. While the role of *Aeromonas* in infectious diarrhea has been controversial, certain isolates of *Aeromonas*, particularly strains of *A. hydrophila*, *A. caviae* and *A. veronii*, have been clearly linked to diarrheal disease[35]. *Yersinia*, *Plesiomonas shigelloides* and *Edwardsiella tarda* are also aquatic organisms but they are relatively infrequent causes of diarrheal disease in the United States. Nevertheless, two outbreaks have been reported where the food items tofu and bean sprouts were tainted with water contaminated with *Yersinia enterocolitica* serotype O:8[36]. *E. tarda* has not been documented to have caused outbreaks, but Bockemühl noted that infants and adults aged 50 years and older were prone to development of protracted, severe diarrheal disease when infected with this agent[37]. All older individuals in this study had bloody diarrhea, most had fever (70%) and 3 (42%) were dehydrated.

The major strains of pathogenic *E.Coli* (ie. *Enterotoxigenic E.Coli, Enteropathogenic E.Coli, Enteroinvasive E.Coli),* are also included as they are among the most important bacterial causes of childhood diarrhea and may be of relevance in the elderly[38, 39]. Their relatively high incidence among participants in the recent English case-control studies on the microbiological causes of gastrointestinal illness also motivates their inclusion[31]. Because of the low infectious dose and the high risk of infection with serious sequelae, studies on diarrheal disease should include *E. coli* O157:H7. This agent was documented as the cause of a large water-borne outbreak involving 243 individuals; 32 (13.2%) persons were hospitalized in this outbreak[40]. Infection with MAC and other non-tuberculous mycobacteria has risen in the last two decades primarily among HIV/AIDS patients, and may be also be of relevance in other immunocompromised populations.

Parasitic Agents. Cryptosporidium parvum has received intense scrutiny because of its role in both large, detected and smaller, undetected outbreaks as well as its severity in AIDS patients[41-43]. Of 17 outbreaks with an infectious etiology that were associated with drinking water in the United States in 1993, 10 (59%) were caused by either *Giardia* or *Cryptosporidium* species[44]. Other organisms such as *Entamoeba histolytica, Cyclospora, Miscrosporidia* and *Giardia lambia* are also established causes of diarrheal illness[43, 45-48]. However, the relative contribution of other organisms such as *Entamoeba Coli, Dientamoeba fragilis, Blastocystis hominis, Iodamoeba bÂtschlii* and *Endolimax nana* to the burden of diarrhea in immunocompromised populations is unclear. We will include both known pathogens and such potential pathogens for microbiological testing and evaluate their association with diarrheal symptoms in our study population.

Viral agents. There are four major families of viruses associated with viral gastroenteritis: rotavirus, enteric adenovirus, calicivirus (including Norwalk and Norwalk-like viruses) and astroviruses. Grohman *et al.* tested stool from HIV infected persons for viral etiology[48]. Viruses were detected in 35 percent of 109 fecal specimens from patients with diarrhea but in only 12 percent of 113 specimens from those without diarrhea ($p < 0.001$). Specimens from patients with diarrhea were more likely than those from patients without diarrhea to have astrovirus (12% vs. 2%, $p = 0.003$); picobirnavirus (9%vs. 2%, $p = 0.017$); caliciviruses, including small round structured viruses (6% vs. 1%, $p = 0.062$); and adenoviruses (9% vs. 3%, $p = 0.047$). They were also more likely to have a mixed viral infection (6% vs. 0%, $p = 0.006$).

Rotaviruses are the leading cause of acute gastroenteritis in newborns and small children throughout the world[1, 49]. Although rotavirus infections typically occur in younger children, disease in older children, adults and the elderly has been reported. A documented rotavirus outbreak in a nursing home of elderly residents was characterized by an attack rate of 66% and some individuals experienced a prolonged illness[49]. Adenoviruses are the second leading cause of severe diarrhea in young children. Although their role in adults is not well established, it is believed that they cause significant disease in the immunocompromised[17]. Adenovirus infections are transmitted by direct contact, small droplet aerosols, fecal-oral route, and by water[50]. About two-thirds of adenoviruses associated with diarrhea are serotypes 40 and 41.

Two new virus families, the Caliciviridae and the Astroviridae, have emerged as important causes of gastroenteritis in both adults and children and will be tested for in our study. The immunocompromised individual with diarrhea is very likely to be infected with either of these enteric viruses[48]. The enteric caliciviruses can be separated into two major groups based on their electron microscopy (EM) morphology. The first is the typical calicivirus (Sapporo-like viruses) that generally infects young children, while the second major group includes Norwalk and Norwalk-like viruses, which are seen most commonly in older children and adults. Norwalk and related viruses are the major causes of epidemic gastroenteritis. The disease is often mild but fatalities have been reported. In a foodborne outbreak in a retirement community, a mortality rate of 1.3% was demonstrated and the agent responsible, Snow Mountain Agent, was a calicivirus[51]. Other key viruses implicated in gastrointestinal illness include Enteroviruses, Parvoviruses and Hepatoviruses[52].

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CHAPTER 2

RATES OF DIARRHEAL ILLNESS RELATED TO HIGHLY ACTIVE ANTI-RETROVIRAL THERAPY:

A SYSTEMATIC REVIEW AND META-ANALYSIS

ABSTRACT

Diarrhea remains a significant cause of morbidity among persons infected with HIV. Incidence estimates reported range between 30-70% in studies from industrialized and developing nations. Gastrointestinal adverse events are the most frequently cited reason for discontinuation for highly active antiretroviral therapy (HAART) and significantly influence patient adherence. As treatment decisions are increasingly made with regard to tolerability, probability of adherence, and patient quality of life, the burden of diarrhea associated with various HAART regimens may be critical in determining an individual's optimal treatment course. The primary objective of this systematic review was to estimate the rates of diarrhea among HIV-infected individuals associated with specific HAART regimens. We conducted a systematic review of the literature using MEDLINE and EMBASE, searching for randomized controlled trials evaluating HAART regimens and reporting the cumulative incidence of diarrhea. We extracted incidence data on diarrhea morbidity and calculated summary estimates where possible. Diarrhea presents a persistent challenge to those living with HIV under anti-retroviral treatment and varies by regimen composition. Rates of diarrhea were highest among protease inhibitor containing regimens: 27.9% (95% CI 20.2, 37.0) of patients in such regimens reported diarrhea. The lowest rates of diarrhea were seen in regimens that include two nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) and a non-nucleoside reverse transcriptase inhibitors (NNRTI) (14.5%, 95% CI 6.9, 21.52).

INTRODUCTION

Diarrheal disease is a significant cause of morbidity and mortality worldwide among children and adults living with HIV/AIDS, with reported incidences of 30-70% in industrialized and developing nations[1-4]. Estimates of incidence and prevalence differ widely, depending on the population being studied, the availability of antiretroviral treatment and the study design and definition of diarrhea utilized. The pathogenesis of diarrhea is associated with multiple etiologies including bacterial, protozoaland viral pathogens, side effects from antiretroviral medications, and underlying impaired immune status[5, 6]. Previous reviews on the global burden of diarrheal illness have focused primarily on HIV-negative children[7]. Diarrheal incidence, duration, severity and mortality are higher among HIV-positive populations compared to immunocompetent populations[6]. However, to date, there has not been a formal systematic review assessing the burden of diarrheal disease among persons living with HIV and AIDS.

Where available, highly active antiretroviral therapy (HAART) has proven to be effective in delaying AIDS onset and has led to substantial reductions in AIDS incidence and mortality, yielding a growing HIV+ population in need of monitoring and treatment management[8]. Acute diarrhea (eg. two or more loose stools per day (Centers for Disease Control)), chronic diarrhea (eg. three or more loose stools per day for more than one month (World Health Organization)) and recurrent diarrhea are the adverse events most often associated with protease inhibitors and HAART[2, 5, 9]. Diarrhea can have a considerable impact on a person's quality of life, contributes to malnutrition and weight loss, and may influence treatment adherence, modification or cessation[3, 10-13]. An estimated one in four patients discontinue antiretroviral therapy within 12 months of initiation because of toxicity and tolerability concerns[14]. HAART regimens require very high levels of adherence (90-100%) for optimal virologic outcomes and prevention of resistance[15, 16]. Given the importance of strict adherence, treatment choices increasingly take into account pill burden, adverse event profiles and other factors likely to influence adherence long-term. Clinicians may tend to avoid recommending regimens associated with high rates of diarrhea.

Trials of antiretroviral agents reporting adverse events offer an accessible body of evidence with which to study the incidence of diarrhea among HIV-infected individuals on various treatment regimens. The purpose of this study is to quantify the burden of diarrheal disease among HIV+ individuals on HAART by conducting a systematic review and meta-analysis from randomized controlled trials to estimate the cumulative incidence of diarrheal disease associated with specific categories of antiretroviral treatment regimens. The regimens reviewed include nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs).

METHODS

Search strategy and study selection

Inclusion criteria were established *a priori* to minimize the potential for selection bias. Our broad search strategy was to query for observational and randomized controlled trials reporting estimates of diarrhea incidence and prevalence. Only results from randomized controlled trials are reported herein. Results from observational studies are to be reported separately.

We searched PubMed (MEDLINE (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi)) and EMBASE (www.embase.com) online electronic databases from January 1980 to April 2006 to identify potentially relevant articles. We developed and crossed separate search strings for diarrheal disease, HIV/AIDS and HAART. Modified versions of published search filters were included to identify randomized controlled trials, cross-sectional and cohort studies[17, 18]. The search terms included key words and Medical Subject heading terms such as "diarrhea", "gastrointestinal illness", "nausea", "vomiting", "Acquired Immunodeficiency Syndrome", "Human Immunodeficiency Virus", "HIV", "AIDS", and "immunodef*". The HAART search string included general terms such as "Highly Active Antiretroviral Therapy" and specific names, abbreviations and brand names for antiretroviral drugs. Three principal antiretroviral drug classes comprising HAART regimens were sought: nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs). Reference lists from included studies as well as other pertinent review articles were scanned to identify additional studies.

All randomized controlled trials (open label and blinded) evaluating antiretroviral efficacy and safety reporting rates of diarrhea were eligible for the study. Even if measures of disease (prevalence, cumulative incidence or incidence density) were not reported directly by the authors the study was included if raw data were available to allow for calculation of an estimate and 95% confidence interval by standard techniques. For inclusion, the studies were required to meet the following criteria:

1. A randomized controlled trial that prospectively examines the effectiveness of different antiretroviral regimens in suppressing plasma viral load among HIV-1-infected individuals;
2. The trial reports regimen-specific data on the cumulative incidence of diarrhea;
3. The study provided information on study design permitting evaluation of its methodological quality (eg. reported information on blinding, randomization techniques, sample size determination).

We excluded (as sources of primary data) review articles, treatment guidelines, correspondence reports, lectures, conference abstracts, letters, case studies, case control studies, controlled-concentration trials, preliminary dosing and pharmacokinetic studies without diarrhea rate data. In addition, trials with a non-randomized allocation of treatment, no comparison group reported, and studies providing rates specific to an outbreak or limited to a particular pathogen were excluded.

Non-English studies were reviewed if an abstract was available in English Only data from full text articles available in English were included. Two independent reviewers (SRS and WTAE) evaluated each of the studies for eligibility for inclusion as well as study validity. If the title or the abstract was judged by either reviewer to be potentially eligible, the full article was examined in detail. All disagreements were resolved by consensus.

Data Abstraction

Reviewers (SRS, KM) abstracted the following information from each eligible study using a standardized abstraction form and spreadsheet: year of publication, the location of the study, whether or not it was a multicenter trial, length of follow-up, the individual drugs comprising the treatment groups (drug, dose and frequency), population type (eg. age group, clinic, hospital or community based), definition of diarrhea utilized , sample size, number allocated to a specific treatment regimen or group, race/ ethnicity, gender, baseline CD4 count, baseline viral load, number

with AIDS defining conditions at baseline, biological sample collection, adverse event scale (if used), and sponsorship by a pharmaceutical company. The primary outcomes for this review were the cumulative incidence of diarrhea related to specific antiretroviral regimens (proportion of patients reporting diarrhea in a specific treatment group). Data on nausea, vomiting, study withdrawal or discontinuation due to diarrhea were also abstracted if available.

As part of the methodologic assessment for randomized controlled trials, data collection included information regarding study design: technique of randomization, generation of the allocation sequence, concealment of the allocation sequence [18,19], whether the trial was reported as "open label" or "double blind", the application of the intention-to-treat principle in the analysis, and a sample size calculation *a priori*. For trials reporting blinded portions and open label extensions, data from blinded segments were utilized when available.

Meta-analysis

Estimates of the cumulative incidence of diarrhea from selected studies were pooled by meta-analysis using StataSE 9.0 (Stata Corporation, College Station, TX, USA). Forest plots illustrating the study specific rates and summary estimates were generated in the R computer program, version 2.2.0 (2005, The R Foundation for Statistical Computing, www.r-project.org). Randomized controlled trials were stratified by regimen classes prior to data analysis to evaluate differences in rates by class.

Summary estimates with 95% confidence intervals were calculated for the outcomes of interest using three approaches: logistic regression using fixed effects, negative binomial regression for random effects and generalized estimating equations (GEE). For logistic regression and GEE models, an expanded data set was created from the diarrhea rates reported from included trials such that each subject within each study was represented individually and coded "0" or "1" for absence or presence of diarrhea during the trial. The fixed effects estimate is the log odds of the overall proportion of diarrhea. Inference is based on a straightforward binomial distribution assuming all subjects have equal probability of diarrhea and are independent.

Random effects and GEE models allow for variability within studies and between study heterogeneity[19]. The random effects and GEE models generally provide wider confidence intervals when between-study heterogeneity is present. The random effects model utilized negative binomial regression specifying a Poisson distribution within each study and a gamma distribution between studies. Given the diversity of study designs, treatment regimens, populations, definitions of diarrhea and time periods of selected studies, significant heterogeneity was expected. In order to statistically test for heterogeneity, we relied on the negative binomial regression which provides a natural likelihood ratio test of the overdispersion which is equivalent to significant heterogeneity.

Non-parametric generalized estimating equations (GEE) methods with robust standard errors and an exchangeable correlation structure were used. The GEE models were utilized for this meta-analysis as they make the fewest assumptions regarding between-study heterogeneity of the three models specified, as well as providing relatively robust inference. Consequently, only GEE summary estimates are reported in Forest plots.

RESULTS

The search strategy yielded 90 studies that satisfied the inclusion/exclusion criteria from among the 337 full-text articles evaluated (Figure 1). Of these 90 studies, 31 randomized controlled trials reporting diarrhea as an adverse event with sufficient data to calculate rates by regimen type were included in this review. The 31 randomized controlled trials were grouped for analysis by their respective treatment classes.

An organization of the meta-analysis by treatment class is presented in Figure 2. Each trial arm was considered an independent cohort followed in time for the incidence of diarrhea. Twenty studies contributing 28 treatment groups reported estimates for protease inhibitor containing regimens. Of these, 13 studies contributing 18 groups were 2 NRTI + PI regimens, and 5 studies contributing 8 groups were 2 NRTI+ 2 PI regimens. Of the eight groups using 2 PIs, two used two different protease inhibitors at full therapeutic doses ("dual PI regimens"), while six included a protease inhibitor plus a low dose ($\leq$400mg per day) of ritonavir to pharmacologically "boost" levels of the primary PI ("boosted PI regimens"). Thirteen studies contributing 17 groups represented NRTI only regimens. Three studies contributing 5 groups represented NRTI+ NNRTI regimens.

The study characteristics for each of the trials are given in Table 1. The 31 trials included in this review reported outcomes for 10,354 individuals on antiretroviral treatment. Mean length of follow-up was 45 weeks. Trials were predominately 24 (20.0%) or 48 (35.5%) weeks in length. All studies identified were multi-center trials. Study participants varied as to their previous treatment histories. Of the 31 studies, 12 (38.7%) were conducted among treatment- naïve patients and 12 (38.7%) among treatment experienced patients. Two studies did not report the treatment history of the population under investigation. The remaining 5 studies were conducted in populations with some previous treatment, but not to the drug under evaluation (eg. ZDV experienced, but PI naive), or among populations with a mixture of previous treatment profiles.

Studies reported the number of patients in each treatment group reporting diarrhea symptoms, however the definition of diarrhea utilized was variable or not reported among the studies. Ten (32.3%) studies did not report the use of any standardized scale for adverse events. Among the 21 studies describing the adverse event scale utilized, the most frequently reported scales were those of the AIDS Clinical Trials Group, National Institutes of Allergy and Infectious Diseases, "mild, moderate or severe" or "Grades 1-4" without further reference. Fifteen (48.3%) studies did not give specific information on severity of diarrhea reported (either by grade or description). Six (19.4%) studies specified that only grades 2-4 or diarrhea of at least moderate severity was reported. Four (12.9%) studies reported all grades of diarrhea. Two (6.5%) studies reported only grades 3 and 4, and three (9.7%) studies reported only "drug related adverse events".

Methodological quality

A summary assessment of methodological quality of the included randomized controlled trials is presented in Table 2. Nearly 26% of trials did not report a method of randomization. Less information was presented on generation and concealment protocols for the allocation sequence. Approximately 42% of trials did not report information on generation of the allocation sequence and approximately 84% of trials did not describe how the allocation sequence assignment was concealed to ensure adequate blinding. Approximately 45% of trials were reported as "double-

blind". The intention to treat principle was applied by 68% of the included trials during analysis. Few studies among this set of trials provided sufficient information to adequately assess their methodological quality. Overall, reporting of key methodological quality markers was poor.

Rates of Diarrhea

Summary estimates of cumulative incidence of diarrhea for all studies and stratified by regimen subgroups are presented in Table 3. We observed significant between study heterogeneity among the included studies as evaluated by a likelihood ratio test, affecting the summary estimates for all trials combined as well as for each subgroup analyzed. Consequently, further discussion is limited to pooled estimates derived from the population averaged GEE models which make the fewest assumptions regarding underlying distributions and allow for between-study heterogeneity. The GEE models yielded estimates of the cumulative incidence of diarrhea similar to those generated by the random effects models.

Sources of heterogeneity cannot be fully determined from theinformation provided in the published studies. However, differences in underlying populations investigated, length of follow-up, reporting or lack of clarity in diarrhea definitions, and variant or mixed previous treatment histories among trial participants may contribute to the observed heterogeneity. Summary estimates adjusted for length of follow-up and scaled to annual cumulative incidence are reported in Table 4. The summary estimates adjusted for length of follow-up provide an easily interpretable annual incidence versus the incidence during the "study period", where study period is variable.

The summary estimate for the cumulative incidence from all trials combined indicated that over 22% of patients on antiretroviral treatment experience diarrhea (22.55%, 95%CI: 18.89%, 26.69%). The annual cumulative incidence for diarrhea provide by the adjusted estimate was nearly identical for all trials combined (22.44%, 95% CI: 18.89, 26.69). Adjustment for length of follow-up did not impact the summary estimates substantially, suggesting this was not a major source of heterogeneity for our meta-analysis, except among the NNRTI regimen group. For this group, the LRT value and summary estimate decreased with adjustment for length of follow-up. Unadjusted estimates are reported in the Forest plots to retain the scale of original data reported from the published trials.

Protease inhibitor containing regimens had higher rates of diarrhea than NNRTI and/or NRTI regimens. For any regimen containing a protease inhibitor (including those combined with newer agents such as fusion inhibitors, or background optimized regimens), the pooled estimate of the diarrhea incidence was 25.79 (95% CI: 21.10, 31.12), compared to 17.35% (95% CI: 10.24, 27.85) for NRTI only groups. NNRTI containing regimens had the lowest rate of diarrhea. For groups treated with 2 NRTIs plus an NNRTI, the cumulative incidence of diarrhea was 14.45 (95% CI: 6.98, 27.85).

HAART regimens with single or double (including boosted regimens) protease inhibitor combinations did not differ significantly with regard to diarrhea rates (single PI: 27.86%, double PI: 27.53%). Among groups treated with 2 NRTIs plus a single PI, the pooled estimate was 27.86% (95% CI: 20.24, 37.01), and 27.53% (95% CI: 15.29,44.39) for 2 NRTIs plus two PIs. The studies contributing to these regimen classes were conducted predominately among treatment naïve patients. The two highest rates of diarrhea reported came from studies conducted in treatment experienced patients[20, 21]. Six of the eight treatment groups in the two PI regimen category included

sub-therapeutic doses (defined as 400mg or less per day) of ritonavir used as a "booster". The pooled incidence from these six boosted PIgroups was 16.44% (95% CI: 10.29, 25.22). The two remaining treatment groups in this category had full dose dual PI regimens which yielded a pooled diarrhea incidence of 60.0% (95% CI: 42.31, 75.55).

In additional sensitivity analysis, we separated out treatment groups containing full therapeutic dose ritonovir or nelfinavir to see if these groups had higher rates than other PI containing regimens. The pooled cumulative incidence estimate was 45.79% (95% CI: 40.67, 51.00) for regimens containing full dose ritonavir or nelfinavir, compared to 20.15% (95%CI: 16.16, 24.85) for all other PI regimens including those containing low-dose ritonavir "boosting", suggesting that these two agents have significantly higher incidences of diarrhea compared to other protease inhibitors.
Though our systematic review included trials of HAART efficacy from the 1990s, no significant relationship between time of publication and magnitude of the cumulative incidence of diarrhea was evident in our analysis (p=0.504).

DISCUSSION

Our meta-analysis of the cumulative incidence of diarrhea derived from randomized trials of anti-retroviral therapies indicates that 22-28% of persons receiving HAART experience diarrheal illness, with the highest rates among those on protease inhibitor containing regimens. Rates of diarrhea were substantial in non-protease inhibitor containing regimens as well (14-17%).

Consistent with previous observations, we found that randomized controlled trials are still not adequately reported[22]. Insufficient description of study design elements prevents rigorous evaluation of methodological quality which can impact validity of study results and inference. Nearly 26% of trials in our systematic review did not report a method of randomization, even fewer described their protocol on generation of the allocation sequence (41.9%) and its concealment (83.9%). Only 67.7% of studies reported applying the intention to treat principle in their analysis. Consensus guidelines such as the Consolidated Standards of Reporting Trials (CONSORT) have been previously drafted and recommended by major journals within their instructions to authors. However our observations suggest that increased standardization and discussion of methods is needed in clinical trials reporting.

We contended with a lack of standardization of reporting of adverse events from the trials reviewed for our meta-analysis. As we reviewed abstracts and full text articles for this systematic review and meta-analysis, often even if adverse events were reported, they were not always specified by symptom, category or severity. We included only trials that provided sufficient data on diarrhea. Twenty-one trials that group gastrointestinal symptoms (nausea, vomiting and diarrhea) were excluded from the review. Consequently, trials that did not delineate adverse events, or those that only reported the most frequent adverse events (and diarrhea was not among the leading AEs) could not be included in our review. Only three of the trials included in our analysis reported treatment arms with no diarrhea reported among participants. In addition, as standards of antiretroviral therapy change rapidly, with new strategies frequently adopted before publication of supportive data, any meta-analysis will be limited due to publication lag:

For those studies included in our meta-analysis, the severity and definition of diarrhea reported was variable or lacked clarity. Over 32% of trials did not report use of a standardized scale for defining diarrhea and over 48% of trials did not specify any definition of diarrhea. This between-study variability as to what grade or definition of diarrhea was reported by various trials likely contributed to the heterogeneity evident in our meta-analysis and the variance of our estimates of diarrhea.

As clinicians aim for more easily tolerable treatment regimens, one might expect an overall trend toward reduction in HAART-associated diarrhea incidence. We were able to test this hypothesis directly. There is no evidence that over time, overall rates of HAART-associated diarrhea are decreasing with selection of newer regimens. Rather, the presence or absence of protease inhibitors within a regimen had the greatest impact on the magnitude of the rate of diarrhea observed regardless of time. The inclusion of protease inhibitors in antiretroviral therapy regimens has led to marked reduction in AIDS morbidity and mortality[23]. However, as opportunistic infections decline in their prevalence among HIV-infected individuals on antiretroviral therapy, medications may emerge as a more common etiology of diarrheal illness.

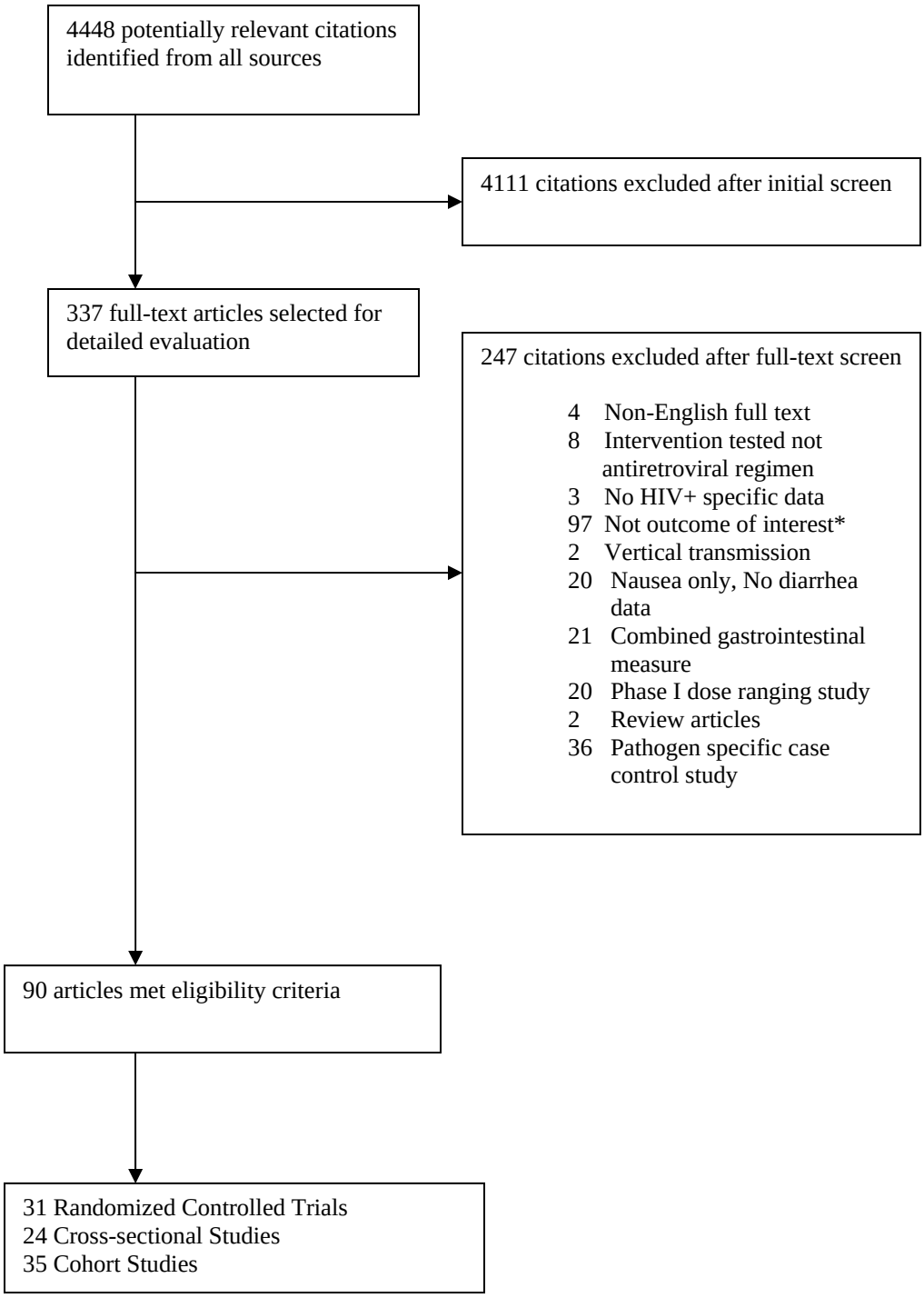
Our analysis also suggests that protease inhibitor regimens may not be uniform in their propensity to cause diarrhea. As clinical experience suggests, regimens including nelfinavir or full therapeutic doses of ritonavir resulted in higher rates of diarrhea. When these regimens are excluded, pooled diarrhea incidence for all other protease inhibitor containing regimens was 20.2%; and for the 6 regimens that included a protease inhibitor boosted with low-dose ritonavir, pooled incidence was 16.4%. These rates compare relatively favorably with the overall diarrhea rates associated with NRTI-only (17.4%) and NNRTI-based regimens (14.5%). Consequently, the data suggest that as a class, protease inhibitors may have acquired a reputation for high rates of diarrhea that is disproportionately driven by a subset of "culprit" drugs.

The magnitude of the diarrhea rates generated by our meta-analysis are lower than previously reported by many observational studies[13, 24, 25]. Difference in definitions and populations may explain this variability. First, our estimates are pooled by specific antiretroviral regimens which significantly differ in their rates of diarrhea. Previous observational studies have either not stratified their data by treatment status, reported diarrhea prevalence for those "on treatment" or "not on treatment". . Second, the diarrhea outcome measure used within the included trials counts an individual only once during the full study period, regardless of the frequency or the number of episodes of diarrhea an individual patient may have experienced. Third, post-market clinical experience may differ from trial experiences, and our analysis is based solely on randomized controlled trial data. In clinical practice, patient populations may have additional co-morbidities or variable adherence which could contribute to differing profiles of diarrhea incidence in practice versus in controlled trial settings. Finally, this review is limited to patients receiving antiretroviral treatment as a part of randomized controlled trials predominately from developed nations in Europe, Australia and North America. Diarrhea rates among HIV-infected individuals in developing nations, where the underlying morbidity and mortality related to gastrointestinal illnesses are greater, are likely to differ considerably, mandating separate assessment.

This meta-analysis indicates that there remains a substantial burden of diarrheal illness among HIV-infected persons on HAART, which varies significantly by treatment composition. As adverse events such as diarrhea impact adherence and quality of life, clinicians and patients are faced with choices in treatment course weighing tolerability, efficacy and probability of adherence. This review

collects the evidence as to the incidence of diarrhea associated with the myriad of treatment options for HIV-1.

Figure 1. Flow of reviewed literature



** NOI category includes studies on diarrhea treatment, diagnostics, endoscopy, pharmokenetics, opportunistic infection treatment, HIV salvage treatment, cost-effectiveness, phrophylaxis, non-HIV population, other HIV related conditions and co-morbidities (eg. Karposi's sarcoma,HPV, etc.)*

Figure 2. Organization of Subgroups in Meta-analysis of Diarrhea Rates

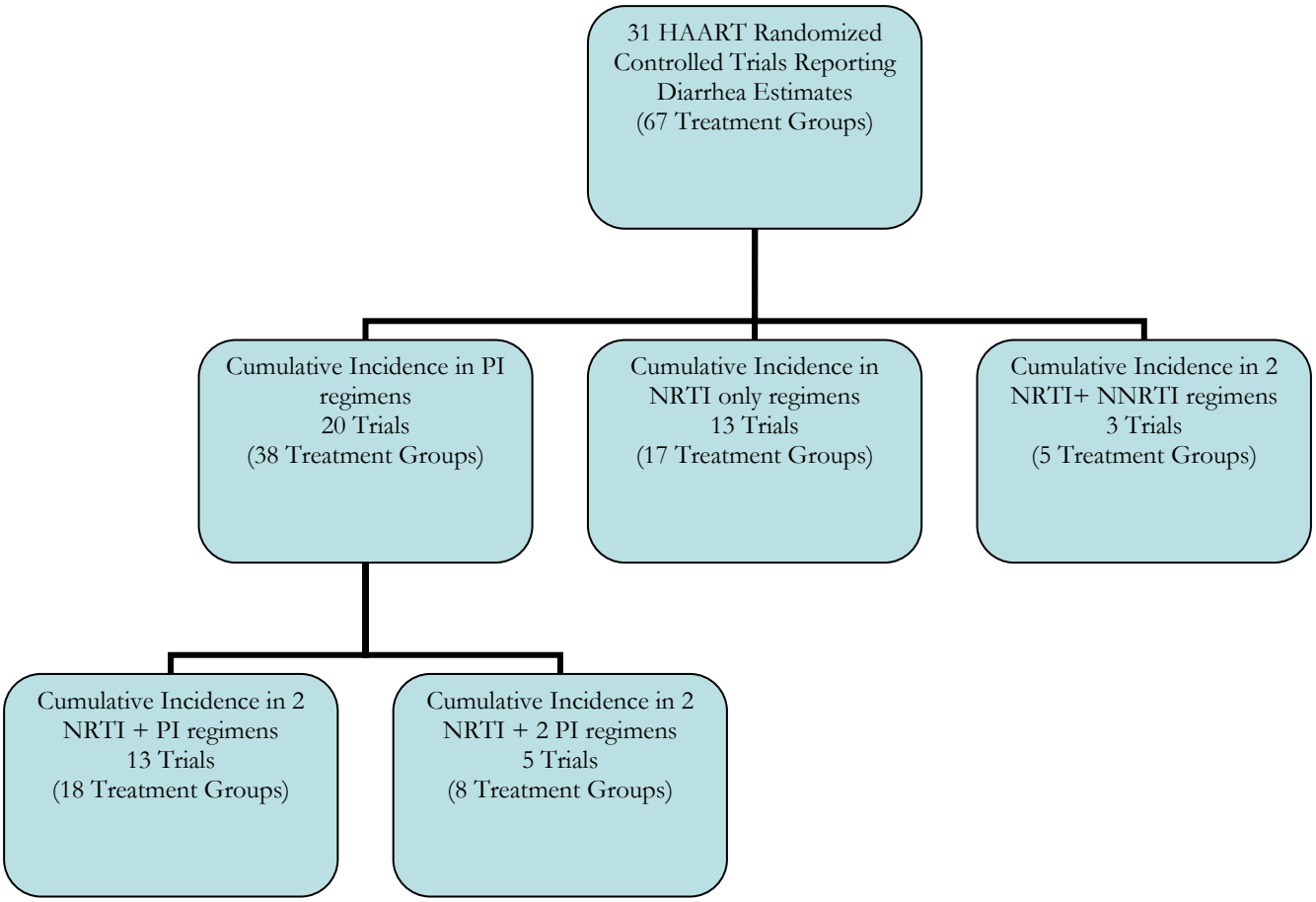


Table 1. Characteristics of included randomized controlled trials (n=31 studies)*

Reference	Total Number of Individuals	Location	Antiretroviral Regimens Compared	Regimen Classes Compared	Follow-up	Previous Treatment History
Cameron et al. 1998[26]	1090	Australia, Europe, North America	RTV+ 2 NRTI vs. 2 NRTI	2 NRTI + PI vs. 2 NRTI	26 weeks	Treatment Experienced
Carr et al. 1996[27]	50	Europe, Australia	ZDV, NVP vs. ZDV	NRTI+ NNRTI vs. NRTI	24 weeks	Treatment Experienced
DeJesus et al. 2004[28]	649	Australia, Europe, North America	ABC, 3TC, EFV vs. ZDV, 3TC, EFV	2 NRTI+ NNRTI vs. 2 NRTI+ NNRTI	48 weeks	Treatment Naive
Eron et al. 2004[29]	38	United States	LPV/RTV(1x), d4T, 3TC vs. LPV/RTV(2x), d4T, 3TC	2 NRTI+ 2 PI vs. 2 NRTI+ 2 PI	48 weeks	Treatment Naive
Fernandez-Cruz et al. 1995[30]	402	Australia, Europe, North America	ZDV vs. ZDV+ IFNA	NRTI vs. NRTI+ IFNA	48 weeks	Treatment Naive
Fischl et al. 1994[31]	48	United States	ZDV vs. ZDV + SC-48334	NRTI vs. NRTI + glucosidase inhibitor	24 weeks	Treatment Naive (ZDV experienced)
Gartland et al. 2001	105	Australia, Europe, Canada	ZDV, 3TC, NFV vs. ZDV, 3TC	2 NRTI + PI vs. 2 NRTI	52 weeks	Treatment Naive
Gatell et al. 1996	159	United States, Europe	ZDV vs. ddI 200mg vs ddI 500mg	NRTI vs. NRTI vs. NRTI	53 weeks	Treatment Experienced
Gathe et al. 2004[32]	649	Australia, Europe, North America	FPV/RTV, ABC, 3TC vs. NFV, ABC, 3TC	2 NRTI + 2 PI vs. 2 NRTI + PI	48 weeks	Treatment Naive
Gerstoft et al. 1997	552	Denmark	ZDV vs. ZDV, ddI vs. ZDV or ddI alternating weekly	NRTI vs. 2 NRTI vs. alt NRTI	88 weeks	47% Treatment Naive
Goodgame et al. 2000[33]	232	Australia, Europe, North America	APV, 3TC, ZDV vs. 3TC, ZDV	2 NRTI + PI vs. 2 NRTI	48 weeks	Treatment Naive
Gruzdev et al. 2003[34]	19	Russia	TMC125 vs. Placebo	NNRTI vs. Placebo	1 week	Treatment Naive
Haas et al. 2001[35]	327	United States, Canada	EFV, IDV + 2 NRTI vs. IDV + 2 NRTI	2 NRTI + PI + NNRTI vs. 2 NRTI + PI	24 weeks	NRTI Experienced, PI& NNRTI Naive
Haas et al. 2003[36]	62	United States	ATV 400mg, SQV+ 2 NRTI vs. ATV 600mg, SQV + 2 NRTI	2 NRTI + 2 PI vs. 2 NRTI + 2 PI	48 weeks	Treatment Experienced

Reference	Total Number of Individuals	Location	Antiretroviral Regimens Compared	Regimen Classes Compared	Follow-up	Previous Treatment History
Johnson et al. 2006[37]	237	Not reported	ATV/RTV+TDF +NRTI vs. LPV/RTV+TDF +NRTI	3 NRTI +2 PI vs. 3 NRTI + 2 PI	96	Treatment Experienced
Kumar et al. 2006[38]	254	United States, Panama, Puerto Rico, Gautemala	ABC/3TC/ZDV (COM)+ABC or ABC/ZDV (TZV) vs. COM/NFV vs. D4T+3TC+NFV	3 NRTI+NRTI vs. 3 NRTI + PI vs. 2 NRTI +PI	96 weeks	Treatment Naive
Lelezari et al. 2003[39]	491	Australia, Europe, North America	Enfurviritide+ LPV/RTV +/or TDF vs. LPV/RTV +/or TDF	Fusion inhibitor + 2 PI +/or NRTI Vs. 2 PI +/or NRTI	48 weeks	Treatment Experienced
Lazzarin et al. 2004[40]	506	Australia, Europe, North America	Enfurviritide + TORO regimen vs. TORO regimen	Fusion inhibitor + 2 PI +/or NRTI vs. 2 PI +/or NRTI	24 weeks	Treatment Experienced
Mauss et al. 1996[41]	67	Germany	ZDV, ddC vs. ZDV, ddI	2 NRTI vs. 2 NRTI	74 weeks	Treatment Experienced
Merigan et al. 1991[42]	193	United States	ZDV vs. Placebo	NRTI vs. Placebo	38 weeks	Treatment Experienced
Michelet et al. 2001[21]	47	France	RTV, SQV + 2 NRTI vs. RTV + 2 NRTI	2 NRTI + 2 PI vs. 2 NRTI + PI	48 weeks	Treatment Experienced
Mitsuyasu et al. 1998[43]	171	United States, Canada	SQV HGC + 2 NRTI vs. SQV SGC + 2 NRTI	2 NRTI + PI vs. 2 NRTI + PI	16 weeks	PI Naive
Murphy et al. 2003[44]	464	Australia, Europe, North America	D4T, 3TC, ATV 400mg vs. D4T, 3TC, ATV 600 mg vs. D4T, 3TC, NFV	2 NRTI + PI vs. 2 NRTI + PI vs. 2 NRTI + PI	28 weeks	Treatment Naive
Nadler et al. 2003[45]	211	United States	APV/RTV + 2 NRTI vs. APV + 2 NRTI	2 NRTI + 2 PI vs. 2 NRTI + PI	24 weeks	Mixed
Podzamczer et al. 2002[46]	142	Spain, Argentina	NFV, ZDV, 3TC vs. NVP, ZDV, 3TC	2 NRTI + PI vs. 2 NRTI + NNRTI	54 weeks	Treatment Naive
Richman et al. 1987	282	United States	ZDV vs. Placebo	NRTI vs. Placebo	22 weeks	Not Reported
Rodriguez-French et al. 2004[47]	249	Europe, North America	FPV, ABC, 3TCvs. NFV, ABC, 3TC	2 NRTI + PI vs. 2 NRTI + PI	48 weeks	Treatment Naive
Saag et al. 2004[48]	571	Latin America, Europe, North America	ddI, d4T, EFV vs. Emtricitabine, ddI, EFV	2 NRTI + NNRTI vs. 2 NRTI + NNRTI	60 weeks	Not Reported
Squires et al. 2000[49]	202	United States	d4T, 3TC, IDV vs. ZDV, 3TC, IDV	2 NRTI + PI vs. 2 NRTI + PI	48 weeks	Treatment Naive

Reference	Total Number of Individuals	Location	Antiretroviral Regimens Compared	Regimen Classes Compared	Follow-up	Previous Treatment History
Squires et al. 2003[50]	550	Europe, North America	TDF + current regimen vs. current regimen	NNRTI+ NRTI +/or PI vs. NNRTI+ NRTI +/or PI	24 weeks	Treatment Experienced
Trottier et al. 2005[51]	997	Australia, Europe, North America	Enfurviritide + TORO regimen vs. TORO regimen	Fusion inhibitor + 2 PI +/or NRTI vs. 2 PI +/or NRTI	48 weeks	Treatment Experienced

Table 2. Quality assessment of antiretroviral randomized controlled trials (n=31 studies)

Quality Assessment Criteria	No. of Studies (%)
Method of Randomization	
Simple	3 (9.7)
Fixed Allocation	3 (9.7)
Blocked	3 (9.7)
Stratified	14 (45.2)
Other	0 (0)
Not Reported	8 (25.8)
Generation of Allocation Sequence	
Adequate	18 (58.1)
Inadequate	0 (0)
Not Reported	13 (41.9)
Concealment of Allocation Sequence	
Adequate	5 (16.1)
Not Adequate	0
Not Reported	26 (83.9)
Described as "open-label"	
Yes	11 (35.5)
No	20 (64.5)
Described as "double-blind"	
Yes	14 (45.2)
No	17 (54.8)
Desired Sample Size Reported	
Yes	21 (67.7)
No	2 (6.5)
Not Reported	7 (22.6)
Intent-to-Treat Principle Applied	
Yes	21 (67.7)
No	1 (3.2)
Not Reported	9 (29.0)

Table 3. Cumulative Incidence (CI) of Diarrhea by HAART Regimen Subgroups

				Fixed Effects		Random Effects		GEE Population Averaged Model	
HAART Regimen	Number of Studies	Number of Treatment Groups	LRT	Incidence[1] (%)	95% Confidence Interval	Incidence[2] (%)	95% Confidence Interval	Incidence[3] (%)	95% Confidence Interval
2 NRTIs + PI	13	18	179.56 p<0.0001*	27.95	26.23, 29.73	27.59	20.96, 36.29	27.86	20.24, 37.01
2 NRTIs + 2 PI	5	8	30.28 p<0.0001*	16.32	13.63, 19.43	26.83	16.03, 44.92	27.53	15.29, 44.39
2 NRTIs + NNRTI	3	5	101.90 p<0.0001*	16.10	14.19, 18.21	14.37	5.42, 38.05	14.45	6.98, 27.52
Any PI containing regimen	20	38	516.53 p<0.0001*	24.22	23.17, 25.31	25.79	20.23, 32.86	25.79	21.10, 31.12
1 or 2 NRTIs only	13	17	237.57 p<0.0001*	21.49	19.87, 23.21	17.30	11.12, 26.82	17.35	10.24, 27.85
All RCTs	31	67	1009.06 p<0.0001*	22.26	21.47, 23.07	22.55	18.22, 27.91	22.55	18.89, 26.69

[1] Fixed Effects Cumulative Incidence (% of participants reporting diarrhea during trial)
[2] Random Effects Cumulative Incidence (% of participants reporting diarrhea during trial)
[3] GEE Population Averaged Model Cumulative Incidence (% of participants reporting diarrhea during trial)
* Dispersion parameter Likelihood ratio test of alpha=0: (no between study heterogeneity present)

Table 4. Annual Cumulative Incidence of Diarrhea by HAART Regimen Subgroups Adjusted for Length of Trial Follow-up

				Fixed Effects		Random Effects		GEE Population Averaged Model	
HAART Regimen	Number of Studies	Number of Treatment Groups	LRT	Incidence [1] (%)	95% Confidence Interval	Incidence [2] (%)	95% Confidence Interval	Incidence [3] (%)	95% Confidence Interval
2 NRTIs + PI	13	18	180.77 p<0.0001*	21.93	21.10, 22.79 p<0.516†	30.14	22.08, 41.12 p<0.226†	30.94	20.27, 43.47 p<0.287†
2 NRTIs + 2 PI	5	8	30.68 p<0.0001*	15.89	12.31, 20.26 p< 0.762†	31.12	16.02, 60.49 p<0.466†	31.63	14.61, 44.43 p< 0.586†
2 NRTIs + NNRTI	3	5	4.47 p<0.017*	10.17	8.32, 12.38 p<0.0001†	8.82	4.80, 16.22 p<0.010†	9.31	6.55, 13.07 p<0.0001†
Any PI containing regimen	20	38	513.46 p<0.0001*	24.95	23.73, 26.22 p<0.020†	26.83	20.74, 34.70 p<0.332	26.77	21.63, 33.62 p<0.261†
1 or 2 NRTIs only	13	17	221.97 p<0.0001*	21.37	19.74, 23.10 p<0.0001†	17.56	11.33, 27.22 p<0.484†	17.66	11.15, 26.81 p<0.414†
All RCTs	31	67	1005.48 p<0.0001*	21.93	21.09, 22.80 p<0.032†	22.45	18.02, 27.96 p<0.866†	22.44	18.69, 26.69 p<0.834†

[1] Fixed Effects [2] Random Effects Cumulative Incidence [3] GEE Population Averaged Model Cumulative Incidence
† p-value associated with coefficient for weeks of study follow-up
* Dispersion parameter Likelihood ratio test of alpha=0: (no between study heterogeneity present)

Figure 3. Forest Plot for Cumulative Incidence of Diarrhea for 2 NRTI + PI Regimens* ? order of studies

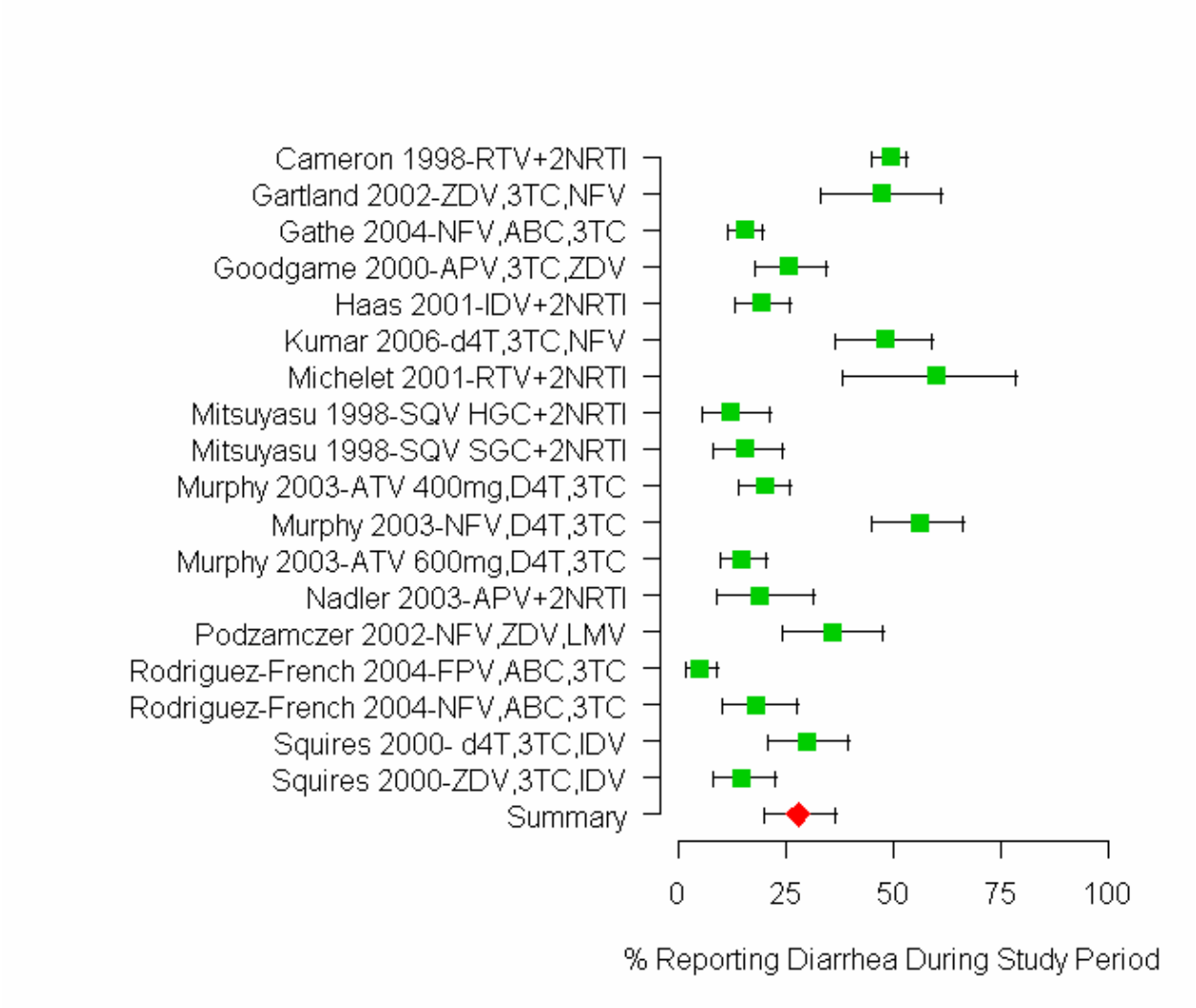


Figure 4. Forest Plot for Cumulative Incidence of Diarrhea for 2 NRTI + 2 PI Regimens

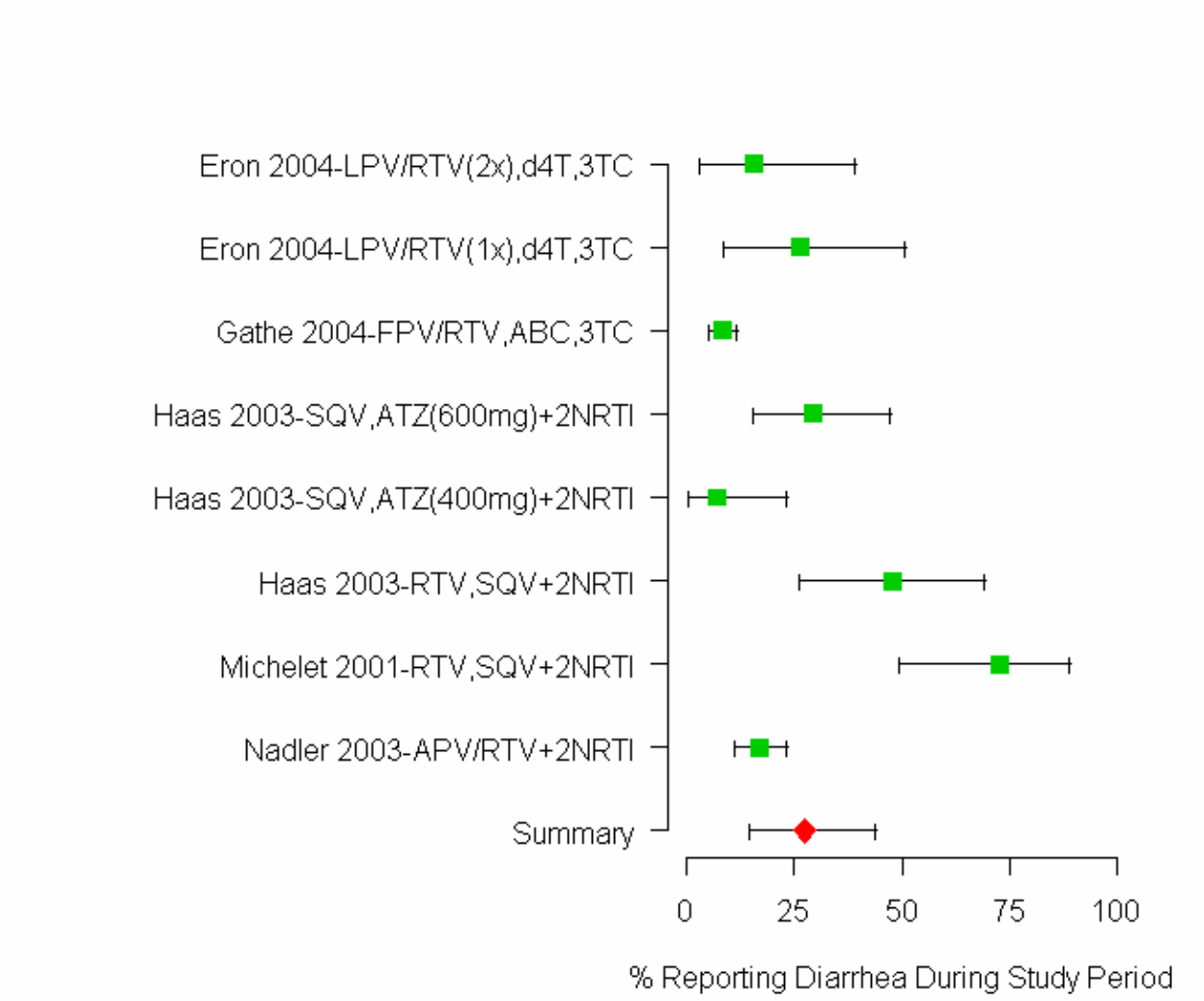
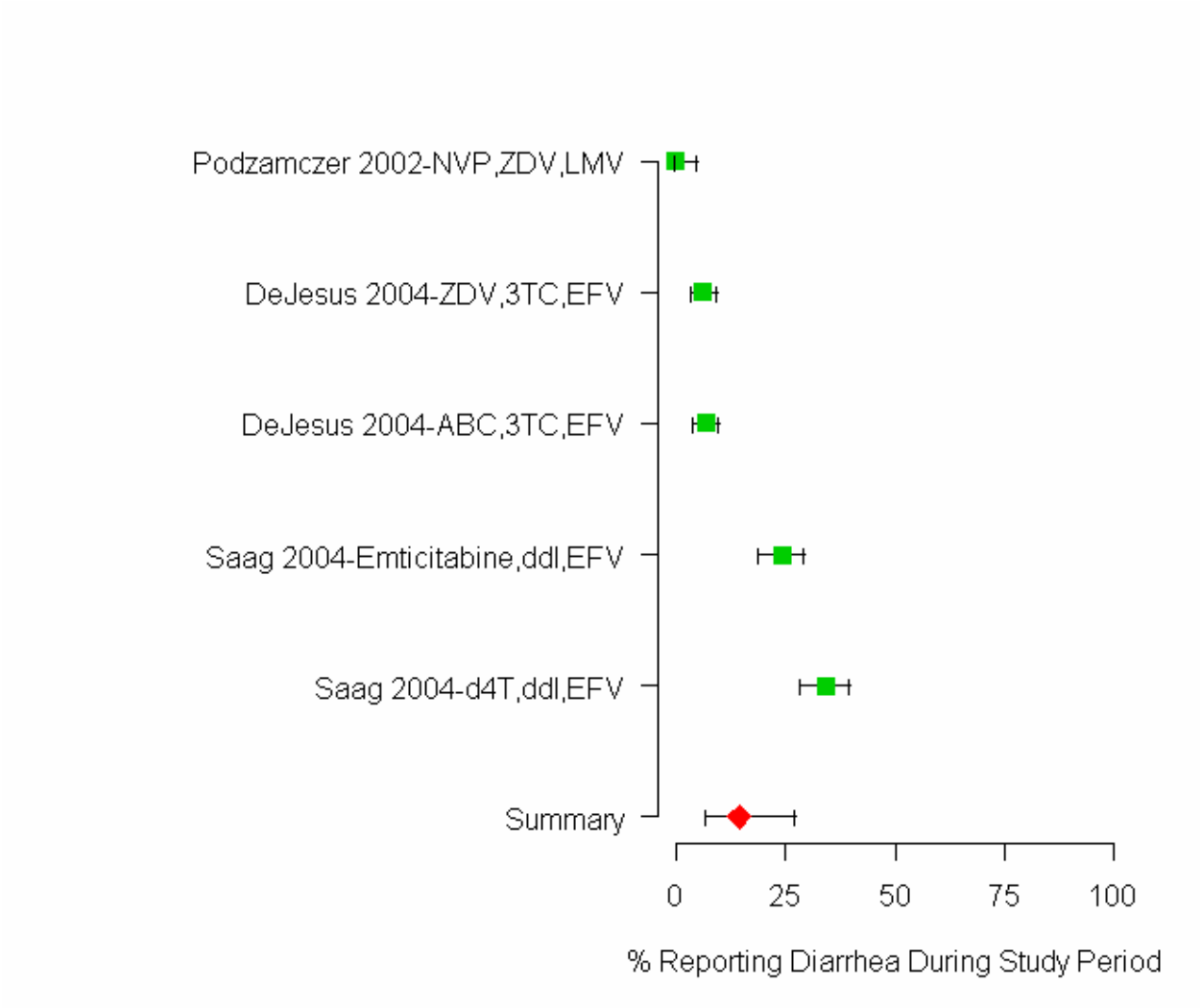


Figure 5. Forest Plot for Cumulative Incidence of Diarrhea for 2 NRTI + NNRTI Regimens



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CHAPTER 3

MAGNITUDE AND ETIOLOGY OF GASTROINTESTINAL ILLNESS AMONG COMMUNITY-DWELLING ELDERLY IN SONOMA COUNTY, CALIFORNIA

Background: Gastrointestinal illness remains an important issue for the elderly given the severity of disease and the disproportionate case-fatality rates seen in this group.

Methods: We conducted a random digit dial serial cross-sectional survey among 2163 adults over 55 years of age in Sonoma County, California from September 2001 to September 2005 to estimate the prevalence of gastrointestinal illness. Respondents were asked if they had experienced vomiting or diarrhea in the month prior to the interview, as well as questions on their water consumption patterns and related behavioral risk factors.

Results: The average monthly prevalence of gastrointestinal illness (vomiting or diarrhea) was 7.34% (6.23, 8.63), corresponding to an incidence rate of 0.99 (95% CI: 0.84, 1.48) episodes per person per year. In the month prior to interview, at least one episode of diarrhea was reported by 6.3% of respondents. Of those reporting gastrointestinal illness, 30.0% experienced vomiting, 23.4% sought medical care, and 10.8% took antibiotics. Average duration of illness was 3.2 days. Overall, seasonal temporal trends were observed with prevalence peaking in summer and winter quarters.

Conclusion: Endemic gastrointestinal illness represents a substantial burden among community-dwelling elderly impacting quality of life and prompting health care utilization.

Introduction

Gastrointestinal (GI) illness is recognized as a significant cause of morbidity and mortality in the elderly, and their case-fatality rate is the highest compared to other age groups[1, 2]. A study reviewing deaths due to diarrhea in the United States over a 9-year period, reported that 78% of such deaths occurred in persons aged 55 years or greater[3]. A recent review of gastrointestinal illness among sensitive populations reported case fatality rates for specific enteric pathogens 10 to 100 times higher in the elderly compared to the general population[4]. Consequently, the elderly represent a sensitive subpopulation at increased risk for infectious gastroenteritis and may also be at increased risk for severe diarrhea and dying of diarrhea because of their increased susceptibility to dehydration, their waning immunity and their frequent hospitalizations[3, 5].

Though various studies focusing on diarrheal illness among elderly in hospitals, acute and long-term care facilities exist, less is known about endemic gastrointestinal illness among elderly adults in community settings. Community prevalence is challenging to estimate since diarrheal illness is often undiagnosed and under-reported through routine surveillance data. Recent national surveys estimated that approximately 375 million episodes of acute diarrhea occur in the United States each year[6]. A considerable burden of these episodes may occur in elderly individuals.

We conducted a random digit dial (RDD) serial cross-sectional survey among 2163 adults over 55 years of age in Sonoma County, California to estimate the period prevalence of gastrointestinal illness, and to identify water consumption patterns and other key behavioral risk factors for waterborne diseases. This random digit dial telephone survey was modeled after the Centers for Disease Control and Prevention's Food NET survey, a nationwide active surveillance tool for foodborne gastrointestinal illness[6]. We adapted the Food NET survey to be more relevant for waterborne disease risk. As respondents were consistently interviewed over a four year period (September 2001-September 2005), we had the opportunity to view trends in the prevalence of gastrointestinal illness, as well as related health care seeking behavior, attitudes towards drinking water consumption and water purification, and potential gastrointestinal illness risk factors (eg. travel, recreational water contact, chronic disease, etc.).

Methods

A cross-sectional random digit dial (RDD) telephone survey was administered from September 2001 to September 2005 to respondents over 55 years of age in Sonoma County, California. Sonoma County has 479,929 residents, with approximately 21.3% over 55 years of age, and contains urban, semi-urban and rural regions[7]. We surveyed residents of Sonoma, Cotati, Rohnert Park, Kenwood, Valley of the Moon, Oakmont and Santa Rosa. Sonoma County was chosen in part for this study for its' high density of households above 55 years of age as illustrated by the distribution map of the over 55 population in Sonoma presented in Figure 1. This survey was conducted in parallel to complement a randomized trial of tap water treatment among households with individuals over 55 years of age.

This sample size was determined from power calculations to detect a community prevalence of gastrointestinal illness between 9% and 15%. This prevalence of gastrointestinal illness is consistent with the levels observed by the investigators in a pilot study in the general population, and in other surveys of gastrointestinal illness.

The sampling frame consisted of households within specified Sonoma County zip codes identified using a list assisted single stage Genesys-ID sampling method. The list-assisted RDD procedure ensured that households with telephone numbers that have been assigned since the publication of the current directories, as well as households with deliberately unlisted numbers, were sampled in their correct proportions, as well as screening for households likely to contain individuals over 55 years of age. The dynamic Genesys listed sample was updated quarterly.

Each quarter (or wave), 665 records were drawn, with a target of 133 interviews to be completed per quarter. A member of the household over 55 years of age was randomly selected for the interview using Computer Assisted Telephone Interviewing (CATI) methods based on the Kish grid of random numbers. We used methods consistent with previous surveys conducted by Centers for Disease Control and Prevention for FoodNet and Behavioral Risk Factor Surveillance System[6, 8]. At least 15 attempts were made to contact the selected household member on different days of the week and times of the day.

Verbal informed consent was obtained prior to the start of each interview. Respondents were informed that the survey was confidential and they may contact the study investigators and/or the University of California, Berkeley Committee for the Protection of Human Subjects for any questions. Ethical approval to conduct this study was obtained by the University of California, Berkeley Committee for the Protection of Human Subjects.

The survey was developed based on previous FoodNet surveys and a recent RDD waterborne disease survey among households with children conducted by our group[9]. During the approximately ten minute survey, participants were asked if they had experienced any vomiting or diarrhea in the past month. If they reported gastrointestinal symptoms, they were queried on secondary symptoms and medical care. In addition, participants were asked about their general health, water consumption patterns (eg. bottled, filter or tap water), recreational water exposures, travel outside the United States and other risk factors for gastrointestinal illness. All surveys were conducted in English.

Data were analyzed in Microsoft Excel 2003 (Microsoft Corporation, Richmond, WA, USA) and STATA 9SE (Stata Corporation, College Station, TX, USA). The primary outcome measure was monthly prevalence of gastrointestinal illness, defined as the number of respondents reporting vomiting or diarrhea in the previous month divided by the total number of respondents. Incidence rates and incidence proportions (or cumulative incidence rates) were calculated using standard methods as outlined by Rothman and Greenland[10]. Crude survey response rates and Council of American Survey Research Organizations (CASRO) rates were calculated for each wave. The CASRO rate is defined as the proportion of completed surveys out of all identified, potentially eligible respondents in the sample, and households where eligibility could not be determined.

Respondent data were weighted using 2000 US Census data for Sonoma County to compensate for the unequal probabilities of selection and to yield a population based estimate of gastrointestinal illness using methods similar to Food Net and the Behavioral Risk Factor Surveillance System[8, 11]. Differences between raw and weighted data were minimal.

Results

Participant Flow and Response Rate

Figure 2 outlines the flow of participants through the study, the response rate and primary outcomes. Over the four-year study period, 2163 interviews were completed, resulting in an overall response rate of 20.4%. Reasons for non-participation are listed in Table 1. Following determination of eligibility, 10.7% of respondents declined to complete the interview. The overall response rate varied negligibly from wave to wave, ranging from 19.8% to 22.7%. Another metric for respondent cooperation is the CASRO (Council of American Survey Research Organizations) rate, defined as the proportion of all eligible respondents in the sample for whom an interview was completed. The CASRO for our study was 38.9% (range: 35.5%-47.6%).

Overall GI prevalence

As indicated in Figure 2, approximately 7.3% of participants reported gastrointestinal symptoms (GI) in the month prior to being surveyed, corresponding to an incidence rate of 0.99 (95% CI: 0.84, 1.48) episodes per person per year. Diarrhea was the more common GI symptom with 6.3% of participants reporting diarrhea, and 1.9% reporting vomiting. Over the sixteen quarters of survey fielding, the reported monthly gastrointestinal prevalence ranged from 3.8% to 8.3% (Figure 3).

Distribution of GI illness by demographic factors

Demographic characteristics of the study population are presented in Table 2. Our sample was predominately white, a reflection of the overall population in Sonoma County (89.9% white 2007 Census), and largely female. GI illness monthly prevalence rates were slightly higher for women than men, but not significantly. Participants aged 55-64 years had the highest rate reported (10.65%, 95% CI: 8.4), and participants aged 75-84 years had the lowest (4.83%, 95%CI: 3.28, 6.82). Interpretation by race or ethnicity is limited by the small numbers of participants in non-white categories. However, disparities in rates of illness were seen for all communities of color compared to whites. The rate for white participants most closely resembled the overall rate for the sample.

Women reported more gastrointestinal symptoms than men in all age strata, except among respondents over 85 years of age where prevalence among men was nearly three times that of women. Rates were higher in younger age groups versus older age groups for all categories except men over 85 years of age. Among those reporting symptoms, regardless of age, participants reported more diarrhea than vomiting (Figure 5).

Temporal distribution of GI illness and trends

Gastrointestinal rates varied over time and are presented by month in Figures 6 and 7. Rates tended to increase generally in late fall of each year into winter. Mid summer peaks or rate increases near July were also seen for 2002, 2003 and 2005.. Annual estimates of the monthly prevalence varied slightly from year to year, ranging from 6.0% in 2004 to 8.4% in 2001. Figure 8 shows each calendar year of fielding separately with a three month moving average curve.

Rates by age group for the entire study period by month are illustrated in Figure 9. For most of the study period from 2001-2004 the 55-64 year age groups exhibited the strongest peaks for gastrointestinal illness regardless of time of year. From late 2004, through 2005, the over 85 year age group exceeded the rates of the younger age groups. Sample size for this study was not stratified by age group, so the number in each age group on any given month varied and could be minimal. The

overall curve of all ages is superimposed on to the series of curves by age group to smooth the fluctuations that may have been influenced by small cell size.

Monthly rainfall plot trends for Santa Rosa in central Sonoma County generally followed those for gastrointestinal prevalence in fall through spring months, especially in 2001, 2002 and 2005 (Figure 10). In 2003 and 2004, GI rates climbed in early fall without major increases in rainfall until later in the season, with offset curves compared to the other years during the study period.

Symptoms and severity

Participants reporting diarrhea or vomiting were also asked about other related symptoms indicative of more severe illness. Prevalence of secondary symptoms among these participants is presented in Table 3. Secondary to their diarrhea or vomiting, the most common other symptoms reported included stomach cramps (46.2%), nausea (35.4) and headache (29.8%). Nausea, headache and fever were significantly more prevalent among those reporting vomiting versus those participants reporting diarrhea. Twenty-nine (18%) participants reporting gastrointestinal illness reported both diarrhea and vomiting.

Mean duration of illness was 3.2 days (±4.8 days). Illness duration was slightly longer for those reporting vomiting with a mean of 4 days (± 7.0 days). Nearly a quarter of participants sought medical care for their illness, with a larger proportion seeking care among those reporting vomiting. Nearly 11% of participants reporting gastrointestinal symptoms took antibiotics or missed work or school. Again, a larger proportion of these cases were those reporting vomiting.

Water consumption patterns

As drinking water may be a risk factor for gastrointestinal illness, participants were queried on their water consumption patterns. The majority of participants reported using municipal tap water at home (84%.7). Water source at home did not vary between those reporting gastrointestinal symptoms and those not reporting symptoms.

Over 30% of participants reported treating their water at home, and nearly 15% of participants reported using bottled water as their primary dinking water source. Type of water treatment used or type of water filter used if any also did not influence risk of gastrointestinal illness in this sample. Bottle water usage as well as usage of faucet mounted and reverse osmosis filters was higher among those reporting gastrointestinal symptoms, however, not substantially higher and not significant for predicting risk (eg. $OR_{bottled}$= 1.2, 95%CI: 0.7, 1.8). Increased usage of water treatment modes among symptomatic individuals may reflect their awareness and concern for waterborne illness, rather than risk related to these products.

Risk factors for GI illness

Several known risk factors for gastrointestinal illness besides drinking water were evaluated for risk in our sample and are presented in Table 5. Foreign travel, recreational water exposures, and intake of untreated recreational water sources did not predict any gastrointestinal risk in this sample. Increased risk for gastrointestinal illness was associated with history of chronic illness with expected gastrointestinal symptoms (OR= 8.9, 95% CI: 5.7, 13.8), and with history of HIV or other immunosuppression (OR= 6.5, 95%CI: 1.9, 19.05).

Though more women reported symptoms than men in our sample, gender was not a significant predictor of gastrointestinal illness in this population (OR=1.2, 95% CI: 0.8, 1.7). African American, Latinos, and Native Americans had higher rates of illness in our sample. Even though these groups were minimally represented in our overall sample, they are likely to be at higher risk for illness compared to whites. African Americans were five times more likely to report symptoms compared to whites in our sample (OR=5.0, 95% CI: 0.8, 21.2, p=0.0086). Similarly, Native American participants were also five times more likely to report symptoms (OR= 5.4, 95%CI: 0.5, 33.08, p=0.0252) and Latinos were over two times more likely to report symptoms than whites (OR= 2.4, 95% CI: 0.8, 5.8, p=0.049). No Asians in our sample reported symptoms.

The younger age groups were more likely to report symptoms in our sample. Participants aged 55-65 years of age were nearly twice as likely to report gastrointestinal illness (OR=1.9, 95%CI: 1.4, 2.7). The lowest risk among participants aged 75 to 85 years old (OR= 0.6, 95% CI: 0.4, 0.9).

Discussion

This survey of community dwelling, non-institutionalized elderly adults found a considerable burden of endemic gastrointestinal illness (diarrhea and/or vomiting) with an overall monthly prevalence of 7%. Though diarrhea was more prevalent than vomiting in our sample, vomiting was indicative of additional secondary symptoms and higher levels of medical care seeking than diarrhea. Our findings are within the 5-10% range for gastrointestinal illness prevalence previously reported by other recent general population studies in North America (Table 6)[8, 12-14].

In the United States, Foodnet random digit dial telephone surveys estimated a monthly prevalence of acute diarrheal illness of 5.1%, with estimates for those over 55 years of age ranging from 2.2% to 3.6% in each cycle, significantly lower than in younger age groups[8]. This study included only diarrhea in the past month in their case definition. We also observed a decrease in rates with age, however, our overall prevalence was higher, with a 6.3% prevalence of diarrhea in the past month for those over 55 years of age.

More closely resembling our gastrointestinal case definition, the Canadian Public Health Agency conducted a cross-sectional telephone survey of 4,612 residents in British Columbia from June 2002 to June 2003, asking participants if they had experienced diarrhea or vomiting in the past 28 days[14]. They estimated a monthly prevalence of GI illness (diarrhea and/or vomiting) of 9.2% (95% CI: 8.4, 10.0). The majority of their sample was adults aged 25-64 who had had a monthly prevalence of 10.4% (95% CI: 9.3, 11.6). Approximately 16% of their sample was over 65 years of age and prevalence estimates were lower in these age groups compared to the younger adults, children and infants surveyed. For those aged 65-69, they reported a monthly prevalence of 7.5% (95% CI: 4.4, 11.7). By comparison, we are reporting a monthly prevalence of 6.5% (95% CI: 4.6, 8.8) for those 65-74 years of age.

Though the rates in adults over 55 years of age may be lower than in infants, children or other younger age groups, the severity of illness may be greater when they do experience GI sypmtoms. The elderly may be particularly susceptible to gastrointestinal infections due to a decline in gastric acid output that is thought to be associated with increasing age[15]. An age-related increase in the incidence of salmonellosis and *Campylobacter* diarrhea has also been recognized[16, 17].

Furthermore, the elderly are at increased risk for severe and fatal gastrointestinal illness. A retrospective analysis describing the disease burden and epidemiology of gastroenteritis hospitalizations in the United States found that the elderly are at highest risk of dying during a gastroenteritis-associated hospitalization, even when compared to infants[1]. Mounts *et al.* reviewed data from the National Hospital Discharge Survey for the years 1979 through 1995. Diarrhea was listed a diagnosis on an average of 452,000 hospital discharges per year, representing 1.5% of all hospitalizations among adults. Persons over 65 years of age accounted for over 75% of hospitalizations due to gastroenteritis and the case-fatality rates were highest in these older age groups; 14.4 deaths/ 1000 discharges among those 65-74 and 24.9 deaths/ 1000 discharges among those over 75 years of age. The mean length of stay increased continuously with age.

This study had several limitations. It was a serial cross-sectional design over a four year period asking about symptoms in the past month, not a prospective study which may provide greater accuracy and precision. Prospective studies have in the past yielded lower estimates of gastrointestinal illness compared to retrospective studies[18]. Our group also had a prospective randomized controlled trial of in home water treatment among adults over 55 years of age conducted in parallel to this telephone survey[19]. Estimates of episodes of diarrhea ranged from 2.26 to 3.64 per person per year, and 1.69 to 2.83 episodes per person per year for "highly credible gastrointestinal illness", a combination of vomiting, nausea and diarrhea[19]. In comparison, in the survey we estimated 0.99 episodes per person year for diarrhea and/or vomiting, lower than the estimates from the prospective study.

Additionally, though most US households have landline telephones, our study could underestimate the burden of illness by missing those without access to such telephones such as the homeless, marginally housed, institutionalized, low income households, or most importantly, households with only cell phones. Cell phones are not included in the phone numbers we purchased for selection of the sampling frame. Prefixes for cell phones are also restricted for random digit dial calling. Also, though we reported rates for minority populations, our sample was largely white limiting its' broad generalizability to more diverse communities. Another limitation of this study is the lack of pathogen specific rate information, and the inability to clearly distinguish etiology of gastrointestinal risk attributable to infectious organisms from due to chronic conditions and diseases.

Nevertheless, among our elderly population, we found a substantial burden of gastrointestinal illness that impacted their quality of life, activities and health care usage. Consequently, the economic, productivity and quality of life impact due to even low prevalence GI related morbidity may be considerable in a national context, as well as the impact on the health care system itself. Estimates from Canada suggest that acute gastrointestinal illness represents an annual per capita cost of $115 Canadian[13]. Comparable costs may occur in the United States.

Figures and Tables

Figure 1. Geographic Distribution of Sonoma County Population Over 55 Years of Age

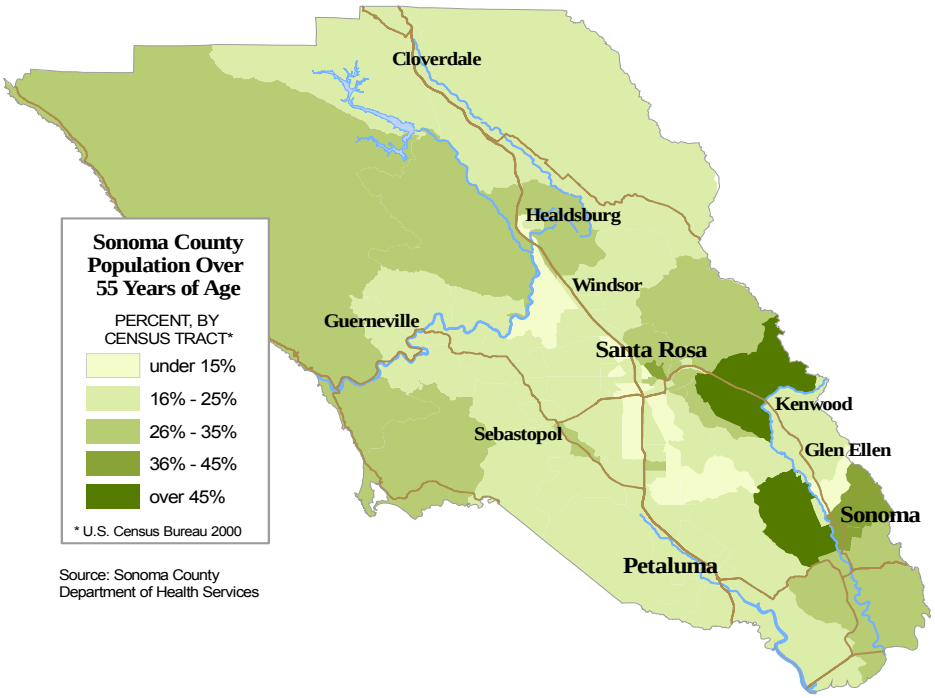


Figure 2. Response Rate and Study Outcomes

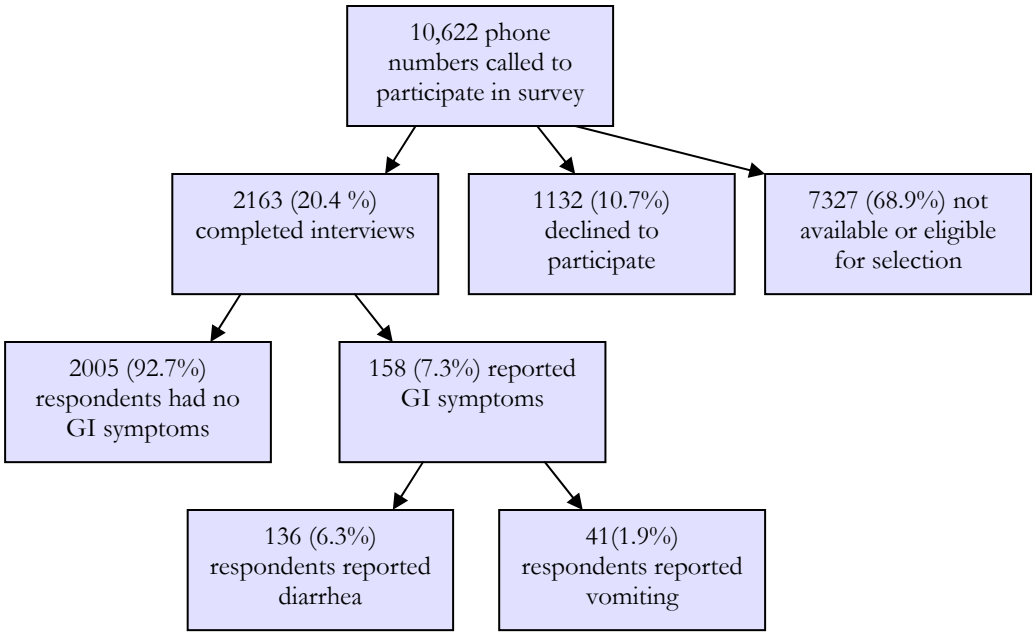


Table 1. Survey Completion Rate and Reasons for Non-Participation (N=10,622)

	N	%
Completed Surveys	2163	20.4
Incomplete by type:		
Declined Participation after Selection	1132	10.7
Non-working phone number	678	6.4
Ring, but no answer	1194	11.2
Not a private residence (ineligible)	460	4.3
No eligible respondent in household	1316	12.4
Selected respondent not available	981	9.2
Prohibitive language barrier	43	0.4
Terminated in middle of call	24	0.2
Busy line	195	1.8
Impairment	91	0.9
Technological barrier	1986	18.7
Hang up before selection process	359	3.4
Total Phone Attempts	10,622	100%

Table 2. Demographic Characteristics and Monthly Prevalence of Gastrointestinal Illness

		Proportion of Survey Respondents (%) N=2163	Monthly Prevalence of GI Illness % (95% CI)	(95% CI)
Gender	M	35.64	6.49	(4.85, 8.46)
	F	64.36	7.76	(6.41, 9.29)
Age	55-64	31.45	10.65	(8.40, 13.27)
	65-74	28.72	6.50	(4.67, 8.78)
	75-84	29.73	4.83	(3.28, 6.82)
	≥85	9.57	6.00	(3.14, 10.25)
	Not available	3.93	-	-
Race/ Ethnicity	White	95.19	6.94	(5.86, 8.13)
	African American	0.51	27.27	(6.02, 60.97)
	Asian/PI	0.87	0	-
	Native American	0.32	28.57	(3.67, 70.96)
	Hispanic/ Latino	1.85	15.00	(5.71, 29.83)
	Other/Multiracial	1.57	18.18	(5.19, 40.28)
	Not available	1.25	6.25	(0.76, 20.81)

Figure 3. Monthly Prevalence of Gastrointestinal Illness by Survey Wave (Quarter)

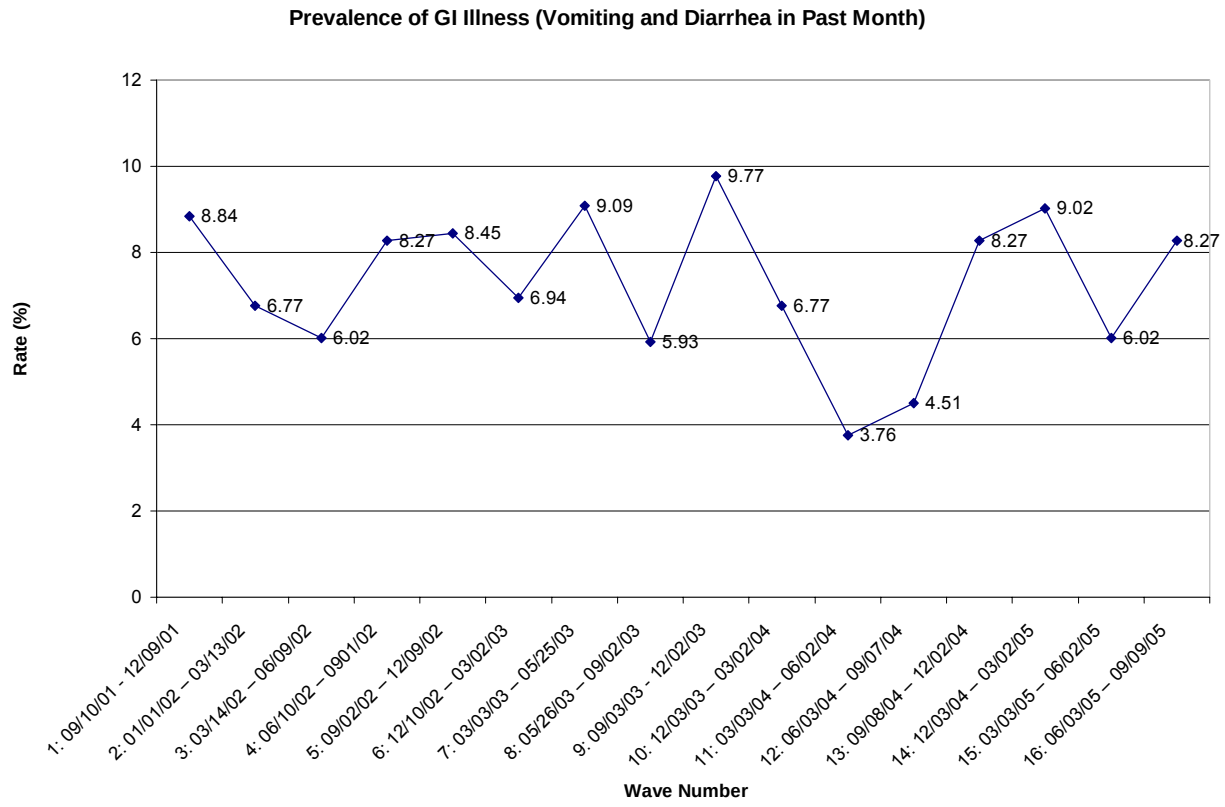


Figure 4. Monthly Prevalence of Gastrointestinal Illness by Age Group and Gender

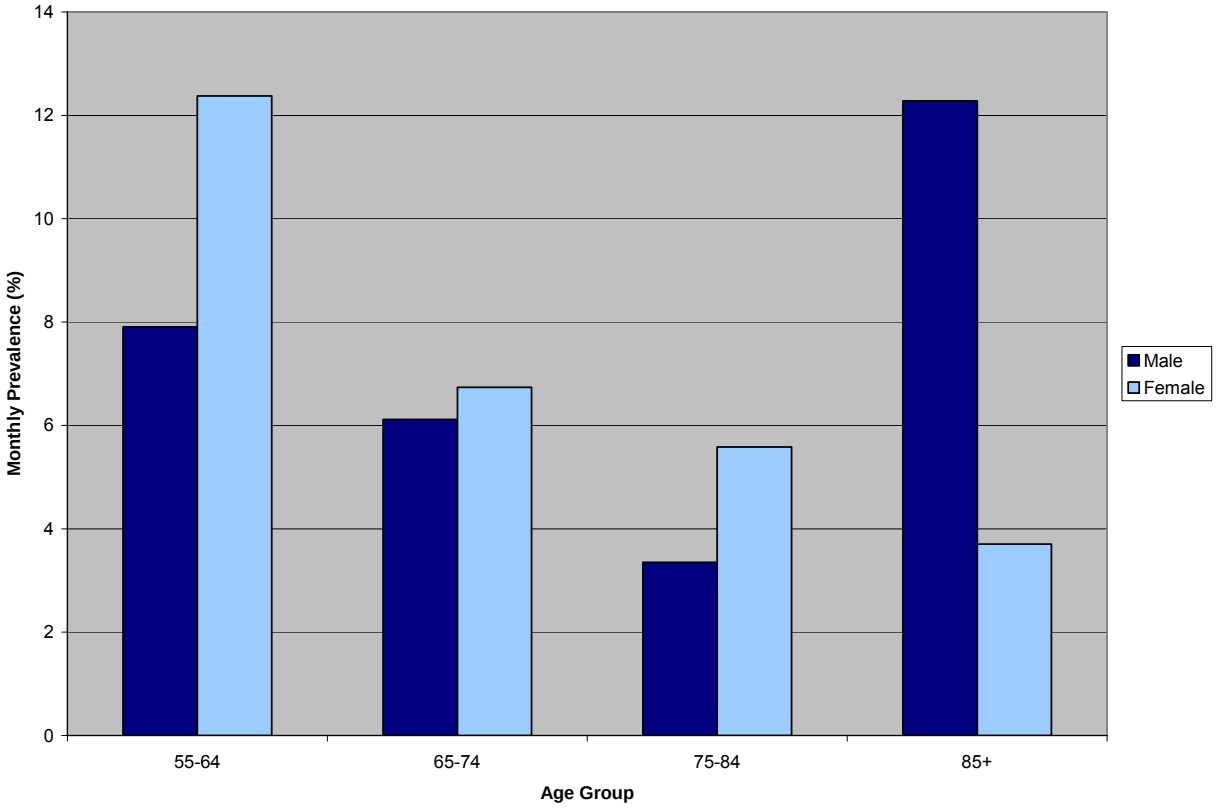


Figure 5. Prevalence of Vomiting and Diarrhea by Age Group among Those Reporting Gastrointestinal Illness (n=158)

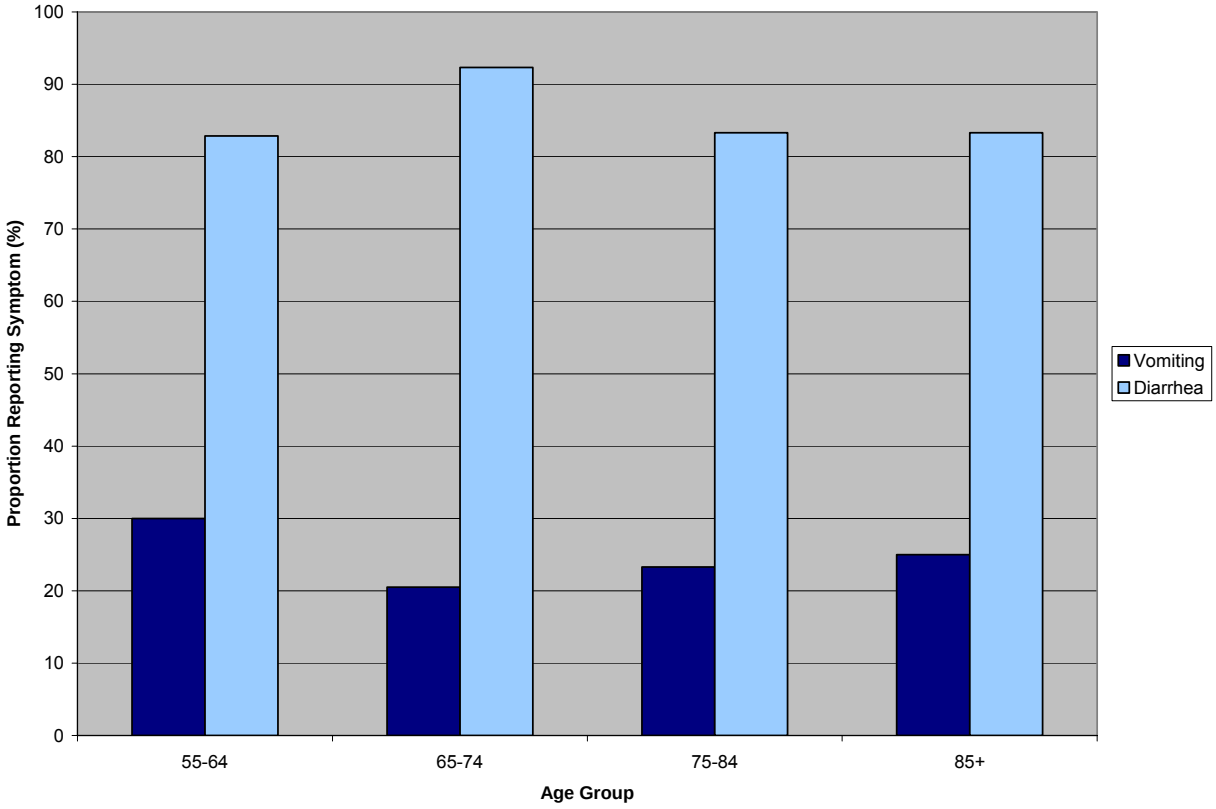


Figure 6. Monthly Prevalence of Gastrointestinal Illness by Month

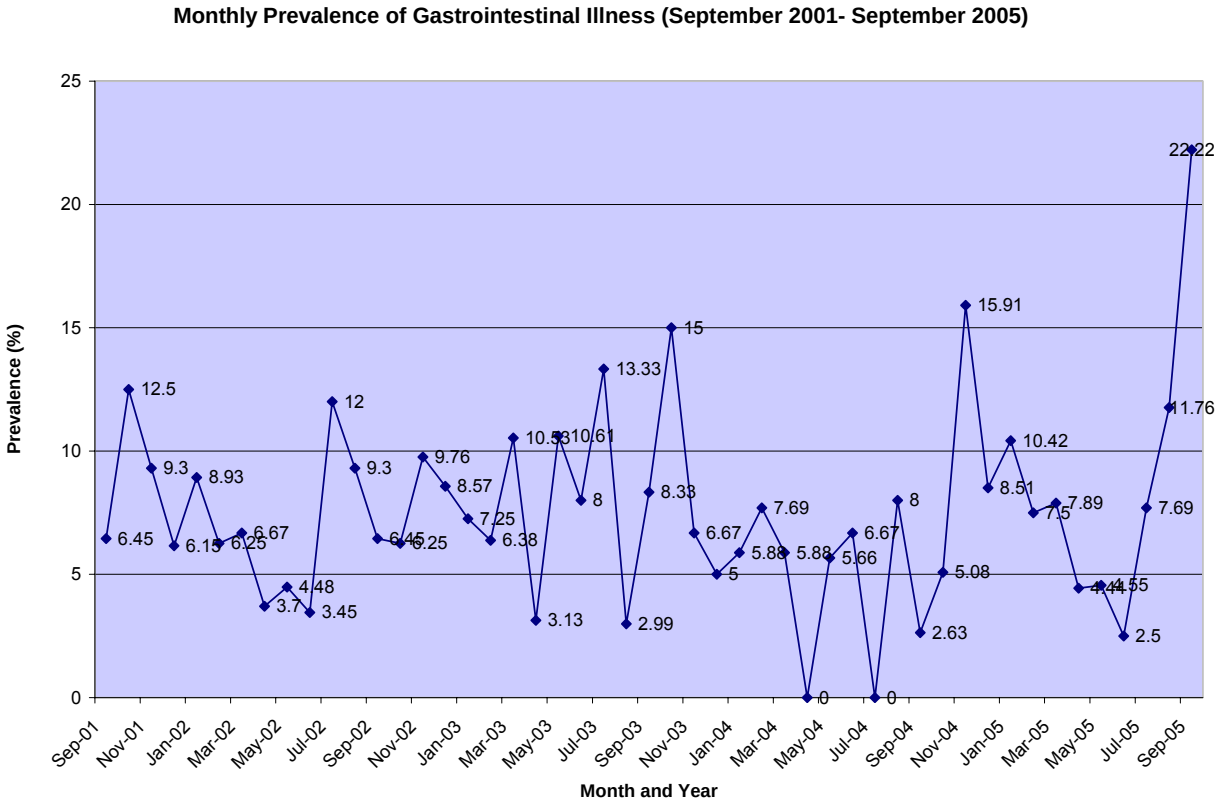


Figure 7. Monthly Prevalence of Gastrointestinal Illness by Month with Three Month Moving Average Trendline

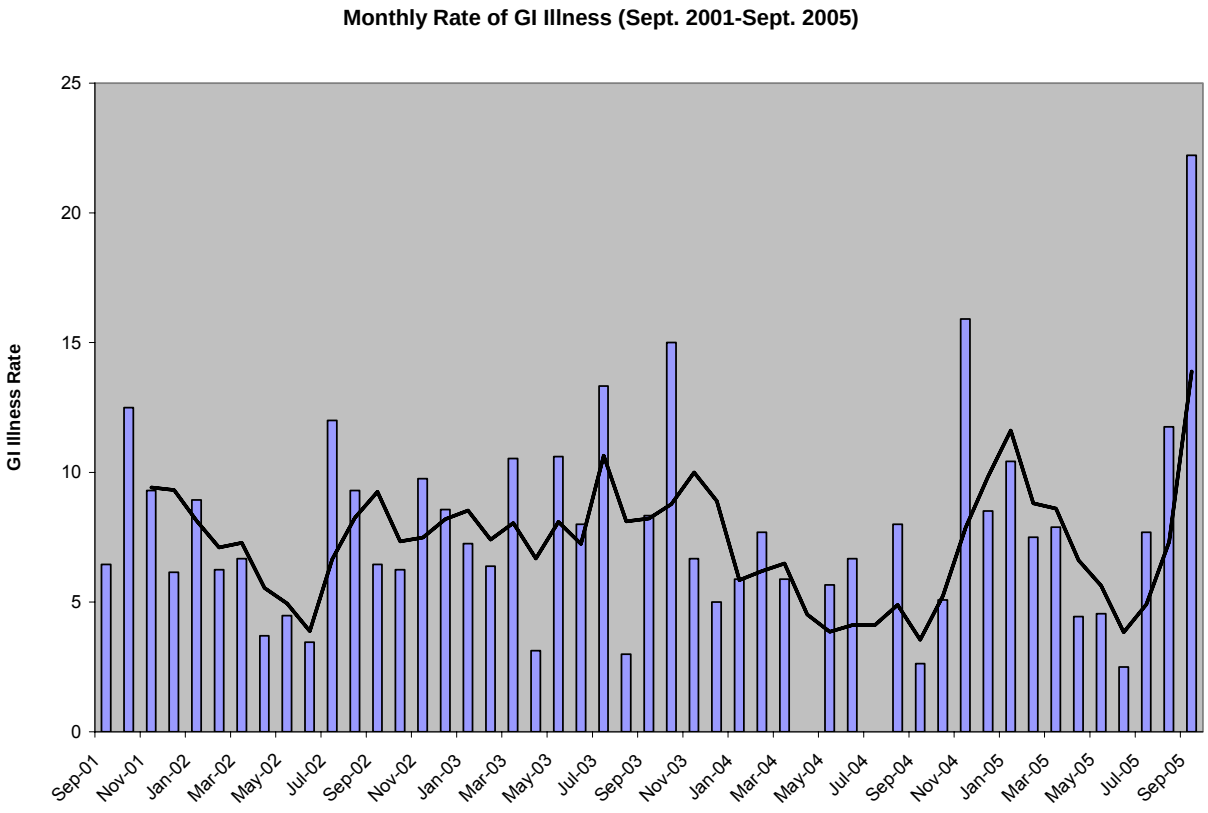
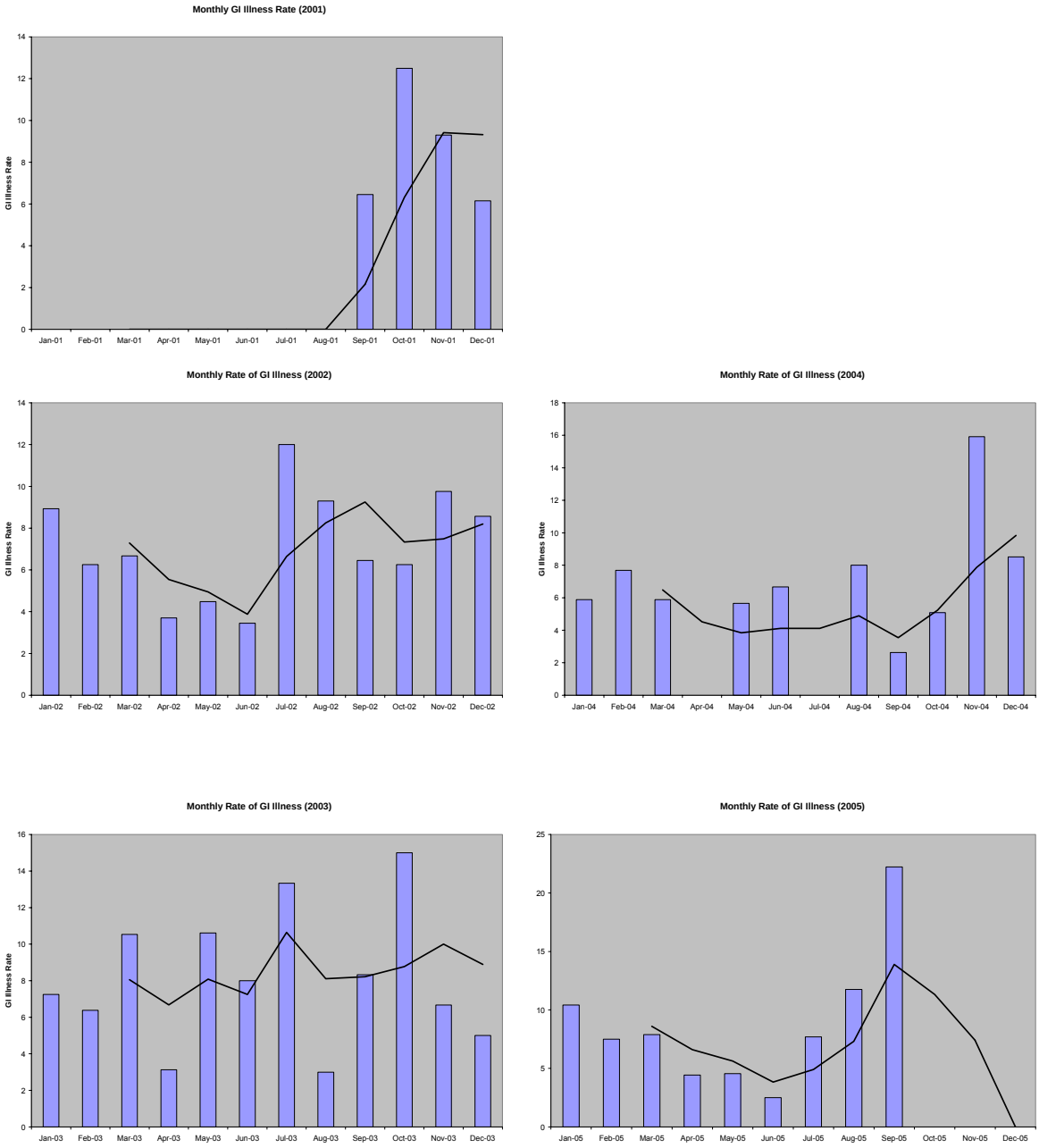


Figure 8. Monthly Prevalence of Gastrointestinal Illness by Month Tiled by Year of Fielding (2001-2005) with Three Month Moving Average Trendline



** Study period was from September 2001 to September 2005, thus partial year data shown above for 2001 and 2005.*

Figure 9. Monthly Prevalence of Gastrointestinal Illness by Age Group by Month with Three Month Moving Average Trendline

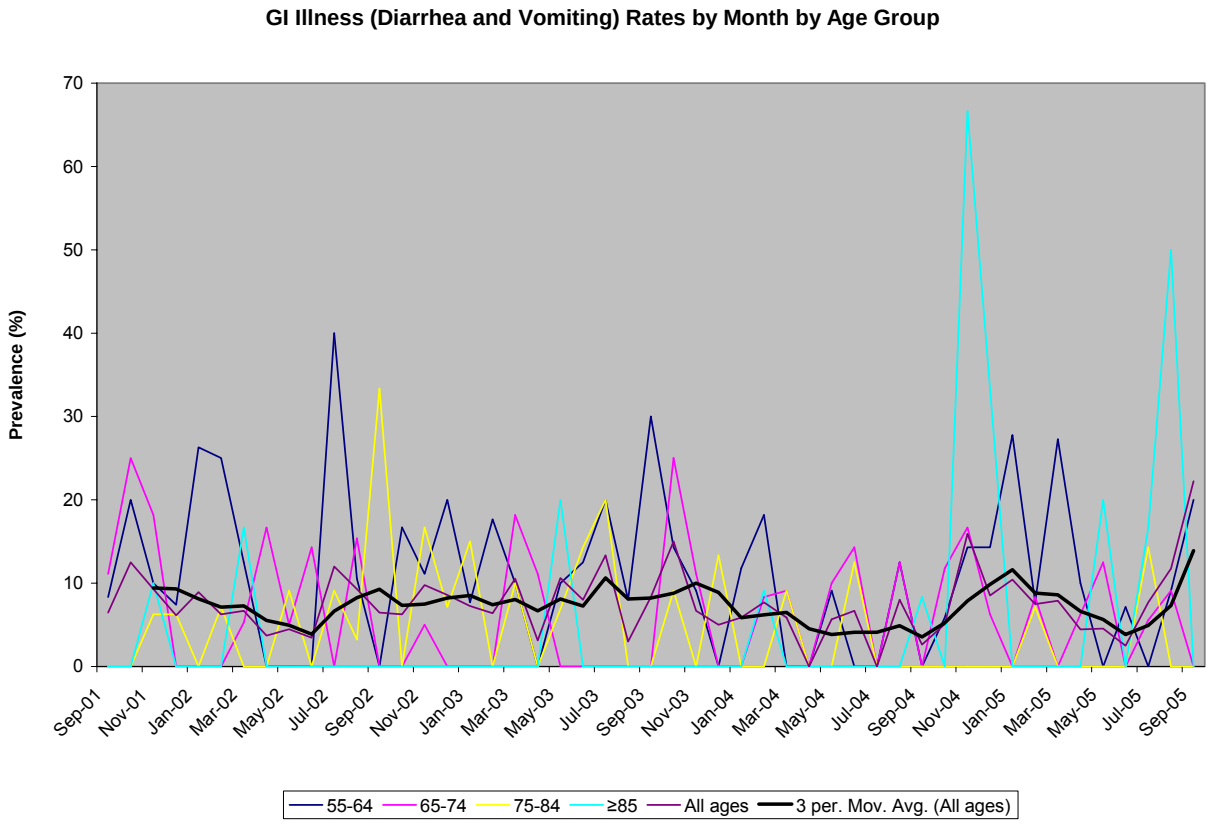


Figure 10. Monthly Prevalence of Gastrointestinal Illness and Rainfall

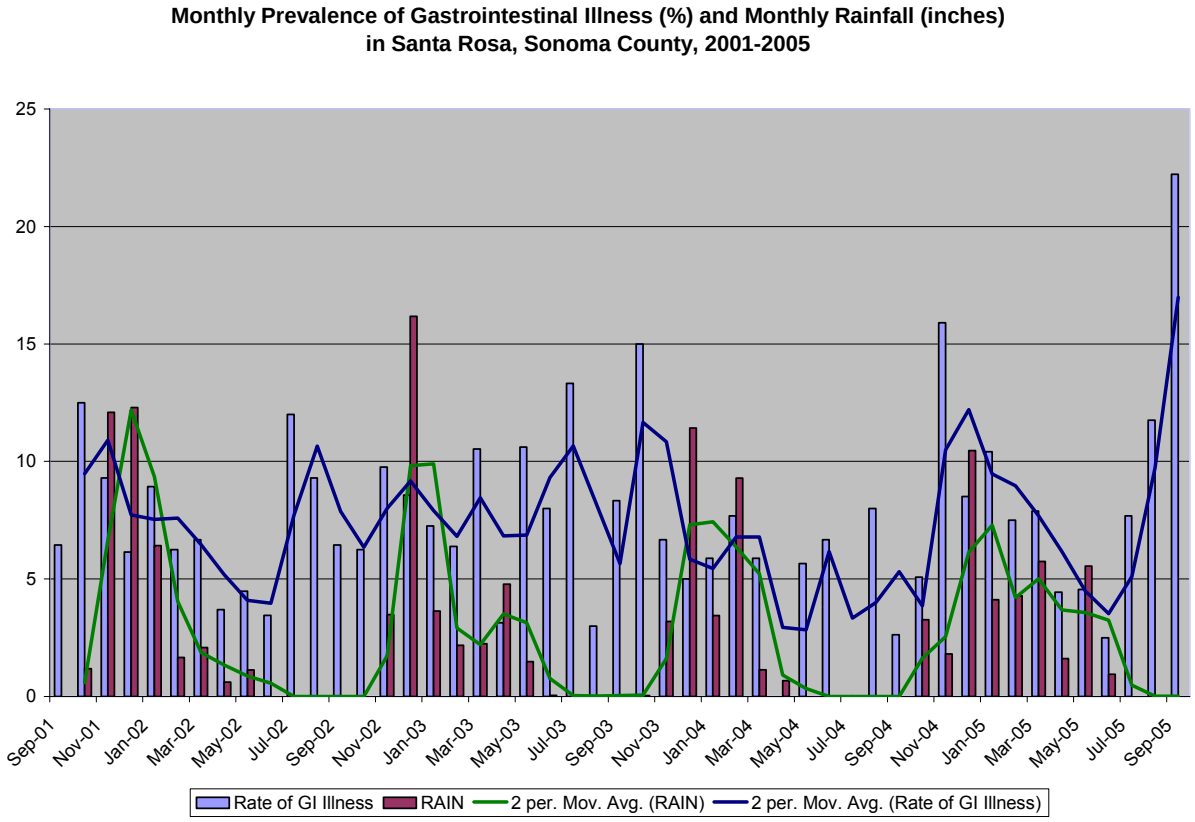


Table 3. Prevalence of Secondary Symptoms Among Those Reporting GI Illness (n=158)

	Prevalence (%) Among All GI Cases (n=158)	Prevalence (%) Among Diarrhea Cases (n=136)	Prevalence (%) Among Vomiting Cases (n=41)
Stomach Cramps	46.20	50.00	34.15
Fever	12.66	13.97	29.27
Nausea	35.44	33.09	82.93
Blood in Stool	8.23	9.56	12.20
Headache	29.75	32.35	48.78
Sore Throat	14.56	15.44	21.95
Sought Medical Care	23.42	23.53	31.71
Took Antibiotics	10.80	8.87	14.63
Missed Work or School	14.55	14.71	24.39

Table 4. Water Consumption Patterns

	Proportion (%) Among All Respondents (n=2163)	Proportion (%) Among those with GI Symptoms (n=158)	Proportion (%) Among those without GI Symptoms (n=2005)
Source of Water at Home			
Municipal Water	84.65	84.18	84.66
Private Well Water	12.94	13.29	12.93
Other	0.79	0.63	0.80
Don't know	1.62	1.90	1.60
Primary Type of Drinking Water			
Unheated tap water	49.51	43.67	49.87
Treated tap water	33.70	36.08	33.58
Bottled water	14.98	17.09	14.88
Other	1.25	1.90	1.20
Don't know	0.55	1.27	0.50
Type of Water Treatment Among those Reporting Treated Water Use	**(n=729)**	**(n=57)**	**(n=672)**
Filtered	83.81	87.72	83.58
Treatment device at house entry	4.80	5.26	4.78
Water softener	5.21	3.51	5.37
Distiller	0.55	0	0.60
Ultraviolet Light	0.41	0	0.45
Boiled	0.27	0	0.30
Other	3.84	1.75	3.88
Don't know		1.75	1.04
Type of Filter Used at Home Among those Reporting Filter Use	**(n=614)**	**(n=51)**	**(n=563)**
Pitcher or jug filter	42.18	35.29	42.70
Faucet mounted filter	17.92	23.53	17.44
Counter top filter	6.51	3.92	6.76
Under the sink filter	14.66	19.61	14.23
Reverse osmosis filter	4.07	0	4.45
Other	1.36	0	1.42
Don't know	13.19	17.65	12.99

Table 5. Potential Gastrointestinal Illness Risk Factors

	Proportion (%) Among All Respondents (n=2163)	Proportion (%) With GI Symptoms (n=158)	Proportion (%) Without GI Symptoms (n=1995)	Odds Ratio (95% CI)
Travel Outside of the United States in Last 30 Days	4.16	6.33	3.96	1.64 (0.74, 3.26)
Drinking Untreated Water				
Lake, pond, river or stream	0.51	1.27	0.45	2.82 (0.29, 13.82)
Private well	14.56	13.92	14.64	0.94 (0.56, 1.52)
Swimming or Entering Water				
Ocean	3.42	3.16	3.46	0.91 (0.28, 2.28)
Lake, pond, river or stream	3.37	4.43	3.31	1.35 (0.51, 3.02)
Hot tub, whirlpool spa or jacuzzi	12.58	14.56	12.43	1.20 (0.72, 1.92)
Swimming pool	12.34	11.39	12.43	0.90 (0.51, 1.51)
Illness				
Chronic disease with GI symptoms	5.59	26.58	3.96	8.91 (5.70, 13.78)*
HIV or other immunosuppression	0.83	3.80	0.60	6.52 (1.98, 19.05)*

** $p>0.0001$*

Table 6. Recent Studies Reporting Gastrointestinal Illness Rates Among Elderly (≥55 years of age)

Reference	Country	Study Period	Study Design	% Sample Size ≥55 years of age	Definition of GI Illness	Monthly Prevalence (%)	Incidence (episodes per person per year)
Jones et al. 2007 (FoodNet)[8]	United States	1996-2003	Cross-sectional (Telephone Survey)	25.8% (n=13632)	Diarrhea in past month	5.1 (95% CI: 4.8, 5.4)	0.6
Imhoff et al. 2004 (FoodNet)[20]	United States	1998-1999	Cross-sectional (Telephone Survey)	45-64: 22% (n=2806) 65+: 12% (n=1530)	Diarrhea in past month	All cases: 10% Acute diarrhea: 6%	All cases: 1.3 Acute diarrhea: 0.72
Thomas et al. 2006[14]	Canada	2002-2003	Cross-sectional (Telephone Survey)	25-64: 64.3% (n=2965) 65+: 16.5% (n=760)	Diarrhea or vomiting in 28 days prior	9.2 (95% CI: 8.4, 10)	1.3 (95% CI: 1.1, 1.4)
Thomas et al. 2008[13]	Canada	2001-2006	Cross-sectional (Telephone Survey)	Not reported, data from 3 regions	Diarrhea or vomiting in 28 days prior	10.0 (95% CI: 9.9, 10.1); 9.8 (95% CI: 8.9, 10.6); 8.6% 9.2(95% CI: 8.4, 10)	1.3 (95% CI: 1.1, 1.4); 1.3 (95% CI: 1.1, 1.4); 1.2 (95% CI: 0.99, 1.4)
Current Study	United States	2001-2005	Cross-sectional (Telephone Survey)	100% (n=2163)	Diarrhea or vomiting in past month	7.3 (95% CI: 6.2, 8.6)	0.99 (95% CI: 0.8, 1.5)

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CHAPTER 4

INFECTIONS OR MEDICATIONS?
ETIOLOGY OF DIARRHEAL ILLNESS AMONG AN URBAN HIV-INFECTED COHORT

ABSTRACT

Background: Persons with Human Immunodeficiency Virus (HIV) infection represent a sensitive subpopulation at increased risk for gastrointestinal illness. Though high prevalences of diarrheal illness ranging from 30 to 100% have been previously reported in this population, limited data exists on pathogen specific prevalence estimates and the relative importance of non-infectious etiologies of gastrointestinal illness. Moreover, less is know regarding the import of pathogens compared to medication related adverse events in both acute and chronic diarrhea patients with HIV infection.

Methods: One hundred and fifty patients with acute, chronic and no diarrhea were recruited from an urban, HIV specialty clinic to provide information on gastrointestinal symptoms, risk factors and a stool sample for examination. HIV indicators including CD4 count and medication profile were abstracted from medical records.

Results: Enteric pathogens were detected in 20% of samples collected. Isolation rates for varied between patients with acute, chronic and no diarrhea, with the highest recovery seen among those with acute diarrhea. Organism isolation was associated with the presence of diarrheal symptoms, anal sex, oral sex and consumption of undercooked meats. Diarrhea was associated with anti-retroviral medication use, with protease inhibitors conferring the greatest risk for diarrhea.

Conclusion: 80% of samples were not able to be linked to a specific pathogen suggesting that non-infectious causes, such as medication or underlying HIV enteropathy, may be relevant etiologies, especially in a post-HAART era, or that perhaps adequate technology for pathogen detection was not applied, for the majority of diarrhea in this population.

Introduction

Diarrhea remains an important cause of morbidity and mortality worldwide among HIV-infected individuals, with cumulative incidence estimates of 30-70% in industrialized nations and up to 100% in developing countries[1]. The pathogenesis of diarrhea is complex and associated with multiple etiologies including bacterial, parasitic and viral pathogens, adverse events from anti-retroviral medications, tumors and underlying immune status. The relative contributions of these risk factors to the overall burden of diarrheal illness is unknown and may vary by geographic location, immune status and access to anti-retroviral medications.

HIV patients also frequently report chronic diarrhea (daily episodes of watery stool lasting 2-4 weeks or more). Chronic diarrhea has a significant impact on patient outcomes, health care utilization and quality of life. Numerous studies on infectious diarrhea in children, hospital patients and travelers have been published, but little is known about the importance of specific viral, bacterial and protozoan agents among HIV-positive individuals in a community setting in the United States[2-4].

Various enteric protozoan infections are seen in HIV+ individuals. Infection with MAC and other non-tuberculous mycobacteria has been frequently reported in the last two decades primarily among HIV/AIDS patients[5, 6]. *Cryptosporidium parvum* has received intense scrutiny because of its role in both large, detected and smaller, undetected outbreaks as well as its severity in AIDS patients[7-9]. Other organisms such as *Entamoeba histolytica, Cyclospora, Miscrosporidia* and *Giardia lambia* are also established causes of diarrheal illness in HIV+ persons[5, 10]. However, the relative contribution of other organisms such as *Entamoeba Coli, Dientamoeba fragilis, Blastocystis hominis, Iodamoeba bÅtschlii* and *Endolimax nana* to the burden of diarrhea in HIV+ population is unclear.

There are a number of non-infectious causes of diarrhea such as side effects due to medications prescribed to HIV+ individuals. This association between diarrhea and medication has increased since the introduction of highly active antiretroviral therapy (HAART) in the last quarter of 199611. Though HAART has led to dramatic decreases in morbidity, mortality and opportunistic infections, adverse events associated with HAART such as nausea, diarrhea, lipodystrophy, and neuropathy, impact quality of life for patients living with HIV and AIDS12. For example, diarrhea is a known complication of Nelfinavir and other protease inhibitors11. Prior to the introduction of HAART, chronic diarrhea affected 50-90% of the HIV+ population, and has been attributed to viral, bacterial, and parasitic infection13, 14. A more recent study suggests that though the prevalence of diarrhea has dropped, it is still notable in the HIV+ population15.

We investigated the relationship between acute, chronic and no diarrhea with enteric pathogens and other risk factors for diarrheal illness among HIV-infected patients from an urban, community clinic.

Methods

HIV-infected patients from the East Bay AIDS Center in Oakland, CA with acute, chronic or no diarrhea were recruited during regular clinic visits for study participation between April 2003 and November 2003. They were asked to provide stool specimens and complete a brief questionnaire on gastrointestinal symptoms and potential risk factors (e.g. travel, sexual behavior, food and water consumption, animal contact). (Study protocols and instruments are included in the appendix).

This sampling design represents a case-control study with two comparison arms (Cases=acute diarrhea; control arm #1=chronic diarrhea; control arm #2=no diarrhea). Recruitment occurred such that control arm groups were selected within 48 hours of the acute case enrollment, to ensure that there was an even distribution of recruitment throughout the study enrollment period to capture any seasonal variations or potential outbreaks.

Diarrhea definitions by group

Clinicians determined enrollment for patients with "acute" diarrhea and requested stool work-up due to the presence of gastrointestinal symptoms (vomiting, diarrhea, abdominal cramps, nausea, bloody diarrhea, fever) that were new, different or more severe than the normal pattern of diarrhea experienced by that patient. Clinicians were instructed to include patients in this category who they would normally order microbiological testing regardless of this study. Diarrhea in these cases was suspected to be of infectious etiology. This group of patients comprise our definition of an acute "case".

Patients with "chronic" diarrhea were defined as those who reported diarrheal symptoms (two or more loose stools per day for two weeks or greater) that were not different from their normal pattern of diarrhea. Diarrhea in such cases may be a side effect of HIV drug treatment and may or may not represent enteric infection. If infectious, it is likely that a unique set of pathogens is associated with diarrheal illness in this group. These patients represent our first comparison group.

The second comparison group was HIV-infected patients with no diarrheal symptoms reported to the clinician. These individuals may have asymptomatic infection with recognized enteric pathogens or other organisms whose pathogenicity is not yet recognized or well defined.

Sample Collection and Testing

Participants were asked to provide a stool sample for microbiological testing. They could choose to provide the sample while at the clinic or in the privacy of their homes within 24 hours of their clinic visit in special containers provided to them. Instructions for safe collection of stool specimens were provided to all participants. If the specimen was collected at the participant's home, study personnel arranged for courier pick-up of the specimen from the participant's home, and delivered the specimen to the clinic. Participants were given $15 for study participation.

Stool specimens were split into aliquots in the clinic by trained staff and sent to a clinical laboratory for standard microbiological work-up (bacterial culture, ova and parasite exam, *C.difficile* toxin and *Giardia* tests) and to UC Berkeley for nucleic acid isolation for future testing. All results from specimens tested were made available to the participant and his or her physician to inform their clinical care.

Clinical Data, Confidentiality and Ethical Approval

HIV viral load, CD4 count and current medications were abstracted from patient records for analysis. To preserve confidentiality, no patient identifying information left the East Bay AIDS Center. All data and specimens were labeled with a unique study identification number for each participant. The code for the unique identification numbers was held in a secure location by the clinical research staff at the East Bay AIDS Center. Ethical approval to conduct this study was obtained by the University of California, Berkeley Committee for the Protection of Human Subjects, and the institutional review board at Alta Bates Summit Medical Center.

Survey and Analysis

The survey was self-administered following enrollment in clinic and took approximately fifteen to twenty minutes to complete. Participants were asked if they had experienced any gastrointestinal symptoms in the past week as well as on the day of enrollment. In addition, participants were asked about their water consumption patterns (eg. bottled, filter or tap water), recreational water exposures, travel outside the United States and other risk factors for gastrointestinal illness. All surveys were conducted in English.

Data were analyzed in Microsoft Excel 2003 (Microsoft Corporation, Richmond, WA, USA) and STATA 9SE (Stata Corporation, College Station, TX, USA). Conditional logistic regression was used to evaluate diarrhea in the past week with risk factors. Pathogen recovery was also evaluated for association with group status and risk factors.

Results

Participant Flow, Organism Isolation Rates and Demographics
Of the 164 participants enrolled, 150 provided stool samples yielding a compliance rate of 91.5% (Figure 1). Participants were evenly distributed into the three groups (acute diarrhea, chronic diarrhea, and no diarrhea). Organisms were isolated in all three groups, with acute diarrhea cases have the highest rate of isolation, 28% compared to 18% among chronic diarrhea patients and 14% among the no diarrhea group.

Participants were recruited from an urban community clinic, and were primarily low income (68% with <$20,000 annual income), male (78%) and African-American (49%, Table 1a). Demographic characteristics for enrolled vs. participants with complete data did not vary significantly (Table 1b).

Spectrum of Organisms Isolated
Bacterial and parasitic organisms were identified in specimens collected. Multiple organisms were isolated form six participants in Group A (acute), five from group B (chronic), and one from Group (no diarrhea). Organisms isolated are presented in Table 2 by group status.

Of the six cases with known pathogenic organisms isolated, four were acute diarrhea cases (Group A). The included three cases of *Shigella species* and one case of *Giardia lamblia*. The chronic diarrhea group (Group B) had one case of *C. difficile*, and the no diarrhea group (Group C) had one case of *Gairdia lamblia.*

Species whose pathogenicity is unclear or not established such as *Endolimax Nana* and *Blastocystis hominis* were isolated from all three groups. *Entamoeba Coli* was also isolated from specimens from all three groups, with greater frequency in chronic and no diarrhea groups compared to the acute diarrhea group. Acute diarrhea and chronic diarrhea groups had the highest frequency of these organisms isolated with sixteen cases each, compared to eight among the no diarrhea group.

Immune Status

Immune status as measured by mean and median CD4 cell count differed between the three groups, with the acute diarrhea group exhibiting the poorest outcomes. CD4 count and HIV viral load data by group is presented in Table 3. Figure 2 displays the CD4 cell count distribution for the three groups with their respective medians.

The acute diarrhea group also had the greatest proportion of patients with CD4 count below 200 cells/mm^3, constituting an AIDS case definition and a threshold for HIV treatment initiation and increased risk of opportunistic infections. Mean HIV viral load burden was also highest in this group compared to the chronic and no diarrhea groups.

Gastrointestinal Symptoms

Gastrointestinal symptoms reported by patients in the survey are presented in Table 4. Group determinations were made by clinicians, however, self-reported symptoms in the past week or on the day of enrollment did not completely match diarrhea group status. For example, 94% of Group A participants (acute diarrhea) and 88% of Group B (chronic diarrhea) participants reported diarrhea in the past week. Moreover, 34% of Group C (no diarrhea) participants reported diarrhea in the past week, and 14% of these participants reported diarrhea on the day of enrollment.

Seventy-six percent of acute diarrhea cases reported that their bowel movements were different from their normal or usual experience, compared to 54% of chronic diarrhea group patients, and 22% of no diarrhea group patients. Eight percent of acute diarrhea cases reported liquid or watery diarrhea, compared to 66% of chronic diarrhea group patients, and 24% of no diarrhea group patients. Fifty percent of acute diarrhea cases took some form of anti-diarrheal medication, compared to 38% of chronic diarrhea group patients, and 8% of no diarrhea group patients.

Potential Risk factors

Various risk factors for gastrointestinal illness such drinking water, foreign travel, recreational water exposure, foods, contact with animals, contact with children and sexual contact were queried in this sample of patients. Prevalence of these exposures are presented in Tables 5 and 6.

The distribution of medications taken by participants is presented in Table 7. Nucloeside reverse transcriptase inhibitors (NRTIs) were taken by 70% of patients in the acute diarrhea group, 56% of patients in the chronic diarrhea group and 60% of no diarrhea group patients. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) were used by 14% of patients in the acute diarrhea group, 22% of patients in the chronic diarrhea group and 32% of no diarrhea group patients. Protease inhibitor (PI) use was most prevalent amongst chronic diarrhea group members (40%), followed by those in the no diarrhea group (30%) and then, the acute diarrhea group (26%)

Associations with Diarrhea

Diarrhea in the past seven days adjusted for group status had borderline or suggestive associations with protease inhibitor use, recreational water contact, oral sex, contact with children in diapers and

consumption of red or pink meat. Odds ratios for medication classes, immune status and other exposures are presented in Table 8.

Participants on protease inhibitor were greater than two times more likely to report diarrhea than those in other groups (OR=2.64, 95% CI: 0.94, 7.40). Though not significant, patients using Non-nucleoside reverse transcriptase inhibitors (NNRTIs) may have a protective effect for diarrhea compared to other antiretroviral class types (OR=0.69, (95% CI: 0.25, 1.87).

CD4 count and viral load did not influence diarrhea reporting. However, data was suggestive that those with CD4 counts below 200 cell/mm^3 may have nearly a two fold increased risk for diarrhea (OR=1.98, 95%CI: 0.62, 6.35).

Potential fecal oral transmission route exposures were associated with diarrhea. This included contact with a child in diapers (OR=3.70, 95% CI: 0.95, 14.34). Anal sex had a non-significant three fold increase in risk for diarrhea (OR=3.13, 95% CI: 0.66, 14.76). However, oral sex was protective for diarrhea (OR=0.36, 95%CI 0.13, 0.99).

Other recreational water contact, food exposures, demographic factors, and bottled, filtered or boiled water use was not predictive of diarrhea in this population.

Associations with Organism Isolation

Associations with isolation of a bacterial or parasitic organism adjusted for group status are presented in Table 9. Similar to diarrhea risk, CD4 count and HIV viral load was not significantly predictive for isolation of an organism from stool samples. However, CD4 count below 200 did exhibit an odds ratio of 1.88 (95% CI: 0.80, 4.45), suggesting this may be a potential factor associated with organism isolation.

Diarrhea in the past seven days and diarrhea on the day of enrollment were associated with organism isolation. Those reporting diarrhea in the past seven days were nearly eight times more likely to have an organism isolated from their stool specimen (OR=7.82, 95% CI: 1.51, 40.48), and those reporting diarrhea on the day of enrollment were thirty times more likely to have an organism isolated (OR=30.57, 95% CI: 3.84, 243.36).

Exposures associated with organism isolation included sex with a man (OR=2.32, 95%CI: 0.99, 5.46), anal sex (OR=4.78, 95% CI: 1.81, 12.58), and contrary to the diarrhea risk model in direction, oral sex (OR=2.40, 95% CI: 1.02, 5.60). Additionally, participants who consumed red or pink meat were three times more likely to have an organism isolated from their specimen (OR= 3.32, 95% CI: 1.40, 7.89). Other food, water or behavioral exposures were not associated with organism isolation.

Group status was not significantly associated with organism isolation, however, the direction of the relationships between isolation and acute, chronic or no diarrhea groups was consistent with expectations. For example, the acute diarrhea group (Group A) had a potentially two fold increase risk compared to other groups. Data for the chronic diarrhea group (Group A) was suggestive for increased risk of organism isolation compared to the no diarrhea group (Group C). Group associations with organism isolation are presented in Table 10.

Discussion

Diarrhea remains a common problem impacting quality of life among HIV-infected patients, often with severe consequences. Summary data from previous studies suggest that adult HIV-infected patients experience 1.15 episodes per person per year, and infected children experience 2.98 episodes of diarrhea per child per year (Table 11, Figures 3 & 4)[1, 16]. In this study, we sought to investigate potential etiologies for acute and chronic diarrhea.

Identification of infectious organisms (pathogenic and possibly pathogenic) occurred in 20% of the samples collected in our study. Though all study groups (acute, chronic and no diarrhea) had organisms isolated from their respective pools of stool samples, acute and chronic diarrhea patients exhibited the highest rates of isolation and multi-organism burden. Pathogenic bacteria such as *Shigella* species were exclusively seen in acute diarrhea patients as anticipated. Our organism isolation rate was lower than those previous reported by cohort studies among HIV-infected persons conducted in Europe and Africa, which ranged from 29 to 46%[1, 16]. However, those studies also recovered pathogens acute diarrhea, chronic diarrhea and control cases in analogous distributions to our sample. Similarly, ova and parasite isolations were more frequent than pathogenic bacteria.

We found limited associations with immune status and anti-retroviral medications classes in our data. However, the data were suggestive of potentially higher risk of diarrhea and pathogen isolation for persons taking protease inhibitors and with CD4 cell counts below 200 cells/mm^3. Though the relationships we found for these parameters were not significant, this may have been due to our limited ability to detect a significant association due to lack of sample size.

Weber et al. identified increasing probability of developing diarrhea with decreasing CD4 cell count among members of the Swiss HIV Cohort Study, especially in those with CD4 cell counts below 0.05x 10^9/L. Brink et al. posited a 3.5 fold increase risk (95% CI: 2.3, 5.3) for diarrhea for individuals with CD4 cell count below 200 cells/mm^3 among 1213 member HIV-infected cohort from community clinics in Uganda[1, 16]. With our sample size an order of magnitude lower, our estimate for diarrhea and CD4 cell count below 200 cells/mm^3 was 1.98 (95% CI: 0.62, 6.35).

Interestingly, we found evidence of possible fecal oral transmission of infectious diarrhea inducing organisms among our sample. Anal sex, oral sex and contact with children in diapers were associated with either pathogen isolation, diarrhea in the past seven days or both. Possible foodborne illness due to undercooked meats was also potentially present in our sample, as this was associated with increased risk for pathogen isolation and diarrhea.

Nevertheless, lack of adequate sample size limited our ability to clearly elucidate etiological associations that may have existed in this cohort between immune status, medications and environmental or behavioral exposures. Pathogen specific risk estimates were similarly limited given the breadth of possible organisms and the low level of pathogen recovery perhaps due to lack of sufficient culture or testing. Future studies with access to sensitive high throughput technologies may provide a wider array of possibilities to explain diarrhea etiology. However, access to such technologies as well as high cost make them prohibitive for utilization in community studies.

Furthermore, we did not assay for viruses in this sample. Enteroviruses, adenoviruses, rotaviruses, noroviruses and astroviruses may be potential causes of diarrhea in this population and have been identified previously as causes of diarrhea in other populations[17-22].

Though we detected organisms in samples from patients with and without diarrhea, 80% of our samples could not be diagnosed suggesting though bacterial and parasitic etiologies remain relevant for HIV related diarrhea, other causes such as anti-retroviral agents, unidentified pathogens, malnutrition or underlying HIV disease may also contribute to the burden of diarrhea in this population.

Figures and Tables

Figure 1. Enrollment, Participant Flow and Organism Isolation Rates

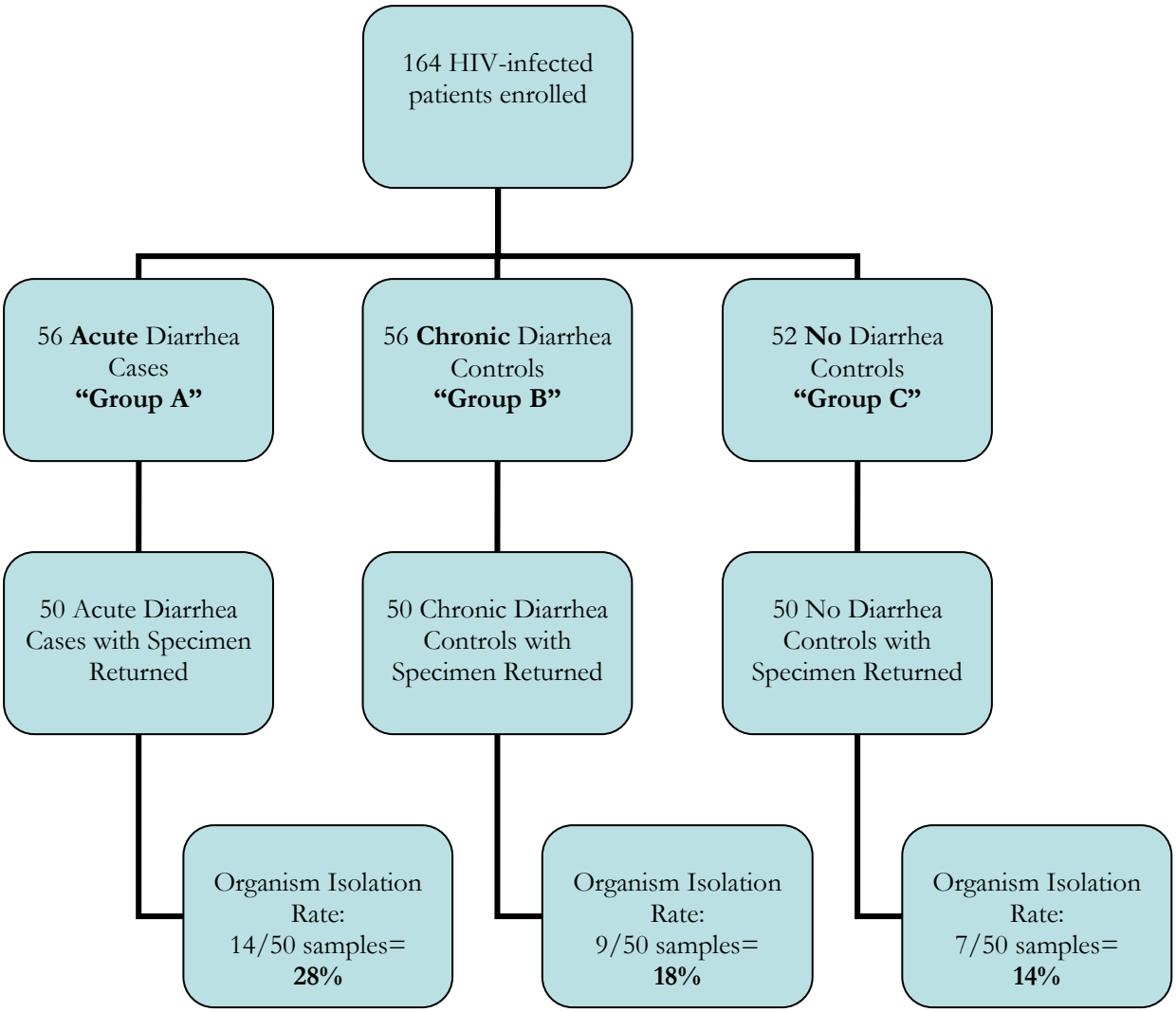


Table 1a. Demographic Characteristics by Group

Characteristic	Group A Acute Cases (n=50)	Group B Chronic Controls (n=50)	Group C Asymptomatic Controls (n=50)
Age (years)	**n (%)**	**n (%)**	**n (%)**
20-29	4 (8.0)	0 (0)	0 (0)
30-39	12 (24.0)	11 (22.0)	7 (14)
40-49	21 (42.0)	18 (36.0)	27 (54.0)
50-59	12 (24.0)	17 (34.0)	15 (30.0)
60-69	1 (2.0)	3 (6.0)	1 (2.0)
70+	0 (0)	1 (2.0)	0 (0)
Gender	**n (%)**	**n (%)**	**n (%)**
Male	37 (74.0)	38 (76.0)	40 (80.0)
Female	13 (26.0)	12 (24.0)	10 (20.0)
Race/ Ethnicity	**n (%)**	**n (%)**	**n (%)**
White	26 (52.0)	17 (34.0)	15 (30.0)
African-American	17 (34.0)	25 (50.0)	31 (62.0)
Hispanic	6 (12.0)	5 (10.0)	2 (4.0)
Asian	1 (2.0)	0 (0)	0 (0)
Native American	0 (0)	2 (4.0)	0(0)
African American/ Native American	0(0)	0 (0)	2(4.0)
Other	0 (0)	1 (2.0)	0 (0)
Highest Level of Education	**n (%)**	**n (%)**	**n (%)**
Junior High School	2 (4.0)	1 (2.0)	3 (6.0)
1-3 years High School	1 (2.0)	7 (14.0)	5 (10)
High School Graduate	12 (24.0)	8 (16.0)	14 (28.0)
1-3 years College	17 (34.0)	21 (42.0)	16 (32.0)
College Graduate	8 (16.0)	4 (8.0)	4 (8.0)
1-2 years Post-Graduate	8 (16.0)	7 (14.0)	8 (16.0)
Not Available	2 (4.0)	2 (4.0)	0 (0)
Annual Income	**n (%)**	**n (%)**	**n (%)**
<$20,000	34 (68.0)	34 (68.0)	34 (68.0)
$20,000-$30,000	1 (2.0)	5 (10.0)	3 (6.0)
$30,000-$40,000	4 (8.0)	3 (6.0)	3 (6.0)
$40,000-$50,000	0 (0)	3 (6.0)	2 (4.0)
$50,000-$100,000	7 (14.0)	2 (4.0)	5 (10.0)
>$100,000	2 (4.0)	0 (0)	2 (4.0)
Not available	2 (4.0)	3 (6.0)	1 (2.0)

Table 1b. Demographic Characteristics of Included vs. Excluded Participants

	N=164 Enrolled	%	N=150 Complete Data	%
Gender				
Female	**37**	**22.56**	**35**	**23.33**
Male	**127**	**77.44**	**115**	**76.67**
Race/ Ethnicity				
White	**63**	**38.41**	**58**	**38.67**
African American	**80**	**48.78**	**73**	**48.67**
Asian	**1**	**0.61**	**1**	**0.67**
Native American	**2**	**1.22**	**2**	**1.33**
African American/ Native American	**2**	**1.22**	**2**	**1.22**
Hispanic	**15**	**9.15**	**13**	**8.67**
Other	**1**	**0.61**	**1**	**0.67**

Table 2. Number of Organisms Isolated by Group
(multiple organisms isolated from some specimens)

Organism	**Group A (acute)**	**Group B (chronic)**	**Group C (control)**	**Total**
Shigella Flexneri	2	0	0	2
Shigella Sonnei	1	0	0	1
Clostridium Difficile	0	1	0	1
Giardia lamblia	1	0	1	2
Endolimax Nana	5	4	4	13
Entamoeba Coli	1	2	2	5
Entamoeba Hartmanni	3	5	1	9
Iodamoeba Buetschili	3	1	0	4
Blastocystis Hominis	3	4	1	8
Dientamoeba Fragilis	1	0	0	1
Total	20	17	9	

Table 3. HIV Indicators

	Group A Acute Cases (n=50)	Group B Chronic Controls (n=50)	Group C Asymptomatic Controls (n=50)
CD4 Count (cells/mm3)			
Mean	324.33	475.98	459.72
Standard Deviation	283.45	373.48	273.40
Range	7 - 1512	44 - 2013	25 - 1221
HIV Viral Load (copies/mL)			
Mean	149,598	75,281.82	41,845.58
Standard Deviation	268,161	185,719.70	92,561.62
Range	50 – 999,999	50 – 750,000	50 – 417,069
Participants with CD4 >200	20 (40%)	11 (22%)	9 (18%)

Figure 2. CD4 Count Distribution by Group with Median (+)

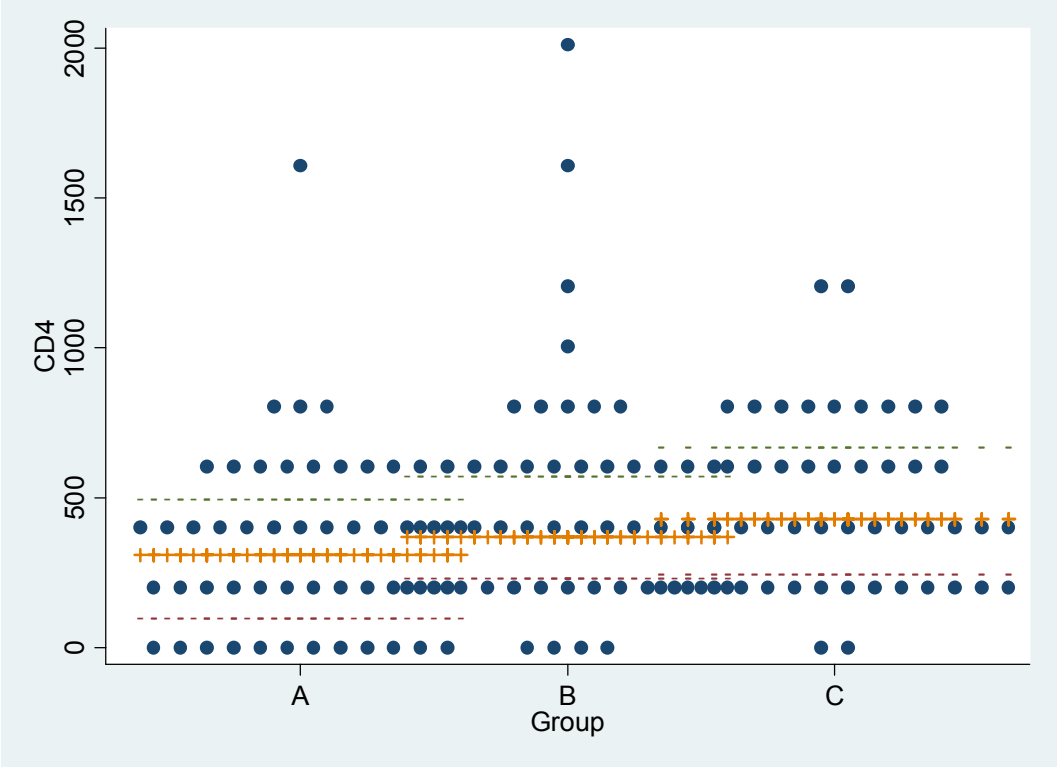


Table 4. Self-Reported Gastrointestinal Symptoms by Group

	Group A Acute Cases (n=50)	Group B Chronic Controls (n=50)	Group C Asymptomatic Controls (n=50)
GI Symptoms in prior 7 days	**n (%)**	**n (%)**	**n (%)**
Cramps	42 (84.0)	33 (66.0)	21 (42.0)
Diarrhea	47 (94.0)	44 (88.0)	17 (34.0)
Nausea	33 (66.0)	26 (52.0)	17 (34.0)
Vomiting	17 (34.0)	12 (24.0)	7 (14.0)
Fever	14 (28.0)	6 (12.0)	4 (8.0)
GI Symptoms today (enrollment day)	**n (%)**	**n (%)**	**n (%)**
Cramps	31 (62.0)	12 (26.0)	6 (12.0)
Diarrhea	31 (62.0)	26 (52.0)	7 (14.0)
Nausea	19 (38.0)	9 (18.0)	5 (10)
Vomiting	4 (8.0)	7 (14.0)	1 (2.0)
Fever	2 (4.0)	0 (0)	2 (4.0)
Diarrhea Related Characteristics	**n (%)**	**n (%)**	**n (%)**
Liquid or watery diarrhea	40 (80.0)	33 (66.0)	12 (24.0)
Blood in diarrhea	6 (12.0)	6 (12.0)	0 (0)
Mucus in diarrhea	12 (24.0)	11 (22.0)	4 (8.0)
Took medicine for diarrhea	25 (50.0)	19 (38.0)	4 (8.0)
Missed day of work or school due to GI symptoms	12 (24.0)	13 (26.0)	4 (8.0)
Bowel movements different from normal or usual experience	38 (76.0)	27.0 (54.0)	11 (22.0)

Table 5. Risk Factors by Group

Potential Exposures in past 7 days	Group A Acute Cases (n=50)	Group B Chronic Controls (n=50)	Group C Asymptomatic Controls (n=50)
	n (%)	n (%)	n (%)
Recreational Water Exposures			
Swam in a Pool	4 (8.0)	2 (4.0)	5 (10.0)
Swam in a Lake, River or Stream	2 (4.0)	1 (2.0)	0 (0)
Swam in Ocean	3 (6.0)	1 (2.0)	1 (2.0)
Swam in Hot Tub	4 (8.0)	4 (8.0)	2 (2.0)
Contact with Children			
Anyone with Diapers	9 (18.0)	4 (8.0)	6 (12.0)
Children under five	6 (12.0)	11 (22.0)	10 (20.0)
Children attending daycare	3 (6.0)	1 (2.0)	2 (4.0)
Contact with Animals			
Dog	22 (44.0)	16 (32.0)	14 (28.0)
Cat	17 (34.0)	16 (32.0)	13 (36.0)
Bird	3 (6.0)	2 (4.0)	6 (12.0)
Goat	1 (2.0)	0 (0)	0 (0)
Rabbit	0 (0)	1 (2.0)	0 (0)
Food Exposures			
Shellfish	6 (12.0)	10 (20.0)	8 (16.0)
Raw fish	3 (6.0)	3 (6.0)	2 (4.0)
Red or Pink Meat	14 (28.0)	18 (32.0)	19 (38.0)
Unpasteurized milk or juice	6 (12.0)	5 (10.0)	3 (6.0)
Traveled Outside the United States	1 (2.0)	3 (6.0)	1 (2.0)
Sexual Contact			
Sexual Contact with Men	19 (38.0)	14 (28.0)	12 (24.0)
Sexual Contact with Women	3 (6.0)	4 (8.0)	4 (8.0)
Oral Sex	19 (38.0)	13 (26.0)	13 (26.0)
Anal Sex	11 (22.0)	8 (16.0)	4 (8.0)
Vaginal Intercourse	6 (12.0)	4 (8.0)	8 (16.0)

Table 6. Drinking Water Consumption

	Group A Acute Cases (n=50)	Group B Chronic Controls (n=50)	Group C Asymptomatic Controls (n=50)
	n (%)	n (%)	n (%)
Bottled Water Consumption			
Always	10 (20.0)	10 (20.0)	10 (20.0)
Often	11 (22.0)	10 (20.0)	19 (38.0)
Sometimes	13 (26.0)	15 (30.0)	11 (22.0)
Rarely	5 (10.0)	9 (18.0)	6 (12.0)
Never	8 (16.0)	3 (6.0)	4 (8.0)
Not Available	3 (6.0)	3 (6.0)	0 (0)
Filtered Water Consumption			
Always	10 (20.0)	8 (16.0)	11 (22.0)
Often	5 (10.0)	6 (12.0)	1 (2.0)
Sometimes	5 (10.0)	6 (12.0)	4 (8.0)
Rarely	5 (10.0)	8 (16.0)	10 (20.0)
Never	22 (44.0)	20 (40.0)	24 (48.0)
Not Available	3 (6.0)	2 (4.0)	0 (0)
Boiled Water Consumption			
Always	4 (8.0)	1 (2.0)	3 (6.0)
Often	0 (0)	3 (6.0)	11 (22.0)
Sometimes	5 (10.0)	5 (10.0)	3 (6.0)
Rarely	3 (6.0)	8 (8.0)	5 (10.0)
Never	35 (70.0)	30 (60.0)	28 (56.0)
Not Available	3 (6.0)	3 (6.0)	0 (0)
Concerned about Drinking Water			
Very Concerned	15 (30.0)	19 (38.0)	17 (34.0)
A Little Concerned	17 (34.0)	16 (32.0)	19 (38.0)
Not Concerned	15 (30.0)	13 (26.0)	14 (28.0)
Not Available	3 (6.0)	2 (4.0)	0 (0)
Heard of CDC Drinking Water Guidelines	7 (14.0)	15 (30.0)	10 (20.0)

Table 7. Medication Profile

Drug Code	Medication	Group A Acute Cases (n=50)	Group B Chronic Controls (n=50)	Group C Asymptomatic Controls (n=50)
		n (%)	n (%)	n (%)
	Nucleoside Reverse Transcriptase Inhibitors			
ABC	Abacvir (Ziagen)	3 (6)	1 (2)	3 (6)
AZT	Zidovudine (Retrovir)	0 (0)	0 (0)	1 (2)
3TC	Lamivudine (Epivir)	12 (24)	12 (24)	13 (26)
DDC	Dideoycytidine (HIVID, Zalcitabine)	0 (0)	0 (0)	0 (0)
DDI	Didanosine (Dideoxydiadosine, Videx)	5 (10)	3 (6)	6 (12)
D4T	Stavudine (Zerit)	6 (12)	8 (16)	6 (12)
AZT/ 3TC	Zidovudine/ Lamivudine (Combivir)	5 (10)	7 (14)	7 (14)
AZT/ 3TC/ ABC	Zidovudine/ Lamuvudine/ Abacavir (Trizivir)	11 (22)	5 (10)	5 (10)
	Any NRTI	35 (70)	38 (56)	30 (60)
	Non-nucleoside Reverse Transcriptase Inhibitors			
DLV	Delavirdine (Rescriptor)	0 (0)	0 (0)	2 (4)
EFV	Efavirenz (Sustiva)	5 (10)	5 (10)	5 (10)
NVP	Nevirapine (Viraimmune)	12 (24)	6 (12)	9 (18)
	Any NNRTI	17 (34)	11 (22)	16 (32)
	Protease Inhibitors			
IDV	Indinavir (Crixivan)	1 (2)	2 (4)	2 (4)
NFV	Nelfinavir (Viracept)	1 (2)	1 (2)	1 (2)
RIT	Ritonavir (Norvir)	4 (8)	7 (14)	7 (14)
SQV	Saquinavir (Fortovase)	2 (4)	1 (2)	3 (6)
APV	Amprenavir (Agenerase)	0 (0)	0 (0)	0 (0)
LPV	Lopinivir (ABT-378)	0 (0)	1 (2)	1 (2)
LPV/ RTV	Lopinavir/ Ritonavir (Kaletra)	7 (14)	11 (22)	4 (8)
	Any PI	13 (26)	20 (40)	15 (30)
	Other			
ADEF	Adefovir (bisPOM-PMEA, Preveon)	0 (0)	1 (2)	0 (0)
HDU	Hydroxyurea	0 (0)	0 (0)	0 (0)
DAP	Dapsone	5 (10)	3 (6)	2 (4)
	Lomotil	10 (20)	8 (16)	5 (10)
SMX-TMP	Sulfamethoxazole-triemthoprim (Bactrim)	10 (20)	11 (22)	8 (16)
TDF	Tenofovir	3 (6)	4 (8)	4 (8)

Table 8. Associations with Self-Reported Diarrhea Controlling for Group

	OR	95 % CI	P value
Medication Classes			
NRTI	1.41	0.56, 3.57	0.47
NNRTI	0.69	0.25, 1.87	0.47
PI	2.64	0.94, 7.40	0.06
Any HIV medication	2.74	0.23, 26.46	0.38
NRTI *(multivariate with all classes)*	1.25	0.44, 3.54	0.67
NNRTI*(multivariate with all classes)*	0.86	0.27, 2.75	0.81
PI *(multivariate with all classes)*	2.41	0.76, 7.61	0.13
Immune Status			
CD4 Count	1.00	0.99, 1.00	0.32
CD4>200	1.98	0.62, 6.35	0.25
HIV Viral Load	1.00	0.99, 1.00	0.08
Exposures			
Female gender	0.36	0.10, 1.31	0.11
Age (increasing)	0.99	0.93, 1.06	0.80
Swam in pool	1.47	0.40, 5.40	0.56
Swam in river, lake or stream	0.14	0.01, 1.45	0.05
Swam in hot tub	0.87	0.04, 17.55	0.93
Contact with a child	1.39	0.47, 4.10	0.54
Contact with children in diapers	3.70	0.95, 14.34	0.04
Contact with child attending daycare	2.73	0.13, 54.40	0.49
Shellfish	1.71	0.60, 4.88	0.31
Red or pink meat	2.48	0.98, 6.28	0.05
Travel outside United States	1.02	0.19, 5.43	0.98
Contact with a dog	0.51	0.19, 1.34	0.16
Contact with a cat	1.09	0.40, 2.98	0.83
Contact with a bird	1.20	0.32, 4.47	0.78
Sex with a woman	0.59	0.11, 3.21	0.54
Sex with a man	0.52	0.19, 1.44	0.21
Oral sex	0.36	0.13, 0.99	0.04
Anal sex	3.13	0.66, 14.76	0.13
Vaginal intercourse	0.42	0.12, 1.53	0.18
Bottled water use	0.89	0.61, 1.29	0.54
Filtered water use	1.15	0.86, 1.53	0.35
Boiled water use	0.90	0.64, 1.26	0.52
Concerned about water quality	1.02	0.58, 1.81	0.94

Table 9. Associations with Organism Isolation Controlling for Group

	OR	95 % CI	P value
Immune Status			
CD4 Count	0.99	0.99, 1.00	0.28
CD4>200	1.88	0.80, 4.45	0.15
HIV Viral Load	0.99	0.99, 1.00	0.88
Gastrointestinal Symptoms			
Diarrhea in past 7 days	7.82	1.51, 40.48	0.01
Diarrhea on day of enrollment	30.57	3.84, 243.36	0.001
Cramps	1.65	0.62, 4.38	0.32
Nausea	0.76	0.33, 1.76	0.53
Vomiting	1.31	0.53, 3.26	0.56
Fever	1.46	0.52, 4.05	0.46
Exposures			
Contact with a child	1.36	0.48, 3.81	0.56
Contact with children in diapers	1.41	0.45, 4.36	0.55
Shellfish	1.56	0.55, 4.44	0.40
Red or pink meat	3.32	1.40, 7.89	0.007
Sex with a woman	0.40	0.05, 3.30	0.40
Sex with a man	2.32	0.99, 5.46	0.05
Oral sex	2.40	1.02, 5.60	0.044
Anal sex	4.78	1.81, 12.58	0.002
Vaginal intercourse	0.21	0.02, 1.65	0.14
Bottled water use	0.85	0.61, 1.18	0.34
Filtered water use	0.95	0.73, 1.22	0.70
Boiled water use	1.12	0.79, 1.59	0.52
Concerned about water quality	1.27	0.75, 2.13	0.37

Table 10. Group Associations with Organism Isolation

	OR	95 % CI	P value
Group A (Acute) vs. other groups	2.04	0.83, 4.98	0.08
Group B (Chronic) vs. other groups	0.82	0.30, 2.09	0.66
Group C (No Diarrhea) vs. other groups	0.54	0.18, 1.45	0.19
Group A (Acute) vs. Group B (Chronic)	1.77	0.62, 5.12	0.23
Group A (Acute) vs. Group C (No Diarrhea)	2.38	0.79, 7.72	0.09
Group B (Chronic) vs. Group C (No Diarrhea)	1.35	0.40, 4.68	0.58

Table 11. Characteristics of Cohort Studies Reporting Incidence Density

Reference	Person Years	Location	Diarrhea Outcome Measure	Population	% ARV Treatment	Total Number of HIV+ Individuals
Attili et al. 2006[23]	630	India	≥3 stools within 24 hours; chronic if >1 month	Outpatient clinic Adults 12-70 yrs	75.7%	470
Brink et al. 2002[16]	779	Uganda	≥3 stools within 24 hours; chronic if >1 month	Outpatient clinic Adults ≥ 18yrs	Not reported	870
Grohmann et al. 1993[24]	29	United States	≥3 stools within 24 hours; chronic if > 28 days	Outpatient clinic Adults ≥ 18yrs	Not reported	91
Mwachari et al. 2004[25]	625	Kenya	≥3 stools within 24 hours (includes acute and chronic)	Outpatient clinics Adults ≥ 18yrs	Not reported	381
Navin et al. 1999[26]	271	United States	≥3 stools within 24 hours; chronic if > 28 days	Outpatient clinics Adults ≥ 20yrs	Not reported	602
Sanchez et al. 2005[27]	115,979	United States	Clinical diagnosis	Hospital, Outpatient, Emergency Rooms Adults ≥ 13 yrs	Not reported	44,778
Weber et al. 1999[1]	3953	Switzerland	≥ 3 stools or ≥ 2 fluid stools within 24 hours; chronic if >28 days	Outpatient clinic Adults ≥ 18yrs	32.2%	1933
Keusch et al 1992[28]	21	Zaire	≥3 stools within 24 hours; persistent if > 14 days	Outpatient clinic Infants and children (birth cohort)	Not reported	35
Kotloff et al. 1994[29]	18.3	United States	≥3 stools within 24	Outpatient clinic Infants up to	Not reported	18

Reference	Person Years	Location	Diarrhea Outcome Measure	Population	% ARV Treatment	Total Number of HIV+ Individuals
			hours; persistent if > 14 days	2yrs (birth cohort)		
Muhe et al 1997[30]	47.5	Ethiopia	Diarrhea (not specified further)	Community orphanage Infants and children	Not reported	33
Pavia et al. 1992[31]	1.04	Zaire	≥3 stools within 24 hours separated by 7 diarrhea free days	Infants and children (10 to 15 months)	Not reported	54
Temple et al. 2001[32]	25.5	United States	Diarrhea (not specified further)	Outpatient clinics Children	100%	21
Thea et al. 1993[33]	43	Zaire	Acute defined as change from normal pattern of diarrhea; persistent if > 14 days; recurrent >2 episodes	Outpatient clinic Infants and children (birth cohort)	Not reported	429

Figure 3. Incidence Density of Acute Diarrhea from Adult Cohort Studies

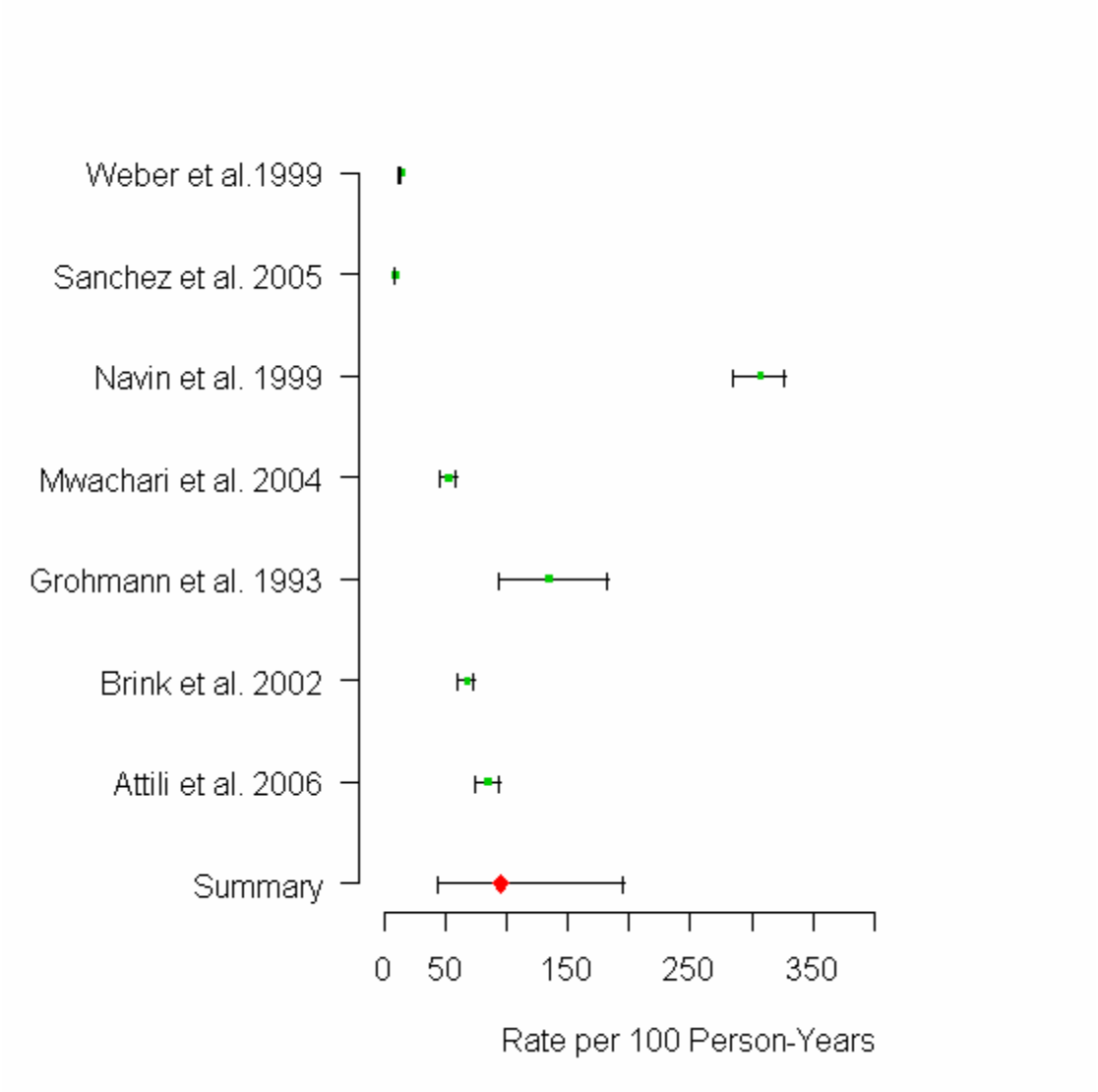
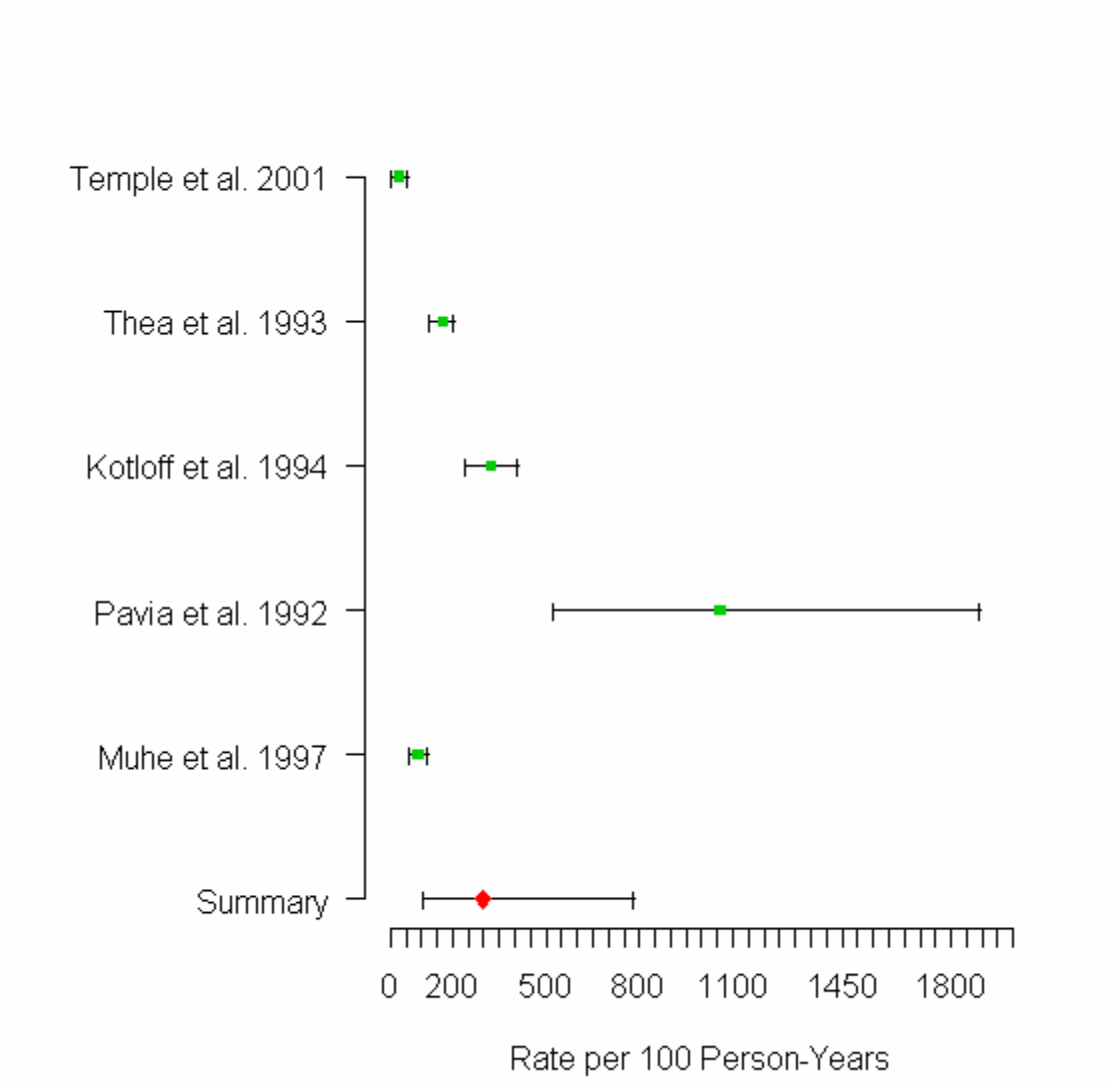


Figure 4. Incidence Density of Acute Diarrhea from Children's Cohort Studies



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AFTERWORD

This dissertation explores gastrointestinal illness in two sensitive subpopulations at increased risk for diarrhea due to some level of immunocompromise, persons infected with the Human Immunodeficiency Virus (HIV) and the elderly. The complexity of possible etiologies, immune status and other related factors are investigated. Each of the three studies comprising this dissertation tackles a particular aspect of the field of gastrointestinal illness in the immunocompromised utilizing a different study design and approach in each case.

The first investigation is via meta-analysis of results from randomized controlled trials of antiretroviral therapies for HIV focusing exclusively on identifying rates of diarrheal illness associated with particular regimen classes. Different highly active anti-retroviral therapy (HAART) regimens are compared and evaluated for reported adverse events for diarrhea and vomiting. Rates of diarrhea ranged from 14.5% to nearly 30% depending on regimen composition. Protease inhibitors, as previous clinical experience has suggested yielded the highest rates of diarrhea, especially regimens including nelfinavir or full (vs. booster) doses of ritonavir. When these regimens were excluded from the protease inhibitor measure, pooled diarrhea incidence for all other protease inhibitor containing regimens lowered to 20.2%.

The lowest rates of diarrhea were seen among nucleotide reverse transcriptase (NRTI) and non-nucleoside reverse transcriptase inhibitor (NNRTI) regimens and ranged from 14-17%, which though lower than the protease inhibitor rates (sans nelfinavir and ritonavir) is not dramatically so. Consequently, the data suggest that as a class, protease inhibitors may have acquired a reputation for high rates of diarrhea that is disproportionately driven by a subset of specific drugs. These drugs may fall out of favor over time as data such as these and clinical experience show which protease inhibitors confer high versus moderate risk for diarrhea, and clinicians increasingly make treatment decisions based on adverse event risk, and potential for discontinuation due to adverse events.

The second study in the dissertation shifts populations to focus on older adults (over 55 years of age), a sensitive subpopulation identified by the Environmental Protection Agency (EPA) of particular risk for drinking water related gastrointestinal illness. A random digit dial (RDD) survey was conducted over a five year period among community dwelling (vs. nursing home or institutional dwelling) elderly to estimate the rate of gastrointestinal illness (diarrhea and vomiting) and identify risk factors for gastrointestinal illness in this population. Annual monthly prevalence of gastrointestinal illness was 7.3%, ranging from 3.8% to 8.3% on a quarterly basis. This corresponded to an incidence rate of 0.99 (95% CI: 0.84, 1.48) episodes per person per year. In the month prior to interview, at least one episode of diarrhea was reported by 6.3% of respondents.

Of those reporting gastrointestinal illness, 30.0% experienced vomiting, 23.4% sought medical care, and 10.8% took antibiotics. Variation by year and season was observed, with some parallels to rainfall in the last two years of the study period. Major predictors of gastrointestinal illness surprisingly were not indicators of infectious etiologies, but rather were underlying chronic illness and immune status. Increased risk for gastrointestinal illness was associated with history of chronic illness with expected gastrointestinal symptoms (OR= 8.9, 95% CI: 5.7, 13.8), and with history of HIV or other immunosuppression (OR= 6.5, 95%CI: 1.9, 19.05).

Interestingly, despite a small sample size for communities of color, racial and ethnic disparities were seen in the gastrointestinal rates. African Americans were five times more likely to report symptoms compared to whites in our sample (OR=5.0, 95% CI: 0.8, 21.2, p=0.0086). Similarly, Native American participants were also five times more likely to report symptoms (OR= 5.4, 95%CI: 0.5,

33.08, p=0.0252) and Latinos were over two times more likely to report symptoms than whites (OR= 2.4, 95% CI: 0.8, 5.8, p=0.049). Foreign travel, recreational water exposures, intake of untreated recreational water sources, and gender were not predictors of risk for gastrointestinal illness in this sample. Nevertheless, given the observed monthly prevalence, endemic gastrointestinal illness represents a substantial burden among community-dwelling elderly impacting quality of life and prompting health care utilization.

The last investigation was again among HIV-positive individuals in the form of a case control design to evaluate infectious and non-infectious (presumably antiretroviral therapy) etiologies for acute, chronic and "no diarrhea" among an urban HIV+ clinic cohort. Risk factors for gastrointestinal illness, medication profiles, CD4 count and HIV viral load were collected to explore the associations between medication risk for diarrhea and the presence of isolatable infectious organisms.

The spectrum of organisms isolated ranged from known pathogens to those whose pathogenicity is unclear. Pathogen isolation was greatest among those reporting acute symptoms; however, pathogens were isolated from all three groups, suggesting that perhaps low level organism burden may contribute to ongoing chronic gastrointestinal illness in this population. Few studies, if any, conducted to date, including this one, have been able to report empirical pathogen specific rates for diarrhea among HIV-positive individuals, given the diversity of gastrointestinal organisms, the sample size needed to measure that within a field setting, and the lack of sufficient detection technology. The relative import of particular pathogens to for immunocompromised populations may be clinically relevant, especially in highlighting the possible pathogenicity of protozoan and bacterial organisms often overlooked for disease severity or underlying burden.

Organisms were detected in 20% of samples collected. Isolation rates for varied between patients with acute, chronic and no diarrhea, with the highest recovery seen among those with acute diarrhea. Organism isolation was associated with the presence of diarrheal symptoms, anal sex, oral sex and consumption of undercooked meats. Diarrhea was associated with anti-retroviral medication use, with protease inhibitors conferring the greatest risk for diarrhea. It remains striking however, that 80% of samples were not able to be linked to a specific pathogen suggesting that non-infectious causes, such as medications, co-morbidities or underlying HIV enteropathy, may be relevant etiologies, for the majority of diarrhea in this population.

In summary, the studies within this dissertation explored gastrointestinal illness in two key immunocompromised populations highlighting the multiple, interwoven etiologies possible, the importance of non-infectious etiologies to the overall burden of illness especially in the developed world, and reiterating the continuing presence of a substantial burden of disease which impacts health status, quality of life, and health care seeking behavior. Though diarrheal illness is often self-limiting in the developed world, it can be severe in these populations and even low levels of diarrhea prevalence can amount to considerable economic impact on a population level.

APPENDICES

PATTERNS AND ETIOLOGIES OF DIARRHEAL ILLNESS AMONG TWO KEY IMMUNOCOMPROMISED POPULATIONS: HIV-INFECTED AND ELDERLY

Appendix I: Systematic Review Supplemental Materials

Review Protocol
PICO: Formulating the Question
Detailed Search History
Data Abstraction Sheet

Estimating the rate of gastrointestinal illness among HIV+ persons

A Systematic Review

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1 BACKGROUND

Diarrhea is a significant cause of morbidity among persons with HIV with cumulative incidence estimates of 30-70% in industrialized nations and up to 100% in developing countries. The pathogenesis of diarrhea is complex and associated with multiple etiologies including bacterial, parasitic and viral pathogens, adverse events from medications, tumors and underlying immune status. The relative contributions of these risk factors to the overall burden of diarrheal illness is unknown and may vary by geographic location, immune status and access to anti-retroviral medications.

HIV+ individuals represent sensitive subpopulations at increased risk for infectious gastroenteritis and may also be at increased risk for severe diarrhea and dying of diarrhea because of their increased susceptibility to dehydration, their waning immunity and their frequent hospitalizations (USEPA 2000; Lew 1991). HIV patients also frequently report chronic diarrhea (daily episodes of watery stool lasting 2-4 weeks or more). Chronic diarrhea has a significant impact on patient outcomes, health care utilization and quality of life.

The association between diarrhea and medications has increased since the introduction of highly active antiretroviral therapy (HAART) in the last quarter of 1996 (McEvoy 1998). For example, diarrhea is a known complication of Nelfinavir and other protease inhibitors (McEvoy 1998). Prior to the introduction of HAART, chronic diarrhea affected 50-90% of the HIV+ population (Janoff 1988), and had been attributed to viral, bacterial, and parasitic infection. More recent studies suggest that though the prevalence of diarrhea has dropped, it is still notable in the HIV+ population (Eisenberg 2002; Bini 1999).

2 PRIMARY RESEARCH QUESTION

The purpose of this study will be to conduct a systematic review to quantify the burden of diarrheal illness among HIV+ individuals and identify key etiological factors related to illness, in particular the relative contributions of medications and infectious pathogens.

3 OBJECTIVES

1. To systematically review the estimates of the incidence of gastrointestinal illness among HIV+ persons.
2. To evaluate the influence of study design and quality on study results.
3. To identify main exposures contributing to the risk of gastrointestinal illness among these population (e.g. medications, waterborne pathogens, food-borne pathogens).
4. To examine sources of heterogeneity in findings between studies.

4 METHODS

4.1 Search Strategy for Identification of Studies

The medical literature published between January 1, 1980 and June 31, 2004 will be searched for studies that examined the rate of gastrointestinal illness HIV+ individuals. The strategy will be

iterative, and no language restriction will be imposed. The literature search will be performed using the following databases:

1. PubMed/Medline
2. EMBASE
3. Experts in the field will be contacted in an attempt to identify unpublished research or studies still underway.
4. Reference lists from included studies and other pertinent review articles (snowballing).

Inclusion Criteria

Inclusion criteria were established *a priori* to minimize the potential for selection bias. All available studies quantifying the rate of gastrointestinal illness or diarrheal illness among HIV+ persons are eligible for this study. Special attempts will be made to include unpublished studies. For inclusion, the studies should meet the following criteria.

The study provides an endemic rate of diarrheal illness or the raw data exists to calculate a rate. Such studies may be:

1. Cohort studies with person-time data
2. RCTs for HAART regimens reporting % of participants reporting diarrhea (n diarrhea/ N total)
3. Case control or cross-sectional studies reporting % of participants reporting diarrhea (n diarrhea/ N total)
4. The study provides enough information to judge its methodological quality.

Studies with information on CD4 count, viral load and % isolation of pathogen are desirable, but not required for primary estimate of the burden of diarrhea in the HIV+ population.

Study eligibility will be judged independently by two reviewers who will review the titles and abstracts identified in the above searches. If the title or the abstract is judged by either reviewer to be potentially eligible, the full article will then be examined in order to resolve the dispute. Reviewers assessing study eligibility will be blinded to the names of the authors, journals, or other publication details.

Exclusion Criteria

Studies will be excluded based on the following criteria:

1. Case reports, letters, lectures, news items, conference abstracts.
2. Treatment guidelines, systematic reviews.
3. Foreign language publications without abstract in English.
4. Studies related to specific outbreaks or limited to specific pathogen.
5. Studies not containing outcomes of interest.

4.2 Methods of the Review

Each reviewer who will extract the data using a standardized, pre-piloted data extraction form. The data extraction form was designed to collect information on study quality, features of study design, sample size, participant characteristics, study population, inclusion and exclusion criteria, study results and potential biases that may affect the overall results as well as quality of reporting of the trial. The inter-rater agreement between reviewers will be assessed.

4.3 Description of Studies

The studies will be summarized according to several key characteristics:

- Study design (case control, cross-sectional, cohort, RCT, etc.)
- Study location
- Definition of gastrointestinal illness and/or diarrhea case definition
- Mean/Median Age (years)
- Proportion of the study sample who are White
- Proportion of the study sample who are male
- Were the study participants symptomatic, asymptomatic, both or not reported?
- Presence of AIDS-defining conditions at baseline
- Baseline CD4 cell count
- Baseline HIV RNA level

4.4 Methodological Quality of Included Studies

Observational studies will be evaluated for their treatment of potential confounders, selection of study subjects (including appropriate controls), and completeness of follow-up. (Stroup 2000)

Quality appraisal for RCTs will be based on the following questions:

1. Was the study described as randomized (includes the use of the words such as randomly, random, and randomization)? (Jadad 1996)
2. Was the study described as double-blind? (Jadad 1996)
3. Was there a description of withdrawals and dropouts? (Jadad 1996)
4. Was the generation of allocation sequences adequate? (Schultz 1995)
5. Was the treatment allocation schedule adequately concealed? (Schultz 1995)
6. Did the analysis include all randomized participants? (Schultz 1995)
7. Was the intention-to-treat principle applied in the analysis? (Schultz 1995)

4.5 Study Factors

Outcome Measures and Definition of Gastrointestinal Illness

The primary outcome is the rate of episodes of diarrhea among HIV+ individuals. There is a diversity of case definitions for diarrhea and gastrointestinal illness in the literature, including composite definitions of diarrhea, vomiting, nausea, abdominal cramps, and other GI symptoms. Case definitions of diarrheal illness also vary by study and may distinguish between acute and

chronic diarrhea. Secondary outcomes will include odds ratios for the association between diarrheal illness and risk factors (eg. medications, infectious agents).

Summary of Outcome Measures

- Rate of diarrheal illness
  - Measures of Incidence
    - Episodes per person per year
  - Measures of Prevalence
    - % of subjects reporting diarrhea in some time period
    - % isolation of pathogens from stool samples
- Etiology of diarrheal illness
  - Odds Ratio
    - Pathogens
    - Medication Adverse Event (HAART)
    - CD4 count

Heterogeneity

Between-study heterogeneity of the estimates of the incidence of gastrointestinal illness will be assessed. If there is evidence of heterogeneity, the sources of this heterogeneity in effect will be explored. The following factors may account for heterogeneity:

- Study quality
- Study design
- Study population (hospital/ clinic based, community based study, retirement/ elder care facility vs. community dwelling cohort, etc.)
- Variation in disease status or immune status among study subjects
- GI illness risk factors: water consumption and contact, food, medications, etc.
- Proportion male
- Proportion non-white
- Mean/median age (years)
- Study location (country)
- Definition of diarrhea/ gastrointestinal illness
- Year of Publication (eg. pre or post HAART)
- Baseline CD4 cell count
- Baseline HIV RNA level

5 STATISTICAL ANALYSES

Our objective will be to provide a summary estimate of diarrheal illness using a model allowing for significant variability between studies. Analyses will be stratified by study design given the distinct outcome measures provided by each study type. Subgroup analyses were conducted to compare and evaluate any underlying differences in rate by location, time period (eg. pre-HAART vs. post-HAART), and by drug class (NNRTI, NRTI, PI).

i. Cohort studies on gastrointestinal illness (person time data-incidence density)

1. Negative Binomial estimate random effects model (assumes Poisson distribution within studies, random effects/ neg binomial between studies)

ii. Cohorts (individual study arms) from HAART RCTs (number of people reporting 1 or more episodes of diarrhea/ total N - cumulative incidence)
    1. GEE adjusted for study period

iii. Cross-sectional surveys (prevalence)
    1. GEE adjusted for study period

If pooling is appropriate, we will calculate pooled rates with corresponding 95% confidence intervals (CIs). Analyses will be conducted in Stata 8.0. All *P* values will be two-tailed with alpha=0.05 level of significance.

Potential subgroups or strata:

- Country - Developing vs. Industrialized countries (eg. World Bank income categories)
- Age - Children under 5 years of age vs. individuals above 5 years
- Medication status – HAART vs. no HAART
- CD4 count levels

6 REPORTING OF RESULTS

In organizing and reporting the results of this systematic review, we will follow the guidelines and specifications checklist drafted by the Meta-analysis of Observational Studies in Epidemiology (MOOSE) which is summarized below (Stroup 2000).

Table. A Proposed Reporting Checklist for Authors, Editors, and Reviewers of Meta-analyses of Observational Studies

Reporting of background should include
Problem definition
Hypothesis statement
Description of study outcome(s)
Type of exposure or intervention used
Type of study designs used
Study population
Reporting of search strategy should include
Qualifications of searchers (eg, librarians and investigators)
Search strategy, including time period included in the synthesis and keywords
Effort to include all available studies, including contact with authors
Databases and registries searched
Search software used, name and version, including special features used (eg, explosion)
Use of hand searching (eg, reference lists of obtained articles)
List of citations located and those excluded, including justification
Method of addressing articles published in languages other than English
Method of handling abstracts and unpublished studies
Description of any contact with authors
Reporting of methods should include
Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested
Rationale for the selection and coding of data (eg, sound clinical principles or convenience)
Documentation of how data were classified and coded (eg, multiple raters, blinding, and interrater reliability)
Assessment of confounding (eg, comparability of cases and controls in studies where appropriate)
Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictors of study results
Assessment of heterogeneity
Description of statistical methods (eg, complete description of fixed or random effects models, justification of whether the chosen models account for predictors of study results, dose-response models, or cumulative meta-analysis) in sufficient detail to be replicated
Provision of appropriate tables and graphics
Reporting of results should include
Graphic summarizing individual study estimates and overall estimate
Table giving descriptive information for each study included
Results of sensitivity testing (eg, subgroup analysis)
Indication of statistical uncertainty of findings
Reporting of discussion should include
Quantitative assessment of bias (eg, publication bias)
Justification for exclusion (eg, exclusion of non–English-language citations)
Assessment of quality of included studies
Reporting of conclusions should include
Consideration of alternative explanations for observed results
Generalization of the conclusions (ie, appropriate for the data presented and within the domain of the literature review)
Guidelines for future research
Disclosure of funding source

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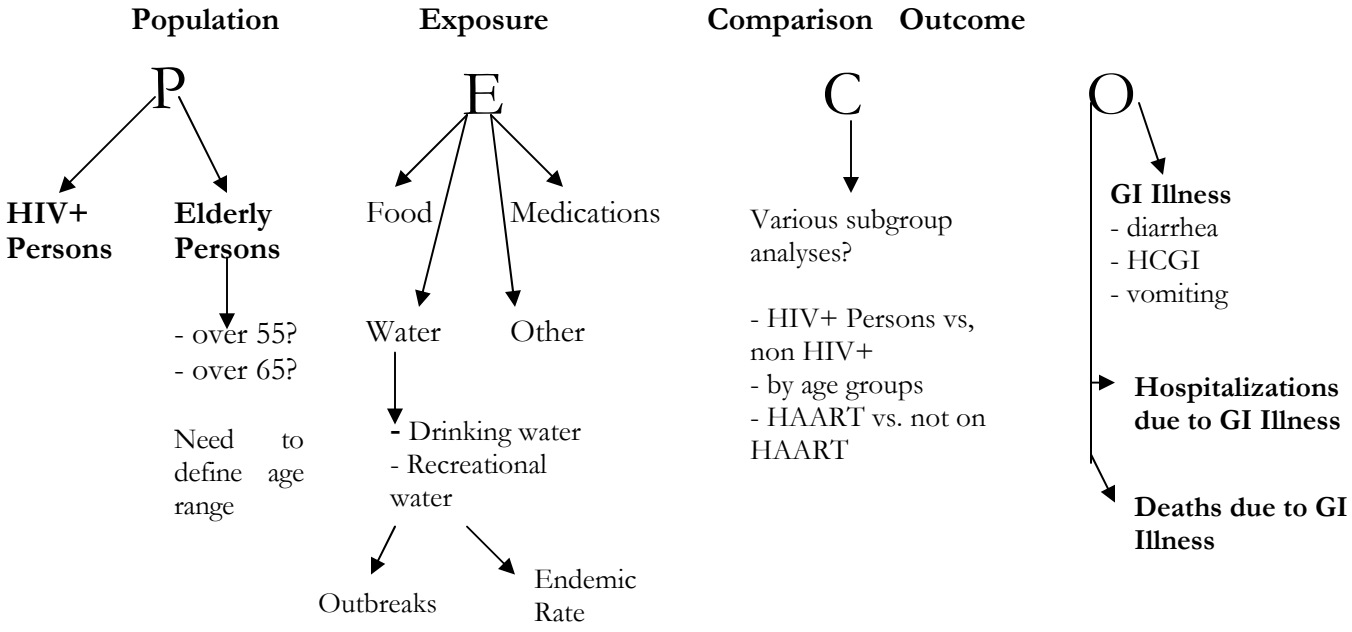
Estimating the rate of gastrointestinal illness among key sensitive subpopulations: the elderly and HIV+ persons

Defining the Question



6.1.1 Possible Questions

1. What is the rate of GI illness among elderly adults above age 55?
2. What is the rate of GI illness among elderly adults above age 55 associated with water exposures?
3. What is the rate of GI illness among elderly adults above 55 compared to adults 25-54?
4. What is the rate of GI illness among HIV+ individuals?
5. What is the rate of GI illness among HIV+ individuals attributable to infectious causes versus medication side effects?
6. What is the rate of GI illness among HIV+ individuals compared to the general population?
7. etc…..!

6.1.2 Studies Designs to consider…

1. Cohort
2. Case-Control/ Nested Case- Control
3. Cross-Sectional
4. don't place limits..?

Potential Search Strings to Identify Studies

P: POPULATION

"Acquired Immunodeficiency Syndrome" [MESH] OR "Acquired Immunodeficiency Syndrome" [tw] OR HIV [MESH] OR "Human Immunodeficiency Virus" [tw] OR HIV [tw] OR HIV [ti] OR AIDS [ti] OR immunodef* [tw] or "Human Immunodeficiency Virus" [ti]"

I: INTERVENTION (or EXPOSURE)

None specified.

C: COMPARISON

None specified.

O: OUTCOME

(((((gastrointestinal[All Fields] AND illness[All Fields]) OR (GI[All Fields] AND illness[All Fields])) OR ((diarrhea[Text Word] OR diarrhoea[Text Word]) OR "diarrhea"[MeSH Terms])) OR ("nausea"[MeSH Terms] OR nausea[Text Word])) OR ("vomiting"[MeSH Terms] OR vomiting[Text Word]))

POSSIBLE METHODOLOGIC FILTERS:

CASE CONTROL, COHORT AND LONGITUDINAL STUDIES (Reference: (by Haynes et al. J Am Med Inform Assoc. 1994 Nov-Dec;1(6):447-58):

"incidence" [MESH] OR "mortality" [MESH] OR "follow-up studies" [MESH] OR "mortality" [SH] OR prognos* [WORD] OR predict* [WORD] OR course [WORD]

"cohort studies" [MESH] OR "risk" [MESH] OR ("odds" [WORD] AND "ratio*" [WORD]) OR ("relative" [WORD] AND "risk" [WORD]) OR "case-control*" [WORD] OR case-control studies [MESH]

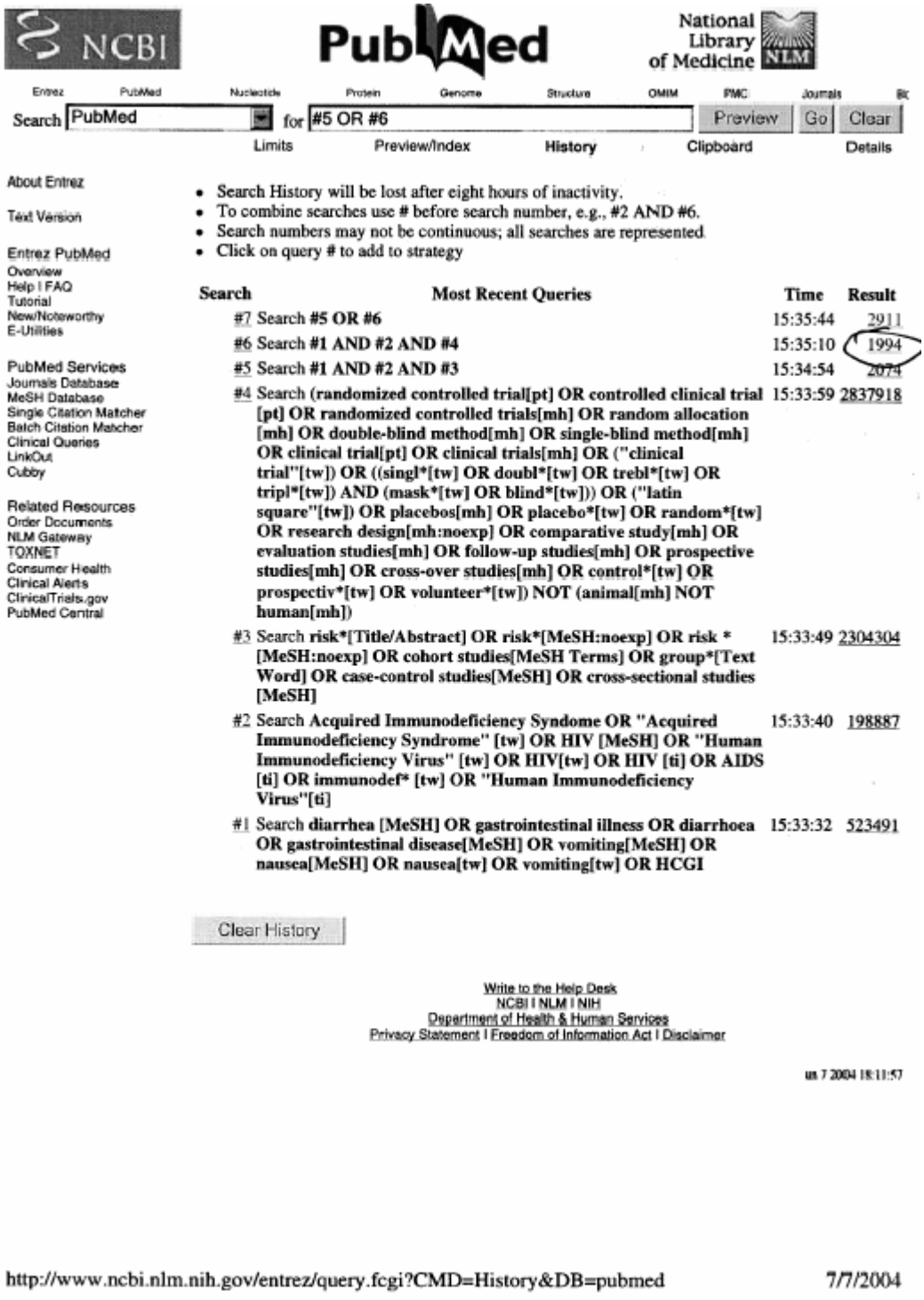
"case-control studies" [MH:NOEXP] OR "cohort studies" [MH:NOEXP]

RANDOMIZED CONTROLLED TRIAL
(REFERENCE: modified Robinson and Dickersin)

Cochrane RCT
(randomized controlled trial [pt] OR controlled clinical trial [pt] OR randomized controlled trials [mh] OR random allocation [mh] OR double-blind method [mh] OR single-blind method [mh] OR clinical trial [pt] OR clinical trials [mh] OR ("clinical trial" [tw]) OR ((singl* [tw] OR doubl* [tw] OR trebl* [tw] OR tripl* [tw]) AND (mask* [tw] OR blind* [tw])) OR ("latin square" [tw]) OR placebos [mh] OR placebo* [tw] OR random* [tw] OR research

design [mh:noexp] OR comparative study [mh] OR evaluation studies [mh] OR follow-up studies [mh] OR prospective studies [mh] OR cross-over studies [mh] OR control* [tw] OR prospectiv* [tw] OR volunteer* [tw]) NOT (animal [mh] NOT human [mh])

Pub Med Search Results



NCBI PubMed National Library of Medicine NLM

Entrez PubMed Nucleotide Protein Genome Structure OMIM PMC Journals Bc

Search PubMed for #5 OR #6 Preview Go Clear

Limits Preview/Index History Clipboard Details

About Entrez
Text Version
Entrez PubMed
Overview
Help | FAQ
Tutorial
New/Noteworthy
E-Utilities

PubMed Services
Journals Database
MeSH Database
Single Citation Matcher
Batch Citation Matcher
Clinical Queries
LinkOut
Cubby

Related Resources
Order Documents
NLM Gateway
TOXNET
Consumer Health
Clinical Alerts
ClinicalTrials.gov
PubMed Central

- Search History will be lost after eight hours of inactivity.
- To combine searches use # before search number, e.g., #2 AND #6.
- Search numbers may not be continuous; all searches are represented.
- Click on query # to add to strategy

Search	Most Recent Queries	Time	Result
#7	Search **#5 OR #6**	15:35:44	2911
#6	Search **#1 AND #2 AND #4**	15:35:10	1994
#5	Search **#1 AND #2 AND #3**	15:34:54	2074
#4	Search **(randomized controlled trial[pt] OR controlled clinical trial [pt] OR randomized controlled trials[mh] OR random allocation [mh] OR double-blind method[mh] OR single-blind method[mh] OR clinical trial[pt] OR clinical trials[mh] OR ("clinical trial"[tw]) OR ((singl*[tw] OR doubl*[tw] OR trebl*[tw] OR tripl*[tw]) AND (mask*[tw] OR blind*[tw])) OR ("latin square"[tw]) OR placebos[mh] OR placebo*[tw] OR random*[tw] OR research design[mh:noexp] OR comparative study[mh] OR evaluation studies[mh] OR follow-up studies[mh] OR prospective studies[mh] OR cross-over studies[mh] OR control*[tw] OR prospectiv*[tw] OR volunteer*[tw]) NOT (animal[mh] NOT human[mh])**	15:33:59	2837918
#3	Search **risk*[Title/Abstract] OR risk*[MeSH:noexp] OR risk * [MeSH:noexp] OR cohort studies[MeSH Terms] OR group*[Text Word] OR case-control studies[MeSH] OR cross-sectional studies [MeSH]**	15:33:49	2304304
#2	Search **Acquired Immunodeficiency Syndome OR "Acquired Immunodeficiency Syndrome" [tw] OR HIV [MeSH] OR "Human Immunodeficiency Virus" [tw] OR HIV[tw] OR HIV [ti] OR AIDS [ti] OR immunodef* [tw] OR "Human Immunodeficiency Virus"[ti]**	15:33:40	198887
#1	Search **diarrhea [MeSH] OR gastrointestinal illness OR diarrhoea OR gastrointestinal disease[MeSH] OR vomiting[MeSH] OR nausea[MeSH] OR nausea[tw] OR vomiting[tw] OR HCGI**	15:33:32	523491

Clear History

Write to the Help Desk
NCBI | NLM | NIH
Department of Health & Human Services
Privacy Statement | Freedom of Information Act | Disclaimer

un 7 2004 18:11:57

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=History&DB=pubmed 7/7/2004

Embase Search Results

Database: EMBASE <1988 to 2004 Week 30>
Search Strategy:
---
1 exp TRAVELLER DIARRHEA/ or exp CHRONIC DIARRHEA/ or diarrhea.mp. or exp CHLORIDE DIARRHEA/ or exp BOVINE DIARRHEA VIRUS/ or exp DIARRHEA/ or exp ACUTE DIARRHEA/ or exp INFANTILE DIARRHEA/ (47175)
2 exp diarrhea/ or exp priority journal/ or exp leaky gut syndrome/ or exp padma lax/ or exp article/ or exp herbaceous agent/ or exp human/ or exp plant extract/ or exp gastrointestinal disease/ or gastrointestinal illness.mp. or exp irritable colon/ (6127482)
3 diarrhoea.mp. or exp Diarrhea/ (44138)
4 gastrointestinal disease.mp. or exp Gastrointestinal Disease/ (9708)
5 vomiting.mp. or exp "POSTOPERATIVE NAUSEA AND VOMITING"/ or exp VOMITING/ or exp "NAUSEA AND VOMITING"/ or exp POSTOPERATIVE VOMITING/ (62944)
6 nausea.mp. or exp "NAUSEA AND VOMITING"/ or exp "POSTOPERATIVE NAUSEA AND VOMITING"/ or exp NAUSEA/ or exp POSTOPERATIVE NAUSEA/ (62314)
7 nausea.mp. or exp "NAUSEA AND VOMITING"/ or exp "POSTOPERATIVE NAUSEA AND VOMITING"/ or exp NAUSEA/ or exp POSTOPERATIVE NAUSEA/ (62314)
8 vomiting.mp. or exp "POSTOPERATIVE NAUSEA AND VOMITING"/ or exp VOMITING/ or exp "NAUSEA AND VOMITING"/ or exp POSTOPERATIVE VOMITING/ (62944)
9 vomiting.mp. or exp "POSTOPERATIVE NAUSEA AND VOMITING"/ or exp VOMITING/ or exp "NAUSEA AND VOMITING"/ or exp POSTOPERATIVE VOMITING/ (62944)
10 exp retrospective study/ or exp drinking water/ or exp water quality/ or exp risk assessment/ or exp chorionic gonadotropin/ or exp hormone action/ or exp first trimester pregnancy/ or exp human/ or exp gastrointestinal disease/ or HCGI.mp. or exp health hazard/ (3930579)
11 1 and 2 and 3 and 4 and 5 and 6 and 7 and 8 and 9 and 10 (402)
12 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 (6141626)
13 (Acquired Immunodeficiency Syndome or "Acquired Immunodeficiency Syndrome" or HIV or "Human Immunodeficiency Virus" or HIV or HIV or AIDS or immunodeficiency or immunodeficient or "Human Immunodeficiency Virus").mp. [mp=title, abstract, subject headings, drug trade name, original title, device manufacturer, drug manufacturer name] (139439)
14 (aids or HIV).mp. [mp=title, abstract, subject headings, drug trade name, original title, device manufacturer, drug manufacturer name] (115745)
15 aids.mp. or exp Acquired Immune Deficiency Syndrome/ (66146)
16 hiv.mp. or exp Human Immunodeficiency Virus/ (100545)
17 13 or 14 or 15 or 16 (149594)
18 Randomized Controlled Trial/ (86433)
19 12 and 17 (144591)

20 Randomized Controlled Trial/ (86433)
21 Clinical Trial/ (301188)
22 Clinical Trial/ (301188)
23 Randomized Controlled Trial/ (86433)
24 random allocation.mp. or exp Randomization/ (11767)
25 Double Blind Procedure/ (47884)
26 Single Blind Procedure/ (4823)
27 Clinical Trial/ (301188)
28 Clinical Trial/ (301188)
29 (double or treble or triple).mp. [mp=title, abstract, subject headings, drug trade name, original title, device manufacturer, drug manufacturer name] (147842)
30 exp human/ or exp article/ or latin square.mp. or exp controlled study/ (5785486)
31 (placebos or placebo).mp. [mp=title, abstract, subject headings, drug trade name, original title, device manufacturer, drug manufacturer name] (84761)
32 placebos.mp. or exp Placebo/ (45623)
33 research design.mp. or exp Methodology/ (640534)
34 comparative study.mp. or exp Comparative Study/ (126101)
35 evaluation studies.mp. or exp Evaluation/ (27068)
36 follow-up studies.mp. or exp Follow Up/ (134344)
37 prospective studies.mp. or exp Prospective Study/ (44602)
38 Crossover Procedure/ (15117)
39 exp LEPROSY CONTROL/ or exp INFECTION CONTROL/ or exp CASE CONTROL STUDY/ or exp MALARIA CONTROL/ or exp CONTROL/ or exp PARASITE CONTROL/ (116728)
40 (prospective or volunteer).mp. [mp=title, abstract, subject headings, drug trade name, original title, device manufacturer, drug manufacturer name] (132845)
41 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 (5875653)
42 19 and 41 (139253)
43 exp RISK FACTOR/ or exp INFECTION RISK/ or risk.mp. or exp RISK ASSESSMENT/ (450583)
44 exp female/ or exp major clinical study/ or exp adult/ or exp acquired immune deficiency syndrome/ or exp article/ or exp human immunodeficiency virus infection/ or exp human/ or exp priority journal/ or exp COHORT ANALYSIS/ or cohort.mp. or exp male/ (6150789)
45 exp female/ or exp major clinical study/ or exp adult/ or exp acquired immune deficiency syndrome/ or exp article/ or exp human immunodeficiency virus infection/ or exp human/ or exp priority journal/ or exp COHORT ANALYSIS/ or cohort.mp. or exp male/ (6150789)
46 exp female/ or exp major clinical study/ or exp adult/ or exp acquired immune deficiency syndrome/ or exp article/ or exp human immunodeficiency virus infection/ or exp human/ or exp priority journal/ or exp COHORT ANALYSIS/ or cohort.mp. or exp male/ (6150789)
47 exp priority journal/ or exp major clinical study/ or exp male/ or exp controlled study/ or exp case control study/ or exp article/ or exp female/ or exp human/ or case-control.mp. or exp adult/ (6171807)

48 cross-sectional studies.mp. or exp Prevalence/ (75143)
49 12 and 17 and 41 (139253)
50 43 or 44 or 45 or 48 (6165235)
51 12 and 17 and 50 (144539)
52 49 or 51 (144545)
53 exp TRAVELLER DIARRHEA/ or exp CHRONIC DIARRHEA/ or diarrhea.mp. or exp DIARRHEA/ or exp ACUTE DIARRHEA/ or exp INFANTILE DIARRHEA/ (46998)
54 exp diarrhea/ or exp gastrointestinal disease/ or gastrointestinal illness.mp. (50515)
55 diarrhoea.mp. or exp Diarrhea/ (44138)
56 gastrointestinal disease.mp. or exp Gastrointestinal Disease/ (9708)
57 vomiting.mp. or exp "POSTOPERATIVE NAUSEA AND VOMITING"/ or exp VOMITING/ or exp "NAUSEA AND VOMITING"/ or exp POSTOPERATIVE VOMITING/ (62944)
58 vomiting.mp. or exp "POSTOPERATIVE NAUSEA AND VOMITING"/ or exp VOMITING/ or exp "NAUSEA AND VOMITING"/ (62944)
59 exp retrospective study/ or exp drinking water/ or exp water quality/ or exp gastrointestinal disease/ or HCGI.mp. (72403)

Diarrhea among HIV+ Persons – Five Sample Studies

Reference	Study Design	Outcomes Rates	Outcomes Risk Estimates	Definition of Diarrhea	Study Setting and Population
Call SA, Hueberdt G, Saag M, et al. (2000) [1]	Cohort (retrospective chart review)	Chronic diarrhea 8-10.5% per year	Opportunistic infection: 53% (1995), 13% (1996, 1997) Medication associated or idiopathic: 32% (1995), 70% (1996) 71% (1997)	Chronic diarrhea- increase in frequency of stools (>3 per day) for longer than 2 weeks	Outpatient HIV Clinic, Univ of Birmingham, AL 1995-1997 HIV patients with CD4 cell counts <200 cells/mm^3 N= 80 cases
Navin TR, Weber R, Vugia D, et al. (1999)[2]	Prospective Cohort	39% reported diarrhea in previous month 832 episodes total- 354 (42.5%) acute, 279 (33.5%) chronic, 195 (23.9%) indeterminate	Parasitic etiology: 14.1% of acute episodes 34.8% of chronic 23.1% indeterminant OR: CD4<100 = 2.5 (1.4, 4.3) CD4 100-199= 1.8 (1, 3.2)	3 or more loose or watery stools within a 24 hour period acute- episodes lasting less than 1 month chronic- episodes lasting more than 1 month indeterminate- time period not able to be determined	ID Clinics affiliated with Emory University 1991-1993 N= 602 HIV+ patients

[1] Call SA, Hueberdt G, Saag M, Wilcox CM. The Changing Etiology of Chronic Diarrhea in HIV Infected Patients with CD4 Cell Counts Less Than 200 cells/m^3. American Journal of Gastroenterology (2000) 95:3142-3146

[2] Navin TR, Weber R, Vugia D, Rimland D, Roberts JM, Addiss DG, Visvesvara GS, Wahlquist SP, Hogan, SE, Gallagher LE, Juranek D, Schwartz DA, Wilcox CM, Stewart, JM, Thompson, SE, Bryan R. Declining CD4+ T-Lymphocyte Counts are Assoiciate with Increased Risk of Enteric Parasitosis and Chronic Diarrhea: Results of a 3 year Longitudinal Study (1999) 20;2:154-159

Weber R, Ledergerber B, Zbinden R, et al (1999)[3]	Prospective cohort	14.2 episodes per 100 person years (95% CI: 13, 15.4) 212 acute episodes 348 chronic episodes	Enteric pathogens in 16.5% of acute cases, 46% of chronic cases	*Need to get paper*	N=1933 Swiss community based cohort
Reference	**Study Design**	**Outcomes Rates**	**Outcomes Risk Estimates**	**Definition of Diarrhea**	**Study Setting and Population**
Brink AK, Mahe C, Watera C, et al. (2002)[4]	Prosepective cohort	Rate of diarrhea: 661 episodes per 1000 person years	Pathogen isolated from 49% of patients with diarrhea, 39% of those without diarrhea OR: Bacteria 1.8 (1, 3.3) Protozoa 1.8 (0.5, 6.3) CD4 <200 3.4 (2.2, 5.3)	3 or more loose stools within 24 hrs acute: episode duration less than 1 month and no diarrhea in the preceding month chronic: episode duration greater than 1 month or recurrent over a period of 2 months	Community based cohort of HIV+ adults in Entebe, Uganda (1995-1997) N=1213 HIV+ adults

[3] Weber R, Ledergerber B, Zbinden R, Alwegg M, Pfyffer GE, Spycher MA, Briner J, Kaiser L, Opravil M, Meyenberger C, Flepp M. Enteric infections in human immunodeficiency virus infected patients: prospective community based cohort study. Swiss HIV Cohort Study (1999) Arch Intern Med, 159(13):1473-80

[4] Brink AK, Mahe C, Watera C, Lugada E, Gilks C, Whitworth J, French N. Diarrhoea, CD4 Counts and Enteric Infections in a Community-Based Cohort of HIV-Infected Adults in Uganda. (2002) Journal of Infection, 45:99-106

Arenas-Pinto A, Certad G, Ferrara G, et al (2003)[5]	Cross-sectional survey	None	OR: Acute diarrhea- I. belli 10.2 (1.2, 88.2) E. histolytica 11.48 1.5, 94) C. parvum 2.6 (0.9, 7.3) Chronic diarrhea- I.belli 16.4 (1.9, 138.4) S.stercoralis 4.3 (1, 17.6) E. histolytica 8.6 (2.6, 29.1) C.parvum 3.4 (1.1, 10.2)	3 or more liquid depositions in 24 hrs acute: duration less than 21 days chronic: duration longer than 21 days	HIV+ adults recruited from HIV clinics in Caracas, Venezuela 1997-2000

Notes: CDC Definition for chronic diarrhea- average of at least two or more watery stools per day for 1 month

[5] Arenas-Pinto A, Certad G, Ferrara G, Castro J, Bello MJ, Nunez LT. Association between parasitic intestinal infections and acute or chronic diarrhea in HIV-infected patients in Caracas, Venuzuela. (2003) International Journal of STD & AIDS, 14:487-492

ESTIMATING THE RATE OF GASTROINTESTINAL ILLNESS AMONG HIV+ PERSONS : A SYSTEMATIC REVIEW

DATA ABSTRACTION SHEET

Reviewer:	Date of Review:

Study:

Study ID Number:
Primary Author:
Journal:
Year of Publication:
Volume:
Issue:
Pages:

For Internal Use Only:

Date Entry Form was Reviewed:
Reviewed By:
Date Entry Form was Entered:
Entered By:

Corresponding Author:
Corresponding Author's Address:
Corresponding Author's Email:

Notes:

Start Time: _____

PART I: STUDY CHARACTERISTICS

7

8 **What type of study design was used?**

☐ RCT ☐ Prospective Cohort
☐ Retrospective Cohort
☐ Case-Control
☐ Nested Case-Control
☐ Cross-Sectional

9 **Was this a multicenter study?**

☐ **Not Reported** ☐ Yes ☐ No

10

11 **Where did the study take place?**

☐ Hospital ☐ Outpatient Clinic
☐ Community Setting
☐ **Not Reported**

_____
Specify country/countries
(enter "NR" if not reported)

12

13 **In what population did the study take place?**

☐ HIV+ general
☐ HIV+ adults
☐ HIV+ children (under 5)
☐ HIV+ children (under 18)
☐ other :_____

14

15 **Did the authors receive informed consent?**

☐ **Not Reported** ☐ Yes ☐ No

16

17 **How long was the follow-up for this study?**

_____
Specify days, weeks, or years

18

19 **What was the recruitment period?**
Start Date: _____

End Date: _____

20

21

22 **List the primary outcome(s) and cutoff(s) of the study.**

23 *If RCT*, **list the drug, dose and frequency for each of the treatment groups.**

DEFINITION OF DIARRHEA/ GI ILLNESS

How was diarrheal illness defined?

- ☐ 2 or more loose stools within 24 hrs
- ☐ 3 or more loose stools within 24 hrs
- ☐ other (list below)
- ☐ Not clear

If applicable, how was acute diarrhea defined?

If applicable, how was chronic diarrhea defined?

Was a composite definition of gastrointestinal illness used? ☐ Yes ☐ No

If yes, please define. ☐ HCGI ☐ Other, defined below

BIOLOGICAL SPECIMEN COLLECTION

Were stool samples collected during the study? ☐ **Not Reported** ☐ Yes ☐ No

Were blood samples collected during the study? ☐ **Not Reported** ☐ Yes ☐ No

ADVERSE EVENTS (for RCTs)

Did the investigators monitor for adverse events? ☐ **Not Reported** ☐ Yes ☐ No

If yes, which scale was used for grading and staging adverse events? ☐ **Not Reported**

PART II: STUDY QUALITY

44

45 SAMPLE SIZE CALCULATION

46 **Was a sample size calculation done *a priori* to determine the minimum sample size needed for confirming a quantitatively significant difference?** ☐ **Not Reported** ☐ Yes ☐ No

47

48 *FOR RCTS:*

49

50 RANDOMIZATION

51 **What was the technique of randomization?**
52 **(Check all that apply)**

☐ **Not Reported**
☐ Simple
☐ Fixed Allocation
☐ Blocked
☐ Stratified
☐ Other

_____

53

54 **How would you describe the generation of the allocation sequence?**

☐ **Not Reported**
☐ Adequate
☐ Inadequate
☐ Not Clear

55

56 **How would you describe the concealment of the allocation sequence?**

☐ **Not Reported**
☐ Adequate
☐ Inadequate

57 BLINDING

58 **Did the authors report that the study was an "open-label" trial?** ☐ Yes ☐ No

59

60 **Was the study described as "double-blind"?** ☐ Yes ☐ No

Were the participants blinded to the intervention? ☐ **Not Reported** ☐ Yes ☐ No

Were the investigators blinded to the intervention? ☐ **Not Reported** ☐ Yes ☐ No

Were the assessors of the outcome(s) blinded to the intervention? ☐ **Not Reported** ☐ Yes ☐ No

Was the success of blinding evaluated? ☐ **Not Reported** ☐ Yes ☐ No

INTENTION-TO-TREAT

Was the intention-to-treat principle applied in the analysis? ☐ **Not Reported** ☐ Yes ☐ No

SOURCES OF FUNDING

Did a pharmaceutical company sponsor the study? ☐ **Not Reported** ☐ Yes ☐ No

Did any of the authors receive any financial support from one or more of the pharmaceutical firms whose products were studied? ☐ **Not Reported** ☐ Yes ☐ No

FOR OBSERVATIONAL STUDIES:

SELECTION AND INFORMATION BIAS

Were the cases (or exposed) representative of the study base?
☐ **Not Reported**
☐ Yes
☐ No
☐ Not Clear

Were the comparison groups (eg. unexposed, controls) drawn from the same community as the cases (or exposed)?
☐ **Not Reported**
☐ Yes
☐ No
☐ Not Clear

Was loss to follow-up the same between the various groups?
☐ **Not Reported**
☐ Yes
☐ No
☐ Not Clear

For case-control studies; what type of controls were used?
☐ Hospital-based
☐ Community-based
☐ Historical
☐ Other: _____
☐ Not Clear

Was follow-up time complete, or if incomplete unlikely to cause bias (small number)? ☐ Yes ☐ No

PART III: BASELINE CHARACTERISTICS

Characteristic	91.1 Group 1	**Group 2**	**Group 3**	**Group 4**	**91.1.1.1 Total**
Treatment Regimen or Exposure Status	_____	_____	_____	_____	_____
Number of Study Participants	_____	_____	_____	_____	_____
Age (Years) Mean (SD)	_____	_____	_____	_____	_____
Median (SD)	_____	_____	_____	_____	_____
Number of Males	_____	_____	_____	_____	_____
Number of White Participants	_____	_____	_____	_____	_____
Number with AIDS-defining conditions	_____	_____	_____	_____	_____
Baseline CD4+ Count ($X10^6$ cells/l)					
Mean (SE)	_____	_____	_____	_____	_____
Median (IQR)	_____	_____	_____	_____	_____
Baseline HIV RNA (log_{10} copies/mL)					
Mean (SE)	_____	_____	_____	_____	_____
Median (IQR)	_____	_____	_____	_____	_____

PART IV: STUDY RESULTS

91.1.1.2 Characteristic	91.2 Group 1	Group 2	Group 3	Group 4	91.2.1.1 Total
Treatment Regimen or Exposure Status					
Number of Study Participants					
91.2.1.1.1 Incidence Measures					
Episodes of Diarrhea (or HCGI)					
Nausea					
Vomiting					
Person-time *(please specify)*					
Rate of Diarrhea					
95% CI					
Prevalence Measures					
92					
93 Diarrhea [n (%)]					
Vomiting [n (%)]					
Nausea [n (%)]					
Discontinuation of Tx [n (%)]					
Withdrawal due to AE [n (%)]					
Study Period					

94 Measures of Association

Association between:

- ☐ Diarrhea and CD4 status
- ☐ Diarrhea and pathogen isolation
- ☐ Diarrhea and medication status
- ☐ Other: _____

(please specify CD4 categories or other relevant specifics next to reported effect measure)

☐ OR ☐ RR ☐ AR

_____

95% CI: _____

☐ Crude ☐ Adjusted

If Adjusted Effect Measure, please list what was adjusted for:

_____

_____

Notes: (eg. % D/C or withdrawal attributed to GI symptoms, etc.)

95 Measures of Association

Association between:
- ☐ Diarrhea and CD4 status
- ☐ Diarrhea and pathogen isolation
- ☐ Diarrhea and medication status
- ☐ Other: _____

(please specify CD4 categories or other relevant specifics next to reported effect measure)

☐ OR ☐ RR ☐ AR
_____
95% CI: _____

☐ Crude ☐ Adjusted
If Adjusted Effect Measure, please list what was adjusted for:
_____
_____

Notes: (eg. % D/C or withdrawal attributed to GI symptoms, etc.)

96 Measures of Association

Association between:
- ☐ Diarrhea and CD4 status
- ☐ Diarrhea and pathogen isolation
- ☐ Diarrhea and medication status
- ☐ Other: _____

(please specify CD4 categories or other relevant specifics next to reported effect measure)

☐ OR ☐ RR ☐ AR
_____
95% CI: _____

☐ Crude ☐ Adjusted
If Adjusted Effect Measure, please list what was adjusted for:
_____
_____

Notes: (eg. % D/C or withdrawal attributed to GI symptoms, etc.)

APPENDIX: DRUG INFORMATION

96.1.1.1 DRUG NAME TRADE NAME ABBREVIATION

96.1.1.2 Nucleoside Reverse Transcriptase Inhibitors

Zidovudine	Retrovir/Azidothymidine	AZT, ZDV
Didanosine	Videx	ddI
Zalcitabine	HIVID	ddC
Stavudine	Zerit	d4T
Lamivudine	Epivir	3TC
Abacavir	Ziagen	ABC

Non-Nucleoside Reverse Transcriptase Inhibitors

Nevirapine	Viramune/Viracept	NVP
Delavirdine	Rescriptor	DLV
Efavirenz	Sustiva	EFV
Loviride		LVR

96.1.1.3 Protease Inhibitors

Indinavir	Crixivan	IDV
Ritonavir	Norvir	RTV
Nelfinavir	Viracept	NFV
Saquinavir	Invirase, hard-gel capsule	SQV-HGC
Saquinavir	Fortavase, soft-gel capsule	SQV-SGC
Amprenavir	Agenerase	APV
Lopinavir	ABT-378	LPV

96.1.1.4 Other

Hydroxyurea		HU
Adefovir dipivoxil		AFD
Tenofovir disoproxil fumarate		TDF

96.1.1.5 Co-Formulations

Lopinavir/Ritonavir	Kaletra	LPV/RTV
Zidovudine/Lamivudine	Combivir	AZT/3TC
Zidovudine/Lamivudine/ Abacavir	Trizivir	AZT/3TC/ABC

Appendix II: Sonoma RDD Survey Materials

Questionnaire

Protocol

A Cross Sectional Survey of Drinking Water and Gastrointestinal Illness in Sonoma, California

May 9, 2001

NOTES TO CLIENT:

I TYPOGRAPHIC CONVENTIONS:

A. [Off-script instructions to the interviewer are shown enclosed in square brackets, as is this sentence.]

B {Instructions to the questionnaire programmer, or notes about material which needs to be added to the questionnaire, are shown enclosed in curly brackets, as is this sentence.} {variable names for the SPSS file are in brackets}

C. ///NOTE TO CLIENT: Special messages to the client are shown enclosed in hash, as is this sentence.///

II. EDITING CONVENTIONS:

A. Macro Additions since previous draft are shown in HIGHLIGHT, as is this entire sentence.

B. Macro Deletions since previous draft are shown in ~~strikeout~~

C. Old variable names have been left beside the question, even though they are in strikeout.

III. CATI SYSTEM SCREENS:

A. Each separately numbered question appears as a single screen the interviewer's computer interviewing workstation. The computer handles all skipping patterns, range checking, question randomization, etc.

INSTRUCTIONS TO CfMC PROGRAMMER

1. QUOTAS

133 Completes per quarter

2. SAMPLE

1. RDD

Zip Codes in sample: Sonoma (95476)
Cotati (94926, 94927, 94928, 94931)
Rohnert Park (94926, 94927, 94928)
Kenwood (94954)
Valley of the Moon (94954)
Santa Rosa/Oakmont (95405, 95409)
Southern portion of 95404 below Hwy 12

III. SPECIAL DISPOSITIONS -- see attached?

IV. VARIABLES (to be used in cross-tabular analysis)

V. LOCATION OF WORD FILE:

A Cross Sectional Survey of Drinking Water and Gastrointestinal Illness in Sonoma, California

{Screening for any household member over 55 years of age. Proxy interviews will be used}
Hello, I'm_____calling from ORC Macro on behalf of researchers at the School of Public Health, University of California, Berkeley. We're doing a research study to find out the amount of illness among people who live in Sonoma County, California. Your phone number has been chosen randomly to be included in this confidential and voluntary study. [Interviewer: please ask to speak with a person 55 years of age or older.]

S1. Is this a private residence?

01. Yes {Continue}
02. No *Thank you very much, but we are only interviewing private residences.* **STOP**
77. Don't know
99. Refused *Thank you very much, but we are only interviewing private residences. STOP*

S2. In what city is this private residence located _____?
{If **NOT City of Sonoma, Cotati, Kenwood, Rohnert Park, Valley of the Moon, Oakmont, or Santa Rosa** resident then:
Thank you very much, but we are only interviewing private residences located within certain counties or states. **STOP}**

{If **City of Sonoma, Cotati, Kenwood, Rohnert Park, Valley of the Moon, Oakmont or Santa Rosa** resident, continue.}

01. City of Sonoma
02. Cotati
03. Kenwood
04. Rohnert Park
05. Valley of the Moon
06. Oakmont
07. Santa Rosa
77. Don't know - *Thank you very much, but we are only interviewing private residences located within certain counties or states.* **STOP}**
99. Refused - *Thank you very much, but we are only interviewing private residences located within certain counties or states.* **STOP}**

S3. Our study requires that we randomly select one person over 55 years of age who lives in your household to be interviewed. How many children and adults, including yourself, are there in your household?

[Interviewer: enter number of household members]

{If S3 is greater than 1 – go to "Household"}
If ">1" **Go to "Household"**

{If "1"}
S4. Are you that person?

01. Yes
02. No {skip to QS6}
77. Don't know {Thank and terminate interview}
99. Refused {Thanks and terminate interview}

S4a. Are you age 55 or older?

01. Yes
02. No [terminate: I'm sorry but we are interviewing residents age 55 or older. Thank you for your time. Have a nice afternoon/evening.
99. Refused

{If S4a = 01}
S5. Then you are the person I need to speak with. May we continue?
{**Go to "Start"}**
{If S4 = 02}
S6. May I speak with him or her? { Go to screening introduction. If S4a = 1 **Go to "Start"}**

{Refer to CDC Foodborne HH selection process}
Household:

H1. How many of the household members are males and how many are females?

01. [Interview: enter number for each gender including children]
77. Don't know
99. Refused

H1a. How many of these household members are 55 years of age or older?

01.[Interviewer: enter number for all household members age 55 or older]
77. Don't know
99. Refused

H1b. How many of these senior members of the household are males and how many are females?

01. [Interviewer: enter number for each gender age 55 or older]
77. Don't know
99. Refused

{Programmer: Household selection "Kish" should include hh members age 55 or older for random selection as qualified respondents}

H1c. The person randomly selected is _____(i.e. The oldest senior male).

H2. What is this person's first name?_____
77. Don't know
99. Refused

H3. What is this person's age?_____
77. Don't know
99. Refused

{If H2 = name/identifier}
H4. "May I please speak with _____(first name),
H401.Yes **–** {**go to "Start"}**
H402. Person not available [Interview: schedule callback]

Call Disposition Codes			
01	Completed interview	07	No eligible respondent could be reached during time period
02	Refused interview	08	Language barrier prevented completion of interview
03	Nonworking number	09	Interview terminated within questionnaire
04	No answer (multiple times)	10	Line busy (multiple tries)
05	Business phone	11	Selected respondent unable to respond because of physical or mental impairment
06	No eligible respondent at this number	12*	Selected respondent does not reside in the catchment area

*Disposition 12 (**Selected respondent does not reside in the catchment area**) is the only disposition that is not in the standard BRFSS dispositions.

INTERVIEW BEGINS HERE:

Start:

Interviewer: If Respondent is different from person that answered screening questions, read both paragraphs. Otherwise, start with second paragraph.

Hello, I'm_____calling from ORC Macro on behalf of researchers at the School of Public Health, University of California, Berkeley. We're seeking information on health related behaviors of individuals like you. One set of behaviors that we are particularly interested in asking about is your consumption of water. Some of the questions we will be asking are personal and sensitive in nature. There may be a slight risk that you will feel uncomfortable or embarrassed answering some of these questions. However, all the information obtained will be kept confidential.

We'd like to ask you some questions about your health and about how much and what kinds of water you drink. We do not ask for your name, address, or other personal information that identifies you. The phone number is erased once we finish all interviews at the end of the year. Your answers may help us make improvements in public health programs that may benefit you. Taking part is up to you. You can skip any parts you don't want to answer, and you are free to end the interview at any time. The interview should take no more than 10 minutes. All information you give us will be kept confidential to the extent allowed by law.

[INTERVIEWER: PLEASE READ THIS PARAGRAPH EVERY TIME]

You may contact Joe Eisenberg at the University of California, Berkeley at 510-643-9257 (collect if necessary) if you have any questions about the study. If you have any questions about your rights as a research subject in this study, you may call the Committee for the Protection of Human Subjects, University of California, Berkeley at (510) 642-7461 (collect if necessary).]

Would you like me to repeat these numbers so that you can write it down?" [Interviewer: If respondent answers 'Yes', repeat preceding paragraph.]

[Interviewer: If asked: this survey will take ten minutes to complete]

Interviewer: Record gender
Male 1
Female 2

{Go to Questionnaire}

Section 1: Health

1. We would like to know about **{your}** medical history. As far as you know, have you ever been told by a physician that **{you}** have any of the following illnesses or conditions?

(Choose all that apply)
{program as separate variables/grid question}

	Yes	No	DK/NS	Refused
1a. Diabetes	1	2	7	9
1b. Heart Disease	1	2	7	9
1c. Hypertension/High Blood Pressure	1	2	7	9
1d. Kidney Disease	1	2	7	9
1e. Organ Transplant	1	2	7	9
1f. Liver Disease	1	2	7	9
1g. Cancer, other than skin cancer	1	2	7	9
1h. Lupus	1	2	7	9
1i. Arthritis	1	2	7	9
1j. Lung Disease, other than asthma	1	2	7	9
1k. Sickle Cell Anemia	1	2	7	9
1l. Spleen Removed	1	2	7	9
1m. HIV or AIDS or other immunocompromising conditions	1	2	7	9
1n. Other illness/condition (specify_____)	1	2	7	9

2. **{ Do you}** have any long lasting or chronic illness or condition in which diarrhea or vomiting is a major symptom, such as Crohn's disease, irritable bowel syndrome, ulcerative colitis, or stomach or esophagus problems?

Yes	1
(Please Specify_____)	
No	2
Don't know/Not sure	7
Refused	9

{If 2 = 1/yes}
2a. Please specify_____

I will be asking you some questions about last month (from _____ {Date 1 month before interview} through _____ {Date of Interview}.

3. In the last 30 days, did you travel outside of the United States?

Yes	1
No	2
Don't know/not sure	7
Refused	9

And now, I would like to ask you some questions about your health.

4. In the past month, **{have you}** had either vomiting or diarrhea?

Yes	1
No	2 **(SKIP to Q22)**
Don't know/not sure	7 **(SKIP to Q22)**
Refused	9 **(SKIP to Q22)**

5. Was this vomiting or diarrhea due to an illness different from any chronic condition **{you}** might have?

ALL: do not include vomiting or diarrhea associated with taking medicines for an illness or condition.

Yes	1
No	2
Don't know/Not sure	7
Refused	9

6. On about what date did this illness begin? If you do not remember the exact date when your illness began, please give me your best guess.

____/ ____/ ____ **(SKIP TO Q8) {if q6 =1, goto q8}**
[Note: If more than one illness, ask about the most recent illness.]

Don't know/not sure	77 **(SKIP TO Q7)**
Refused	99 **(SKIP TO Q7)**

7. Did this illness begin in the last month?

Yes	1
No	2
Don't know/Not sure	7
Refused	9

{if q3 = 1}
8. Did this illness begin before, during, or after **{your}** return from travel outside of the United States?

Before	1
During	2
After	3
Don't know/Not sure	7
Refused	9

9. During this illness, for how many days altogether did **{you}** have either diarrhea or vomiting?
__ __ days **[Interviewer: If more than one illness, ask about the most recent illness.]**

Don't know/not sure	77
Refused	99

10. During this illness, which of the following symptoms did **{you}** have?

	Yes	No	DK/NS	Refused
10a. Stomach cramps	1	2	7	9
10b. Fever	1	2	7	9
10c. Headache	1	2	7	9
10d. Sore throat	1	2	7	9
10e. Cough	1	2	7	9
10f. Nausea	1	2	7	9
10g. Muscle/body aches	1	2	7	9
10h. Stiff neck	1	2	7	9
10i. Runny nose/nasal discharge	1	2	7	9

10j. Sneezing	1	2	7	9
10k. Chills	1	2	7	9
10l. Vomiting	1	2	7	9 For how many days __ __
10m. Diarrhea	1	2	7	9 For how many days __ __

{If Q10L = 1}
11. For how many days did you have vomiting?

Record number of days_____
77. Don't know/not sure
99. Refused

{If Q10m = 1}
12. During this illness, what was the maximum number of stools or bowel movements **{you}** had in any 24-hour period? Was it....

	1 - 2	1
	3- 5	2
	6 - 10	3
	11 - 20	4
	>20	5
[don't read these responses]	DK/NS	7
	Refused	9

{If Q10m = 1}
13. Did **{you}** have blood in your stool?

Yes	1
No	2
Don't know/Not sure	7
Refused	9

14. Did **{you/}** go to a doctor, nurse, or other medical person for this illness?

Yes	1
No	2 **(SKIP TO Q17)**
Don't know/Not sure	7 **(SKIP TO Q17)**
Refused	9 **(SKIP TO Q17)**

Note to client: This Q15 series is asked in a loop. If the respondent selects Emergency Room, they will be asked how many times did they visit a particular facility. Then the verification question, 15c will be asked ONLY if they say they visited a particular facility 5 times or more.
15a. Where did **{you}** go for **{your}** illness? Did **{you}** go to an emergency room?

01. Yes
02. No
77. Don’t know/Not sure
99. Refused

15b. Where did **{you}** go for **{your}** illness? Did **{you}** go to a doctor’s office?

01. Yes
02. No
77. Don’t know/Not sure
99. Refused

15c. Where did **{you}** go for **{your}** illness? Did **{you}** go to a clinic?

01. Yes
02. No
77. Don’t know/Not sure
99. Refused

{If q15a = 2,7,or 9 AND q15b = 2,7, or 9 AND q15c = 2,7.or 9}
15_1. Did you go to any other health care facility for this illness?

01. Yes
02. No
77. Don’t know/Not sure
99. Refused

15_2. Where did you go for your illness?

01. Record response_____

{If 15a = 1/yes}
15d. How many times did **{you}** go to an emergency room for this illness?
~~visit that each facility?~~

[Interviewer: enter the number of times for each]

01. Doctor’s office/clinic _____
02. Emergency room _____
77. Don’t know
99. Refused

{If 15b = 1/yes}

15e. How many times did **{you}** go to a doctor's office for this illness?

[Interviewer: enter the number of times]

01. Doctor's office _____
77. Don't know
99. Refused

{If 15c = 1/yes}
15f. How many times did **{you}** go to a clinic for this illness?

[Interviewer: enter the number of times]

01. Clinic _____
77. Don't know
99. Refused

{Restore the # of times visited if responses in Q15 > 5}

check3. [If respondent says that they have visited a emergency room, doctor's office, or other more than five times,

[Interviewers please ask, "I want to make sure that I heard you correctly, you said that you visited an emergency room, doctor's office, clinic, or Other ____# of times?"]

a. Emergency room	1	2	7	9	_____	
b. Doctor's office/clinic		1	2	7	9	_____
c. Clinic	1	2	7	9		
d. Other (specify_____)1		2	7	9	_____	

16. Did **{you}** take any antibiotics for this illness?

Yes	1
No	2
Don't know/Not sure	7
Refused	9

{ASK TO ALL RESPONDENTS?}

17. Were you employed at a job or business in the past month (between _____ {[Date 1 month before interview]} through _____ {[Date of Interview] }?

Yes	1

No	2 **(SKIP TO Q20)**
Don’t know/Not Sure	7 **(SKIP TO Q20)**
Refused	9 **(SKIP TO Q20)**

18. In the last month, did you miss any time from work because of this illness, for example because you called in sick or took time off to see a doctor?

Yes	1
No	2 **(SKIP TO Q20)**
Don’t know/Not Sure	7 **(SKIP TO Q20)**
Refused	9 **(SKIP TO Q20)**

19. In the last month, how many days altogether did you miss more than half of the day from work due to this illness?

___ ____DAYS	
Don’t know/Not Sure	77
Refused	99

20. Did this illness prevent **{you}** from performing school, recreation, or vacation activities, or work in the home?

Yes	1
No	2 **(SKIP TO WATER)**
Don’t know/Not Sure	7 **(SKIP TO WATER)**
Refused	9 **(SKIP TO WATER)**

21. As a result of this illness, for how many days **{were you}** unable to perform these activities?
____ ____DAYS

Less than a day	55
Don't know/Not sure	77
Refused	99

Section 2: Water

I’d now like to ask you a few questions about the water that you use and drink.

22. Which of the following is the source of tap water in your home?
[Interviewers: Please Read]

	Municipal, city, or county water	1	
	Private well water	2	
	Other (Please specify_____)		3
Do not read	Don't know/Not sure	7	
Do not read	Refused	9	

23. At home, what is the primary type of water **{you use}** most often for drinking? Untreated tap water means tapwater without ADDITIONAL treatment at home with special filters.
[Interviewers: Please Read]

	Untreated tap water	1 **(SKIP TO Q28)**
	Treated tap water (for example, with a filter, softener, UV, or whole house point-of entry device)	2
	Commercially bottled water	3
	Other (Please specify_____)	4
Do not read	Don't know/Not sure	7 **(SKIP TO Q28)**
Do not read	Refused	9 **(SKIP TO Q28)**

24. What is the primary reason why you choose not to **{drink}** untreated tap water ?
[Interviewers: Please Do Not Read]

Bad taste or odor	1
Concern of harmful chemicals or cancer causing agents	2
Concern of germs (infectious agents)	3
Other (Please specify _____)	4
Don't know/Not sure	7
Refused	9

{Ask Q25, if Q23=2, else skip to Q28}
25. How do you treat your tap water? **(~~Open-ended~~ Multi response - choose all that apply)**
{MUL = 7} **[Interviewers: Please Read]**

	Filtered	1
	Ultraviolet (UV) light	2 **(SKIP TO Q28)**
	Boiled	3 **(SKIP TO Q28)**
	Treatment device at point where all water enters the house (such as filter, UV, distiller, softener)	4 **(SKIP TO Q28)**
	Softener	5 **(SKIP TO Q28)**
	Distiller	6 **(SKIP TO Q28)**
	Other (Please specify _____)	8 **(SKIP TO Q28)**
Do not read	Don't know/Not sure	7 **(SKIP TO Q28)**
Do not read	Refused	9 **(SKIP TO Q28)**

26. There are different types of water filters. Of the following types of water filters which one(s) do you use in your home? **[Interviewers: Please Read] {MUL=6}**

	A pitcher or jug filter	1 **{LABEL CARAFE}**
	An end of faucet mounted filter	2 **{LABEL FAUCET}**
	A counter top filter (usually connected to the faucet)	3 **{LABEL COUNTER}**
	An under-sink model	4 **{LABEL SINK}**
	A reverse osmosis unit	5 **(SKIP TO Q28)**
	Other _____	8 **(SKIP TO Q28)**
Do not read	Don't Know	7 **(SKIP TO Q28)**
Do not read	Refused	9 **(SKIP TO Q28)**

27. Please estimate how many days prior to today's interview that the filter element was changed.
[Interviewers: Please Do Not Read]

less than (<) 30 days ago	1
1 to 3 months ago	2
more than (>)3 to 6 months ago	3
more than 6 months ago	4
Never/Can't be changed	5
Unknown	7
Refused	9

28. At home, what is the primary type of water that is used for cooking and food preparation?
[Interviewers: Please Read]

	Regular tap water	1
	Filtered tap water	2
	Commercially bottled water	3
	Other (Specify_____)	8
Do not read	Don't know/not sure	7
Do not read	Refused	9

29. At home, when you make juices or other cold drinks that require the addition of water do you usually use?
[Interviewers: Please Read]

Don't make cold drinks	1
Regular tap water	2

	Water that you filtered	3
	Water that you boiled	4
	Bottled water	5
Do not read	Don't know/not sure	7
Do not read	Refused	9

30. At home, when you make coffee, tea, or other hot drinks that require the addition of water do you usually use?

[Interviewers: Please Read]

	Don't make hot drinks	1
	Regular tap water	2
	Water that you filtered	3
	Water that you boiled	4
	Bottled water	5
Do not read	Don't know/not sure	7
Do not read	Refused	9

We are interested in knowing how much tap water and bottled water **{you drink}** per day. Please include in your estimate juices or other cold drinks that require the addition of water, but not hot beverages such as coffee.

31. Please estimate the number of 12 ounce glasses of water you drink in a day? As a comparison, a soft drink can has 12 ounces. **(RECORD THE NUMBER FOR HOME AND OUT OF HOME CONSUMPTION SEPARATELY.)**

31a. **At home** {bold & flash}

None	1
1-2	2
3-5	3
>5	4
Unknown	7
Refused	9

31b. **Out of home** {bold & flash}

None	1
1-2	2
3-5	3
>5	4
Unknown	7
Refused	9

32a. In the past month, did you drink any water from the following sources?

					IF YES, Was this in the past 7 days?			
					Yes	No	DK/NS	Refused
1. Lake, pond, river, or stream	1	2	7	9	1	2	7	9
2. Private well	1	2	7	9	1	2	7	9

{If 32a = 1/yes}
32b. Was this in the past 7 days? {Restore responses from 32a}

	Yes	No	DK/NS	Refused
1. Lake, pond, river, stream	1	2	7	9
2. Private well	1	2	7	9

33.1 In the past month, did you swim in, wade in, or enter any of the following types of water?

					IF YES, Was this in in the past 7 days?				**IF YES,** Did you put your face or head under water?			
	Yes	No	dk/ns	Refused	Yes	No	dk/ns	Refused	Yes	No	dk/ns	Refused
33.1a. Ocean, beach	1	2	7	9	1	2	7	9	1	2	7	9
33.1b. Lake, pond, river, or stream	1	2	7	9	1	2	7	9	1	2	7	9
33.1c. Hot tub, whirlpool, jacuzzi, spa	1	2	7	9	1	2	7	9	1	2	7	9
33.1d. Recreational water park	1	2	7	9	1	2	7	9	1	2	7	9
33.1e. Swimming pool	1	2	7	9	1	2	7	9	1	2	7	9

{If 33.1 = 1/yes}{Restore yes response categories from q33.1}
33.2 Was this in the past 7 days?

1.Yes
2. No
7. Don't know/Not sure
9. Refused

{If 33.2 = 1/yes} {Restore yes response categories from q33.2}
33.3 Did you put your face in or head under water?

1. Yes
2. No
7. Don't know/Not sure

9. Refused

Section 3: Demographics

Now I would like to ask you some basic questions about {you}

34a. What is **{your}** month and year of birth?

{PROGRAMMER: RECORD MONTH AND YEAR ONLY}

____ /____/ _____ **{record response}** **(SKIP TO 35)**
mm yyyy

Don't know/Not sure	777 **(ASK 34B)**
Refused	999 **(ASK 34B)**

34b.What is **your** age? ____ **{record response}**

35. What would you consider **{your}** race to be? **(Choose all that apply)**
{MUL =14} **[Interviewers: Read only if necessary]**

97	**White**	**1**	
	Black, African American, Negro	2	
	American Indian/Alaska Native		3
	Asian Indian	4	
	Chinese	5	
	Filipino		6
	Japanese	7	
	Korean	8	
	Vietnamese	9	
	Native Hawaiian	10	
	Guatemalan/Chamorro	11	
	Samoan	12	
	Some other race, (specify _____)	14	
	Don’t Know	77	[Do not read]
	Refused	99	[Do not read]

36a. Are you/ Spanish/Hispanic/Latino?

Yes	1	
No	2	~~SKIP TO Q37~~
Don’t Know/Not Sure	7	
Refused	9	

{if q36a = 1}

36b. Are you Puerto Rican, Dominican, Guatemalan, Columbian, Cuban, Spanish, Honduran, Mexican/Mexican American/Chicano, Nicaraguan, Panamanian, Salvadoran, Ecuadorian, Peruvian,or something else? {MUL=14}

Puerto Rican	01
Dominician	02
Guatemalan	03
Columbian	04
Cuban	05
Spanish	06
Honduran	07
Mexican	08
Nicaraguan	09
Panamanian	10
Salvadoran	11
Ecuadorian	12
Peruvian	13
Other	14
Don’t Know/Not Sure	77
Refused	99

Closing statement:
That's my last question. The answers of everyone in the study will be combined to give us information about people’s health and if health is related to water consumption behavior. Thank you very much for your time and cooperation.

CPHS PROTOCOL NARRATIVE FORM

Instructions: ***Complete all applicable sections of this form****. (If requesting Exempt Status, see instructions on Exempt Request form).* ***Please type,*** *using a different font than the one in this form****. Handwritten or incomplete forms will be returned.*** *Use language that is clear, concise, and non-technical wherever possible, and define all acronyms.* ***For renewals or amendments, highlight all changes from the previously approved version on one copy.*** *A grant proposal or thesis will not be accepted in place of a protocol written according to this format.*

Lead Investigator: John M. Colford, Jr.

Protocol Title: A cross-sectional survey of drinking water and G.I. illness in Sonoma, CA **CPHS #:** 2004-5-108

Related CPHS Project(s)? Title: A randomized trial of tap water treatment in the elderly CPHS #:

Title: CPHS #:

SECTION 1: PURPOSE AND BACKGROUND OF STUDY

- **Purpose:** Provide a brief explanation of the proposed research, including specific *study hypothesis*, *objectives*, *and rationale*.

The primary objective of this study is to gather information to aid the formulation of an estimate of the incidence of gastrointestinal illness among elderly individuals, and to provide information on drinking water consumption and community diarrhea incidence that will complement our intervention trial being conducted in Sonoma, California. A cross-sectional telephone survey will be administered to approximately 2100 randomly chosen individuals over a 4 year period (i.e. about 133 per quarter for 16 quarters). The survey will only sample persons 55 years of age and older, since we are particularly interested in studying G.I. illness and water consumption in elderly persons. The survey (Appendix I) will take about ten minutes and will ask questions about water consumption and swimming activities. The questions are based on the year 2000 Centers for Disease Control and Prevention's FoodNet survey.

- **Background:** Give relevant background (e.g., summarize previous/current related studies) on condition, procedure, product, etc. under investigation, including citations (with attached bibliography) if applicable.

There is heated debate in the United States about the extent to which waterborne infectious diseases may be transmitted to human beings through drinking water that meets federal standards for pathogen removal. These concerns have been heightened by the findings of Payment *et al*. in Canada which suggested that approximately 25% of "highly credible" gastrointestinal illness in a community might be due to drinking water. The potential burden of gastrointestinal symptoms attributable to waterborne pathogens may be significant in terms of both economic productivity and individual discomfort and disease. Additionally, widespread reports of outbreaks of disease linked to public water supplies meeting federal drinking water standards (Mac Kenzie *et al*, Goldstein *et al*) have generated extensive media coverage.

Furthermore, gastrointestinal illness is recognized as a significant cause of morbidity and mortality in the elderly, and their case fatality rate is the highest compared to other age groups (Scwartz et al 2000, Mounts et al 1999). A study reviewing deaths due to diarrhea in the

United States over a 9 year period, reported that 78% of such deaths occurred in persons aged 55 years or greater (Lew 1991). The elderly represent a sensitive subpopulation at increased risk for gastrointestinal illness, with increased likelihood of severe illness (Gerba 1996). Although many infectious diseases are more problematic in the elderly because of a decline in immune function and a higher incidence of pre-existing malnutrition and dehydration, it is still not known what the principal modes of transmission are relevant and which infectious agents are responsible.

This project is funded by the National Institutes of Health, National Institute on Aging. The telephone survey will be conducted by ORC Macro, on residents in Sonoma County, California. We are currently conducting a randomized intervention trial in Sonoma, California and have full IRB approval for that study (CPHS #2000-11-111).

- *International research*: If research will be done outside the U.S., see CPHS Guidelines on Conducting Research Abroad—Demonstrating Knowledge of "Local Research Context."

- *Collaborative research*: If any non-UCB institutions or individuals are collaborating in the research, discuss here and complete CPHS Cover Sheet, Part IV, *attaching any relevant IRB approvals*.

SECTION 2: QUALIFICATIONS OF STUDY PERSONNEL

- **Expertise:** Explain expertise of Lead Investigator, Faculty Advisor (if applicable), any co-investigators or other key personnel listed in the application, and how it relates to their specific roles on the study team.

Jack Colford, MD Ph.D, Associate Professor of Epidemiology, University of California, Berkeley, School of Public Health. Principal Investigator. Dr. Colford is a physician (board-certified in Internal Medicine and Infectious Diseases) and epidemiologist (PhD in epidemiology). Dr. Colford was the Principal Investigator of the preliminary recreational water study conducted as background work for this proposal in Mission Bay during the summer of 2003. Additionally, he is the Principal Investigator of four distinct federally funded (NIH, CDC, USEPA) separate randomized intervention trials (total > $6M) investigating drinking water and health effects in the elderly, in HIV+ individuals, and in general populations. He has more than seven years of experience in the design and management of large-scale federally-financed epidemiology trials and field studies (described above in preliminary work). He has published numerous peer-reviewed articles on drinking and recreational water topics. He is the sole instructor of epidemiologic methods courses (advanced epidemiology, systematic review and meta-analysis, and Clinical Trial Design at UC Berkeley and basic epidemiology courses in the summer sessions each year at the University of Michigan and the University of Zurich, Switzerland).

- **Training:** For *graduate or undergraduate students* who are Lead Investigator or key personnel of the study, confirm training to conduct research with human subjects (required for all student

researchers—see CPHS Cover Sheet, Part VI). *Attach copy of completion report for each individual, unless submitted previously*.

SECTION 3: SUBJECTS (Persons/Records/Specimens)

- **Eligibility:** Describe proposed subject population, including criteria for study inclusion and exclusion (e.g., age, health status, language). If any inclusion/exclusion criteria are based on gender, race, or ethnicity, explain rationale for the restrictions. Indicate how, when, and by whom prospective subjects will be identified and eligibility determined (provide fuller discussion of recruitment, screening, and consent process in Sections 4-6). Describe randomization or other assignment method for intervention and control groups.

Households within the cities of Sonoma, Cotati, Kenwood, Valley of the Moon, Oakmont, Santa Rosa, Petaluma and Rohnert Park will be included. Only persons who are 55 years of age and older will be included in the survey.

- **Number:** State total number of subjects planned for the study and how many must be recruited to obtain this sample size. Explain how number of subjects needed to answer the research question was determined.

A cluster sampling procedure will be used to select households within the cities of Sonoma, Cotati, Kenwood, Valley of the Moon, Oakmont, Santa Rosa and Rohnert Park. Approximately 133 randomly chosen individuals who are 55 years of age and older, will be selected to interview during each quarter, for 16 consecutive quarters, for a total of 2100 interviews. This sample size was determined from power calculations to detect a community prevalence of gastrointestinal illness between 9% and 15%. This prevalence of gastrointestinal illness is consistent with the levels observed by the investigators in apilot study in the general population, and in other surveys of gastrointestinal illness.

- **Vulnerable Subject Groups:** Indicate whether any proposed subjects are children/minors, prisoners, pregnant women, those with physical or cognitive impairments, or others who are considered vulnerable to coercion or undue influence.

SECTION 4: RECRUITMENT

- **Summary:** Explain how, where, when, and by whom prospective subjects will be identified/selected and approached for study participation. NOTE: If researcher is subject's instructor, physician, or job supervisor, or if vulnerable subject groups will be recruited, explain what precautions will be taken to minimize potential coercion or undue influence to participate.

ORC Macro will be conducting this survey based on our specific guidelines. Eligible households will be identified from telephone banks with working residential lines and one individual in each household will be randomized to answer the questionnaire. Steps in the recruitment process will be:

1. Generation of sampling frame
2. Random selection of households by random digit dialing

3. Determination of household willingness to participate
4. Random selection of one individual age 55 or older in each household
5. Questionnaire completion, by telephone, for selected household member
6. If the selected member is not available at the time of the first call, the head of the household will be asked to identify times when we can contact the selected member. No more than 15 return phone calls will be made to the home to contact the selected member.

- **Recruitment Materials:** Describe and *attach samples of any recruitment materials* (e.g., letters, flyers, advertisements [note type of media/where posted], scripts for verbal recruitment, etc.).

None, this is a telephone only survey.

- **Permissions:** If applicable, describe and *attach IRB approval or letter of permission/ cooperation* from institutions, agencies or organizations where off-site subject recruitment will take place (e.g., another UC campus, clinic, school district).

SECTION 5: SCREENING PROCEDURES

- **Summary:** If prospective subjects will be screened via tests, interviews, etc., prior to entry into the "main" study, explain how, where, when, and by whom screening will be done. NOTE: *Consent must be obtained for screening procedures as well as "main" study procedures. As appropriate, either: 1) create a separate "Screening Consent Form;" or 2) include screening information within the consent form for the main study* (see Section 6).

In order to be eligible to participate in the cross sectional survey, the household contacted must be:

1. A private residence;
2. Located in the City of Sonoma, Cotati, Kenwood, Oakmont, Valley of the Moon, Santa Rosa or Rohnert Park, California
3. Include at least one household member who is 55 years of age or older.

Once the household meets the inclusion criteria, one household member who is 55 years of age or older will be randomly selected to be interviewed.

- **Identifiable Personal Information:** Indicate if identifiable personal information will be obtained as part of the screening process. (Confidentiality issues should be addressed in Section 11).

No

SECTION 6: INFORMED CONSENT

NOTE: See CPHS Informed Consent Guidelines before completing this section.

- **Summary:** Explain how, where, when, and by whom informed consent and/or assent will be obtained. NOTE: If any vulnerable subject groups/other special circumstances are involved (e.g., use of surrogate consent), address considerations appropriately.

Informed consent for the cross sectional survey will be obtained orally over the telephone. The informed consent statement is located on the questionnaire itself. In order to select the survey participant in each household, all household members will only be identified by first name or initials. No other identifying information will be collected on survey participants or their household members.

- **Consent Materials:** Describe any consent/assent form(s) to be used, and *attach copies.*

  If *screening procedures* will be done for the study, see above. Whichever method is used (separate consent or part of the main consent), the form should include a statement regarding what will happen to screening information collected for individuals who do not enter the study.

  If any *vulnerable subject groups* will be involved, address appropriately (e.g., if study includes minors, both an assent form for the child and a consent/permission form for the parent(s) may be required).

  For *international research*, provide for and describe *local contacts* in the area.

Oral consent script is at the beginning of the body of the telephone questionnaire.

- **Request for Waiver of Consent:** If you are requesting waiver of any of the required elements of informed consent, or waiver of documented consent, or waiver of parental consent or child's assent, provide justification and describe plans for any additional safeguards. (See CPHS Informed Consent Guidelines).

N/A

SECTION 7: STUDY PROCEDURES

- **Summary:** Describe how the research will be conducted, providing information about all study procedures (e.g., interventions/interactions with subjects, randomization, photographing, audio- and/or videotaping, data collection), including follow-up procedures. (Screening procedures should be discussed in Section 5).
  Be sure to make clear what the sequence of study procedures is (i.e., describe in chronological order).

Once a household member is selected and agrees to take part in the survey, they will be asked to answer a set of questions over the telephone (see Appendix I). These questions will ask the participants about their water consumption patterns, medications they may be taking, and other questions regarding their health.

- **Study Personnel, Location, Time:** Explain who will conduct the procedures, where and when they will take place. Indicate frequency and duration of visits/sessions, as well as total time commitment for the study.

Telephone interviews will be conducted over the phone by trained interviewers employed by ORC Macro. The phone call will last about 15 minutes, and is a one time contact with the participant.

- **Experimental vs. Standard Procedures:** Identify any procedures that are experimental/ investigational and explain how they differ from standard procedures (medical, psychological,

educational). If applicable, distinguish between procedures that the subject would undergo regardless of enrollment in the study and procedures done specifically for study purposes.

N/A

- **Deception:** This includes both *"active deception"* (deliberately giving false information about study purpose and/or procedures to subjects) and *"lack of full disclosure"* (withholding complete information about the study from subjects.) If any type of deception will be used, explain what it will entail, why it is justified, and what the plans are to debrief subjects. Also, attach debriefing forms(s)/materials. (NOTE: If study involves significant deception at time of subject enrollment/consent, the CPHS may require a post-study re-consent as part of debriefing process).

N/A

- **Drugs/Devices:** If study involves an experimental drug or device, complete IND/IDE information on CPHS Cover Sheet. Describe any study drug here, including generic and/or chemical name, how it is supplied (e.g., powder, capsule, liquid), administration method and schedule, etc.

N/A

- **Placebo:** If placebo will be used, provide rationale and explain why active control is not appropriate.

N/A

- **Data Collection Instruments:** If interviews, questionnaires, surveys, or focus groups will be conducted for the study, provide citations for standard instruments and *attach 1 copy of any non-standard instruments to be used*.

Survey is based on EPA and CDC's FoodNet Survey

- **Identifiable Personal Information:** Indicate if identifiable personal information will be obtained from/about subjects. (Confidentiality issues should be addressed in Section 11).

First name or initials only

SECTION 8: RISKS/DISCOMFORTS

- **Summary:** Describe all known risks, discomforts, and/or side effects of study procedures, whether physical, psychological, or social (e.g., pain, stress, invasion of privacy), noting probability and magnitude of potential harm. Include risks of randomization and placebo if applicable.

There is minimal risk associated with completing the cross sectional survey. A potential risk is the loss of confidentiality from the information that the participant provides for the study, but we will take every precaution to make certain that this risk is minimized (see below). In order to select the survey participant in each household, all household members will be identified by first name or initials only. No other identifying information will be collected on survey participants or their household members.

- **Measures to Minimize Risks/Discomforts:** Discuss measures that will be taken to minimize risks or discomforts to subjects.

In order to select the survey participant in each household, a list of household members' first names or initials will be collected. No other identifying information will be collected on survey participants or their household members.

- **Currently Unknown Risks:** If applicable, indicate if a particular study treatment or procedure may involve risks to the subject (or to the embryo or fetus, if the subject is or may become pregnant) that are currently unforeseeable.

SECTION 9: BENEFITS

- **Summary:** Describe any potential benefits to the individual subject, group of subjects, and/or society.
  If subjects will not benefit directly from study procedures, this should be stated. NOTE: Do not include compensation/ payment of subjects in this section, as remuneration is not considered a "benefit" of participation in research (compensation/ payment should be addressed in Section 12).

There may be no substantial benefit to the participants directly from the research. At the end of the study, the results will be made available to participants, the local health department, and the local water department. It is hoped that the research will benefit society by determining whether the quality of water treatment is enough to prevent the transmission of infectious agents in drinking water.

SECTION 10: ALTERNATIVES TO PARTICIPATION

- **Summary:** Describe appropriate alternative resources, procedures, courses of treatment, if any, that are available to prospective subjects. If there are no appropriate alternatives to study participation, this should be stated. If the study does not involve treatment/intervention, put "N/A" here.

N/A

SECTION 11: CONFIDENTIALITY
NOTE: See CPHS Data Security Policy before completing this section.

- **Summary:** Explain how subject privacy will be protected and how confidentiality of subject information will be maintained.

All information collected during the research should be kept in a locked file. The key to the code of names of individual subjects should be kept in a separate locked file. No individuals will be identified in any publications

- **Access to/Security of Study Records:** Discuss who will have access to study records/specimens and how the records will be secured. Address all applicable points below:

  - Will subjects be asked to give permission for release of identifiable data (e.g., information, videotapes), now or in future? If so, explain here and include appropriate statements in consent materials.

  No

  - Will data be collected anonymously (i.e., no identifying information from subjects will be collected/ recorded that can be linked to the study data)? (NOTE: Data is not collected anonymously if there is a code linking it to personally identifiable information).

  Yes, other than first name

- If using existing data/biological specimens, will the researchers have access to a code linking the data to personally identifiable information?

N/A- no specimens being collected.

- If identifying information will be collected and linked to data/specimens, explain at what stage identifiers will be removed from the data/specimens.

N/A

- If identifiers will be retained, explain why this is necessary and how confidentiality will be protected.

- If the data is coded, explain where the key to identifiers will be stored, how it will be protected, and who will have access to it.

- Indicate whether research data/specimens will be destroyed at the end of the study. If data will not be destroyed, explain why, where, in what format, and for how long it will be retained.

- Explain how data collection instruments, audiotapes, videotapes, photographs, etc. will be stored and who will have access to them. Indicate at what point they will be transcribed and/or destroyed (if ever).

Instruments will be destroyed after all analyses are complete

NOTE: The CPHS does not require that researchers destroy their human subjects data at the completion of their research. *Whenever appropriate, researchers may retain study data for future use/ other research purposes as long as they make provision in the protocol and consent documents for such use.* Researchers must spell out in the protocol how confidentiality will be maintained vis-à-vis long-term storage of data and/or granting of access to other researchers, and the consent forms must clearly ask subjects for permissions in this regard.

- **HIPAA:** If any of the study data sources are covered entities under HIPAA (Health Insurance Portability and Accountability Act), explain what arrangements have been made to comply with the Privacy Rule regarding subjects' "protected health information." (See CPHS website for HIPAA guidance).

N/A

- **Reportable information:** If it is reasonably foreseeable that the study will collect information which state or federal law requires to be reported to other officials (e.g., child or elder abuse) and/or ethically requires action (e.g., suicidal ideation), discuss here and reference reporting requirements in consent documents.

N/A

- **Certificate of Confidentiality:** In certain circumstances, researchers may plan to protect research records from subpoena by seeking a Certificate of Confidentiality (http://grants.nih.gov/grants/policy/coc/index.htm). If a Certificate of Confidentiality will be sought for this study, indicate here and reference in consent documents.

N/A

SECTION 12: FINANCIAL CONSIDERATIONS

- **Compensation/payment:** Describe plan for compensation of subjects by addressing points below. If no compensation will be provided, this should be stated.

  N/A

  - If subjects will be compensated for their participation, explain in detail about the amount and methods/ terms of payment.
    -Include any provisions for partial payment if subject withdraws before study is complete.
    -When subjects are required to provide Social Security number in order to be paid, this data must be collected separately from consent documentation. If applicable, describe security measures that will be used to protect subject confidentiality.

  N/A

  - If non-monetary compensation (e.g., course credit, services) will be offered, explain how it will be provided.

  N/A

  - Discuss reasoning behind amount/method/terms of compensation, including appropriateness of compensation for the study population and avoiding undue influence to participate.

  N/A

- **Costs to Subjects:** If applicable, describe any costs/charges which subjects or their insurance carriers will be expected to pay. (If there are no costs to subjects or their insurers, this should be stated.)

  N/A

- **Treatment and Compensation for Injury:** *If the study involves more than minimal risk*, indicate that the researchers are familiar with and will follow University of California policy in this regard, and will use recommended wording on any consent forms (see CPHS Informed Consent Guidelines).

  N/A

SECTION 13: ADVERSE EVENT MANAGEMENT/REPORTING

- Explain how unanticipated negative outcomes/experiences or serious adverse events will be managed. (NOTE: This may apply in social-behavioral as well as biomedical research (e.g., undue stress or anxiety of subject, breach of confidentiality via loss of laptap computer with study data.) Provisions should be made and described here *if applicable*.)

N/A

- Describe plans for provision of treatment for study-related injuries, and how costs of injury treatment will be covered (see "Treatment and Compensation for Injury" above).

N/A

- Discuss plans for reporting unanticipated or serious adverse events to CPHS (see CPHS Adverse Events). (This applies to all types of research.)

N/A

SECTION 14: ATTACHMENTS

- Please list all attachments (e.g., consent forms, survey instruments, recruitment materials, appendices) included with your submission.

Appendix III: Enteric Pathogens Study Materials

Consent Form
Questionnaire
Protocol
Recruitment Flyer
Abstracts from Bay Area Clinical Research Symposium & University-wide AIDS Research Program Annual Investigators Meeting

East Bay AIDS Center at the Alta Bates Summit Medical Center and the University-Wide AIDS Research Program

INFORMED CONSENT

An enhanced approach for studying the causes of diarrheal disease in an HIV+ cohort: The Enteric Pathogens Microarray Study

Jeffrey Burack, MD MPP, Assistant Adjunct Professor
Alta Bates Medical Center and School of Public Health, University of California, Berkeley

John M. Colford, MD, PhD, Associate Professor
Joseph Eisenberg, PhD Assistant Adjunct Professor
School of Public Health, University of California, Berkeley

IRB Protocol No. 021005

Participant's Initials:_____ Study Patient Identification Number:_____

Voluntary Participation: Before volunteering to participate in this research study, I agree to carefully read this Informed Consent document. It describes the purpose, procedures and precautions of the study and the possible benefits and risks of participating in this study. I may withdraw from the study at any time and my refusal to participate in this study will not influence my present or future medical care in any way.

Study Background and Purpose: Dr. John Colford and Dr. Joseph Eisenberg from the School of Public Health at the University of California, Berkeley and Dr. Jeffrey Burack from the East Bay AIDS Center (EBAC) and the School of Public Health at the University of California, are doing a research study on diarrheal illness in HIV-infected patients.

Participation and Study Procedures: If I agree to participate in this study, the following will happen:

1. I will be asked to fill out a questionnaire that will take about 5-10 minutes. This will contain questions about any diarrhea I have had in the past few weeks. It will also ask about my health, sexual practices, and the kind of food I eat and water I drink, my pets, and recent travel outside the San Francisco Bay Area.
2. I will be asked to collect a stool specimen. Study staff will test the specimen for common causes of diarrhea and digestive problems.

Specimen Testing: My stool will be tested for bacteria and parasites that commonly cause diarrhea, using a new diagnostic technology called a "microarray", as well as the standard microbiology tests as would be ordered by my physician. The microarray is a glass slide that uses DNA detection techniques to look for 30-50 microbes at a time. Clinical labs run multiple tests that look for one microbe at a time.

Specimen Storage and Future Testing: The investigators would like my permission to freeze part of my stool specimen for future testing. At this point, they are not sure what studies might be done. But if any of the test results seem to have meaning for my health, they will inform my clinicians at the East Bay AIDS Center (EBAC). The samples will not be used for human genetic testing unless I agree to such testing in the future. I may participate in the study even if I don't want to have my samples stored for future testing. Also, I may agree to have my samples stored and later decide that I want to withdraw them from storage. If so, I should call the study office at 510-204-1870 and the samples will be discarded as I instruct.

NOTE: If I do not wish to agree to the storage and future testing of my stool, my samples will be destroyed once testing for this study is complete.

Risks and Precautions: If I agree to give a stool sample, there are minor risks that I should be aware of when samples are taken. Giving stool samples, although done in private, might cause some minor embarrassment. Giving samples for the study also carries the risk of loss of privacy, but this chance will be made as small as possible (see below).

Benefits: If I agree to test my stool specimen, my doctors and I will be told any results that could affect my health care. The investigators will also tell me about the findings from the study when it has finished. The investigators hope that this research will help in learning about causes of diarrhea in HIV-infected persons.

Confidentiality of Records: All the facts collected from me during the research will be kept private to the full extent allowed by law. Each participating person will be assigned a code number. The key to that code will be kept in a locked file. My name and identifying information will not be attached to any specimen or document that leaves EBAC. This will make the chance of loss of privacy very small.

The results from tests of my stool will be given to me to discuss with my doctor, since these results might improve my health care. If the tests find that I have Salmonella, Giardia, or certain other infections, the law requires the study to tell my local public health department about it (as would be required of your regular doctor if he/she found that I have these infections). This is the same procedure that my regular doctor is required to follow.

Compensation: I will receive $15 for participating in this study. (There will be no charge to me for study testing.)

Injury: If I am injured as a result of taking part in this study, medical care and treatment will be available to me as a participating subject. The costs of this care may be covered by the University of California depending on a number of factors. If I have any questions regarding this assurance, I may consult study staff or call the Committee for Protection of Human Subjects, 101 Wheeler Hall, University of California, Berkeley, California 94720-1340, 510-642-7461. I may also contact the Alta Bates Summit Medical Center Institutional Review Board at 510-204-1414.

Questions: If I have any questions about my rights as a research subject, I may talk to the Committee for the Protection of Human Subjects, 101 Wheeler Hall, MC 1340, University of California, Berkeley, CA 94720-1340, 510-642-7461or I may also contact the Alta Bates

Summit Medical Center Institutional Review Board at (510) 204-1414.If I have any questions about the research, before or during the study, I may call Dr. Jeffrey Burack or Ms. Jamie Mandelke at 510-204-1870.

Research Participant's Bill of Rights: A copy of the Research Participant's Bill of Rights is attached to this consent form.

Consent (Specimen Donation): "I have read this form and understand this research study. I agree to provide a stool specimen and participate in this research study. I have had a chance to ask questions, and all my questions have been answered. "

Participant Signature: _____ Date: _____

Participant Name: _____

Consent (Storage and Future Testing): "My stool samples may be stored and may be tested in the future when new tests become available. My samples will not be used for human genetic testing unless I agree to this in the future. Any results that may affect my health or health care will be communicated to my doctors at EBAC. I agree to take part in the research."

Participant Signature: _____ Date: _____

Participant Name: _____

Signature of Person Obtaining Consent: _____

Name of Person Obtaining Consent:_____ Date:_____

Physician/Investigator Statement:

"I have explained this research study in an understandable and appropriate language to the patient and/or to his/her authorized representative. I believe that I have fully informed this patient of the nature of this study. its possible benefits, and the risks of participation."

Investigator Signature: _____ Date:_____

Investigator Name:_____

Research Participant's Bill of Rights

The rights below are the rights of every person who is asked to be in a medical research study. As a research participant, you have the following rights:

1. To be told what the study is trying to find out.

2. To be told what will happen to you and whether any of the procedures, drugs, or devices are different from what would be used in standard practice.

3. To be told about the frequent and/or important risks, side effects or discomforts of the things that will happen to you for research purposes.

4. To be told if you can expect any benefit from participating and, if so, what the benefit might be.

5. To be told the other choices you have and how they may be better or worse than being in the study.

6. To be allowed to ask any questions concerning the study, both before agreeing to be involved and during the course of the study.

7. To be told what sort of medical treatment is available if any complications arise.

8. To refuse to participate at all or to change your mind about participating after the study is started. This decision will not affect your right to receive the care you would receive if you were not in the study.

9. To receive a copy of the signed and dated consent form.

10. To be free of pressure when considering whether you wish to agree to be in the study.

If I have other questions, I should ask the study doctor or the research staff. In addition, I may contact the University of California, Committee for the Protection of Human Subjects (UCB CPHS) or the Alta Bates Medical Center Institutional Review Board (ABIRB). They are concerned with the protection of volunteers in research studies. I may reach the UCB CPHS at 101 Wheeler Hall, University of California, Berkeley, California 94720-1340, 510-642-7461. I may reach the AB IRB's office by calling: 510-204-1414 or writing to the Alta Bates Institutional Review Board, Suite 1150, Alta Bates Summit Medical Center, 2450 Ashby Avenue, Berkeley, CA 94705.

An enhanced approach for studying the causes of diarrheal disease in an HIV+ cohort: The Enteric Pathogens Microarray Study

Section 1: Background Information

1. What is your date of birth? (Month/Date/Year) _____
2. What is your gender (please circle your answer)? Male Female

3. Please circle the highest year of school you have completed:
   Elementary: 1 2 3 4 5 6 7 8
   High School: 9 10 11 12
   College: 1 2 3 4 5 6+

4. Please check the box that best indicates your racial background.

☐ White ☐ Native American/American Indian
☐ Black/African American ☐ Asian or Pacific Islander
☐ Hispanic ☐ Other (please indicate)_____

5. Please check the box that best indicates your total combined household income during the past 12 months. Include money from jobs, social security, retirement income, and public assistance. Also include income from interest, dividends and any other sources.

☐ $20,000 or less ☐ Between $40,001 and $50,000
☐ Between $20,001 and $30,000 ☐ Between $50,001 and $100,000
☐ Between $30,001 and $40,000 ☐ Greater than $100,000

Section 2: Questions about your health.

6. During the past seven days have you had any of the following symptoms? (Please circle Yes or No)

A.	Cramps in your stomach or abdomen	Yes	No
B.	Diarrhea (2 or more loose or unformed stools in a day)	Yes	No
C.	Nausea	Yes	No
D.	Throwing up or vomiting	Yes	No
E.	A fever (➔ If Yes, what was your temperature? _____)	Yes	No
F.	Bloating or Gas	Yes	No

7. Do you have any of these symptoms TODAY? (Please circle Yes or No)

A.	Cramps in your stomach or abdomen	Yes	No
B.	Diarrhea (2 or more loose or unformed stools in a day)	Yes	No
C.	Nausea	Yes	No
D.	Throwing up or vomiting	Yes	No
E.	A fever (➔ If Yes, what was your temperature? _____)	Yes	No
F.	Bloating or Gas	Yes	No

8. If you had diarrhea today or in the past seven days… (➔ If not, SKIP to Question 9)

A.	Was your diarrhea ever liquid or watery?	Yes	No
B.	Was there ever blood in your diarrhea?	Yes	No
C.	Was there ever mucus in your diarrhea?	Yes	No
D.	Did you take any medicines for your diarrhea?	Yes	No

9. Did you miss a day or more of work or school because of nausea, abdominal cramps, vomiting, fever or diarrhea (Please circle the best answer)?

Yes No Not Applicable

10. Were your bowel movements in the past week different from your normal or usual experience?

Yes No

Section 2: Questions about general behaviors. Please circle all answers that apply or check the appropriate box.

11. During the past week (7 days), did you swim in any of the following:
Swimming Pool River, Lake or Stream Ocean Hot Tub None

12. During the past week (7 days), did you have contact with any of the following:
Children under five Anyone with Diapers Children attending daycare None

13. During the past week (7 days), did you eat any of the following:
Shellfish Raw Fish Red or Pink Meat Unpasteurized milk or juice None

14. During the past week (7 days), did you travel outside of the United States?

☐ Yes ☐ No

If Yes, which country did you travel to? _____

15. During the past week (7 days), did you have any contact with the following animals:
Dog Cat Bird Horse Goat Rabbit None
Other farm animal or pet: (please specify _____)

16. During the past week (7 days), have you had any sexual contact with a woman?

ρ Yes ρ No

17. During the past week (7 days), have you had any sexual contact with a man?

ρ Yes ρ No

18. During the past week (7 days), did you have any of the following types of sexual contact?
Oral Sex Anal Sex Vaginal Intercourse None

Section 3: Questions about your drinking water. Please circle the best answer or check the appropriate box.

19. How often do you drink bottled water?

ρ Always ρ Often ρ Sometimes ρ Rarely ρ Never

20. Excluding bottled water, how often do you filter or treat water before drinking it?
ρ Always ρ Often ρ Sometimes ρ Rarely ρ Never

21. Excluding bottled water, coffee, tea and other hot drinks, how often do you boil water before you drink it?
ρ Always ρ Often ρ Sometimes ρ Rarely ρ Never

22. How concerned or worried are you about the quality of your drinking water and its possible effects on your health?
ρ Very concerned ρ A little concerned ρ Not at all concerned

23. Have you heard of the federal drinking water guidelines (from the Centers for Disease Control) for people infected with HIV?
ρ Yes ρ No

Thank you for completing this survey.

Application to the University of California, Berkeley
Committee for the Protection of Human Subjects

Joseph Eisenberg, PhD Assistant Adjunct Professor (PI)
John M. Colford, MD, PhD, Associate Professor
Sona R. Saha, MPH, Graduate Student Researcher
School of Public Health, University of California, Berkeley

August 8, 2002

Research Protocol

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1. Title

An enhanced approach for studying the causes of diarrheal disease in an HIV+ cohort: The Enteric Pathogens Microarray Study

2. Related Studies

Several of the core study personnel (including Joseph Eisenberg (PI) and John M. Colford (Co-Investigator)) have received CPHS approval for related studies on gastrointestinal illness in immunocompromised individuals, including "A Randomized Trial of Tap-water Treatment in HIV+ persons" (CPHS# 2001-11-50) and “A Randomized Trial of Tap Water Treatment in the Elderly” (CPHS#2002-6-90). The consent form, questionnaire, recruitment and participant materials for this study are based on those previously approved for the two studies mentioned above.

3. Nature and Purpose

Microarray technology has been applied in a variety of arenas including the characterization of mRNA populations in tumor and normal cells, the identification of virulence genes in *M.tuberculosis* and the analysis of temporal changes in gene expression during cellular events (1-2). Although microarrays have been employed for transcript profiling and gene expression analysis, the potential power of microarrays as diagnostic tools for infectious pathogens has been largely untapped. We propose to apply this technology to detect the presence of infectious agents associated with gastrointestinal illness in fecal specimens collected from HIV+ patients with acute, chronic and no diarrhea from a community AIDS clinic.

This application is of particular relevance to HIV-infected individuals as they represent a sensitive subpopulation at increased risk for infectious gastroenteritis (3). Numerous studies on infectious diarrhea in children, hospital patients and travelers have been published, but little is known about the importance of specific viral, bacterial and protozoan agents among HIV+ individuals in a community setting in the United States (4-7).

The epidemiology of diarrhea in the immunocompromised population is very different than that in the general population and can be potentially life threatening (3). There are a number of non-infectious causes of diarrhea such as side effects due to medications prescribed to HIV+ individuals. This association between diarrhea and medication has increased since the introduction of highly active antiretroviral therapy (HAART) in the last quarter of 1996 (8). For example, diarrhea is a known complication of Nelfinavir and other protease inhibitors (8). The other major cause of diarrheal disease in this population is infection with infectious pathogens. Prior to the introduction of HAART, chronic diarrhea affected 50-90% of the HIV+ population (9), and has been attributed to viral, bacterial, and parasitic infection. A more recent study suggests that though the prevalence of diarrhea has dropped, it is still notable in the HIV+ population (10).

A cross-sectional study recently conducted by our group with support from UARP and the CDC found that 47% of HIV+ participants (n=226) reported diarrhea in the 7 days prior to being surveyed (11). The aim of this study was to measure the occurrence of diarrhea among HIV+ individuals, and to examine the relationship of diarrhea to drinking water consumption patterns, risk behaviors, immune status, as well as medication use after the introduction of HAART. Our data suggested that only 30% of the diarrhea reported was attributable to side effects from the HAART medication. An increase in CD4 count was protective only for those with a low risk of diarrhea associated with medication (OR = 0.6 [0.6, 0.9]). Chronic or idiopathic HIV-related diarrhea may be associated with medication or may potentially be of infectious etiology with unrecognized pathogens.

Efficient, rapid identification of specific bacterial and protozoan organisms in stool specimens collected from HIV+ persons with acute diarrhea, chronic diarrhea and no diarrhea using microarray technology is the central goal of this pilot study. We propose to compare the results of the microarray to that of standard microbiological tests to evaluate its performance and validate its use for epidemiological research purposes. To limit the scope and costs of this pilot project, the microarray will be limited to bacterial and protozoan organisms. The results of the microarray and standard analyses will enable us to evaluate the association of specific organisms with the different profiles of diarrhea seen in HIV+ persons.

Though recent developments in molecular analysis techniques have increased the sensitivity with which enteric pathogens can be detected, they remain inefficient since each organism needs to be tested for individually. Microarrays are uniquely suited to mass screening and offer the potential to maximize efficiency as various PCR products, each representing an individual hybridization test, can be spotted on a single microarray and assayed simultaneously.

A microarray is a glass slide onto which single stranded DNA fragments are adhered at fixed points (spots). A single slide may hold up to tens of thousand of targets, each related to a single gene (12,13). Microarrays exploit the preferential binding of complementary single stranded nucleic acid sequences. Samples of fluorescently labeled mRNA or cDNA are washed over the microarray as in a nucleic acid hybridization test, except on a substantially larger scale. Gene sequences from the sample hybridize to their complementary sequences in the “spots”. To quantify hybridization, the array is scanned, excited by a laser and the relative fluorescent intensities of each spot measured.

This approach offers a rapid, highly sensitive method that ultimately may be less costly than conventional tests. This microarray can also serve as a prototype for testing all types of infectious

agents, beyond enteric pathogens, and provide a model to apply to other infectious diseases prominent in HIV+ individuals. If successfully developed, such an approach would also alter the way in which epidemiologic studies can evaluate stool specimens for the presence of pathogens.

The principal objectives of this study are:
To develop a pilot "enteric pathogens microarray" to detect various bacterial and protozoan organisms from fecal specimens.
To validate the microarray by:
directly comparing microarray results with results from standard microbiological tests and evaluating agreement of the two techniques on the same clinical specimens;
determining the specificity, sensitivity, predictive value (positive and negative) and ROC characteristics of the microarray using nucleic acid products from known pathogens;

1) To evaluate the association of specific infectious organisms with acute, chronic and no diarrhea in HIV+ individuals using standard clinical microbiology and the novel microarray.
2) To determine if infectious agents previously unrecognized as potential pathogens are associated with clinical symptoms of gastrointestinal illness.

4. Subjects

One hundred and fifty HIV+ patients will be recruited from the East Bay AIDS Center at Alta Bates Medical Center, Berkeley, CA to participate in this study. Participants will be asked to provide a stool specimen and complete a brief questionnaire on gastrointestinal symptoms and potential risk factors. Stool specimens will be tested for bacterial and parasitic organisms using standard microbiological methods and by the enteric pathogens microarray developed for this study.

5. Recruitment

Participants will be recruited during regular clinic visits for study participation by study investigators and staff at the East Bay AIDS Center (EBAC). Participants will be selected based on the presence or absence of diarrheal symptoms at the time they enter the clinic. Our goal is to enroll fifty participants each in three groups: acute diarrhea, chronic diarrhea and no (asymptomatic) diarrhea.

Patients with "acute" diarrhea are those for whom their clinicians at the East Bay AIDS Center would request stool work-up due to the presence of gastrointestinal symptoms (vomiting, diarrhea, abdominal cramps, nausea, bloody diarrhea, fever) that are new, different or more severe than the normal pattern of diarrhea experienced by that patient. Diarrhea in such cases is suspected to be of infectious etiology. The patients comprise our definition of a "case".

Patients with "chronic" diarrhea are defined as those with diarrheal symptoms (two or more loose stools per day for two weeks or greater) that are not different from their normal pattern of diarrhea. Diarrhea in such cases may be a side effect of HIV drug treatment and may or may not represent enteric infection. If infectious, it is likely that a unique set of pathogens is associated with diarrheal illness in this group. These patients represent our first comparison group.

The second comparison group is HIV+ patients with no diarrheal symptoms. These individuals may have asymptomatic infection with recognized enteric pathogens or other organisms whose pathogenicity is not yet recognized or well defined.

Comparison groups will be matched on time to the cases (within one week of case specimen collection), to ensure that there is an even distribution of recruitment throughout the study enrollment period to capture any seasonal variations or potential outbreaks. If a patient meets any of the above definitions, a physician or clinic staff member will refer the patient to a research study staff member.

The study staff member will describe the study and ask whether the patient may be interested in participating in such a study. The staff member will go through the eligibility criteria with those who indicate an interest in the study. If the patient is both willing and eligible, the researcher will go through the consent form with them. The consent form will contain all information about eligibility criteria, design of the study potential risks and benefits. Signing of the consent form by the patient will document consent.

A flyer and brochures describing the study and inviting participation will be displayed in the clinic. To enhance in-clinic recruitment if needed, clinic patients may be sent a letter and recruitment To enhance in-clinic recruitment if needed, clinic patients may be sent a letter and recruitment flyer by mail describing the study, eligibility criteria and contact information of study staff. Interested individuals can call study staff or come into the clinic to learn more about the study

6. Screening Procedures

Individuals meeting the inclusion criteria below will be requested to provide a stool sample and complete a brief questionnaire. Each individual must meet the following inclusion criteria:

(a) HIV+
(b) A patient of the East Bay AIDS Center
(c) Have either acute, chronic or no diarrhea

A study staff member will review the case and comparison group definitions (see above) with the potential participant and evaluate which group the patient would be eligible for. If an individual matches the “acute diarrhea” case definition, they will be enrolled in that group and within the following week two additional patients (one for the chronic diarrhea group and one for the no diarrhea group) will be recruited and screened for participation to match this case. After a case episode, a six-day symptom free period will be required before a participant could be enrolled again as a case or in one of the two comparison groups.

The study staff member will describe the study purpose and procedures and inquire whether the patient may be interested in participating in the study. The staff member will go through the eligibility criteria with those who indicate an interest in the study. If the patient is both willing and eligible, the researcher will go through the consent form with them. The consent form will contain all information about eligibility criteria, design of the study potential risks and benefits. Signing of the consent form by the patient will document consent.

7. Procedures

Once an individual agrees to take part in the study and the consent form has been signed, the participant will be asked to complete a brief questionnaire while at the clinic. This questionnaire will ask the participants about their gastrointestinal health, water consumption patterns, and exposure to other risk factors for GI illness. (The questionnaire and recruitment materials are currently being developed.)

Participants will also be asked to provide a stool sample for microbiological testing. They may provide the sample while at the clinic or in the privacy of their homes within 24 hours of their clinic visit in special containers provided to them. Instructions for safe collection of stool specimens will be provided to all participants. If the specimen is collected at the participant's home, study personnel will arrange for a courier to pick-up the specimen from the participant's home, and deliver the specimen to the clinic.

Stool specimens will be split into aliquots in the clinic by trained staff and sent to a clinical laboratory for standard microbiological work-up (bacterial culture, ova and parasite exam, *C.difficile* toxin and *Giardia* tests) and to UC Berkeley for nucleic acid isolation and preparation for microarray analysis. A portion of the sample sent to UCB will also be stored for future testing at the UCB laboratories; participants will sign a consent indicating that they have donated their specimens for any future testing and may ask for their stored specimens to be destroyed at any time by contacting study staff. The study participant will be instructed to contact their own health care provider in the event of any perceived need for medical care or a medical emergency.

HIV viral load, CD4 count and HIV medications will also be abstracted from patient records for analysis and statistical modeling. To preserve confidentiality, no patient identifying information will leave the East Bay AIDS Center. All data and specimens will be labeled with a unique study identification number for each participant. The code for the unique identification numbers will be held in a secure location by the clinical research staff at the East Bay AIDS Center.

All results from specimens tested will be made available to the participant and his or her physician to inform clinical care. Individual participants will be notified of the results of all tests by letter, which will include a recommendation to discuss the results with their own medical practitioner. In addition, subjects will be notified by telephone if any treatable conditions are identified, with advice to notify their own medical practitioner as soon as possible. Subjects will be given a telephone number at the study office to call if they have any questions.

Study personnel will notify affected subjects and appropriate county health department for reportable infectious diseases. If a subject tests positive for any pathogenic organism, the test result notification letter will be mailed to the subject along with a CDC fact sheet. These CDC fact sheets are in the public domain, and can be found at the CDC's web site (www.cdc.gov/ncidod/diseases). If a subject tests positive for an organism with unclear clinical relevance, the test result notification letter will be mailed to the subject along with a general information sheet.

8. Benefits

There may be no substantial direct benefit to the participants from the research. It is hoped that the research will benefit society by determining which infectious agents are most associated with diarrheal symptoms in HIV+ patients. Patients will receive the results of their stool tests to discuss with their physician.

9. Risks

There are minor risks associated with the procedures. Collection of stool samples, although conducted in private, might cause the individual some embarrassment and inconvenience. Giving samples for the study also carries the risk of loss of privacy, but this chance will be made as small as possible (see below).

10. Confidentiality

All the facts we collect from subjects during the research will be kept private to the full extent allowed by law. Each participating person will be assigned a unique code number. The code for the unique identification numbers will be held in a secure location by the clinical research staff at the East Bay AIDS Center. No identifying information will leave the clinic. Furthermore, the East Bay AIDS Center will not provide any information to U.C. Berkeley researchers that would enable the identification of individual subjects. This will minimize the risk of loss of privacy.

The results from tests of stool samples will be given to subjects to discuss with their doctor, since these results might improve their health care. If we find participants to have Salmonella, Giardia, or certain other infections, the law requires us to tell the participant and the local public health department about it. This is standard procedure that any regular medical practitioner is required to follow.

11. Informed Consent

The informed consent form is included in Appendix I.

12. Financial Aspects

Each participant will be given $15 for submitting a stool sample and completing the questionnaire.

97.1.1

13. Written Materials

The consent form, stool collection instructions and test result notification letters are attached. The questionnaire and recruitment materials are currently being developed and will be submitted for IRB approval once completed.

14. Signature

Joseph Eisenberg, PhD
(Principal Investigator)

15. Contact Information

Joseph Eisenberg, PhD (PI)	ph: 510 643-9257	fax: 510 642-5815
	Email: eisenber@socrates.berkeley.edu	
John M. Colford, MD PhD	ph: 510 643-1076	fax: 510 643-5163
	Email: jcolford@socrates.berkeley.edu	
Sona R. Saha, MPH	ph: 510 642-6265	fax: 510 643-7315
	Email: ssaha@uclink4.berkeley.edu	

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Enteric Pathogens Microarray Study
Stool Collection Instruction Sheet

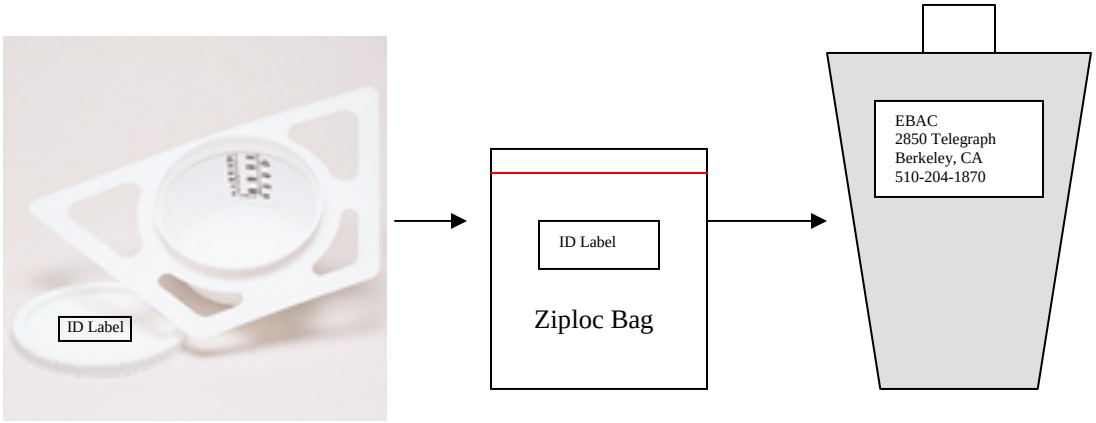
You may collect your stool sample while at the clinic or at your convenience once you get home. If you choose to collect your sample at home, please collect it within 24 hours of your clinic visit. Please call our study staff at 510 204-1291 to arrange for the sample to be picked up by courier. Alternatively, you may drop the sample at the clinic.

The Stool Kit
Stool collection kit was given to you at the clinic. In the stool collection kit you will find:
One plastic stool collection container with ID label;
One large zip-lock plastic bag;
One stamped paper bag with study name and clinic delivery address.

To Collect a Stool Sample
Please write the following information on the stool collection container label:
Date and time specimen was collected;
Your Participant ID Number should already be pre-written on the label.

Use the container provided to collect the stool specimen:
Remove the lid from the stool kit collection container; be sure the frame around the container is in place.
Lift the toilet seat.
Place the stool collection container and frame on the toilet bowl towards the back of the bowl and lower the toilet seat cover over the container/frame to secure it in place.
Pass stool directly into the container (do not scoop stool out of toilet water).
Place the lid back onto the stool collection container and make sure it is secure.
Do not allow any urine to fall into the container.

How to Prepare the Sample for pick-up
Please place the stool collection container into the Ziploc bag and the labeled paper bag. Please call the study staff immediately to arrange for a convenient pick-up time (510 204-1291). If you are going to be away from your house, you may leave the paper bag on your front porch for pick-up.



ENTERIC PATHOGENS MICROARRAY STUDY

Specimen Processing and Transport

Participants in the EPM Study will be providing stool samples for microbiological analysis. Specimens will need to be split into aliquots to forward to Unilab and UC Berkeley.

UNILAB
Vial 1-3: O&P formalin
Vail 4: Cary Blair
Vial 5: clean vial
↓
Give to Unilab courier with requisition slip

UC BERKELEY
Vial 1: clean vial
↓
Store in –70C freezer until ready for shipment to UCB

- Please label all vials with participant ID, date and time of collection.
- Please fill vials about ¾ full, to the bottom line of the label.

Courier Information

An account for this study has been created with:

Modern Express Courier
1-800-4000-7874
510 444-6245
510 444-5418 (fax)
2525 Mandela Parkway, Oakland, CA 94706
www.OaklandCourier.com

Please mention the "EPM Study" when you call to request a pick-up. The study's account number is 31962. The courier should arrive at the pick-up location (either the clinic or participant's residence) in 30-45 minutes from the time of the call. Regular service guarantees delivery within 3-4 hours and "Rush" service guarantees delivery within 2 hours.

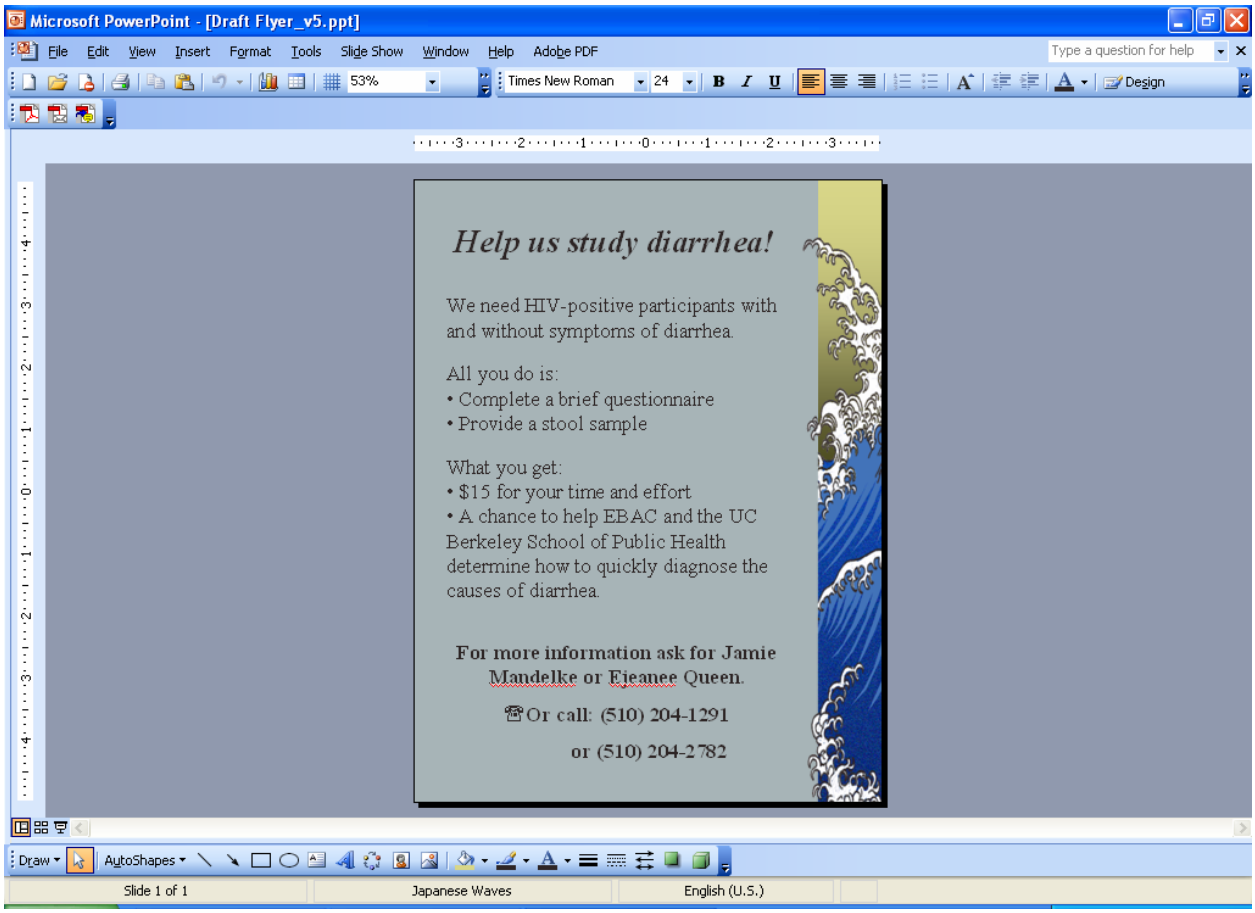
- All packages need to be labeled "DIAGNOSTIC SPECIMEN".
- Packages to UC Berkeley will need to be packed in dry ice and also labeled "UN 3373" and "UN 1845 DRY ICE".

Please send specimen packages to UC Berkeley to:

Amy White, Riley Lab - Infectious Disease, 140 Warren Hall, Berkeley, CA 94720
First (not ground) Floor in Warren Hall.

Please call Amy to let her know when the samples will leave the office and approximately when they will arrive (Amy White @310-643-2949, or cell @415-407-6958). Amy will call EBAC to confirm that the package has arrived.

Shipments to UCB will be done periodically and coordinated by Jamie Mandelke and/or Sona Saha.


Microsoft PowerPoint - [Draft Flyer_v5.ppt]
File Edit View Insert Format Tools Slide Show Window Help Adobe PDF
Type a question for help
Times New Roman 24 Design
Help us study diarrhea!
We need HIV-positive participants with and without symptoms of diarrhea.
All you do is:
• Complete a brief questionnaire
• Provide a stool sample
What you get:
• $15 for your time and effort
• A chance to help EBAC and the UC Berkeley School of Public Health determine how to quickly diagnose the causes of diarrhea.
For more information ask for Jamie Mandelke or Ejeanee Queen.
☎Or call: (510) 204-1291
or (510) 204-2782
Draw AutoShapes
Slide 1 of 1
Japanese Waves
English (U.S.)

Abstract for Bay Area Clinical Research Symposium

Although microarrays have been employed for transcript profiling and gene expression analysis, the potential power of microarrays as diagnostic tools for infectious pathogens has been largely untapped. Using such an array, we would need to test a clinical specimen only once to detect the presence or absence of numerous pathogens or differentiate pathogenic from non-pathogenic strains. We are currently designing a pilot diagnostic microarray to detect the presence of a broad range of infectious agents associated with gastrointestinal illness in stool specimens. This enteric pathogens microarray is composed of 40mer oligonucleotides derived from species specific and conserved rRNA sequences from approximately 45 bacterial and protozoan organisms including pathogenic E.Coli, Shigella, Salmonella, Mycobaterium, Cryptosporidum, Entamoeba and Microsporidia species.

In parallel, we are conducting a case control study through the East Bay AIDS Research Institute (EBARI) investigating the etiology of GI illness among HIV+ individuals. Stool specimens are being collected from 150 HIV+ patients with and without diarrheal symptoms from a community AIDS clinic; we will compare the performance of this novel microarray to that of standard clinical microbiological analysis. This application of the array is of particular relevance to HIV+ individuals as they represent a sensitive subpopulation at increased risk for infectious gastroenteritis.

HIV+ patients from the East Bay AIDS Center with acute, chronic or no diarrhea are being recruited to provide stool specimens and complete a brief questionnaire on gastrointestinal symptoms and potential risk factors (e.g. travel, sexual behavior, food and water consumption, animal contact). This sampling design represents a case-control study with two comparison arms (Cases=acute diarrhea; control arm #1=chronic diarrhea; control arm #2=no diarrhea). The stool specimens will be analyzed by a clinical laboratory using standard methodologies and by the enteric pathogens microarray in development. Information on medications, HIV viral load and CD4 count is also being abstracted from participant medical records. Recruitment began in April 2003 and over 80 participants have been enrolled as of August 2003.

Our principal objectives for this pilot enteric pathogens microaray study are to: 1) design, develop and validate an enteric pathogens microarray to detect bacterial and protozoan organisms from fecal specimens, 2) evaluate the association of specific organisms with acute, chronic and no diarrhea in HIV+ patients using standard microbiological methods and the enteric pathogens microarray, and 3) determine if organisms previously unrecognized as pathogens are associated with symptoms of gastrointestinal illness. Study results are anticipated in summer 2004. Although this pilot study focuses on bacterial and protozoan organisms, we plan to develop a more comprehensive microarray in the future that will include important enteric viruses.

UARP 2004 Investigators Meeting and Conference on AIDS Research

ABSTRACT

Title: An enhanced approach for studying the causes of diarrheal disease in an HIV+ population: The Enteric Pathogens Microarray Study

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Abstract Text:

Although microarrays have been employed for transcript profiling and gene expression analysis, the potential power of microarrays as diagnostic tools for infectious pathogens has been largely untapped. Using such an array, we would need to test a clinical specimen only once to detect the presence or absence of numerous pathogens or differentiate pathogenic from non-pathogenic strains. We are currently designing a pilot diagnostic microarray to detect the presence of a broad range of infectious agents associated with gastrointestinal illness in stool specimens. This enteric pathogens microarray will be composed of 40mer oligonucleotides derived from species specific and conserved rRNA sequences from various bacterial and protozoan organisms including pathogenic E.Coli, Shigella, Salmonella, Mycobaterium, Cryptosporidum, Entamoeba and Microsporidia species.

In parallel, we are conducting a case control study investigating the etiology of gastrointestinal illness among HIV+ individuals. Stool specimens are being collected from 150 HIV+ patients with and without diarrheal symptoms from a community AIDS clinic; we will compare the performance of the microarray to that of standard clinical microbiological analysis. This application of the array is of particular relevance to HIV+ individuals as they represent a sensitive subpopulation at increased risk for infectious gastroenteritis.

HIV+ patients from the East Bay AIDS Center with acute, chronic or no diarrhea are being recruited to provide stool specimens and complete a brief questionnaire on gastrointestinal symptoms and potential risk factors (e.g. travel, sexual behavior, food and water consumption, animal contact). This sampling design represents a case-control study with two comparison arms (Cases=acute diarrhea; control arm #1=chronic diarrhea; control arm #2=no diarrhea). The stool specimens will be analyzed by a clinical laboratory using standard methodologies and by the enteric pathogens microarray in development. Information on medications, HIV viral load and CD4 count is also being abstracted from participant medical records. Recruitment began in April 2003 and 142 participants have been enrolled and submitted specimens as of November 15, 2003.

Our principal objectives for this pilot enteric pathogens microarray study are to: 1) design, develop and validate an enteric pathogens microarray to detect bacterial and protozoan organisms from fecal specimens, 2) evaluate the association of specific organisms with acute, chronic and no diarrhea in HIV+ patients using standard microbiological methods and the enteric pathogens microarray, and 3) determine if organisms previously unrecognized as pathogens are associated with symptoms of gastrointestinal illness. Study results are anticipated in summer 2004. Although this pilot study focuses on bacterial and protozoan organisms, we plan to develop a more comprehensive microarray in the future that will include key enteric viruses.