THE ROLE OF FOMITES IN THE TRANSMISSION OF NOROVIRUS

by

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DEDICATION

This work is dedicated with much love and appreciation to my parents, Emmanuel and Suzanne Fankem, who have always been there for me, have always believed in me, and have consistently encouraged me. I am sure they'll be happy to see that it is finally done.

Mother, thank you for always being the pillar I can always rely on, come what may.

MERCI POUR TOUT

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ABSTRACT

Introduction: Acute gastroenteritis is a common illness in humans. It has a high morbidity worldwide and in the United States (U.S); a sizeable mortality is reported in developing countries. Viruses are a common cause of acute gastroenteritis and noroviruses are the leading cause of non-bacterial gastroenteritis (1). Fomites play an important role in the infection cycle of norovirus. This study explored the comparability between a quantitative microbial risk assessment (QMRA) model to predict the probability of illness from norovirus due to environmental contamination with actual observed attack rates, the impact of appropriate cleaning procedures on fomites contamination, and the role of fomites in the transmission of norovirus

Method: Data from three different norovirus illness outbreaks occurring from 2004 to 2005 in Arizona in different venues were used. Questionnaires were used to determine demographics, symptoms associated with gastrointestinal illness, medical visits, and potential exposures such as food items consumed. When available, stool samples were collected from ill participants. Fomites samples were also gathered from the different venues; houseboats, college summer camp living spaces, and a restaurant. These samples were tested for norovirus using reverse transcriptase polymerase chain reaction (RT-PCR). Viral concentration on fomites was calculated using the most probable number methodology.

Results: Norovirus was found on fomites from all three outbreaks. Improper cleaning of fomites increased the proportion of norovirus positive fomites from 40 percent to 70 percent in contrast, after cleaning and disinfection with 5000 mg/L free chloride, the proportion of norovirus positive fomites decreased to 33%. The predicted values from the quantitative microbial risk assessment model were consistent with the observed epidemiological attack rates.

Conclusion: This study showed unequivocally that fomites play an important role in the transmission cycle of norovirus, and that improper cleaning and disinfection procedures only serve to spread the virus to previously uncontaminated fomites. In the case of outbreaks where people are not always available to answer questionnaires, QMRA should be considered as a worthwhile alternative to a full-scale epidemiological study.

CHAPTER ONE – INTRODUCTION

DISSERTATION FORMAT

This doctoral student worked as a graduate student (Research Associate) in Dr. Gerba's laboratory and was involved in all the studies described in this dissertation. Consequently, this researcher has detailed knowledge of the data and was involved in several aspects of the conduct of each of three specific study outbreaks. This dissertation consists of three manuscripts prepared for publication and presented as appendices.

By using this dissertation format, the candidate's research is presented in five chapters and Appendices A-F. The first chapter introduces the topic of the dissertation research and the specific aims that are associated with three distinct manuscripts. The second chapter provides a review of the literature. The third chapter presents methods of the research. The fourth chapter presents the results of the study. The final chapter consists of a discussion of the relationship between the role of fomites in the spread of norovirus and the use of quantitative microbial risk assessment as an alternative to a full scale epidemiological study, including limitations of the overall research and specific studies and recommendations for future research. The first three appendices of this dissertation contain the manuscript drafts of three related studies designed and undertaken by the candidate: 1) Assessment Of Quantitative Microbial Risk

Assessment to Predict Attack Rates During an Outbreak of Norovirus Based Upon Fomites Exposure; 2) Outbreak of Norovirus Illness in a College Summer Camp: Impact of Cleaning on Occurrence of Norovirus on Fomites; 3) Norovirus Outbreak During a Christmas Banquet. All were written primarily by the degree candidate, with input from the coauthors and reviewed by the dissertation committee.

The dissertation author was responsible for all of the research presented in the manuscripts, with the following exception: Marlene Gaither collected all environmental and stool samples.

EXPLANATION OF THE PROBLEM AND ITS CONTEXT

A. Background and Significance

Acute gastroenteritis is a common illness in humans. It has a high morbidity worldwide and in the United States (U.S); a sizeable mortality is reported in developing countries (2). Viruses are a common cause of acute gastroenteritis, especially in children, and noroviruses are the leading cause of non-bacterial gastroenteritis (1).

Noroviruses are transmitted via the fecal-oral route. The average incubation period for norovirus-induced gastroenteritis is 12-48 hours with symptoms that usually resolve in 12-72 hours. Illness is characterized by acute-onset projectile vomiting, watery non-bloody diarrhea with abdominal cramps, low grade fever, headache, and malaise (3-5). The infectious dose required for symptomatic illness is considered to be low (10-100 virions) (6) and this very low infectious dose of norovirus may be one reason why it is the most common cause of diarrheal illness (1). Two other factors also contribute to the considerable impact of disease caused by norovirus: a large human reservoir of infection (7), and the ability to be transmitted by a variety of routes.

Noroviruses are particularly contagious due to their low infective dose, 10-100 virions (6). Norovirus gastroenteritis causes rapid dehydration, which is of

particular concern among the elderly and very young as well as those who are engaged in physically demanding activities that hasten dehydration, characteristic of outbreaks among soldiers during deployment (8). There are no known non-human reservoirs for human norovirus, and no long-term immunity is gained from infection (9). It is not yet possible to grow noroviruses in conventional cell culture systems. Reverse transcriptase polymerase chain reaction (RT-PCR) identification of norovirus has only been possible since the early 1990s, and the test has only recently become widely available.

Outbreaks of norovirus acute gastroenteritis due to fomite transmission have lately become of great interest. Several outbreaks have been linked to fomite contamination in various settings, such as houseboats, a concert hall, and nursing homes (10-12). Fomites comprise porous and nonporous inanimate surfaces or objects that can become contaminated with pathogenic microorganisms and may serve as vehicles in disease transmission (13-15). Fomites become contaminated with pathogenic microorganisms by direct contact with body secretions or fluids, contact with soiled hands, contact with aerosolized virus (large droplet spread) generated via talking, coughing, vomiting, sneezing, or airborne viruses that settle after disturbance of a contaminated fomite (13, 14, 16). Once the fomite is contaminated, the transfer of infectious organisms can readily occur between inanimate and animate objects and vice versa, or between fomites if they are brought together (15, 17).

The significance of this study is two-fold. First, there is the potential for reduction in morbidity related to norovirus outbreaks in venues where large number of people tend to convene. The role of fomites in the spread of microorganisms is sometimes belittled as we do not realize the impact of proper cleaning and disinfection procedures to curtail the morbidity associated with acute gastroenteritis such as norovirus illness. Second, we show that using quantitative microbial risk assessment as a surrogate for a full scale epidemiological study is valid as it is less time consuming, less costly, and can reasonably predict attack rates observed in actual outbreaks.

B. Study Aims

Human noroviruses spread very easily via person-to-person contact, contaminated surfaces, food, water, and by airborne spread through vomiting. The overall goal of this research is to understand the effects and impact of proper cleaning and disinfection on the spread of noroviruses via fomites in everyday venues. Because of the nature of the infection (acute infection of short duration) we are addressing, data from a variety of actual documented outbreaks are used to show how the appropriate cleaning and disinfection procedures can reduce the amount of microorganisms found on fomites, hence reducing the probability of infection. Although the aims of the three studies differ slightly from each other, they all deal with the role of fomites in norovirus outbreaks in different venues.

The three aims of this project are:

1) Assess the comparability between a quantitative microbial risk assessment model to predict the probability of illness from norovirus due to environmental contamination on houseboats and actual observed attack rates (Manuscript #1: A norovirus outbreak on houseboats).

2) Determine the impact of improper cleaning on the prevalence of fomites in an outbreak setting. An evaluation of an outbreak of norovirus in a college summer camp provided the opportunity to identify the source of infection, the causes of disease spread, to underline the importance of environmental clean up and recommend strategies for prevention of future outbreaks in such settings (Manuscript #2: Norovirus illness in a college summer camp).

3) Evaluate the role of fomites and human behaviors in the spread of the disease outbreak (Manuscript #3: Norovirus outbreak during a Christmas banquet).

CHAPTER TWO - LITERATURE REVIEW

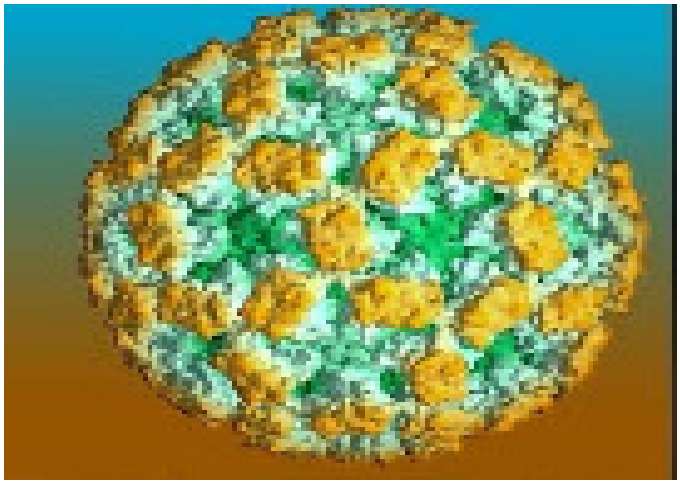
A. Overview of Norovirus Outbreaks

Outbreaks of norovirus gastroenteritis have occurred in various settings such as schools, daycare centers, nursing homes, hospitals, cruise ships. Hamano et al (18) studied samples from 60 acute non-bacterial gastroenteritis outbreaks over an eight year period (February 1997 - March 2004) in Okayama, Japan; noroviruses were detected in 59.1% of cases from 77% of those outbreaks. The outbreaks mainly occurred in restaurants (50%), schools (20%), and healthcare institutions (15%). These results were similar to those of Hedlund et al (19) who studied the epidemiology of calicivirus infections in Sweden from 1994 to 1998. Caliciviruses were responsible for 60% of the outbreaks in all settings (hospitals, nursing homes, schools/daycare centers, catering), and 60% of infected patients were elderly, 70-90 years (19). In the US, the Centers for Disease Control and Prevention (CDC) analyzed 8,271 foodborne outbreaks reported from 1991 to 2000 (20). They noticed an increase in the proportion of norovirus-confirmed outbreaks, from 1% in 1991 to 12% in 2000.

B. Genetic Variability of Noroviruses

Noroviruses (NoV), formerly known as small round structured viruses (SRSVs) or Norwalk-like viruses (NLVs), were discovered in 1972 (21, 22) and belong to the genus *Norovirus* in to the large family of *Caliciviridae* (23). Noroviruses, also called human noroviruses, mainly cause acute gastroenteritis in humans. They are called caliciviruses after the cup-shaped depression (Figure 1) in their surface (from the Greek *calyx*, meaning cup) (24). They are positive sense (i.e., the viral RNA genome can be directly translated by the host cell to make viral proteins) non-enveloped viruses possessing a single-stranded RNA genome of 7.5-7.7 kb, and are icosahedral in shape (25, 26). Human noroviruses are a genetically diverse and belong to one of two genera, the Noroviruses and the Sapoviruses (previously known as Sapporo-like viruses), of human caliciviruses.

Figure 1: X-ray crystallographic structure of norovirus capsid (24)



Classification of noroviruses is, due to lack of cell culture system, based on phylogenic grouping of complete Open Reading Frames (ORF2) sequences (26, 27). The genome of norovirus encodes for one major structural capsid protein called viral protein (VP1) of about 56 K, a minor capsid protein (VP2) at the 3'-end of the genome and the 5'-end encodes a large polyprotein that is transitionally processed into nonstructural proteins including RNA polymerase (POL) which is needed for viral replication (25, 26). Based on the sequence from the POL gene (ORF1) or VP1 gene (ORF2), noroviruses can be divided into five separate genogroups (GI, GII, GIII, GIV, and GV) of which genogroups I, II, and IV infect humans. Within genogroups, norovirus strains can be further delineated into at least 22 genetic clusters or genotypes based on grouping by genetic relatedness in the complete ORF2 sequence (3, 4, 23, 28).

C. Laboratory Diagnosis of Norovirus

Until recently, diagnosis of norovirus infection relied on insensitive methods such as electron microscopy (29) and serological testing (30) with human reagents that were mainly available in research settings. In the early 1990s, sensitive and simpler methods were developed to detect norovirus by identifying its viral RNA after reverse transcriptase polymerase chain reaction (31). In 1993, the CDC adopted reverse transcriptase polymerase chain reaction (RT-PCR) for the

routine detection of norovirus (32). Polymerase chain reaction (PCR) is a diagnosis tool that was invented in 1985 to amplify specific DNA sequences.(33)

Identification of norovirus as the cause of outbreaks of acute gastroenteritis has improved with the increasing use of the reverse transcriptase polymerase chain reaction (RT-PCR). Polymerase Chain Reaction is an in vitro technique that allows for the amplification of a specific deoxyribonucleic acid (DNA) region that lies between two known regions of DNA sequences (33). RT-PCR is a two step variation of PCR that is used when the genetic material of the organism of interest is ribonucleic acid (RNA), such as is the case for norovirus. RT-PCR first converts RNA to complementary DNA (cDNA) from messenger RNA (mRNA) using deoxynucleotide triphosphates (dNTPs) and the enzyme reverse transcriptase (RT). This step is then followed by PCR which allows for the amplification of the cDNA. RT-PCR is used as a diagnostic tool to test for the presence of norovirus RNA in environmental and stool samples. Identification of the virus can be best made from stool specimens taken 48 to 72 hours after onset of symptoms, although good results can be obtained by using RT-PCR on samples taken as long as five days after symptom onset. Viruses can still be detected in stool samples taken as late as two weeks after patient recovery.

The RT-PCR procedure used for all analyses in the dissertation studies has a sensitivity of 85% and was optimized to detect noroviruses GI and GII by

targeting a relatively well conserved region at the 3'-end of the major capsid (VP1) gene (28).

D. Burden of Norovirus Illness

Gastroenteritis is a common illness worldwide both in developed and developing countries. Foodborne diseases are estimated to cause 76 million illnesses, 325,000 hospitalizations, and up to 5,000 deaths annually in the United States (US) (1). In the US, the proportion of norovirus confirmed foodborne outbreaks rose from 1% in 1991 to 12% in 2000 (20). In 1997, gastroenteritis contributed to the death of more than 6,000 persons in the US (20). Human noroviruses are the leading cause of nonbacterial gastroenteritis in the US. It is estimated that of all foodborne illnesses, Norwalk-like viruses account for over 67% of all cases (23 million), 33% of hospitalizations, and 7% of deaths (1). It is also estimated that more that 90% of non-bacterial acute diarrhea episodes are due to noroviruses (34-37).

Outbreaks of norovirus gastroenteritis typically occur in settings where a large number of people congregate, such as schools, daycare centers, restaurants, nursing homes, hospitals, and cruise ships (12, 38-47). Gastroenteritis caused by noroviruses is mild and self-limiting in the absence of other factors. Unlike rotavirus, noroviruses affect all age groups (7). The highest incidence is in

children less than five years of age (48), but the greatest impact of noroviruses is probably an economic one among the elderly in health-care settings (19, 49, 50).

Although different types of caliciviruses cause illness in humans throughout the world, there is evidence that one strain of norovirus (GII.4 95/96 US) has caused more than 50 geographically distinct outbreaks in the US. This strain was also found in seven other countries on five continents between August 1993 and July 1997 (51). In a study of 284 outbreaks of non-bacterial gastroenteritis in the US between July 1997 and June 2000, the Center for Disease Control and Prevention (CDC) determined that NLVs were responsible for 93% of the outbreaks reported in 29 states, the District of Columbia, the US Virgin Islands, and cruise ships docking in American ports (52). The most common settings reported were restaurants (39%), nursing homes and hospitals (25%), schools, day care centers, and camps (13%), and vacation destinations such as cruise ships (10%). Norovirus surveillance activity in the US from 2006 to 2007 suggested a national increase has occurred in the frequency of acute gastroenteritis outbreaks caused by norovirus (including fatal cases in long-term-care facilities). The CDC reviewed 126 outbreaks of acute gastroenteritis: 91% were of norovirus origin, and 76% of those were associated with two new GII.4 norovirus variants known as Minerva and Laurens (28). The majority of these outbreaks occurred in nursing homes (29.4%), at restaurants and at catered events (10.3%) (53).

In Europe, established surveillance systems found that between 1995 to 2000, more than 85% of reported non-bacterial outbreaks of gastroenteritis were due to norovirus (36). The incidence of norovirus infections increased in Germany fivefold from 2001 to 2002, peaking during the winter (54). Norovirus related outbreaks steadily increased from 1992 to 2000 in England and Wales. Reports of norovirus outbreaks in England and Wales peaked in 1995 with 367 outbreaks, and then decreased to 139 in 1997. Since then, the frequency of outbreaks has steadily increased; 281 outbreaks were reported in 2000 (55). The outbreaks were reported as “infectious intestinal diseases” and because noroviruses are the most commonly identified cause of intestinal diseases in Western European communities, the authors assumed all outbreaks included in their report to be due to noroviruses (55). Once again, the most common settings were health-care institutions: 754 (40%) outbreaks occurred in hospitals and 724 (39%) in residential-care facilities. The outbreaks were most common in elderly care and geriatric units in 251 (39%) of the 648 hospital outbreaks and 169 (89%) of the 190 residential home outbreaks. A total of 147 (7.8%) outbreaks occurred in hotels, 73 (4%) occurred in schools, and 105 (6%) were linked to food outlets. Seventy-four outbreaks (3.9%) occurred in other settings such as private homes, holiday camps, and military bases. More than 50,000 people were affected by these outbreaks, which accounted for both morbidity and mortality. There were 128 hospitalizations (case-hospitalization rate = 33/10,000 cases) from 52 outbreaks; forty-three deaths (case-fatality rate 7.5/10,000 cases) in 38

outbreaks; all were associated with outbreaks in hospitals (24 deaths) and residential-care facilities (19 deaths) (55). There is a scarcity of economic impact data related to norovirus. However, the economic cost of norovirus infections in healthcare settings is quite significant. One study that these infections were associated with estimated the economic burden to be well over $180 million from 2002-2003 in the United Kingdom alone (5).

E. Populations at Risk

There is no specific therapy against norovirus gastroenteritis other than re-hydration. Unfortunately, there is still no vaccine to prevent this illness. Studies of outbreaks indicate that noroviruses infect patients of all ages, a feature that distinguishes them from other agents of viral gastroenteritis such as rotaviruses and astroviruses, which primarily affect children. This difference suggests that immunity to noroviruses may not be long lasting. The most vulnerable to such illness are usually the elderly and people with underlying illness e.g., the immune-suppressed.

Mechanisms of immunity to norovirus are unclear. It seems that immunity may be strain-specific and lasts only a few months (56); therefore, given the genetic variability of noroviruses, an individual is likely to be repeatedly infected throughout their lifetime, explaining the high attack rates in all ages reported in

most outbreaks. Parino et al (9) investigated clinical immunity due to Norwalk like virus infections. They challenged and then re-challenged 12 volunteers with Norwalk virus and evaluated symptoms, jejunal biopsies, and serum antibody. After the first challenge, gastroenteritis developed in 50% of the volunteers who, when re-challenged 27 to 42 months later, again had gastroenteritis with jejunal lesions. In the six previously immune volunteers illness or jejunal lesions did not develop. Four of five ill volunteers had increases in serum antibody to norovirus after both challenges. Serum antibody did not increase in three immune volunteers after either challenge. Four volunteers who had twice become ill underwent a third challenge four to eight weeks after their second illness (9). The level of serum antibody before initial challenge could not be used to predict whether or not an individual would develop illness. Another norovirus human volunteer study showed that 82% of the subjects became infected (57). Of those infected, 68% were symptomatic and 32% were asymptomatic. The proportion of subjects infected was similar for those with (82%) and without (60%) pre-existing antibody ($P > 0.2$) (57).

These findings indicate two forms of immunity for viral gastroenteritis may exist, one of short and the other of longer duration. Seemingly, factors other than serum antibody appear important in immunity to norovirus gastroenteritis. Recent evidence also suggests that susceptibility to infection may be genetically determined, with people of blood group O being at greatest risk for severe

infection (58). Noroviruses seemingly recognize histo-blood group antigens as receptors for infection. In a study that investigated the relationship between a person's ABO histo–blood group type and the risk of norovirus infection and symptomatic disease after clinical challenge, the authors found that individuals with an O phenotype were more likely to be infected with norovirus (OR = 11.8, 95% CI: 1.3–103), whereas persons with a B histo–blood group antigen had decreased risk of infection (OR = 0.096, 95% CI: 0.16–0.56) and symptomatic disease (OR = 0, 95% CI: 0–0.999) (58).

a. The Immune-Compromised and the Elderly

Norovirus can affect individuals of all ages in various settings, but are a predominantly important cause of acute gastroenteritis in elderly care settings and in hospitals (38, 40, 43, 45, 54, 59-74). Norovirus infections in immune-compromised populations, such as hospitalized patients, are more severe than seen in healthy individuals. A study of a norovirus outbreak in a university hospital in Germany showed that there were severe clinical features in patients with underlying diseases (54). In five outbreak wards, 84 patients and 60 nurses were affected with an attack rate of 32% observed in patients and 76% in nurses. The authors found that patients suffering from underlying illnesses such as cardiovascular disease, renal transplant, and immunosuppressive therapy were more likely to have severe complication associated with norovirus infection, odds ratio varying from OR = 17.1, 95% CI: 2.17-403; OR = 13.0, 95% CI: 1.63-281;

and OR = 5.7, 95% CI: 1.78-20.1 respectively. The underlying illnesses coupled with norovirus infection led to severe outcomes typified by decreased potassium level, increased levels of C-reactive protein and creatine phosphokinase in these patients (54). In this study, participants that were 65 years or older were 11.6 times more likely to have longer lasting diarrhea (>2 days) compared to participants that were less than 65 years old.

A study on the long term features of norovirus gastroenteritis in the elderly (73) showed that although acute symptoms typically abated after three to four days of illness, non-specific symptoms such as thirst, anorexia, lethargy, and vertigo could persevere for up to 19 days (73). The occurrence of these non-specific, but longer lasting symptoms, raises the concern of delayed recovery and potential falls due to vertigo in the elderly (73). In another study, the median duration of norovirus excretion was 8.6 days with a range from 2-15 days (57), which is slightly longer than the average seven days observed in a volunteer study (57), indicating that a long excretion time can occur in the elderly. Longer excretion time in the elderly should be acknowledged so that the appropriate cleaning and disinfection procedures are put in place to curtail further spread of norovirus in this susceptible population.

F. The Role of Fomites in the Spread of Norovirus

Fomites are defined as porous and non-porous surfaces or objects that can become contaminated by pathogens and serve as a vehicle in the transmission of pathogens (14, 75-77). The role of fomites in disease transmission was first recognized long before the identification of pathogenic organisms, when smallpox outbreaks were traced to imported cotton in 1908 (14). Fomites come to play a role in the transmission of pathogens when they become contaminated by aerosolized pathogens (e.g. droplets from vomitus or coughing), contact with soiled hands or objects, or direct contact with body secretions. Several epidemiology studies support laboratory research by indicating environmental contamination as a potential vehicle for virus transmission. An outbreak in a Honolulu nursing home indicated that influenza virus was spread by staff hands or fomites (e.g.. towels, medical cart items) (78). Epidemiological studies in daycare centers have detected rotavirus on various surfaces including toys, phones, toilet handles, sinks and water fountains (79). Also, the transmission of hepatitis A virus (HAV) by contaminated drinking glasses was associated with an outbreak of gastroenteritis in a public house when an ill bartender with HAV served drinks (80). In a study of Respiratory Syncitial Virus (RSV) infected infants, nursing volunteers who touched infected infants or the surrounding fomites developed RSV while nurses with no infant or fomite contact did not develop symptoms (17, 81). Barker et al showed that norovirus could be

transferred from contaminated fomites to clean hands and then the contaminated hands could transfer norovirus to another surface, such as a door handle, a tap handle, or a telephone receiver (82). They also found that the contaminated fingers could sequentially contaminate up to seven additional clean surfaces without the need to re-inoculate the hands (82). The ability to transfer microorganisms from fomites to hands and vice versa provides a vehicle for further spread of pathogens to previously uncontaminated surfaces; pathogens which, in turn, could be transferred to unsuspecting healthy individuals hence feeding a contamination cycle.

G. Survival of Norovirus

The ability of norovirus to be spread via fomites depends on its ability to survive in the environment. Several studies (Table 1) have shown that noroviruses can survive for long periods of time on environmental surfaces (16, 83, 84). In the absence of methods for in vitro cultivation of noroviruses, feline calicivirus (FCV) has been used as a surrogate for norovirus (16, 83, 85-89) in laboratory studies. Feline calicivirus is a respiratory virus (unlike norovirus which is an enteric virus) that is used as surrogate for human norovirus. FCV is used as surrogate because it has similar resistance to dehydration as human norovirus and is more stable at varying pH than enteric canine calicivirus (90).

Clay et al (83) studied the survival of FCV on uncommon surfaces such as computer mouses, keyboard keys, brass, telephone buttons, telephone receivers, and wires. They found that at room temperature, FCV survives on telephone receivers and buttons for up to three days (2-3 days). In another study, D'Souza et al (84) showed that FCV at room temperature could survive up to seven days on various surfaces, such as stainless steel, Formica, and ceramics. The survival of FCV seems to be temperature dependent; it survives for longer periods of time at room temperature (22±2℃) than at body temperature (37℃) or in cooler temperature (4℃) (16).

Table 1: Studies assaying survival of Feline calicivirus (F9), a surrogate for norovirus, on fomites

Author (Ref)	Fomite	Temp (°C)	Log_{10} reduction	Survival (hrs)	Survival (days)
Clay, S et al (83)	Computer keys	Room Temp		8-12	<1
	Computer mouse			24-48	1-2
	Brass			8-12	< 1
	Telephone buttons			48-72	2-3
	Telephone receiver			48-72	2-3
	Telephone wire			24-48	1-2
D'Souza, DH et al (84)	Stainless steel	Room Temp (22±2)	8.5	168	7
	Formica		7	168	7
	Ceramic		8	168	7
Doultree, JC et al (16)	Glass	4	4.75	1,344	56
		Room Temp (20)	9	504-672	21-28
		37	1	< 24	<1

Room Temp = Room temperature

H. Inactivation of Norovirus

The ability of norovirus to resist environmental stresses, coupled with its low infective dose, make this virus a great concern for public health professionals. In order to reduce the incidence and morbidity associated with norovirus related gastroenteritis, methods to reduce its occurrence in the environment, hence

decreasing its spread, are needed. Noroviruses are very resistant to environmental stress, and there are not many conventional cleaners that will effectively inactivate them (16, 90-95). Several disinfectants and procedures have been studied to assess their potential for norovirus inactivation. Inactivation of norovirus by ultra violet (UV) and gamma radiation, heat, chlorine, ethanol, quaternary ammonium compounds, and pressure have been studied (16, 90-95).

The Environmental Protection Agency (EPA), considers a test agent acceptable if a minimum of 4-$\log_{10}$ reduction in viral cytopatic effect (CPE) of FCV (complete inactivation of the virus occurs at all dilutions tested) observed in cell culture is demonstrated compared to plate recovery control (PRC) (96). This means that when added to a cell culture containing the microorganism of interest, the test agent should show a minimum of 4-$\log_{10}$ reduction in cell damage due to the microorganism when compared to a control cell culture that does not contain the test agent. When cytotoxicity (the quality of being toxic to living cells, usually assessed using molecular assays) of the cell culture used to assay the virus occurs, at least a 3-$\log_{10}$ reduction from the PRC must be demonstrated beyond the cytotoxic level with complete inactivation of the virus at all dilutions tested (96). Cytopathic effects are visible morphological changes observed in infected cells due to viral replication. These changes comprise altered cell shape, detachment from substrate, cell lysis, membrane fusion, altered membrane permeability, to inclusion bodies.

a. Quaternary Ammonium Compounds

Quaternary ammonium compounds (QACs) are amphiphilic surface active (surfactants) compounds used as disinfectants (97). QACs are both hydrophilic, or water-loving, and lipophilic, or oil-loving. This property of QACs makes them soluble in both water and oil-based mediums. The ability of quaternary ammonium compounds disinfectant cleaners (such as, R-82 and Pinoclean) to inactivate FCV have been studied (16, 93). Doultree et al (16) studied the inactivation potential of Pinoclean (1.0 w/w; Samuel Taylor, Ermington, NSW), a QAC based cleaner, on FCV in suspension. They found that even at double the manufacturer's recommendation, 1:10 concentration, Pinoclean failed to inactivate FCV.

In another study, a different QAC, R-82 (Lonza, Inc, Allendale, NJ) was tested for effectiveness against feline calicivirus a surrogate for norovirus. Petri dishes were inoculated with FCV stock, let dry and then treated with 2 mL of 1:256 diluted R-82 (93). The dishes were left for 10 minutes at room temperature (20±2℃) after which 2 mL of a neutralizing agent were applied. The mixture was collected and added to cultured monolayers of Crandell-Reese feline kidney cells to assess virus specific cytopathic effect (CPE). After 10 minutes contact time, the diluted R-82 formulation showed a 6.6 –6.4 $\log_{10}$ reduction in CPE of FCV.

The results obtained with the 1:256 dilution formulation of R-82 increase its choice as a norovirus disinfectant.

b. Chlorine

Chlorine-based products have long been regarded as the disinfectant of choice against norovirus. Several studies have looked at the inactivation potential of these products against FCV (16, 90, 91, 93, 95). Doultree et al (16) looked at two different types of chlorine-based disinfectants, Det-Sol 5,000 (Eucalip Bio Chemical Group Pty Ltd, East Melbourne, Victoria) and White King (Kiwi Brand Pty Ltd, Clayton South, Victoria), at concentrations varying from 100 to 5,000 ppm. No infectious virus was detected after exposure to 5,000 ppm of both disinfectants (5 $\log_{10}$ reduction). Hence, at a concentration of 5,000 ppm hypochlorite, FCV was inactivated. Duizer et al (90) showed that 3,000 ppm (or higher) concentration of sodium hypochlorite was needed to completely inactivate (> 5 $\log_{10}$) FCV at room temperature in suspension for 30 minutes. In another study (93) concentrations of 100 ± 10 and 1,000 ± 10 ppm sodium hypochlorite showed average 3.2 and 6.6 $\log_{10}$ reduction against FCV .

The pH and temperature can also affect virucidal (virus killing capability) activity of chlorine against FCV. With a concentration of 0.50 mg/liter free chlorine at pH 6 and 5°C for 15 seconds, a greater than 4 $\log_{10}$ reduction of FCV was observed;

while at pH 8 and 5°C, a 4 $\log_{10}$ reduction of FCV was observed between 15 seconds to 3.5 minutes (91). Thus, chlorine-based products at the appropriate concentration (≥ 5,000 ppm) could be used to effectively disinfect surfaces contaminated by norovirus.

c. Ethanol

Ethanol (ETOH) is a disinfectant commonly used in cleaning formulations and in laboratory settings, making it a good candidate in inactivation studies of FCV. Unfortunately, ETOH has so far proven to be an inadequate disinfectant against norovirus. ETOH at final concentration of 70% or 75% failed to completely inactivate FCV, with a maximum 2 $\log_{10}$ reduction after 8 minutes and 1.25 $\log_{10}$ reduction after 1 minute respectively (16, 90).

d. Ozone

The use of ozone to inactivate norovirus was studied by Hudson et al (94) on different surfaces in an office, a cruise liner cabin, and a hotel room. Fomites (plastic surfaces, fabric, carpet, cotton) were inoculated and the prototype ozone generator turned on, reaching levels of 20-25 ppm for 20 min, followed by a burst of water vapor for 5 minutes. On most surfaces, the concentration of FCV was

reduced by more than 3 $\log_{10}$, and in some cases, the inactivation was complete (> 4 $\log_{10}$ reduction in FCV titer).

e. Heat

Heat has been used over the years as one of the primary methods of inactivation of pathogens; for example, it is used to sterilize equipment in laboratory settings and in hospitals. In an inactivation study, FCV stocks in sealed tubes were heated at various temperatures (56ºC, 70ºC, boiled for 1 minute, 3 minutes, 5 minutes, or 1 hour) then inoculated in Crandell Feline Kidney (CRFK) cells and incubated at 37ºC for two to four days to CPE. Heating at 56ºC for 1 or 3 minutes had no effect on FCV, but after heating for 1 hour, no virus could be detected in cell culture (7.5 $\log_{10}$ reduction). Exposure of FCV to 70ºC for 1 or 3 minutes resulted in a partial reduction in virus (3 and 6.5 $\log_{10}$ respectively); no virus could be recovered after exposure to 70ºC for 5 minutes, and boiling for one minute completely inactivated FCV (16). Duizer et al (90) found comparable results with a 3 $\log_{10}$ reduction in FCV titer after heating for 71.3ºC for 1 minute.

I. Quantitative Microbial Risk Assessment

Risk is an inherent reality of everyday life. Because of this, it is important to account for it when making decisions that may have unwanted consequences. Quantifiable risk assessment was first developed primarily to assess health risks to humans associated with chemical exposures (98), but it has been expanded to include other health related risks. Risk assessment offers a framework for determining the relative urgency of problems and the proper distribution of resources to mitigate risks. The results of risk analyses can help us effectively target interventions to areas in which the greatest risk reduction can be achieved given the available resources (99). Despite this, we have to keep in mind that risk assessment is not an absolute, rather it is an evaluative, multifaceted, and comparative process (99).

Quantitative Microbial Risk Assessment (QMRA) is a method that allows the probability that an event such as an infection will occur to be estimated numerically. QMRA has been previously used to describe the dose-response relationship for infection with bacteria such as *Campylobacter jejuni, and Shigella* (100, 101).

a. The components of Risk Assessment

The elements of risk assessment as depicted in Figure 2 are as follows:

a. **Hazard identification** – This step in risk assessment defines the hazard and the nature of its harm, identifying a pathogen (such as norovirus) and documenting its effects on human health.

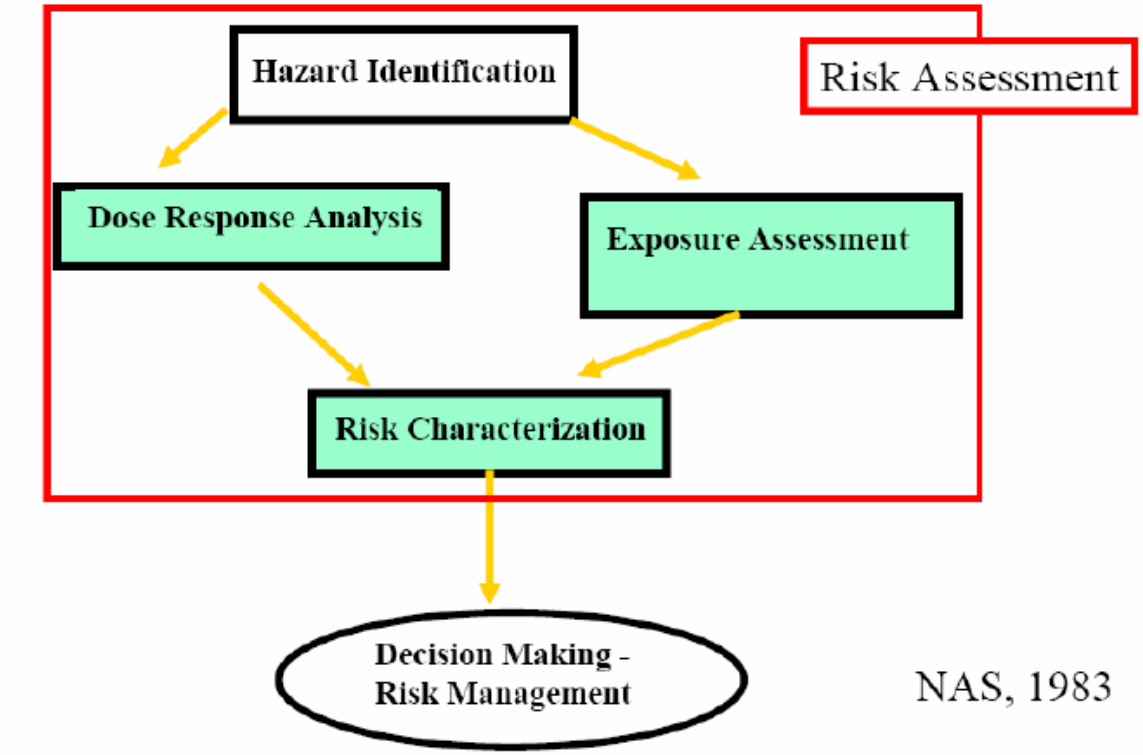
b. **Exposure Assessment** – This step is the process of measuring or estimating the intensity, duration and frequency of exposure to environmental agents such as viruses or bacteria. We can be exposed to contaminants via the fecal-oral route, by inhalation, or on the skin. In exposure assessment, the type, location, and activity patterns of the exposed population are as important as the exposure pathway of the contaminant. An exposure pathway is the course that the contaminant takes from its source to the receptors, in human or animals, via the environment.

c. **Dose-response Assessment** – This assessment step is used to characterize the relationship between various doses of exposure and the incidence of health effects (102). This assessment is usually displayed as a plot showing the response to increasing doses of an infectious agent. In QMRA, human dose-response studies are available for many microorganisms and can thus be used to estimate the effects of various levels of exposure to the microorganism. There has never been a study on the dose-response relationship of norovirus to infection.

d. **Risk Characterization** – This process estimates the potential adverse events given exposure and dose-response to an infectious agent.

The outcomes of risk characterization are then used in risk management, which include consideration of economic, political, social issues, and problems inherent to the proposed solution (99). Risk communication is a pivotal component of risk management as it is the interactive process of information and opinion exchange between various stakeholders and the lay public.

Figure 2: The risk analysis process (103)



A major advantage of QMRA is that it has the potential to be used as a surrogate for a full-scale epidemiological study. QMRA is cheaper and faster than a full-scale epidemiological study and eliminates the impact of confounding factors, i.e. multiple routes of exposure, as it uses models to predict probability of illness, which can be compared to actual attack rates. Despite this obvious quality of the QMRA method, only a few studies have used it to model predicted outcomes resulting from outbreaks. The few studies that validated the use of QMRA models used data from foodborne studies in which information on exposure and outcomes were available (100, 104-107).

Quantitative microbial risk assessment has some limitations and controversies. As noted by Cothern (108) “one of the main impediments to the development and use of quantitative risk assessment is the lack of complete information and data as input to this process” (p. 3). One of the more controversial areas of microbial modeling is the potential for a single microorganism to initiate infection. This “independent-action” theory may actually be flawed as the probability that one pathogen will evade all the body’s immune defense to actually cause illness is very small (109).

Dose-response is aimed at the mathematical characterization of the relationship between the dose administered and the probability of infection or disease in the

target population. Usually, pathogens are measured in doses that are routinely used to count the specific microbe in laboratory settings, such as plaque counts in cell culture for viruses, colony counts for bacteria, and direct microscopic count of cysts for protozoa. This means that for viruses and bacteria, viable but non-culturable organisms are not counted, while for protozoa, the results are particle counts (nonviable organisms viewed through the microscope could be counted in the dose) (99).

Dose-response data are an important component of risk analysis and these data are often obtained from human feeding studies. The volunteer studies use humans with normal functioning immune systems. Hence, one could argue that in real life, the immune status of the various individuals would influence the model and the models could actually be less conservative and underestimate the risks associated with vulnerable or sensitive populations (99). Despite the fact that there are several human data sets that can be used for modeling in a dose-response model, they are not exhaustive; for example, there is currently no available dose-response model for noroviruses. More human and animal studies will be needed to address more completely the hazard and dose-response parameters, including strain variation, virulence, immunity, and multiple exposures. During the risk characterization step of QMRA, assumptions made at each level influence the outcome and variability of the risk outcome. Despite

these limitations, QMRA has proven to be an invaluable tool in predicting health effects.

b. Historical Perspective of Risk Assessment

Microbial risk assessment is a relatively new field that has been in practice for about 38 years (110). Risk assessment is used when it was impossible to conduct epidemiological studies looking at very low levels of chemicals in the environment. Risk assessment can also be used to evaluate risk through a broad spectrum, from outbreaks to low level risk, and it can also allow us to look at risk over a given time period. The US Environmental Protection Agency (EPA) first used risk assessment based on dose-response models for the development of the Surface Water Treatment Rule (SWTR) for the parasite *Giardia lamblia* (111). Several microorganisms such as *Giardia lamblia, Cryptosporidium*, and *Shigella* were used in order to set what are now accepted standard procedures for quantitative microbial risk assessment (111-113).

About 18 years ago, QMRA evolved from risk assessment (110). QMRA evolved because the US Environmental Protection Agency's (EPA) was attempting to address the issue of setting standards and guidelines for treating drinking water in the US National Primary Drinking Water Regulations (NPDWRs), to reduce the incidence of *Giardia* and *Cryptosporidium* (114). Based on their risk analysis, the

EPA estimated that if a person was to consume two liters of water per day, his/her daily risk of *Giardia* infection would range from $10^{-3.5}$ to 10^{-5} if the *Giardia* cysts concentration in 100 L varied from 0.75 to 0.025 respectively (111). When the first regulations were set for the Safe Water Treatment Rule (SWTR), a risk of 1 *Giardia* infection per 10,000 was deemed acceptable (114) and unlikely to be associated with an illness outcome.

The Safe Water Drinking Act (SDWA), originally passed by Congress in 1974 to protect public health by regulating the nation's public drinking water supply, mandated the control of *Giardia lamblia* in water (113). *G. lamblia* is a parasite that is considered to be one of the most common causes of waterborne disease in human, and found in both drinking and recreational water in the US (115). The Surface Water Treatment Rule (SWTR), an amendment to the SDWA, was put in place in 1996 and aimed to strengthen protection against microbial contaminants such as *Giardia* and *Cryptosporidium*. The SWTR mandated that all surface waters be sufficiently treated to reduce the source water concentration of *Giardia* cysts by at least 99.9% (3 logs) (112). The new rules and regulations demanded that health departments take appropriate steps to protect the public against waterborne giardiasis. A risk assessment model, using data from published reports of outbreaks, was used to estimate the risk of infection after exposure to treated water of different levels of *Giardia* cysts (106). The final model assumed a 2L consumption of water daily and was based on human studies for *Giardia*. All

these outbreaks were associated with unfiltered chlorinated surface water and levels of *Giardia* cysts concentrations ranged from 0.6 to 21 per 100L. The duration of the outbreaks ranged from 30 days to 60 days. The observed attack rates for the outbreaks ranged from 0.5% to 16 % and the final exponential risk assessment model estimated infection rates after 30-60 days exposures ranging from 0.6 to 38% (106) which approximated the observed attack rates.

Shigella was another example of a microorganism studied using QMRA to assess potential health risk. According to the Centers for Disease Control and Prevention's 2005 annual report, there were a total of 10,484 laboratory confirmed cases of shigellosis in the US with a rate of 3.5 infections per 100,000 population (116). Shigellosis is a disease characterized by fever, stomach cramps, and often bloody diarrhea; it usually resolves after five to seven days (117). Although mortality due to shigellosis is now rare in the US, shigellosis due to *Shigella dysenteriae* type 1 has been linked to deathly epidemics in developing countries (118, 119). Crokett et al, compared the prevalence of shigellosis, due to *Shigella dysenteriae* and *Shigella flexneri*, in the US with dose-response information using quantitative microbial risk assesmment models (100). The beta-poisson model showed an overlap of the estimated 95% confidence interval with the observed *Shigella* data, suggesting that the model could be used to describe shigellosis in general (100). The Crockett et al study was conducted in 1996 when there were an estimated 300,000 annual cases of shigellosis in the

US. Assuming that the US population at that time was 250 million, that everyone had an equal risk of contracting shigellosis during the year, and that 30%-40% of illness was foodborne and waterborne, the average individual risk assessed by the final risk model would range from $9.9x\ 10^{-7}$ to $1.3x10^{-6}$ with an average exposure range of 0.07 to 0.1 *Shigella* for 1 day per year of exposure (100).

Another study that compared estimates based on risk assessment methodology and data from a prospective epidemiological study on waterborne infectious disease prevalence was conducted by Haas et al in 1993 (104). The authors estimated the daily and annual risk of waterborne viral infection with a Monte-Carlo analysis using data from a prospective epidemiological study. The risk assessment model estimated that a 0.000717 (95% CI: 0.0000317-0.00188) daily risk of illness (or an annual risk of 0.23) was associated with the ingestion of contaminated drinking water (2L/day). The results of this risk assessment coincided with the results of an epidemiological study conducted in suburban Montreal Canada from March 1988 to June 1989 by Payment et al, where the daily risk of infection was observed at 0.00082 or the annual risk of illness was 0.24 cases/person year (120). The predictions of the risk model in this study could be used to shape decision-making in drinking water standards.

Risk assessment has also been used as part of an approach to facilitate informed decision making and control food safety hazard (121). Although the

efficiency of handwashing has been studied, the risk associated with different handwashing techniques had not been studied. This gap in knowledge was explored in a study by Montville et al (107). Experimental and literature data were used to develop a quantitative risk assessment model to assess the potential risk related with different handwashing techniques. The risk was expressed as log colony forming units (CFU) on hands at the end of the handwashing process. When antimicrobial soap, no sanitizer, paper towel drying, no rings, and touch free faucet were used, experimental data showed a –1.75 to 9 final logs CFU count while the model, based on separate literature data collected from different studies under similar conditions, predicted a –0.5 to 8 final log CFU count on hands. The model also predicted that when hands were washed using chlorohexidine gluconate soap, in conjunction with alcohol-free sanitizer, no rings, and a conventional faucet, that the use of paper towels to dry the hands resulted in a better (decreased CFU) final log CFU (-4 to 9) versus using hot air dryer to dry the hands (-2 to 10) (107). Furthermore, the final log CFU count on hands for the experimental data overlapped with the distribution predicted by a model for handwashing under similar conditions that had been based on the literature (122-128). The high concordance between quantitative risk assessment models and prospective data analysis allowed increased confidence in the method and suggested that QMRA could be used to provide sound information for the management of contamination via hands during food preparation.

Another infectious disease that has been explored through QMRA is salmonellosis. Salmonellosis is an infection caused by the bacteria *Salmonella*. Salmonella is a group of bacteria that can cause diarrheal illness in humans; there are many serotypes of the bacterium but *Salmonella typhimurium* and *Salmonella enteritidis* are the most common in the US (129). *Salmonella* alone accounts for 27% of reported foodborne cases, 26% of hospitalizations, and 31% of food related deaths in the US (1). This disease is currently front page news as it has been associated with multi-state outbreaks potentially linked to consumption of tomatoes. According to the CDC, from April to July 2008, more than 800 people have been infected with *Salmonella* Saintpaul in 36 states and the District of Columbia (130).

Rose et al, developed a quantitative risk assessment model to evaluate the potential risk of disease from exposure to food contaminated with *Salmonella* and then compared the model to outbreak data (105). The authors estimated that exposure to 1 CFU of *Salmonella* would be associated with a risk of infection equal to $7.5x10^{-3}$ and a probability of mortality equal to $7.5x10^{-6}$ (105). The attack rates from various outbreaks were then compared to the probability of infection predicted by the risk assessment model as shown in Table 2. These final model predictions were similar to the observed attack rates. Quantitative risk assessment again could be useful to make informed decisions about risk management.

Table 2: Comparison of outbreak data to model predictions for assessment of risks associated with exposure to *Salmonella*

Food	Dose CFU	Attack rates (%) (131)	Predicted probability of infection (%)*
Water	17	12	12
Pancretin	200	100	77
Hamburger	60-230	?	36-82
Ice cream	102	52	54
Cheese	100-500	28-36	53-98
Chocolate	100-250	?	53-85
Cheese	10^5	100	>99.99
Ham	10^6	100	>99.99

*Per final risk model developped by Rose et al. Modified from Rose et al (105)

CHAPTER THREE - PRESENT STUDY

A. Methodology

The overall goal of this research is to understand the effects and impact of proper cleaning and disinfection on the spread of noroviruses via fomites in everyday venues. Data sets from three outbreaks were analyzed to determine if the number of cases of illness could be predicted by knowledge of the concentration of norovirus on fomites, to demonstrate the importance of fomite disinfection after an outbreak in preventing further spread of norovirus, and to assess the potential role of fomites in the spread of norovirus in the food service industry. Three manuscripts are attached in appendices that describe in more detail the methodology and results. The following section highlights the general methods used in the collection and analysis of the data.

a. Human Subjects Approval

The Institutional Review Board of The University of Arizona approved this dissertation work prior to its commencement (Appendix D). First, this researcher passed the Social and Behavioral Research Investigators and the Biomedical Research Investigators tests offered online by The University of Arizona as part

of the Human Subjects Protection Program (HSPP) through the Collaborative Institutional Training Initiative (CITI).

The candidate then submitted a project review application to The University of Arizona Institutional Review Board that contained the following components: 1) verification of human subjects training, 2) project summary, 3) summary of study population, 4) recruitment and consent procedures, 5) methodology and data collection procedures, 6) confidentiality of personal identifying information, and 7) benefits, costs, compensation, and risks. Many of the above sections were not applicable to the current study, since no participants were specifically recruited for this project. Rather, data were used from the pre-existing surveillance data from outbreak investigations. The University of Arizona HSPP granted exempt status for this study (see Human Subjects Approval Form in Appendix D).

All files were kept on computers and were password-protected, as specified under the approval. There were no risks to participants in this study as we are using anonymous surveillance data from outbreaks. Although these studies did not provide an immediate benefit to the participants, the results will, however, provide valuable information for planning future prevention programs and determining where and in what manner limited resources should be focused to reduce the spread of norovirus, a viral gastroenteritis which occurs worldwide and has no treatment. The findings of these studies could also help in

recommendations for proper cleaning of fomites, which in turn will reduce the incidence of acute gastroenteritis.

b. Laboratory Methods for Norovirus Detection

The same sampling and fomites analyses were used for all three outbreaks. Environmental samples were obtained by swabbing fomites. The swabbing method used was the same developed for and incorporated into previous studies by the laboratory (132). Briefly, fomites samples were obtained by swabbing 100 cm^2 of each individual surface with a sterile polyester fiber-tipped transport system collection swab moistened in transport medium (BBL Culture swabs, Becton, Dickinson and Company, Sparks, NJ). All samples were transported to the laboratory on ice and frozen at -80°C until assayed.

Viral RNA was purified from the samples using QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA). Reverse transcriptase polymerase chain reaction was performed on the purified viral RNA followed by semi-nested PCR. Ten microliters of PCR product was detected using agarose gel-eletrophoresis. The 2% agarose gel was stained by adding 10 microliters of molecular grade ethidium bromide solution 10 mg/ml (Promega, Madison, WI) to the liquid gel buffer mixture. An Alpha Imager 2000 Documentation and Analysis system (Alpha Innotech Corp.) was used to visualize and photograph the resulting RT-PCR

product bands. To genotype the norovirus positive samples, thirty microliters of positive sample was purified using a QIAquick PCR Purification Kit from Qiagen Inc. (Valencia, CA). Resulting samples were then sequenced for verification of positives. The sample sequencing was conducted at the University Of Arizona Genomic Analysis Technical Center using a 377 ABI sequencer from Applied Biosystems.

Determination of Norovirus Concentration on Fomites Samples

The concentration of norovirus (per cm^2) on fomites was estimated by the most probable number (MPN) method using dilutions series (133) after detection of norovirus on fomites using the nested-PCR method (described in the section on Laboratory methods for norovirus of this dissertation). The MPN general-purpose program used was adapted from the method of Hurley and Roscoe (134). Ten fold dilutions of nucleic acid extracts (10^1 to 10^5) were amplified in triplicate for each dilution. The most probable number of viral genomes, continuous variables, was assessed for each surface.

c. Data Collection

1. ***Study #1:*** *Assessment of Quantitative Microbial Risk Assessment to Predict Attack Rates during an Outbreak of Norovirus based upon Fomites Exposure*

Source and Study Population

Participants in three consecutive 5-night educational boating trips who became ill with symptoms of nausea and vomiting between May 9 and May 29, 2004 were included in this study. Of the total 54 boaters, the United States Public Health Service was able to contact twenty-seven (50%) for interview, and 20 of those interviewed (74%) fit the case definition of vomiting and/or diarrhea with onset between May 9 and May 30. The age of the trips' participants ranged from 50-85 years (mean age = 68.2 years). The boating trips took place on a large recreational lake in northern Arizona. Participants were all senior citizens taking part in educational trips organized in conjunction with a local community college.

- **Data Collection**

This project required the collection of two separate categories of data: data from the houseboat trips participants and environmental data. First, information was needed for each houseboat guest both with regard to the acute illnesses, potential exposures, medical visits and demographic and historical medical data.

Second, microbial data from environmental swabs were required. Data from trip participants was acquired via questionnaires; Table 3 lists the variables of interest obtained through the questionnaires.

Table 3: Questionnaire variables

Variables	Categories
Date of event	Month, Day, Year
Time of interview	AM, PM
Age	
Gender	Male, Female
Number of people in houseboat	
Date of trip	Month, Day, Year
Ill	Yes, No
Symptoms	
Nausea	Yes, No
Vomiting	Yes, No
Diarrhea	Yes, No
• Diarrhea episodes per day	
Date of illness onset	Month, Day, Year
Duration of illness	Yes, No
Physician visit	Yes, No
Exposure	Yes, No
Other ill people	Yes, No
Food preparation	Yes, No
Cleaning	Yes, No
Lake water	Yes, No
Other	Yes, No

Environmental Data

After the last trip, a total of twelve fomites samples were obtained from the houseboats involved in the outbreak (Table 4). The swabbing method used was as used in previous studies by the laboratory (132). All samples were transported to the laboratory on ice and frozen at -80℃ until assayed. Table 4 lists the actual surfaces swabbed.

Table 4: Fomites sampled in houseboats

Sample Location per Houseboat*
Houseboat # 2
Kitchen sink
Door handles
Toilet lid
Houseboat # 10
Door handles
Toilet lid
Kitchen sink & tap handle
Refrigerator door
Houseboat # 13
Bathroom toilet lid
Refrigerator door handle
Kitchen sink & tap handle
Door handles
Restroom lavatory door

*Area sampled (for each houseboat) = 100cm2

2. ***Study # 2****: Outbreak of Norovirus Illness in a College Summer Camp: Impact of Cleaning on Occurrence of Norovirus on Fomites*

Source and Study Population

The study population for this study included all persons attending a summer camp at a Northern Arizona summer camp from 7/18/2005 to 7/31/2005 inclusive. The outbreak affected a total of 115 individuals (103 cases); ages ranged from fourteen (14) to fifty-four (54) years old. The case definition for norovirus illness was as follows: acute onset of nausea, vomiting, and/or diarrhea (i.e., no prodromal period before gastrointestinal symptoms). The inclusion criteria into the study were:

1) Participation in any of four summer camps (A, B, C, D), or
2) Having contact with members of the camps within 48 hours before illness onset, or
3) Visiting one of a variety of locations at the summer camp within 48 hours before onset of illness.

On July 20, 2005, the local Health Department Communicable Disease program coordinator received a call from the infection control practitioner at Flagstaff Medical Center about three emergency department patients with similar symptoms: vomiting, diarrhea, and dehydration. The three patients (2 participants and a staff member) were members of Camp A from an area summer camp. Further investigation by the communicable disease staff identified forty other

Camp A participants with similar symptoms. On July 21, 2005, an outbreak investigation was initiated. An employee from the health department interviewed participants attending the summer camps on July 21. They were given questionnaires to determine the demographics and symptoms associated with the gastrointestinal illness. Figure 1, Appendix B shows a schematic of the study population for this analysis

- **Data Collection**

This study required the collection of two separate categories of data: data from the summer camps participants and environmental surfaces (fomites). We collected demographic, illness data from all summer camps participants and employees who complained of illness. Table 5 lists the questions of interest.

Table 5: Variables collected through self-administered questionnaires

Variables	Categories
Date of event	Month, Day, Year
Time of event	AM, PM
Date of Birth	Month, Day, Year
Date of illness onset	Month, Day, Year
Gender	Male, Female
Time of illness onset	AM, PM
Date of Illness recovery	Month, Day, Year
Time of illness recovery	AM, PM
Type of participant	Resident, Staff, Other
Nausea	Yes, No
Vomiting	Yes, No
• Vomiting episodes per day	1-2, 3-5, ≥6
Diarrhea	Yes, No
• Diarrhea episodes per day	1-2, 3-5, ≥6
• Bloody diarrhea	Yes, No
Fever	Yes, No
Sweats/Chills	Yes, No
Abdominal Pain or Cramps	Yes, No
Severe weakness	Yes, No
Headache	Yes, No
Backache	Yes, No
Muscle aches	Yes, No
Physician visit	Yes, No
Other	Yes, No
Environmental Data	

For this study, samples were collected at three different dates July 21 and 22, August 1, and August 15. These dates were associated with initiation of investigation and the two cleaning protocols that were utilized, one in July and the other in August, (Table 3, Appendix B).

Ten fomites (doorknobs and toilet seats) were sampled during July 21-22 before any cleaning took place by facilities management personnel. The residence halls that housed norovirus cases from Camp A, a multi-purpose athletic facility, and the student union were cleaned on July 30. The cleaning crew did not have specific cleaning instructions and used their common cleaning solutions composed of soap and water. After the cleaning, 51 fomites in residence halls and Camp A facilities were swabbed for norovirus again on August 1, the day after cleaning. The surfaces included toilet handles and seats, bathroom sink faucet handles, bathroom doorknobs, walls, mattresses, urinal handles, chairs, drinking fountains, and floors. The third and final fomite sample (10 samples) collection took place on August 15 after cleaning of fomites with a bleach (sodium hypochlorite) solution containing 5000 mg/L free chlorine in conjunction with detergent (soap and water), and separate disinfecting wipes (containing a quaternary ammonium disinfectant; Clorox Company, Oakland, CA). Table 3, Appendix B shows the timeline of the environmental investigation and disinfection for this study. Table 6 lists the fomites sampled.

Table 6: Fomites sampled in summer camps*

July 21/22	August 1	August 15
Doorknobs	Bathroom doorknobs	Lavatory handles
Toilet seats	Bathroom sink faucet handles	Toilet seats
	Chairs	Toilet handles
	Doorknobs	
	Drinking fountains	
	Floors	
	Ice chest lid	
	Ice machine lid	
	Mattresses	
	Toilet handles	
	Toilet seats	
	Urinal handles	
	Walls	
	Water jug	

* Once it was determined that fomites sampled on July 21 and 22 were norovirus positive, extensive sampling took place on August 1, and only those fomites that tested positive on August 1 were re-sampled on August15.

3. ***Study # 3***: *Norovirus Outbreak during a Christmas Banquet*

Source and Study Population

Persons included in this study had taken part in Christmas party at a local restaurant on December 1, 2006. On December 6, 2006, the local Health Department Communicable Disease program was notified of a cluster of illness associated with a Christmas party. This outbreak affected 38 people (30 cases) with age ranging from 5 to 64 years old (mean age = 43 years.) The case definition was as follows: acute onset of nausea, abdominal cramps within three days of having eaten at the party, vomiting, and/or diarrhea (i.e. no prodromal

period before gastrointestinal symptoms). On December 6, the outbreak investigation was initiated. Participants in the Christmas party were located and were given questionnaires to determine the demographics and symptoms associated with the gastrointestinal illness. Participants' consumption of food and various beverages during the party was obtained.

Data Collection

Questionnaires were used to collect data from the Christmas banquet participants. Attempts were made to interview all participants about illness status, symptoms, previous exposure to an ill individual, travel history, mode of transmission, and food history from November 28 to December 1, 2006. Some variables for personal demographic variables and disease experience were used as in study # 2 (Table 5). While we were able to obtain a food history for all participants, there were no leftovers of the food items available for norovirus testing. Table 7 lists the menu's food items available during the banquet.

Table 7: Menu of food items served at the Christmas banquet

Food Item	Eaten
Fresh vegetables	Yes, No
Fresh fruits	Yes, No
New York strip	Yes, No
Chicken piccata	Yes, No
Orange ruffe	Yes, No
Potatoes	Yes, No
Steamed vegetables	Yes, No
Roll & butter	Yes, No
Chocolate cake	Yes, No
Cheese cake	Yes, No
Pumpkin pie	Yes, No
Ranch dressing	Yes, No
Pesto dressing	Yes, No
Fresh greens	Yes, No

d. Data Management

Data from all three outbreaks were initially recorded on paper data collection forms and were subsequently transferred to an electronic format using an Excel database. Questionnaires for all three outbreaks were forwarded to the investigator who was responsible for data entry. The principal investigator was responsible for all quality control related to this study. Methods of quality control included printing periodic summaries of the data to compare the different versions, and checking every 10^{th} data collection form against the database for accuracy of data entry. There was less than one error per 10 values entered. Potential errors were double-checked against the original interviews and

questionnaires and were corrected where necessary. Missing data, which were assigned a "missing" value during data entry, were left as missing. Computers and files used for data collection were securely locked, password-protected and backed up on removable data storage media. Norton antivirus software was installed on the computer and updated weekly. All data utilized in this dissertation project were de-identified prior to being submitted to the investigator and responses could not be linked to a participant. The links to the participants were maintained by the environmental health manager who collected the original questionnaires during the outbreaks.

e. Data Analysis

For all three outbreaks included in this study, Completed questionnaires were entered into an Access file, and imported into Stata version 9.0 (StataCorp LP, College Station, TX, USA) (135). Descriptive statistics were calculated for participants' demographics and illness symptoms for each outbreak.

Attack rate was calculated as the number of interviewed participants who fit the case definition for norovirus illness during the trip period or meal divided by the number of interviewed participants who took part in the trips, attended camp, or attended the banquet.

The concentration of norovirus (per cm^2) on fomites was estimated by the most probable number (MPN) method using dilutions series (133). The MPN general-purpose program used was adapted from the method of Hurley and Roscoe (134). Ten fold dilutions of nucleic acid extracts (10^1 to 10^5) were amplified in triplicate for each dilution.

For the second outbreak of norovirus in a college summer camp, in order to determine whether or not the cleaning regiments were effective at properly cleaning norovirus contaminated fomites, the percentage of norovirus positive samples during the three sampling periods was calculated. The percent of rooms in which norovirus was detected on fomites was also calculated for each collection period (Figure 3, Appendix B). Table 4, Appendix A shows the detection of norovirus positive fomites after each cleaning/disinfection regiment. Percentage was calculated as the number of positive samples divided by the number of samples and multiplied by 100.

For the third outbreak of norovirus illness during a Christmas banquet at a restaurant, to identify menu food items that were associated with more illness, crude odds ratio and 95% confidence intervals were calculated.

1. Quantitative Microbial Risk Assessment of Risk Prediction

For Manuscript #1 and Aim 1 of the dissertation, two QMRA models to predict the risk of illness during the houseboat trips were ran using similar assumptions. The first model attempted was a beta-Poisson model using dose-response data from rotavirus due to the lack of dose-response data for norovirus. The second and final model used was a simple discrete time exposure model using variables gathered from literature reviews. The exponential model assumes constancy of the pathogen-host survival probability while the beta-Poisson dose-response model modifies the exponential model by allowing distribution of pathogen-host interaction probabilities (102).

We first used the beta-Poisson model based on rotavirus dose-response data because the infectious dose fifty (ID_{50}) of norovirus is believed to be around 10–100 virions (32, 136). Another reason we used the beta-Poisson model was because when Haas (137) examined mathematical models which could best estimate the probability of infection from existing databases associated with human feeding studies, he found that for viruses the beta-Poisson model best described the probability of infection. Another reason for using the beta-Poisson model was that is a dynamic exposure model. It provided a statistically significant improvement in fit over the exponential model given the fact that, in general, there is variability on infectivity and variability in the host susceptibility. With the beta-Poisson model, the probability of infection per exposure event is then

described as: $P_i = 1 - (1 + N/\beta)^{-\alpha}$, where N is the number of noroviruses ingested per exposure, and β and α are parameters that characterize the host-virus interaction (dose-response curve) (99). We calculated the cumulative probability of infection assuming a Poisson distribution of noroviruses on the fomites (assuming a daily exposure to a constant concentration of virus), as follows:

Probability of infection: $Pr(Inf) = 1-(1- P_i)^{(\text{number of occurrences})}$

The second model was the discrete time exposure model. This model , based on assumptions gathered from literature reviews, was preferred because when there is no dose-response data, one cannot fit most of the Beta-Poisson model's parameters and the exponential is simpler to use and only has one parameter. There is currently no available dose-response data on human norovirus. The only available information is that "as few as 10 to 100 virus particles have been reported to cause infection" (83); hence, the simplest model for QMRA with norovirus is an exponential single-hit model with parameter α, the risk of infection per virus ingested is:

$$Pr(Inf) = 1 - \exp(-\alpha \times Dose)$$

where Pr(Inf) is the probability of infection, Dose is the expected number of viruses ingested, and exp is the base of the natural logarithm (the number 2.718…). A continuous-time (dynamic) model for estimating the ingested dose of norovirus via the fomite transmission pathway is diagrammed in Figure1, Appendix A. The discrete-time model equation for the total dose of viable

norovirus virus delivered to the lips due to the initial contamination of a finger pad with N viable virus is as follows:

$$\text{Dose} = 0.34 \times \mathrm{N} \times \sum_{i=1}^{\infty} \left[(0.7)^{\text{nsurface}} \times \exp\left(-0.03 \times \mathrm{T}_{\text{lips}}\right) \right]^{i} \times (0.66)^{i-1}$$

The lack of data from norovirus studies led us to use estimates from other microorganisms to build our QMRA model. The assumptions, gathered from literature reviews of other microorganisms such as bacteriophages, rotaviruses, and rhinoviruses, for this dose equation are listed in Table 1, Appendix A. The value 0.34 is the finger-to-lips transfer efficiency per touch (138). The complementary value 0.66 is the fraction of virus remaining on a finger pad following a touch to the lips. The value 0.7 is the fraction of virus remaining on a finger pad following a touch to a room surface, given that the finger-to-room surface transfer efficiency per touch is 0.3 (139). The value 0.03 is the die-off rate of norovirus on the fingers (139). The term T_{lips} is the time between successive touches to the lips. For 6 finger touches to the lips per hour, T_{lips} = 10 minutes. The derivation of Equation 1 is provided in the Appendix of Manuscript 1 (Appendix A).

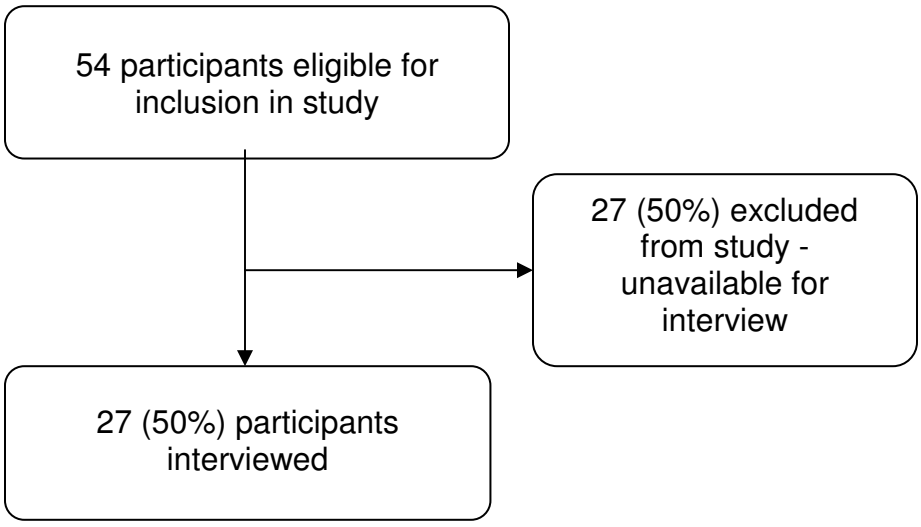
CHAPTER FOUR - RESULTS

This chapter summarizes the results of the outbreaks analyses associated with the three manuscripts.

1. ***Study #1****: Assessment of Quantitative Microbial Risk Assessment to Predict Attack Rates during an Outbreak of Norovirus Based Upon Fomites Exposure*

This section presents the results of analyses conducted for the first study. Detailed results and discussion of the analysis are found in Appendix A. Overall, during this outbreak there were 54 potential participants on the three boat trips, we were only able to reach 27 (50% response rate) for interview, and a total of 20 participants (74% attack rate) fit the case definition for noroviruses gastroenteritis. Figure 3 below shows a schematic of the study population for this analysis. Of the 12 fomites samples collected, 7 (58%) tested positive for noroviruses. The viruses that were typed were all identified as belonging to genogroup GII (Table 3, Appendix A), and the number of viral genomes found on fomites ranged from 1.029 to 53,725 with a mean of 16,239. .

Figure 3: Flow chart of study participation



A Quantitative Microbial Risk Assessment model was conducted to estimate risk prediction. In order to conduct theses analyses, we assumed that the seven sampled norovirus concentration values constituted a discrete probability distribution of all the viable norovirus concentrations that were present on all contaminated houseboat surfaces.

Analyses using the beta-Poisson model resulted in a daily probability of infection from noroviruses ranging from 49% to 65%, while the overall probability of infection during the entire trip (5 days) was 1 for all the concentrations of noroviruses detected on the fomites. Although the true distribution of concentrations was surely not limited to the available set of values, using the final discrete time exponential model avoids making assumptions about the

parametric form of the distribution, does not extrapolate concentrations beyond the observed range, and maintains the observed mean sample concentration of 16,239 viruses per 100 cm^2.

In addition, the final dose (and risk) calculations assumed that: (i) a person touches 4 cm^2 of contaminated surface (approximately twice the surface area of a finger pad) per contamination episode; (ii) 30% of the virus in that touched area is transferred to the finger pad (139); and (iii) the finger-to-clean room surface touch rate is one per minute, such that only 0.72% of the viable virus transferred to the finger pad is eventually ingested (per Table 2, Appendix A). For example, if the touched contaminated surface had 16,239 viable noroviruses per 100 cm^2 (the expected value of the discrete probability distribution of concentrations based on the sampling), that single contamination episode is expected to result in ingesting two viable noroviruses.

Given that 7 of the 12 commonly touched fomites (primarily door handles) were found to be contaminated with norovirus, and that deposition of viable norovirus was likely ongoing due to developing illness among different houseboat occupants. It is reasonable to expect that the number of contamination events over four days was at the high end of those displayed in Table 4, Appendix A. Given 32 or more contamination events in four days (one event or more every

two waking hours), the cumulative probability of infection is estimated to be at least 0.89 (89%).

In conclusion, socializing and sharing meals amongst houseboat trip participants, combined with improper cleaning lead to the spread of noroviruses. The quantitative microbial risk assessment model estimated an 89% risk of illness for the duration of the houseboat trip for each participant, and the actual observed attack rate was 74%. Given the fact that 32% of people infected with norovirus are asymptomatic (57), our model's estimate was a fairly good approximation of the actual attack rate.

2. ***Study # 2****: Outbreak of Norovirus Illness in a College Summer Camp: Impact of Cleaning on Occurrence of Norovirus on Fomites*

Detailed results and discussion of the analysis are found in Appendix B. The summer camps at the highest risk of infection included Camp A that began on July 17 and other camps that shared a residence hall or dining facility with the wrestlers. The attack rate among participants of Camp A was higher at 30.3% than for all other affected summer camps (Table 2, Appendix B). The high number and rate of acute gastroenteritis in Camp A was used to verify the existence of an outbreak.

From July 18-July 28, 115 individuals became ill at the summer camps; 61 (53%) of whom were participants or staff of Camp A, a wrestling camp, that began on July 17. There were initially 1,110 eligible participants from the pool of staff and students in the summer camps; of those, 995 (89.6%) were excluded because they did not present with any of the norovirus illness symptoms. We identified 115 cases (10.4%) that potentially fit the case description for norovirus illness; of those 11 were unavailable for interview. We interviewed 104 (9.4%) participants for inclusion in the study. Figure 1, Appendix B shows a schematic of the study population's selection.

Figure 2, Appendix B shows the epidemiological curve starting on July 18 and ending on July 29. No cases were reported to have onset dates before July 18 or after July 28. The single case on July 18 was the probable index case. The outbreak peaked on July 20 with 50 cases and subsided after that date. Out of the 103 cases for which gender information was available, 24 were women (23%) and 79 were men (77%). Age ranged from 14 to 54 years old. Out of the 103 people for which symptom information was available, the symptoms included nausea in 20 (19.4%), vomiting in 83 (80.6%) and diarrhea in 91 (88.3%). Nausea, vomiting, and diarrhea were reported by 14 (13.6%) of the individuals (Table 1, Appendix B). Four individuals required emergency care for severe dehydration, one of whom was hospitalized for two days. No fatalities occurred during this outbreak.

Fomites samples were collected on July 21 and 22 and noroviruses were detected on 17% of these samples. More extensive sampling took place on August 1 after cleaning with rags, soap and water, of which 11 (22%) were positive for norovirus. Forty five percent (45%) of the fomite samples of August 1 from toilet seats and toilet handles in the wing of the residence hall that housed ill individuals from Camp A were positive for norovirus. Surfaces that were supposed to be cleaned and disinfected still tested positive for norovirus. Table 3 in Appendix B summarizes the timeline for environmental investigation and disinfection during the outbreak. The second cleaning and disinfection occurred on August 14 before re-sampling of the same fomite locations on which norovirus was detected on August 15. After this round of cleaning and disinfecting, the percentage of dorm rooms testing positive was reduced to less than 35% (Figure 3, Appendix B).

In conclusion, the low observed attack rate during this outbreak may have been due to the wide distribution of campers and staff across a large campus, and the fact that interviewers themselves got ill and could not reach all camp participants before they departed from the summer camp. Improper cleaning and disinfecting regimen was most likely the culprit in the spread of norovirus to fomites. Proper cleaning and disinfection effectively reduced the number of norovirus positive fomites in the summer camp.

3. ***Study # 3****: Norovirus Outbreak during a Christmas Banquet*

Detailed results and discussion of the analysis are found in Appendix C. From December 1 to December 4, gastrointestinal illnesses affected a total of 30 individuals (75% infection rate). There were 40 participants at the banquet; 38 individuals were interviewed for a response rate of 95%. Figure 1, Appendix C shows the flow chart of study participation. The median age of the outbreak participants was 51 years (range 13-64 years) and 55% were male. Out of the 30 cases reported, 10 (33%) were women and 20 (67%) were men. The median time from the Christmas dinner to onset of symptoms was 33 hours (range: 8-55.5 hours). Most cases reported diarrhea (80%), vomiting (73%), nausea (67%), and abdominal pains (63%) (Table 1, Appendix C). Of the 30 cases, 23 (77%) had recovered at the time of completing the questionnaire. Although one case required medical attention, no fatalities occurred during the outbreak.

Analysis of food consumption records implicated two food items, the chicken piccata and the mashed potatoes, as possible causes of the outbreak. Univariate analysis of all the surveys showed no statistically significant (*P*-value < 0.05) association of illness with the consumption of food items, including the chicken piccata and the mashed potatoes (Table 2, Appendix C). However, the magnitude of the effect for chicken piccata was large, (OR = 4.0, *P*-value = 0.09, 95% CI: 0.55-27.89). People who attended the Christmas banquet at the restaurant and suffered from norovirus illness were 4.0 times more likely to have

eaten chicken piccata than those attendees who did not eat the chicken piccata. The chicken piccata and the mashed potatoes are food items not usually associated with norovirus illnesses as they are both cooked, kept and served warm. This suggests a fomite driven infection route for this norovirus outbreak. The wide 95% confidence interval observed with the chicken piccata might be due to a small sample size, hence a type I error. The sample size available in this outbreak was only 38 participants; in order to have and odds ratio of 4.0 that would be statistically significant, with an 80% minimum power, one would need to have had at least 74 participants.

Fomite samples were collected on December 20, and noroviruses were detected on 12% (n = 4) of the samples. The surfaces that tested positive included the public banquet room door handles, the ice machine handles in the kitchen, the main walk-in cooler door handle, and the men's restroom lavatory handles. We did not conduct genotyping for norovirus positive fomite samples. Three out of the four (75%) stool samples of ill persons were positive for norovirus. All three cases whose samples were sequenced were infected with the same genogroup I (GI.4) strain of norovirus. Table 3, Appendix A (Study #1) shows the estimated amount of viral genomes per fomites.

In conclusion, this outbreak of norovirus illness during a Christmas banquet at a local restaurant may have been fomite driven. The fomites that tested positive were the banquet public doors, the kitchen ice machine handles, the main kitchen walk in handle, and the employee men's restroom lavatory handles. The food item that seemed associated with the outbreak was the chicken piccata (OR = 4.0, *P*-value = 0.09, 95% CI: 0.55-27.89) although this was not statistically significant.

CHAPTER FIVE - OVERALL DISCUSSION AND CONCLUSIONS

This chapter discusses the results from the three specific outbreaks investigations and how they apply to the research questions. The studies' limitations and significance are also presented, and areas for future research are suggested.

A. Re-statement of Study Aims

The overall goal of this research was to understand the effects and impact of proper cleaning and disinfection on the spread of noroviruses via fomites. The present research consisted of three inter-related studies. The aim of the first study was to assess the comparability between a quantitative microbial risk assessment model to predict the probability of illness from norovirus due to environmental contamination on houseboats and actual observed attack rates (Manuscript #1: A norovirus outbreak on houseboats). The second study aimed to determine the impact of improper cleaning on the prevalence of fomites in an outbreak setting. An evaluation of an outbreak of norovirus in a college summer camp provided the opportunity to identify the source of infection, the causes of disease spread, to underline the importance of environmental clean up and recommend strategies for prevention of future outbreaks in such settings (Manuscript #2: Norovirus illness in a college summer camp). Our third and final

study aimed to evaluate the role of fomites and human behaviors in the spread of the disease outbreak (Manuscript #3: Norovirus outbreak during a Christmas banquet).

The results of these analyses support the role of fomites in the transmission of disease. Fomites were implicated in the outbreaks of norovirus illness in all these settings, on houseboats, the restaurant hosting the Christmas banquet, and in the college summer camp. These findings were consistent with previous epidemiological studies that show that outbreaks of norovirus illness usually occur in settings where a large number of people tend to congregate (38, 44, 140, 141). The attack rates, ranging from 30% to 75%, and the type of affected individuals observed in the three studies that make up this project were also consistent with previous findings. Norovirus is known to affect all age groups with a higher incidence observed in the elderly and immune compromised (70, 73, 142, 143) such as the individuals who were affected by the outbreak of norovirus during the houseboats trips.

In our quantitative microbial risk assessment model, the epidemiological data were consistent with the estimate that 100% of the houseboat occupants were infected, which is an attack proportion higher than usually observed in epidemiological studies (144-146). Given moderate assumptions for an estimate of norovirus infectivity (an infectious dose 50% corresponding to about 30 virus),

the final exposure or risk model estimated a high proportion of infections (at least 89%). Although the model indicated that only a small fraction of virus contaminating a finger pad would be ingested, the substantial norovirus concentrations found on contaminated surfaces, combined with a plausibly frequent number of contacts with those surfaces produced a high likelihood of infection. The attack rate predicted by our quantitative microbial risk assessment model (89%) was higher than the actual observed attack rate (74%). However, given the age range (50-85 years, mean age = 68.2 years) of those who took part in the houseboat trips (these were mainly elderly who are more susceptible to norovirus illness) the low infectious dose of norovirus (10-100 virions) and the amount of virions on fomites (median = 5,392 genomes per 100 cm^2 of fomite), we would expect to have all the participants get sick. When we take into account the fact that 32% of people suffering from norovirus illness were asymptomatic (57), our predicted attack rate matched the observed attack rate.

The outbreak of norovirus illness in a summer camp was associated with lower attack rates (2-30%) than the other two outbreaks (50-85%); this may have been due to the wide distribution of campers and staff across a large campus. The low attack rate observed might also have been due to the fact that during the outbreak investigation, two health department interviewers got infected with norovirus and could not perform their duty. The interviewers had to take some days off and by the time they came back to complete the interviews, some of the summer camps'

participants had already left, hence the low number of available participants (89.6% of the summer camp participants could not be reached). Hygiene and disinfection intervention studies have demonstrated two concepts which support transmission of viral illness via fomites. One is the cleaning of hands decreases viral illness (147-149). The second is disinfection of fomites decreases surface contamination, and interrupts disease spread (noroviruses, coronaviruses, and rotavirus) (150, 151). As expected, because noroviruses are resistant to environmental stressor, cleaning of contaminated fomites with the usual methods; soap and water was conducive to the contamination of other fomites. Improper cleaning procedures lead to an increase in the number of contaminated fomites. The percentage of norovirus contaminated fomites increased from 40% to 73%, but decreased to 33% after proper cleaning with 5000 mg/L free chlorine (Figure 3, Appendix B). This improper cleaning procedure after the start of the outbreak lead to an increase in cases; from three ill subjects to more than 38 new cases in the span of 24 hour as depicted by the epidemic curve (Figure 2, Appendix B).

B. Limitations

A major limitation of this project is the small sample size available due to the use of outbreak data. This is a major issue with outbreak data because one cannot control the sample size. The small sample size (N = 38, power = 30%) in the Christmas banquet outbreak was likely responsible for the lack of statistical

significance of the chicken piccata as a source of the outbreak. In all the outbreaks, we were not able to obtain food items to test for norovirus. This would have been important because not finding norovirus in the food items would have conclusively eliminated food as a source of the outbreaks; and we would be certain that all the outbreaks were not foodborne but rather fomites initiated.

Because the health department is always notified after an outbreak has occurred, as was the case in these three outbreaks, localizing people who attended the different events is not always feasible. Also, the retrospective time line, adds an element of recall bias. Those who experience illness are probably more likely to remember their exposures compared to those who did not get ill. This might be misleading in the case of norovirus illness as 30% of those infected are usually asymptomatic.

Another weakness of the current study is reliance on self-reported disease status, rather than confirmed physician diagnoses. Epidemiologic research often relies on self-administered questionnaires to determine health status, and accurate self-reporting of disease and exposures is critical for obtaining valid estimates of disease incidence. Stool samples were not obtained for all those who presented with norovirus illness symptoms. The fact that people were not willing to submit stool samples, the self limiting nature of norovirus illness was

most likely a factor in the low attack rates (3-30%) observed in the college summer camp outbreak.

Quantitative microbial risk assessment is not an exact science and is based on assumptions due to a lack of data. Several important sources of uncertainty in the risk estimates will be described. First, the virus transfer efficiency between finger pad and fomite was assumed to be 30%. This value was based on prior, published work with adenovirus, and may not hold for norovirus (139). In addition, transfer efficiency likely varies with the nature of the touched surface and the degree of pressure applied so, the true transfer efficiency of norovirus could either be greater or lower than the assumed value. Second, we assumed that all the viruses quantified via RT-PCR were viable (infectious). The die-off rate on inanimate nonporous surfaces of feline calicivirus (a norovirus surrogate) is in the approximate range of 0.5% to 2% per minute (83), which corresponds to a half life of approximately 30 to 140 minutes. Thus, it is certain that not all sampled norovirus was viable. On the other hand, sampling was done after houseboat surfaces had been cleaned, in which case the sampling underestimated (likely substantially) the concentrations of norovirus present prior to cleaning. There is no way to precisely reconstruct the concentrations of viable norovirus during the periods of occupancy. Given the high concentrations found via RT-PCR after cleaning, and given presumed ongoing deposition of norovirus

due to developing illness among different houseboat occupants, viable norovirus concentrations on fomites were likely high.

Third, we assumed an exponential dose-response function with an estimated infectivity parameter $\alpha = 0.022$. The true nature of the dose-response function may be different, and the α value was estimated using the information that "as few as 10 to 100 virus particles have been reported to cause infection" (83). The latter statement does not specify the *proportion* of individuals infected by any given dose and permits different interpretations. For example, if infection occurs among 90% of individuals each ingesting 10 norovirus, then $\alpha = 0.23$; in contrast, if infection occurs among 10% of individuals each ingesting 100 norovirus, then $\alpha = 0.0011$. There is a 210-fold difference in the infectivity parameter between these interpretations. In turn, the virus dose required to impart a high probability of infection (say, 99%) is 210-fold different; for $\alpha = 0.23$, a dose of 20 viruses is required, while for $\alpha = 0.0011$, a dose of 4,200 virus is required. Clearly, the α value is an important source of uncertainty in our modeling. However, in the context of the heavily contaminated houseboat surfaces, even the moderate estimate of $\alpha = 0.022$ leads to a significant risk. For example, an ingested dose of 3 viruses following one contamination event imparts a 7% probability of infection.

C. Conclusion and Future Work

In summary, this study demonstrated the important role that fomites play in the transmission of norovirus in different settings. The study also underscores the importance of proper cleaning and disinfection procedures in norovirus outbreak, due to the virus resistance to environmental stressors. In the outbreak of norovirus during a Christmas banquet, we can not pinpoint the specific source of infection. However, the investigation seems to point to an asymptomatic food handler; this calls to attention the need to emphasize proper hygiene in the food industry.

The values predicted by the quantitative microbial risk assessment epidemiological model are consistent with the observed epidemiological data; such information could be used to establish goals for the reduction of pathogens on fomites to reduce the probability of infection in outbreaks. QMRA could also be used as a surrogate for a full scale epidemiological study, which would reduce the cost associated with outbreaks investigation.

In conjunction with epidemiology and other data sources, risk assessment can be a very powerful tool. As well as being used in partnership with epidemiology it can also provide useful insights into areas such as rare events and severe disease outcomes where epidemiology is not appropriate. The ease with which

parameters can be changed within a risk assessment model makes it ideal to inform both international guidelines and standards. It can also be used to test ‘what if’ scenarios, which may help target management interventions.

Future research should focus on finding environmental cleaning and disinfecting solutions effective against norovirus that are widely available to the public. Education of the public about norovirus illness should also be emphasized as many people are unaware of the morbidity associated with norovirus illness. Human feeding studies should also be undertaken in order to construct a dose-response curve for norovirus, which could then be used in quantitative microbial risk assessment models; this would allow for a better estimate of risk.

APPENDIX A: MANUSCRIPT #1

Assessment of Quantitative Microbial Risk Assessment to Predict Attack Rates during an Outbreak of Norovirus based upon Fomites Exposure

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Abstract

Fomites are believed to play a significant role in the transmission of many viral infections, but information on pathogen occurrence and concentration has seldom been documented. The goal of this study was to determine the concentration of norovirus on fomites on shared houseboats and compare probability of illness predicted by a quantitative microbial risk model with epidemiological data. Norovirus genomes were detected on eight of eleven fomites tested with a concentration ranging from 256 to 60,664 per 100 square cm with a mean of 10,678. A risk model was developed in which fomite contact frequency, efficacy of transfer of the virus from the fomites to the hands, and then to the mouth were used to predict the risk of infection. The model predicted a probability of illness of 100% in susceptible individuals. The actual attack rate based upon the epidemiological investigation of the houseboats ranged from 50-85%; thus the model predicted value is higher than the range of observed attack rates. This may be due to the advanced age of infected individuals, and the low infectious dose of norovirus (10-100 virions.) Such an approach can be used to determine the level of fomites decontamination that may be needed to minimize the risk of norovirus infection from fomite exposure.

Keywords: Fomites, Norovirus, Outbreak, Quantitative microbial risk assessment

I. Introduction

Gastroenteritis due to human noroviruses is common worldwide in both developed and less developed countries. It is estimated that of all foodborne illnesses in the United States, Norwalk-like viruses account for over 67% of all cases (23 million), 33% of hospitalizations, and 7% of deaths (1).

Noroviruses, formerly known as Norwalk-like viruses or small round structured viruses, are members of the Norovirus genus in the Caliciviridae family. They are non-enveloped, positive sense, icosahedral, single-stranded RNA viruses that cause gastroenteritis. Human noroviruses are genetically diverse and belong to one of three genogroups (GI, II, or IV); each of which is further divided into more than 25 genetic clusters (2, 3). Outbreaks of norovirus gastroenteritis occur in multiple venues such as schools, daycare centers, nursing homes, hospitals, and cruise ships (4-14). Noroviruses are transmitted via the fecal-oral route.

The average incubation period for norovirus caused gastroenteritis is 12-48 hours with symptoms that usually resolve in 12-72 hours. Illness is characterized by acute-onset projectile vomiting, watery non bloody diarrhea with abdominal cramps, low grade fever, headache, and malaise (2, 3, 15). The concentration of norovirus found in feces is quite high: 8.4 $x10^5$/gram for genogroup 1 (16), and 3 x 10^8/gram to $10^{8.22} – 10^{10.24}$/gram for genogroup 2 (16, 17).

Quantitative Microbial Risk Assessment (QMRA) is a method that allows quantitative estimate of the probability that an event, such as an infection will occur. QMRA has been previously used to describe dose-response relationship for infection with bacteria such as *Campylobacter jejuni, and Shigella* (18, 19). It has not been used to compare theoretical infection probability to actual outbreak data involving fomites nor norovirus. We investigated an outbreak of gastrointestinal illness that occurred among participants in three consecutive 5-night educational boating trips. Our specific aim was to assess the comparability between a quantitative risk assessment model to predict the probability of illness from norovirus due to environmental contamination on houseboats and actual observed attack rates.

Methods

Description of Outbreak

Participants in three consecutive 5-night educational boating trips became ill with symptoms of nausea and vomiting between May 9 and May 29, 2004. Of the 54 total trip participants, the United States Public Health Service was able to contact 27 (50%) for interview, and 20 of those interviewed (74%) fit the case definition of vomiting and/or diarrhea with onset between May 9 and May 30. The age of the trips' participants ranged from 50-85 years (mean age = 68.2 years.) The boating

trips took place on a large recreational lake in northern Arizona. Participants were all senior citizens taking part in educational trips organized in conjunction with a local community college. The college rented four 52-foot houseboats from a local vendor. During the first trip, one boat was exchanged with the vendor due to mechanical problems. The new boat was then also exchanged, again due to mechanical problems. In total, six boats were used during the first trip, however the four boats in use by the end of the first trip were the four boats used for the following two trips. The first trip began on May 9. The subsequent investigation revealed that one of the participants on this trip arrived displaying symptoms of gastroenteritis, and this participant appeared to have been the index case for the outbreak. Two days after the start of the first trip, other participants on the trip began to become ill. The next trip began on May 16, and the same four boats were used.

Participants on this trip began to show symptoms of gastroenteritis one day later on May 17. The final trip began on May 23, again the same four boats were used, and participants on that trip began to show symptoms of gastroenteritis on May 24. Among those interviewed the illness attack rate by trip ranged from 50% to 86%, with an overall attack rate of 74%. If those interviewed are representative of the group that participated in the trips, up to 40 illnesses may have resulted from this outbreak.

Environmental Investigation

The source of drinking water for trip participants was large onboard tanks that had been filled with tap water prior to departure. The houseboats were equipped with onboard kitchen and bathroom facilities. Participants casually cleaned the boats during the trips (i.e. rags were used to wipe counter tops and surfaces), but surfaces were not disinfected. Between trips, and after the first outbreak of illness, instructors from the community college wiped down surfaces such as vinyl tablecloths, doorknobs, vinyl mattresses, food containers, and other hard surfaces with a diluted bleach solution of unknown concentration. The boats were returned to the vendor for refill of the potable water tanks and emptying of the waste holding tanks between each trip. While there is not a record of which passengers were on which boats, the groups frequently interacted and shared meals. The trip participants prepared their own meals; lunch and dinner were often communal with all of the boats participating in food preparation. This high level of interaction between participants on different boats, especially food preparation and sharing, could explain transfer of the virus from boat to boat. Additional details of the epidemiological investigation are contained in a previously published paper (20)

Virus concentration on fomites

The fomites were sampled after the last trip. Virus detection on fomites has been previously described by Boone (21). For this outbreak, fomites samples were obtained by swabbing each individual surface with a sterile polyester fiber-tipped transport system collection swab moistened in transport medium (BBL Culture swabs, Becton, Dickinson and Company, Sparks, NJ). All samples were transported to the laboratory on ice and frozen at -80℃ until assayed. Samples were homogenized using a vortex mixer followed by viral RNA extraction.

RNA Extraction

Viral RNA was purified from the samples using QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA), and the Mini Spin Protocol (22) was followed with the following modifications: the total sample volume was doubled to 280µl, and a double elution using two consecutive 40µl volumes of Buffer AVE was performed. The purified RNA samples were stored at -20℃.

The efficiency of the QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA) extraction was evaluated using a known initial concentration of poliovirus. Known concentrations of poliovirus were heat shocked and detected by reverse transcription polymerase chain reaction (RT-PCR) in serial dilutions (10^{-1} to 10^{6}).

The same concentration and volume of poliovirus was then RNA extracted and detected by RT-PCR. The concentration of norovirus particles in each sample was then determined using the MPN method (23).

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

RT-PCR was performed on the purified viral RNA using Qiagen OneStep RT-PCR kit (Qiagen Inc, Valencia, CA). The primers MJV12 (5'-TAY CAY TAT GAT GCH GAY TA-3') and RegA (5'-CTC RTC ATC ICC ATA RAA IGA-3') (24), (modified JV12/JV13 primers) (24), are specific for human norovirus genogroup GI and GII originating from region A of the NV capsid gene. All reagents used for the reverse transcriptase and the amplification steps were obtained from Applied Biosystems (Roche Molecular Systems Inc. Branchburg, NJ). PCR was performed using 22.25 µL of Rnase free water (Promega Madison, WI), 5.0 µL of 25 mM $MgCl_2$ solution, 0.25 µL of 5unit/mL Amplitaq gold, 5 µL of GeneAmp 10 × PCR buffer, 4.0 µl of 2.5 mM of dNTP mix, 1.0 µL of 50 µM (upstream and downstream) primers (Reg A and MJV12), 0.5 µL of 50 nM random hexamers, 0.5 µL of 20 units/µL RNAse inhibitor, and 0.5 µL of 50 units/ µL RT per sample. A 10µl volume of purified RNA template was used in a total reaction volume of 50µl. The reverse transcriptase reaction mixture was placed in an Applied Biosystems Gene Amp PCR System 9700 thermacycler (Roche Molecular Systems Inc. Branchburg, NJ). Thermal cycling conditions were as follows:

reverse transcription of viral RNA for 60 minutes at 42˚C; activation of Taq polymerase for 15 minutes at 95˚C; 40 cycles: 30 seconds at 94˚C, 30 seconds at 50˚C, 30 seconds at 72˚C; and final extension for 10 minutes at 72˚C.

Semi-nested Polymerase Chain Reaction

Semi-nested PCR was performed using: 5.0 µL of 10 x PCR gold buffer, 5.0 µL of 2.5mM MgCl, 4.0 µL of 2.4 mM dNTP, 1.0 µL of 50mM of internal primer MP 290 and primer Reg A, 0.25 µL of 5 units/ml of Amplitaq Gold, and 2µl volume of PCR product from RT-PCR was used in a total reaction volume of 50µl. The reaction mixture was placed in an Applied Biosystems Gene Amp PCR System 9700 thermacycler (Roche Molecular Systems Inc. Branchburg, NJ). Thermal cycling conditions were as follows: 5 minutes at 95°C, 40 cycles of: 30 seconds at 94°C, 30 seconds at 49°C, 30 seconds at 72°C, and a final elongation step for 10 minutes at 72°C.

Rnase-free water negative controls and known positive norovirus controls were done concurrently with the unknown samples. The semi-nested PCR product was visualized using ethidium bromide stained 2% agarose gel run in 0.5X TBE buffer. An Alpha Imager 2000 (Alpha Innotech Company, San Leandro, CA) was used to visualize the resulting product bands. Norovirus positive semi-nested PCR product was purified using the QIAquick PCR purification kit (Qiagen Inc,

Valencia, CA), and sequenced at the University of Arizona's Genomic Analysis Technical Center using a 377 ABI sequencer from Applied Biosystems (Roche Molecular Systems Inc. Branchburg, NJ). Sequencing is a commonly used method for the confirmation of PCR positive products to prevent false-positive results (24).

Sequences were compared to known sequences in the National Center for Biotechnology Information's nucleotide-nucleotide BLAST database for confirmation of positive samples as human norovirus. All amplicons reported as norovirus positive were confirmed as human noroviruses by sequencing.

Determination of Norovirus Concentration on Fomites Samples

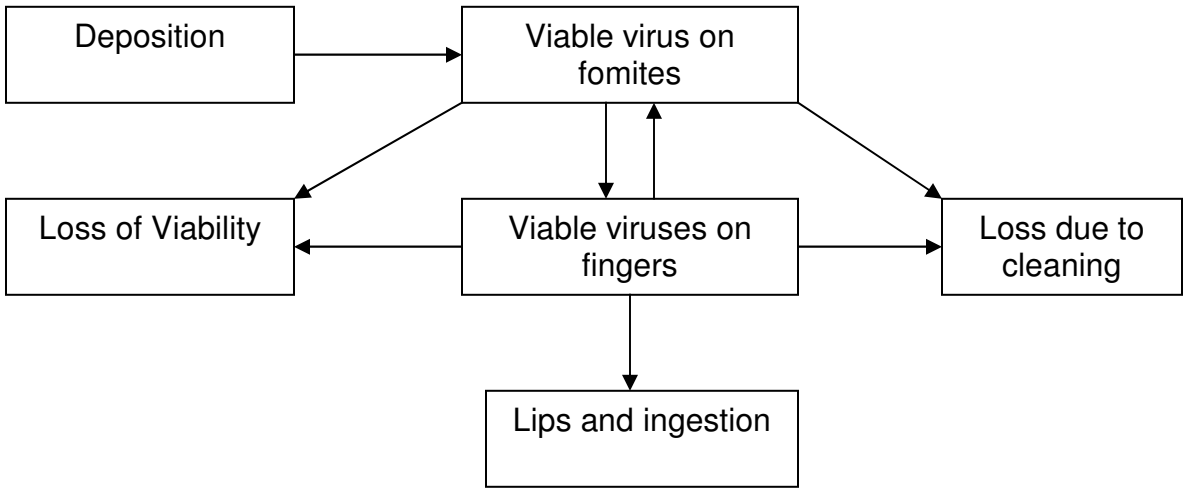
The concentration of norovirus (per cm^2) on fomites was estimated by the most probable number (MPN) method using dilutions series (23). The MPN general-purpose program used was adapted from the method of Hurley and Roscoe (25). Ten fold dilutions of nucleic acid extracts (10^1 to 10^5) were amplified in triplicate for each dilution.

Rationale for Risk Assumptions

An Exposure Model

A continuous-time (dynamic) model for estimating the ingested dose of norovirus via the fomite transmission pathway is diagrammed in Figure 1. In general terms, one considers (i) the rate of viable virus deposition onto fomite surfaces, (ii) the rate of removal of viable virus from fomite surfaces via die-off due to environmental stress (for example, desiccation) and cleaning, (iii) the rate of transfer to the finger pads via touching the fomite surfaces, (iv) the rate of loss of viable virus from the finger pads via die-off, cleaning, and return to fomite surfaces during touching, and (v) the rate of transfer from the finger pads to the lips (followed by ingestion) via hand-to-lip touches. An algebraic description of this continuous-time model has been published previously (26).

Figure 1: A continuous-time (dynamic) exposure model



Unfortunately, outside of a research setting, the rate of norovirus deposition onto fomite surfaces and the rate of touches to these contaminated surfaces are usually unknown and difficult to estimate. Therefore, we used a simpler discrete-time exposure model to answer the following question: Given that an assumed number of viable norovirus are transferred to a finger pad at some arbitrary time zero, what number of viable virus will ultimately be transferred to the person's lips (and ingested)? The general idea is that a person will make a series of finger pad touches to the lips before the finger is overtly washed. In between these touches to the lips, the person will touch the finger pad to clean room surfaces (which will transfer some norovirus from the finger pad onto those surfaces), and a fraction of the norovirus remaining on the finger pad will die due to environmental stress. When the person touches the finger pad to the lips, some of the remaining viable norovirus will be transferred to the lips. These discrete interwoven events are tracked over a reasonable time period until all viable norovirus are predicted to be lost from the finger pad, or until the finger is presumed to be washed.

The information needed for the model is the following: (i) the rate of touching the finger pad to the lips (# per time), (ii) the rate of touching the finger pad to non-contaminated room surfaces (# per time), (iii) the die-off rate of norovirus on the finger pad (fraction per time), (iv) the percent of norovirus on the finger pad that is transferred to a clean room surface during a touch, and (v) the percent of

norovirus on the finger pad that is transferred to the lips during a touch. With regard to the rate of touches to the lips, an observational study (26) of ten volunteers performing office-type work reported an average rate of 6 per hour (range of 0 to 24 per hour across the subjects). The rate of touching the fingers to room surfaces is not a quantity for which we have found published data; in the alternative, we will consider the rates 2, 1, 0.5, and 0.2 per minute (i.e., twice a minute, once a minute, once every two minutes, once every five minutes). Others rates could be considered.

With regard to the die-off rate of human norovirus on the hands, we could not locate specific data. However, a study with human rotavirus (a gastrointestinal pathogen) found that the die-off rate on the hands was in the approximate range of 1% to 5% per minute (27), and a study with a feline calicivirus strain (a surrogate for human norovirus) found that the die-off rate on inanimate nonporous surfaces was in the approximate range of 0.5% to 2% per minute (28). Because the die-off rate of human *Influenzae A* virus is more rapid on the hands than on inanimate nonporous surfaces (29), we judged that the die-off rate of norovirus on the hands would also be greater than on inanimate nonporous surfaces. For our exposure model, we used the midpoint of the range for rotavirus die-off on the hands, 3% per minute (0.03 min^{-1}).

With regard to the percent of norovirus on a finger pad that are transferred to an inanimate surface from the pad during a touch, we located only two studies that directly provided such data. For human rotavirus, approximately 30% of the virus on a finger pad were transferred to a stainless steel disk (and vice versa) during a touch (27). For human rhinovirus, approximately 56% (range 28% to 70%) of the virus seeded onto two to three fingers were transferred to a doorknob or a faucet handle during a normal touching event (30). Other studies have demonstrated the transfer of virus from contaminated fingers to room surfaces, but they were not designed to quantify transfer efficiency per se (31, 32). In contrast to the 56% finger-to-surface transfer efficiency found for rhinovirus, a different study reported that only 0.6% of rhinovirus on a stainless steel disk were transferred to a finger pad during a touch (33). Although we judge that transfer efficiency is a fairly uncertain input, we assumed that the finger-to-room surface transfer efficiency per touch was 30%.

Finally, a study using a bacteriophage found that 34% of the phage on a fingertip were transferred to the lower lip during a 10-second contact (34). Thus, we assumed the finger-to-lips transfer efficiency per touch was 34%. Table 1 summarizes the input values we used for the model.

The discrete-time model equation for the total dose of viable norovirus virus delivered to the lips due to the initial contamination of a finger pad with N viable virus is as follows:

Eq. (1) $$\text{Dose} = 0.34 \times N \times \sum_{i=1}^{\infty} \left[(0.7)^{nsurface} \times \exp\left(-0.03 \times T_{lips}\right) \right]^{i} \times (0.66)^{i-1}$$

Table 1: Assumptions used in discrete-time exposure model

Input	Assumed Values
Rate of finger touches to lips	6 per hour
Rate of finger touches to room surfaces	2, 1, 0.5, 0.2 per minute
Die-off rate of norovirus on the fingers	0.03 min^{-1}
Finger-to-room surface norovirus transfer efficiency per touch	30%
Finger-to-lips norovirus transfer efficiency per touch	34%

The value 0.34 is the finger-to-lips transfer efficiency per touch. The complementary value 0.66 is the fraction of virus remaining on a finger pad following a touch to the lips. The value 0.7 is the fraction of virus remaining on a finger pad following a touch to a room surface, given that the finger-to-room surface transfer efficiency per touch is 0.3. The value 0.03 is the die-off rate of norovirus on the fingers. The term T_{lips} is the time between successive touches

to the lips. For 6 finger touches to the lips per hour, T_{lips} = 10 minutes. The derivation of Equation 1 is provided in the Appendix.

Table 2 displays the output of the Equation 1 model given N = 1,000 initial viable viruses on a finger pad (a value chosen for illustration) for four different rates of finger touches to clean room surfaces. The number of viable viruses transferred to the lips on each of the first six touches to the lips (which would occur over an one-hour period given T_{lips} = 10 minutes), and the overall dose to the lips due to these six touches are shown. Because there is an assumed 30% loss of viable virus from the finger pad for each touch to a clean room surface, an increase in the finger-to-room surface touch rate substantially decreases the overall dose to the lips following a single finger pad contamination event. The overall dose delivered given one finger-to-room surface touch every five minutes is 162. The overall dose decreases to 46, 7.2, and 0.2 viable virus as that touch rate increases respectively to, once every two minutes, once every minute, and twice every minute. Table 2 also shows that essentially all of the final dose delivered to the lips will occur on the first two or three touches to the lips following a finger pad contamination event. The fraction of the initial viable viruses transferred per touch, and transferred overall, is the listed dose divided by 1,000.

Table 2: Number of viable norovirus transferred to the lips on the first six touches to the lips following a single finger pad contamination event for four different touch rates of the finger to clean room surfaces. It is assumed that N = 1,000 for illustration purposes

Order of Touch to the Lips	Rate of Touching the Contaminated Finger Pad to Clean Room Surfaces			
Touch Number	**Two per minute**	**One per minute**	**One per two minutes**	**One per five minutes**
1	0.20	7.1	42	123
2	0	0.1	3.5	30
3	0	0	0.3	7.1
4	0	0	0	1.7
5	0	0	0	0.4
6	0	0	0	0.1
Dose due to six touches	0.20	7.2	46	162

In terms of translating the norovirus ingested dose per finger pad contamination event into a cumulative probability of infection during a four-day stay on a houseboat, three more pieces of information are required. First, what is the actual value of N, the number of viable noroviruses transferred to the finger pad from a contaminated surface during a touch? This value is informed by the results of the swab sampling of the houseboat surfaces for norovirus genomes, which are described subsequently. Second, how many contamination events occurred during the four-day period? The number of contacts with norovirus-

contaminated fomites is not known for any houseboat occupant. In the alternative, we will consider a range of contacts over 16 waking hours a day for four days (64 hours total). The assumed contact frequencies are 64, 32, 16, 8 and 4 (i.e., once every hour, once every two hours, once every four hours, once every eight hours, once every 16 hours). The third required information item is the dose-infection response relationship for norovirus, which is now discussed.

Dose-Response Relationship

We could not locate dose-infection response data for human norovirus. The only available information is that "as few as 10 to 100 virus particles have been reported to cause infection" (28). We reason as follows. The simplest dose-response model is an exponential single-hit model with parameter α, the risk of infection per virus ingested (35):

Eq. (2) $$\Pr(\text{Inf}) = 1 - \exp(-\alpha \times \text{Dose})$$

where Pr(Inf) is the probability of infection, Dose is the expected number of viruses ingested, and exp is the base of the natural logarithm (the number 2.718…). The statement, "as few as 10 to 100 virus particles have been reported to cause infection" does not specify the proportion of individuals each receiving 10 to 100 norovirus who become infected. If this proportion is 50%, and if 32 is

taken as the virus dose associated with Pr(Inf) = 0.5, it can be shown $\alpha = 0.022$. Note that 32 is the geometric mean of the cited range endpoints of virus doses that cause infection, or $\sqrt{10 \times 100} = 32$.

The Equation 2 dose-response function has the property that the norovirus doses received due to different episodes of finger contamination are additive. For example, if there were four contamination events (for example, one a day on four days), the Dose term in Equation 2 would be the sum of the four doses from the respective contamination events. If there are k contamination events, the cumulative probability of infection given these k events is:

Eq. (3) $$\Pr(\text{Inf} \mid \text{k events}) = 1 - \exp\left(-\alpha \times \sum_{j=1}^{k} \text{Dose}_j\right)$$

where Dose_j is computed by Equation 1.

Results

Overall, there were 54 participants on the boat trips of which 27 were interviewed and 20 (74% attack rate) fit the case definition for noroviruses gastroenteritis. Of the 12 fomites samples collected, 7 (58%) tested positive for noroviruses. The

viruses that were typed were all identified as belonging to genogroup GII (Table 3).

Table 3: Adjusted concentration of norovirus genomes on fomites in houseboats

Sample Location – (Houseboat Number)	Number of Genomes (MPN)* / 100 cm^2	Norovirus Genogroup†
Kitchen sink (2)	**53,725**	N/S
Door handles (2)	**24,314**	Not typed
Toilet lid (2)	0	N/A
Door handles (10)	**24,314**	Norovirus GII Uppsala/IV1348/2003
Toilet lid (10)	**1,029**	Not typed
Kitchen sink & tap handle (10)	0	N/A
Refrigerator door (10)	0	N/A
Bathroom toilet lid (13)	0	N/A
Refrigerator door handle (13)	**5,392**	Not typed
Door handles (13)	**2,451**	Norovirus GII Strain Hu/Nov Farmington Hills/2002
Restroom lavatory door (13)	**2,451**	N/S
Kitchen sink & tap handle (13)	0	-
Mean	**16,239**	

*MPN (most probable number) for samples that were Norovirus positive. †Genome typing for samples with strong PCR bands. N/S = No sequence matching, N/A = Not applicable.

We assumed that the seven sampled norovirus concentration values constitute a discrete probability distribution of all the viable norovirus concentrations that were present on all contaminated houseboat surfaces. Although the true distribution of concentrations was surely not limited to this set of values, our formulation avoids making assumptions about the parametric form of the distribution, does not extrapolate concentrations beyond the observed range, and maintains the observed mean sample concentration of 16,239 viruses per 100 cm^2.

The following dose (and risk) calculations assume that: (i) a person touches 4 cm^2 of contaminated surface (approximately twice the surface area of a finger pad) per contamination episode; (ii) 30% of the virus in that touched area is transferred to the finger pad (27); and (iii) the finger-to-clean room surface touch rate is one per minute, such that only 0.72% of the viable virus transferred to the finger pad is eventually ingested (per Table 2). For example, if the touched contaminated surface had 16,239 viable noroviruses per 100 cm^2 (the expected value of the discrete probability distribution of concentrations based on the sampling), that single contamination episode is expected to result in ingesting two viable noroviruses

Expected Dose due to One Contamination Episode =

$$(1 \text{ touch}) \times (16{,}239 \text{ per } 100 \text{ cm}^2) \times (4 \text{ cm}^2 \text{ per touch}) \times 0.3 \times 0.0072 = 1.40$$

It happens that the expected total dose received over k contamination events is the product of k and the expected dose due to one contamination event, or $k \times 1.40$. In turn, the cumulative probability of infection given k contamination events is a function of the expected total dose received, per Equation 3. Table 4 shows the probability of infection given k number of finger pad contamination events over the four-day period on the house boat, where k = 4, 8, 16, 32, 64.

Table 4: Cumulative probability of infection over fours days on a houseboat assuming different numbers of finger pad contamination episodes. The cumulative probability of infection was computed by Equation 3 with α = 0.022.

Number of finger pad contamination events (k) in four days	Expected total dose given k contamination events	Cumulative probability of infection given k contamination events
4	12	0.23
8	25	0.42
16	49	0.66
32	99	0.89
64	197	0.99

Table 4 shows the intuitively reasonable result that as the number of contamination events increases, so does the expected total dose and the cumulative probability of infection. An immediate issue is the likely value of k, the number of contamination events, for an individual houseboat occupant. There is no direct information on this value. However, given that 7/12 commonly touched fomites (primarily door handles) were found to be contaminated with norovirus, and that deposition of viable norovirus was likely ongoing due to developing illness among different houseboat occupants. It is reasonable to expect that the number of contamination events over four days was at the high end of those displayed in Table 4. Given 32 or more contamination events in four days (one event or more every two waking hours), the cumulative probability of infection is estimated to be at least 0.89 (89%). This estimate is in line with the finding that 74% (20/27) of interviewed houseboat occupants had symptoms fitting the case definition of norovirus-induced gastrointestinal illness, and that approximately 30% of individuals infected with norovirus do not exhibit clinical symptoms of illness. In other words, the interview findings suggest that 100% of the houseboat occupants were infected, and our exposure/risk model predicted that at least 89% would become infected.

Discussion

No previous studies have reported the concentration of enteric viruses detected on fomites during outbreaks. Such information can be useful in estimating risks to exposed populations and assessing the efficacy of cleanup methods. Although the number of sites sampled for norovirus was limited, norovirus was detected on 58% (7/12) of the surfaces tested at concentrations as high as 53,725 viral genomes per 100 cm^2, with a median value of 5,392 genomes per 100 cm^2. The RT-PCR method that was used for the detection of norovirus is 95% sensitive, i.e., it correctly detects norovirus in positive sample 95 times out of 100 (36). The efficiency of virus recovery for the swab method that was used is 15% on laminar surfaces for the coliphage MS-2 (37).

The epidemiological data were consistent with the estimate that 100% of the houseboat occupants were infected, which is an attack proportion higher than usually observed in epidemiological studies (38-40). Given a moderate estimate of norovirus infectivity (an infectious dose 50% corresponding to about 30 virus), our exposure/risk model also estimated a high proportion of infections (at least 89%). Although our model indicated that only a small fraction of virus contaminating a finger pad would be ingested, the substantial norovirus concentrations found on contaminated surfaces, combined with a plausibly

frequent number of contacts with those surfaces produced a high likelihood of infection.

We comment on several important source of uncertainty in our risk estimates. First, we assumed the virus transfer efficiency between finger pad and fomite to be 30%. This value was based on work with adenovirus, and may not hold for norovirus (27). In addition, transfer efficiency likely varies with the nature of the touched surface and the degree of pressure applied. Second, we assumed that all the sampled virus quantified via RT-PCR was viable (infectious). As discussed, the die-off rate on inanimate nonporous surfaces of feline calicivirus (a norovirus surrogate) is in the approximate range of 0.5% to 2% per minute (28), which corresponds to a half life of approximately 30 to 140 minutes. Thus, it is certain that not all sampled norovirus was viable. On the other hand, sampling was done after houseboat surfaces had been cleaned, in which case the sampling underestimated (likely substantially) the concentrations of norovirus present prior to cleaning. There is no way to precisely reconstruct the concentrations of viable norovirus during the periods of occupancy. Given the high concentrations found via RT-PCR after cleaning, and given presumed ongoing deposition of norovirus due to developing illness among different houseboat occupants, viable norovirus concentrations on fomites were likely high.

Third, we assumed an exponential dose-response function with an estimated infectivity parameter $\alpha = 0.022$. The true nature of the dose-response function may be different, and our α value was estimated using the information that "as few as 10 to 100 virus particles have been reported to cause infection" (28). The latter statement does not specify the *proportion* of individuals infected by any given dose and permits different interpretations. For example, if infection occurs among 90% of individuals each ingesting 10 norovirus, then $\alpha = 0.23$; in contrast, if infection occurs among 10% of individuals each ingesting 100 norovirus, then $\alpha = 0.0011$. There is a 210-fold difference in the infectivity parameter between these interpretations. In turn, the virus dose required to impart a high probability of infection (say, 99%) is 210-fold different; for $\alpha = 0.23$, a dose of 20 viruses is required, while for $\alpha = 0.0011$, a dose of 4,200 virus is required. Clearly, the α value is an important source of uncertainty in our modeling. However, in the context of the heavily contaminated houseboat surfaces, even the moderate estimate of $\alpha = 0.022$ leads to a significant risk. For example, an ingested dose of 3 viruses following one contamination event imparts a 7% probability of infection.

In conclusion, our study suggests that the numbers of norovirus detected on fomites following an outbreak were sufficiently high to lead to ingested doses causing infection among all susceptible individuals. The epidemiological data confirm that the attack rate was consistent with the values predicted by the

QMRA model. Such information can be used to establish goals for the reduction of pathogens on fomites to reduce the probability of infection after outbreaks.

APPENDIX

Derivation of the Discrete-Time Exposure Model

Consider that at time zero, N infectious viruses are transferred to a finger pad due to a touch. Assume that the person will touch that same finger pad to the lips T_{lips} minutes later (for example, T_{lips} = 10 min). Assume that in the intervening 10-min period, the person touches the contaminated fingertip to $n_{surface}$ number of clean room surfaces (for example, $n_{surface}$ = 2). Assume that each touch to a clean room surface transfers 30% of the virus particles on the finger pad back to the surface. Thus, the fraction of the initial N virus that remain on the finger pad when the touch to the lips occurs is $0.7^{nsurface}$; for example, if $n_{surface}$ = 2, then 0.7^2 = 0.49. Of the virus remaining on the finger pad when the touch to the lips occurs, a fraction $\exp(-0.03 \times T_{lips})$ is still viable, where 0.03 min^{-1} is the first-order die-off rate of norovirus on the hands. If T_{lips} = 10 min, $\exp(-0.03\ min^{-1} \times 10\ min)$ = 0.74. The touch to the lips transfers 34% of the viable virus on the finger pad to the lips. Thus, upon the first touch to the lips, the number of viable virus transferred to the lips ($Dose_1$) is:

Eq. (A1) $$Dose_1 = 0.7^{nsurface} \times \exp(-0.03 \times T_{lips}) \times 0.34 \times N$$

For example, if $n_{surface} = 2$ and $T_{lips} = 10$ min, then Dose 1 = $0.49 \times 0.74 \times 0.34 \times N = 0.123$ N. After the first touch to the lips, the number of viable virus remaining on the fingertip (Remainder 1) is:

Eq. (A2) $$\text{Remainder 1} = 0.7^{nsurface} \times \exp(-0.03 \times T_{lips}) \times 0.66 \times N$$

Consider that the next touch of the contaminated finger pad to the lips occurs T_{lips} minutes later, and that in the intervening period of length T_{lips}, the person makes another $n_{surface}$ touches of the fingertip to clean surfaces. It follows that of the Remainder 1 viable virus, a fraction $0.7^{nsurface} \times \exp(-0.03 \times T_{lips})$ is still present on the fingertip and viable when the second touch to the lips occurs. Again, 34% of that viable virus is transferred to the lips. Thus, upon the second touch to the lips, the number of viable virus transferred to the lips ($Dose_2$) is:

Eq. (A3) $$Dose_2 = [0.7^{nsurface} \times \exp(-0.03 \times T_{lips}) \times 0.66] \times 0.7^{nsurface} \times \exp(-0.03 \times T_{lips}) \times 0.34 \times N$$

$$= [0.7^{nsurface} \times \exp(-0.03 \times T_{lips})]^2 \times 0.66 \times 0.34 \times N$$

After the second touch to the lips, the number of viable virus remaining on the fingertip (Remainder 2) is:

Eq. (A4) $$\text{Remainder 2} = [0.7^{nsurface} \times \exp(-0.03 \times T_{lips})]^2 \times 0.66^2 \times N$$

If T_{lips} and $n_{surface}$ are fixed numbers, and if no explicit hand cleaning occurs, the number of viable virus transferred to the lips on the i^{th} touch subsequent to the original contamination of the fingertip is:

Eq. (A5) $$Dose_i = [0.7^{nsurface} \times \exp(-0.03 \times T_{lips})]^i \times 0.66^{(i-1)} \times 0.34 \times N$$

The total dose of viable virus delivered to the lips due to the original contamination of the fingertip is the sum of the doses delivered by each touch to the lips:

Eq. (A6) $$Dose = Dose_1 + Dose_2 + Dose_3 + \ldots.$$

$$= 0.34 \times N \times \sum_{i=1}^{\infty} \left[(0.7)^{nsurface} \times \exp\left(-0.03 \times T_{lips}\right)\right]^i \times (0.66)^{i-1}$$

The percent of the original N viable virus transferred to the lips is simply: $\frac{Dose}{N} \times 100\%$

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APPENDIX B: MANUSCRIPT # 2

Outbreak of Norovirus Illness in a College Summer Camp: Impact of Cleaning on Occurrence of Norovirus on Fomites

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Summary

During the summer of 2005 an outbreak of norovirus acute gastroenteritis occurred in a college summer camp and was reported to the local health department. The outbreak spread rapidly to several other groups concurrently sharing the same facilities. During the investigation fomites were sampled, at different times, in dorm rooms and tested for norovirus. The number of norovirus positive rooms increased after the first room cleaning, from 40% to 73%. After the initial cleaning, the staff was instructed on proper cleaning and disinfection procedures and provided disposable disinfecting wipes to reduce cross contamination, the number of norovirus positive rooms decreased to 30%. These findings reinforce the need for appropriate cleaning and disinfection procedures during a norovirus outbreak.

Keywords: Fomites, Norovirus, Summer camp

INTRODUCTION

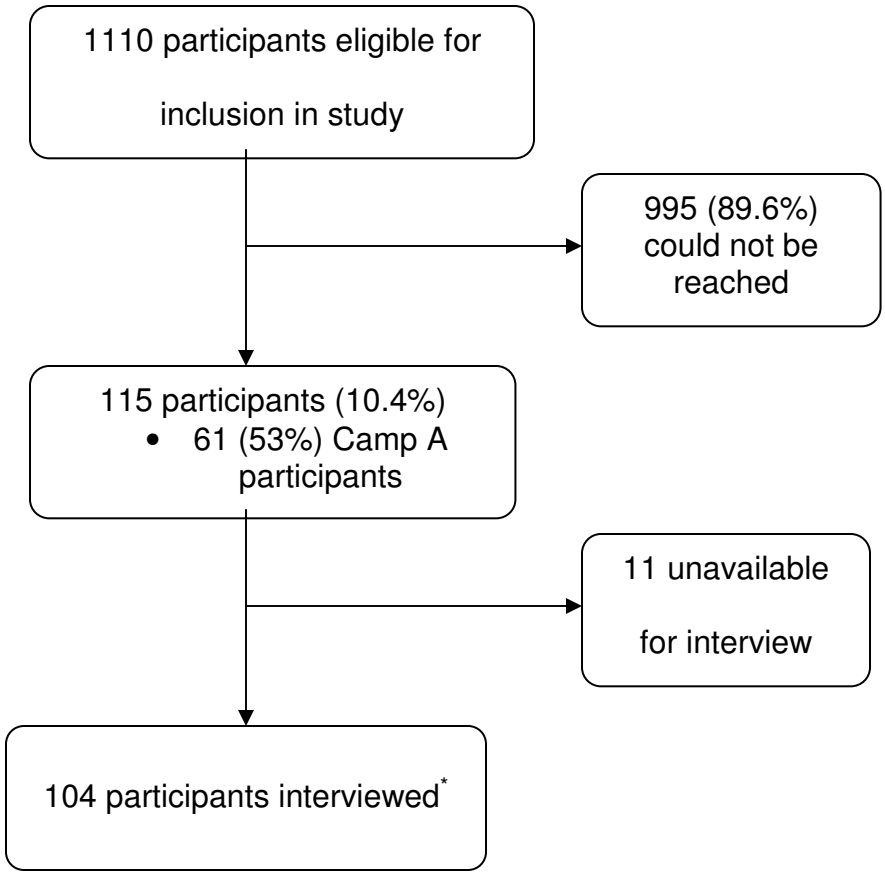
Norovirus is the leading cause of nonbacterial gastroenteritis worldwide. Norovirus was formerly known as the Norwalk-like virus or small round structured virus, and is a member of the *Norovirus* genus in the *Caliciviridae* family of viruses. They are non-enveloped, positive sense, icosahedral, single-stranded RNA viruses. Human norovirus is genetically diverse and belongs to one of three genogroups (GI, II, or IV); each of which is further divided into more than 25 genetic clusters (1, 2). Outbreaks of norovirus gastroenteritis occur most frequently in various settings such as schools, daycare centers, nursing homes, hospitals, and cruise ships (3-13). Noroviruses are transmitted via the fecal-oral route. The average incubation period for norovirus induced gastroenteritis is 12-48 hours with symptoms that usually resolve in 12-72 hours. Illness is characterized by acute-onset projectile vomiting, watery non-bloody diarrhea with abdominal cramps, low grade fever, headache, and malaise (1, 2, 14). The very low infectious dose of norovirus may be one reason why it is the most common cause of diarrheal illness (15). This study investigated a summer camp associated outbreak of norovirus illness to identify the source of infection, the causes of disease spread, and to recommend strategies for prevention of future outbreaks in such settings.

METHODS

Background

On July 20, 2005, the local Health Department Communicable Disease program coordinator received a call from the infection control practitioner at Flagstaff Medical Center about three emergency department patients with similar symptoms: vomiting, diarrhea, and dehydration. All three patients (2 participants and a staff member) were also members of Camp A (wrestling camp) from an area summer camp. Further investigation by the communicable disease staff identified 40 other Camp A members with similar symptoms. On July 21, 2005, an outbreak investigation was initiated. An employee from the health department interviewed participants attending the summer camps on July 21. They were given questionnaires to determine personal demographic information and symptoms associated with the gastrointestinal illness. Outbreak investigation participant selection is shown in Figure 1.

Figure 1: Flow chart of study participation



Outbreak Case Definition

A retrospective cohort study of all persons attending the summer camp from 7/18/2005 to 7/31/2005 inclusive was conducted. The case definition for norovirus illness was acute onset of nausea, vomiting, and/or diarrhea (i.e. no prodromal period before gastrointestinal symptoms). The inclusion criteria into the study were:

1) Participation at any of the four summer camps (A, B, C, D), or
2) Having contact with members of the camps within 48 hours before illness onset, or
3) Visiting one of a variety of locations at the summer camp within 48 hours before onset of illness.

Environmental Investigation and Disinfection

Samples were collected at three different dates July 21 and 22, August 1, and August 15. In Table 1 are shown the dates of the environmental investigation and cleaning/disinfection interventions. Ten fomites (doorknobs and toilet seats) were sampled during July 21-22 before any cleaning took place by facilities management personnel.

Table 1: Timeline of environmental investigation and disinfection

Date	Action
July 20	• Local health department contacted
July 21 & 22	• Outbreak investigation initiated o Participants questionnaires o Fomites and fecal samples collected
July 31	• Cleaning with soap and water
August 1	• Fomite samples collection
August 14	• Cleaning and disinfection with soap and water followed by 5000 mg/l free chlorine and disposable disinfecting wipes
August 15	• Re-sampling of fomites that tested positive for norovirus on August 1

The residence halls that housed norovirus cases from Camp A, a multi-purpose athletic facility, and the student union were cleaned on July 30. The cleaning crew did not have specific cleaning instructions and used their common cleaning solutions composed of soap and water. After the cleaning, 51 fomites in residence halls and Camp A facilities were swabbed for norovirus again on August 1, the day after cleaning. The surfaces included toilet handles and seats, bathroom sink faucet handles, bathroom doorknobs, walls, mattresses, urinal handles, chairs, drinking fountains, and floors.

After review of the results the Environmental Health Program Manager (EHPM) for the local health department instructed facilities management personnel to again clean and disinfect contaminated fomites. The EHPM advised the summer camp facilities staff to clean and disinfect surfaces in bathrooms, bedrooms, and common areas using a bleach (sodium hypochlorite) solution containing 5000 mg/L free chlorine. The surfaces were cleaned with detergent (soap and water) prior to disinfection. Separate disinfecting wipes (containing a quaternary ammonium disinfectant; Clorox Company, Oakland, CA) were used for each surface to reduce the possibility of cross contamination, and the chlorine solution was often refreshed after cleaning each room. The third and final fomite sample (10 samples) collection took place on August 15 after cleaning of fomites as per instructions of the EHPM. Only the surfaces that tested positive for norovirus from the fomite sampling on August 1 were re-sampled.

Virus concentration from stool

Noroviruses were concentrated from stool by suspending 1 gram of stool in 7 ml phosphate buffered saline. Suspensions were then vortexed for 60 seconds, and centrifuged for 30 minutes. The supernatant was removed and aliquoted for storage at -20℃ until further processing.

Virus concentration from fomites

Methods for virus detection on fomites was previously described by Boone (16). Briefly, the fomites samples were obtained by swabbing each individual surface with a sterile polyester fiber-tipped transport system collection swab moistened in transport medium (BBL Culture swabs, Becton, Dickinson and Company, Sparks, MD). All samples were transported to the laboratory on ice and frozen at -80℃ until assayed. Samples were homogenized using a vortex mixer followed by viral RNA extraction.

RNA Extraction

Viral RNA was purified from fomite and stool samples using QIAamp Viral RNA Mini Kit (Qiagen Inc, Valencia, CA) (17). The Mini Spin Protocol was followed with the following modifications: the total sample volume was doubled to 280 µl,

and a double elution using two consecutive 40 µl volumes of Buffer AVE was performed. The purified viral RNA samples were stored at -20℃.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

RT-PCR was performed on the purified viral RNA using Qiagen OneStep RT-PCR kit (Qiagen Inc, Valencia, CA). The primers MJV12 (5'-TAY CAY TAT GAT GCH GAY TA-3') and RegA (5'-CTC RTC ATC ICC ATA RAA IGA-3') (18), (modified JV12/JV13 primers) (18), are specific for human norovirus genogroup GI and GII originating from region A of the NV capsid gene. All reagents used for the reverse transcriptase and the amplification steps were obtained from Applied Biosystems (Roche Molecular Systems Inc. Branchburg, NJ). PCR was performed using 22.25 µL of Rnase free water (Promega Madison, WI), 5.0 µL of 25 mM $MgCl_2$ solution, 0.25 µL of 5unit/mL Amplitaq gold, 5 µL of GeneAmp 10 × PCR buffer, 4.0 µl of 2.5 mM of dNTP mix, 1.0 µL of 50 µM (upstream and downstream) primers (Reg A and MJV12), 0.5 µL of 50 nM random hexamers, 0.5 µL of 20 units/µL RNAse inhibitor, and 0.5 µL of 50 units/ µL RT per sample. A 10µl volume of purified RNA template was used in a total reaction volume of 50µl. The reverse transcriptase reaction mixture was placed in an Applied Biosystems Gene Amp PCR System 9700 thermacycler (Roche Molecular Systems Inc. Branchburg, NJ). Thermal cycling conditions were as follows: reverse transcription of viral RNA for 60 minutes at 42˚C; activation of Taq

polymerase for 15 minutes at 95˚C; 40 cycles: 30 seconds at 94˚C, 30 seconds at 50˚C, 30 seconds at 72˚C; and final extension for 10 minutes at 72˚C.

Semi-nested Polymerase Chain Reaction

Semi-nested PCR was performed using 5.0 µL of 10 x PCR gold buffer, 5.0 µL of 2.5mM MgCl, 4.0 µL of 2.4 mM dNTP, 1.0 µL of 50mM of internal primer MP 290 and primer Reg A, 0.25 µL of 5 units/ml of Amplitaq Gold, and 2µl volume of PCR product from RT-PCR in a total reaction volume of 50µl. The reaction mixture was placed in an Applied Biosystems Gene Amp PCR System 9700 thermacycler (Roche Molecular Systems Inc. Branchburg, NJ). Thermal cycling conditions were as follows: 5 minutes at 95°C, 40 cycles of: 30 seconds at 94°C, 30 seconds at 49°C, 30 seconds at 72°C, and a final elongation step for 10 minutes at 72°C.

Rnase-free water negative controls and known positive norovirus controls were done concurrently with the unknown samples. The semi-nested PCR product was visualized using ethidium bromide stained 2% agarose gel run in 0.5X TBE buffer. An Alpha Imager 2000 (Alpha Innotech Company, San Leandro, CA) was used to visualize the resulting product bands. Norovirus positive semi-nested PCR product was purified using the QIAquick PCR purification kit (Qiagen Inc, Valencia, CA) and sequenced at the University of Arizona's Genomic Analysis

Technical Center using a 377 ABI sequencer from Applied Biosystems (Roche Molecular Systems Inc. Branchburg, NJ). Sequencing is a commonly used method for the confirmation of PCR positive products to prevent false-positive results (18).

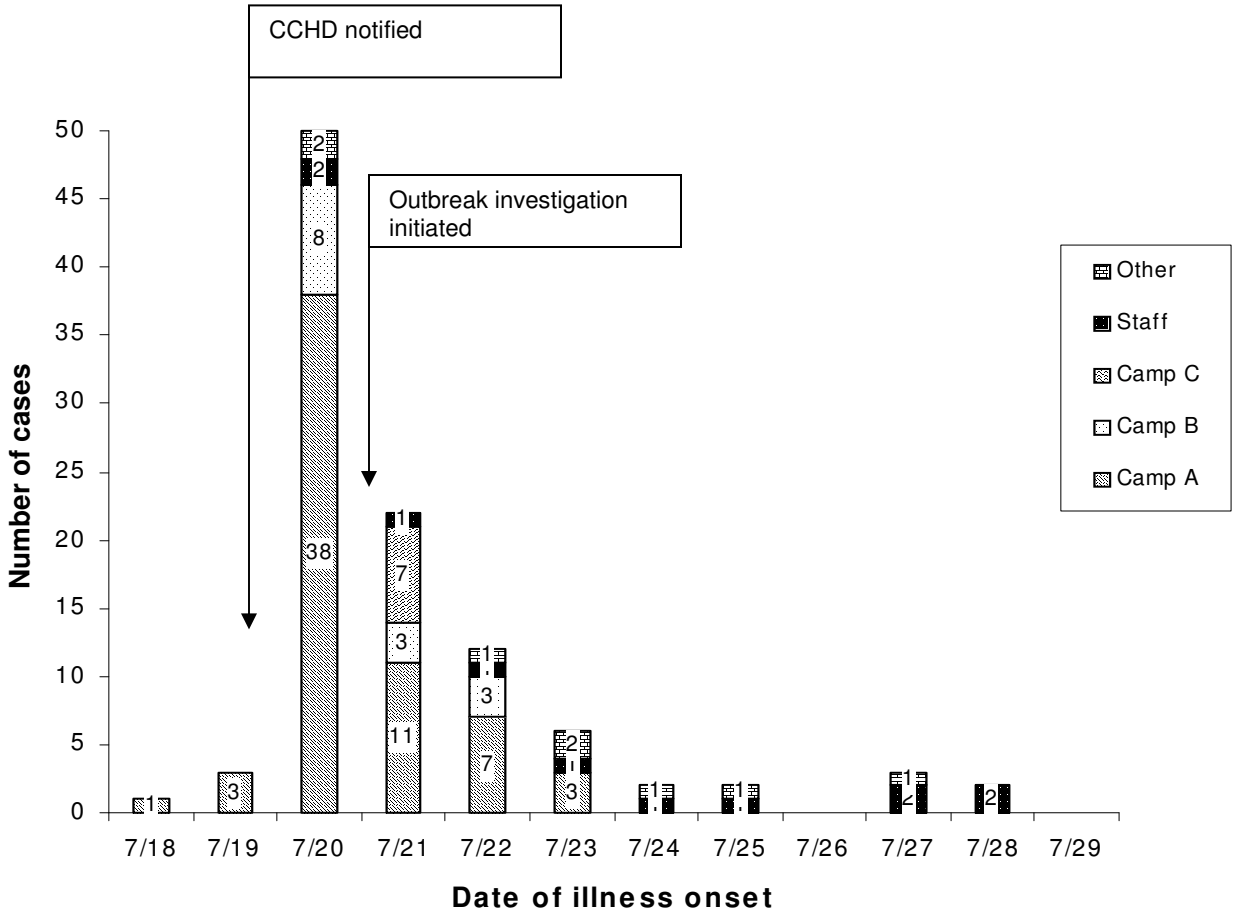
Sequences were compared to known sequences in the National Center for Biotechnology Information's nucleotide-nucleotide BLAST database for confirmation of positive samples as human norovirus. All amplicons reported as norovirus positive were confirmed as human noroviruses by sequencing.

RESULTS

Epidemiological Findings

From July 18-July 28, the illness affected a total of 115 individuals, 61 (53%) of which were participants or staff of Camp A that began on July 17. During the investigation, 104 cases were interviewed. Eleven of the 115 left the state before they could be interviewed. Figure 2 below shows the epidemiological curve starting on July 18 and ending on July 29. No cases were reported to have onset dates before July 18 or after July 28. The single case on July 18 is the probable index case. The "Other" category includes regular students at the camp's location and two health department employees who interviewed cases in person.

Figure 2: Epidemiological curve of the outbreak



The outbreak peaked on July 20 with 50 cases and subsided after that date. After July 23rd, the 0-2 cases per day may have been part of the normal background of acute gastroenteritis for a college campus with dormitory housing. This is difficult to prove, because not all causes of acute gastroenteritis are reportable diseases, and most cases do not seek medical attention.

Out of the 103 cases for which gender information was available, 24 were women (23%) and 79 were men (77%). Age ranged from 14 to 54 years old. Out of the 103 people for which symptom information was available, the symptoms included nausea in 20 (19.4%), vomiting in 83 (80.6%) and diarrhea in 91 (88.3%). Nausea, vomiting, and diarrhea were reported by 14 (13.6%) of the individuals (Table 2). Four individuals required emergency care for severe dehydration, one of whom was hospitalized for two days. No fatalities occurred during this outbreak.

Table 2: Demographics of individuals

Gender	**Total (%)**
Male	79 (77)*
Female	24 (23)*
Symptoms	
Nausea	20 (19.4)
Vomiting	83 (80.6)
Diarrhea	91 (88.3)
All symptoms	(14) 13.6

* Based on 103 available surveys

Environmental Findings

The summer camps at the highest risk of infection included Camp A that began on July 17 and other camps that shared a residence hall or dining facility with the wrestlers. The attack rate among participants of Camp A was higher at 30.3%

than for all other affected summer camps (Table 3). The high number and rate of acute gastroenteritis in Camp A was used to verify the existence of an outbreak.

Table 3: Attack rates among camps participants

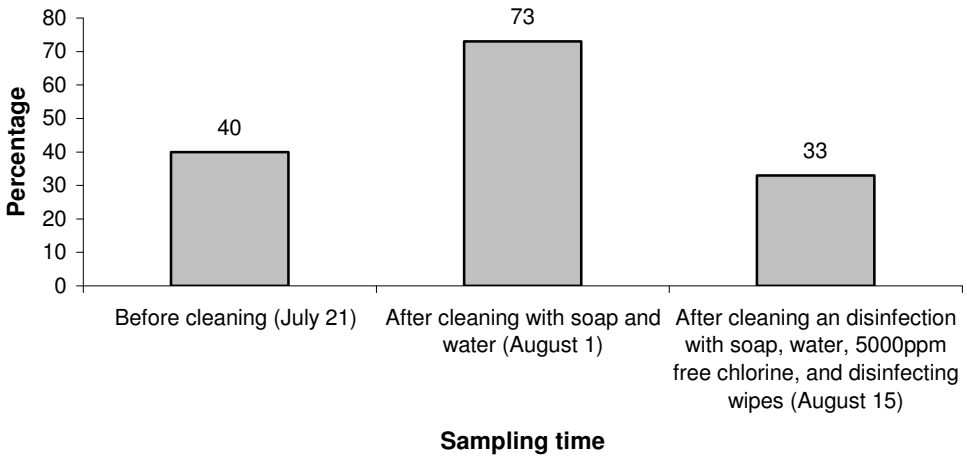
Camp	Number Ill (n=84)	Total Number (n=1,026)	Attack Rate (% Ill)
A*	61	201	30
B	14	683	2
C	7	75	9
D	2	67	3

*Includes only camp A that began on July 17.

Laboratory Findings

Of the fomites samples collected on July 21 and 22, norovirus was only detected on 17% of the samples. More extensive sampling took place on August 1 after cleaning with rags, soap and water, of which 11 (22%) were positive for norovirus. Forty five percent (45%) of the fomite samples of August 1 from toilet seats and toilet handles in the wing of the residence hall that housed ill individuals from Camp A were positive for norovirus. Surfaces that were supposed to be cleaned and disinfected still tested positive for norovirus The second cleaning and disinfection occurred on August 14 before re-sampling of the same fomite locations on which norovirus was detected on August 15. After this round of cleaning and disinfecting, the percentage of dorm rooms testing positive was reduced to less than 35% (Figure 3).

Figure 3: Percent of rooms in which norovirus was detected on fomites



DISCUSSION

This outbreak of noroviruses in a summer camp was associated with attack rates varying from 3 to 30% that may have resulted from poor disinfection procedures or cross contamination from the use of the same cleaning/disinfecting tools (i.e. clothes, rags, mops, etc) during the outbreak.

A total of 115 cases were identified as part of this outbreak, but complete data were available for only 84 cases. Over half of the cases occurred among participants and staff of Camp A, which also had the highest attack rate (30%). Stool specimens were collected from four Camp A participants, and three of them (75%) tested positive for norovirus confirming this virus as the causative agent of

the outbreak. The illness spread very quickly through this group (with three participants ill on one day, and 38 more ill on the next day). The close physical contact that takes place during camp activities and associated mats, in the wrestling camp, probably led to much of the spread to other participants. The virus also spread to a limited degree to three other summer camp groups that shared space with Camp A. Other cases included college employees and students. Other sources of spread may have included being in close proximity to someone who was vomiting (during which the virus can become airborne and contaminate fomites over a large area), and touching contaminated surfaces in the dormitory, Camp A facilities, or other locations on campus. Sink faucet handles, toilet handles, and toilet seats in the dormitory that housed Camp A participants tested positive for the virus.

A study by Baker et al (19) found that twenty-eight percent (28%) of surfaces cleaned and sanitized using 5,000 mg/L free chlorine were still positive for norovirus. After the second round, the following steps were undertaken to reduce the number of surfaces on which norovirus was detected:

1. All surfaces that had previously tested positive for norovirus were washed with a detergent solution prior to disinfection (to improve the effectiveness of the disinfectant).

2. A solution of 5,000 mg/L free chlorine was used to disinfect surfaces by mixing two cups of bleach with one gallon of water
3. The chlorine solution was kept at 5,000 mg/L by frequently making fresh solutions. The summer camp employees were instructed on the cleaning and disinfection procedure but were not closely monitored.

Separate chlorine solutions and disposable disinfecting wipes (to reduce cross contamination) were used to clean the toilet surfaces, lavatory surfaces, and doorknobs.

The number of dorm rooms testing positive for norovirus increased from 40% at initial sampling to 73% after the first cleaning and then decreased to 33% after proper cleaning and disinfection (Figure 3). The increase in toilet handles testing positive (Table 4) might also have been due to improper initial disinfection of surfaces with 5000 mg/L free chlorine: or the cleaning solution was not appropriately refreshed, or wiping cloths and/or sponges were indiscriminately used to clean and/or disinfect surfaces which could lead to cross contamination.

Table 4: Detection of norovirus (% positive) on fomites after cleaning/disinfection

Fomites	Before cleaning (July 21)	After cleaning with soap and water (August 1‡)	After cleaning with soap, water, chlorine, and disinfecting wipes (August 15*)
Toilet seat	50	NT	NT
Toilet handle	NT	27	67
Lavatory handle (Tap)	NT	64	29
Total	50	45	40

NT = not tested. ‡ Sampling after cleaning with rags, soap and water. * Sampling after cleaning and disinfecting with soap and water followed by 5000 mg/l free chlorine and disinfecting wipes.

No common food or meal was shared by the cases prior to the first illnesses. However, a dining facility was shared by Camps A and B. At least one participant in Camp A was reported to have vomited during dinner. Thus, the most likely means of viral spread was airborne spread from vomiting and fomites contaminating surfaces in the dining room or bathrooms.

The viral RNA was sequenced, and found to be GII.2. This included the probable index case, who became ill on July 18. This was the first report of this norovirus genotype in Arizona. Thus, the virus was most likely brought to Arizona from someone who was infected in another state prior to coming to the camp.

The Centers of Disease Control and Prevention has previously reported that norovirus may be spread via fomites (20). The virus may also be aerosolized

during vomiting and when diarrhea stools are flushed in a toilet. This virus has a very low infective dose, and may remain viable on surfaces for more than a month (21). This virus is also resistant to free chlorine concentrations of 1000 part per million in laboratory studies using feces (21).

During the outbreak, two health department employees became ill 24-36 hours after interviewing cases of Camp A in their residence hall. Several of the cases were vomiting at the time of the interviews or had recently vomited. One of the two health department employees entered a bathroom to sample fomites very soon after an individual vomited. Exposures may have occurred from ingestion of airborne virus particles due to close proximity of bathrooms to bedrooms. Contaminated surfaces in the residence hall rooms could have been another source of exposure. To prevent future norovirus illness among staff, health department employees were directed, in the future, to wear gloves and masks when interviewing cases that are vomiting or have recently vomited.

The health department's recommendations for outbreak control included:

1- Confining ill camp participants to their rooms.
2- Confining all participants of the affected summer camps to their residence halls.
3- Recommending frequent hand washing by camp participants and university employees.

4- Excluding food service employees experiencing acute gastroenteritis symptoms from work.
5- Thorough cleaning and disinfection of the affected areas.

On July 23, university officials canceled all remaining summer conference camps on campus. The summer camp staff thoroughly disinfected all residence halls and facilities that were likely to have been contaminated with norovirus. These infection control measures likely slowed the spread of the virus. No cases of norovirus illness connected to this outbreak were reported after July 28.

Measures taken to prevent future norovirus outbreaks included the following: summer camp officials reviewing contracts with summer conference camp organizers to determine the feasibility of excluding sick camp participants, the summer camp adopting new disinfection protocols based on the recommendations described above, multiple press releases about the outbreak, the health department developing and distributing a norovirus informational brochure, and the inclusion of a norovirus article in the summer issue of the health department's epidemiology newsletter.

Our study was limited by several factors. In outbreaks of norovirus illness, the onset of secondary illness often overlaps the late onset of primary cases, which may lead to misclassification of primary and secondary cases. We were not able

to sequence stool and fomites positive samples in order to ascertain whether or not the outbreak was due to a single source. Also, given that 30% of norovirus cases are asymptomatic, the full extent of the outbreak might have been underestimated. Mild and self-limiting infections were probably not reported.

This outbreak of norovirus illness in a summer camp underscores the importance of understanding the spread of the virus via fomites, especially in area where people tend to congregate. The closed environment of summer camp, combined with inadequate cleaning procedures spurred the spread of this outbreak. When outbreaks occur in institutional settings, such as a college summer camp, it is important to reinforce the advice about proper hygiene in order to curtail the spread of the outbreak to other settings such as households. This study has produced evidence that fomites are important in the transmission of norovirus and that using a combination of standard cleaning procedures and 5000 mg/L free chlorine can effectively reduce cross contamination of fomites due to cleaning during a norovirus outbreak.

On the basis of our findings, we recommend that in institutional settings, people with acute gastroenteritis be excluded in order to avoid transmitting the disease to other people. The appropriate cleaning and disinfecting procedures should also be applied depending on the infectious agent as standard cleaning procedures do not always kill all microorganisms.

DECLARATION OF INTEREST

None.

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APPENDIX C: MANUSCRIPT #3

Norovirus Outbreak during a Christmas Party

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I. SUMMARY

On December 2005, 28 persons who had attended a buffet style Christmas dinner at a restaurant in Arizona fell sick with diarrhea, nausea, and vomiting. To determine the risk factors of illness, questionnaires were administered to the participants, food preparation flow was investigated, and stool and fomite samples were collected. The samples were tested for norovirus using RT-PCR. Analyses of the questionnaires showed no statistically significant association between illness and any of the food items served during the Christmas banquet. Genomic analysis of norovirus positive stool samples showed that all samples belonged to norovirus genogroup I (GI.4). The epidemiological curve for this outbreak was reminiscent of a point source contamination for norovirus. None of the food handlers acknowledged any illness in the days preceding the banquet, during the banquet or afterwards.

It is likely that one or more asymptomatic food handlers in the restaurant contaminated serving utensils through direct and indirect contact. Generally, food handlers are oblivious of their potential for transmitting norovirus and this is compounded when the probable point source food handler is asymptomatic, which may have been the case in this outbreak. These findings underscore the need to emphasize hygiene among food handlers to prevent future foodborne outbreaks.

Keywords: Fomites, Norovirus, Outbreak

II. INTRODUCTION

Norovirus is the leading cause of nonbacterial gastroenteritis worldwide. This virus is formerly known as the Norwalk-like virus or small round structured virus, and it is a member of the *Norovirus* genus in the *Caliciviridae* family of viruses. These viruses are non-enveloped, positive sense, icosahedral, and single-stranded RNA viruses. Human norovirus is genetically diverse and belongs to one of three genogroups (GI, II, or IV); each of which is further divided into more than 25 genetic clusters (1, 2). Outbreaks of norovirus gastroenteritis have occurred in various settings such as schools, daycare centers, nursing homes, hospitals, and cruise ships (3-13). Noroviruses are transmitted via the fecal-oral route. The average incubation period for norovirus induced gastroenteritis is 12-48 hours with symptoms that usually resolve in 12-72 hours. Illness is characterized by acute-onset projectile vomiting, watery non-bloody diarrhea with abdominal cramps, low grade fever, headache, and malaise (1, 2, 14). The very low infectious dose(15) of norovirus may be one reason why it is the most common cause of diarrheal illness (16). Two other factors also contribute to the considerable impact of disease caused by norovirus: a large human reservoir of infection (17), and the ability to be transmitted by a variety of routes.

It is estimated that more that 90% of non-bacterial acute diarrhea episodes are due to norovirus (18-21). In the US, the proportion of norovirus confirmed foodborne outbreaks rose from 1% in 1991 to 12% in 2000 (22). It is estimated

that of all foodborne illnesses, Norwalk-like viruses account for over 67% of all cases (23 million), 33% of hospitalizations, and 7% of deaths (16). Some of the noroviruses related outbreaks have been linked to improper hygiene in food handlers (23-28). This study investigated a Christmas party in a restaurant-associated outbreak of norovirus illness to identify the source of infection, the causes of disease spread, and to recommend strategies for prevention of future outbreaks in such settings.

III. METHODS

Epidemiological Investigation

On December 6, 2006, the local Health Department Communicable Disease program was notified of a cluster of illness associated with a Christmas party. The symptoms reported by the patients were consistent with acute gastroenteritis: vomiting, diarrhea, and dehydration. The Christmas party had taken place in a local restaurant on December 1, 2006 with a dinner served buffet style starting at about 7:30 pm. The outbreak investigation was initiated, and a retrospective cohort study of all persons attending the Christmas party at the restaurant on December 1 was conducted on December 6. The banquet took place at a restaurant, and the food had been prepared and served by employees from the restaurant. Immediate action was taken by the health department to control and investigate the outbreak.

Participants at the Christmas party were localized and given questionnaires to determine the demographics and symptoms associated with the gastrointestinal illness. Dinner participants were asked about their illness status, symptoms, previous exposure to an ill individual, travel history, mode of transmission, and food history from November 28 to December 1. It was possible to assess their consumption of food and various beverages during the party. The restaurant's employees were not interviewed because the food and beverage director stated that no employees had reported illness before, during, or after the banquet. The food and beverage director mentioned that some of the evening shift employees ate the same food that was prepared for the banquet, but the identity of those employees was not available.

A case was defined as a person who attended the Christmas dinner at the restaurant on December 1, and had any of the following symptoms up to three days after the dinner: acute onset of nausea, abdominal cramps within three days of having eaten at the party, as well as vomiting, and diarrhea.

Completed questionnaires were entered into an Access file, and imported into Stata version 9.0 (StataCorp LP, College Station, TX, USA) (29). Univariate analysis calculating odds ratio for different food items consumed was performed.

Environmental Investigation

The environmental investigation focused on the process flow of suspected foods, looking at storage, washing, and sanitizing of food equipment. No leftovers of the foods served during the Christmas dinner could be tested. We were only able to secure four stool samples from ill individuals. Given that the clinical picture suggested a norovirus outbreak, the stool samples were tested for norovirus by reverse transcriptase polymerase chain reaction (RT-PCR) by the Arizona State Public Health Laboratory. Genotyping of the norovirus positive samples was conducted and confirmed by genomic sequencing. After detection of norovirus positive stool sample results, on December 20, 32 fomite samples from the restaurant's kitchen were collected.

a. Environmental Sampling

Virus detection

Methods for virus detection on fomites was previously described by Boone (30). Briefly, the fomite samples were obtained by swabbing each individual surface with a sterile polyester fiber-tipped transport system collection swab moistened in transport medium (BBL Culture swabs, Becton, Dickinson and Company, Sparks, NJ). All samples were transported to the laboratory on ice and frozen at -80℃ until assayed. Samples were homogenized using a vortex mixer followed by viral RNA extraction.

Noroviruses were detected in stool samples by suspending 1 gram of stool in 7 ml phosphate buffered saline. Suspensions were then vortexed for 60 seconds, and centrifuged for 30 minutes. The supernatant was removed and aliquoted for storage at -20℃ until further processing.

Viral RNA was purified from fomite and stool samples using QIAamp Viral RNA Mini Kit (Qiagen Inc, Valencia, CA) (31). The Mini Spin Protocol was followed with the following modifications: the total sample volume was doubled to 280 µl, and a double elution using two consecutive 40 µl volumes of Buffer AVE was performed. The purified viral RNA samples were stored at -20℃.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

RT-PCR was performed on the purified viral RNA using Qiagen OneStep RT-PCR kit (Qiagen Inc, Valencia, CA). The primers MJV12 (5'-TAY CAY TAT GAT GCH GAY TA-3') and RegA (5'-CTC RTC ATC ICC ATA RAA IGA-3') (32), (modified JV12/JV13 primers) (32), are specific for human norovirus genogroup GI and GII originating from region A of the NV capsid gene. All reagents used for the reverse transcriptase and the amplification steps were obtained from Applied Biosystems (Roche Molecular Systems Inc. Branchburg, NJ). PCR was performed using 22.25 µL of Rnase free water (Promega Madison, WI), 5.0 µL of

25 mM $MgCl_2$ solution, 0.25 µL of 5unit/mL Amplitaq gold, 5 µL of GeneAmp 10 × PCR buffer, 4.0 µl of 2.5 mM of dNTP mix, 1.0 µL of 50 µM (upstream and downstream) primers (Reg A and MJV12), 0.5 µL of 50 nM random hexamers, 0.5 µL of 20 units/µL RNAse inhibitor, and 0.5 µL of 50 units/ µL RT per sample. A 10µl volume of purified RNA template was used in a total reaction volume of 50µl. The reverse transcriptase reaction mixture was placed in an Applied Biosystems Gene Amp PCR System 9700 thermacycler (Roche Molecular Systems Inc. Branchburg, NJ). Thermal cycling conditions were as follows: reverse transcription of viral RNA for 60 minutes at 42˚C; activation of Taq polymerase for 15 minutes at 95˚C; 40 cycles: 30 seconds at 94˚C, 30 seconds at 50˚C, 30 seconds at 72˚C; and final extension for 10 minutes at 72˚C.

Semi-nested Polymerase Chain Reaction

Semi-nested PCR was performed using: 5.0 µL of 10 x PCR gold buffer, 5.0 µL of 2.5mM MgCl, 4.0 µL of 2.4 mM dNTP, 1.0 µL of 50mM of internal primer MP 290 and primer Reg A, 0.25 µL of 5 units/ml of Amplitaq Gold, and 2µl volume of PCR product from RT-PCR in a total reaction volume of 50µl. The reaction mixture was placed in an Applied Biosystems Gene Amp PCR System 9700 thermacycler (Roche Molecular Systems Inc. Branchburg, NJ). Thermal cycling conditions were as follows: 5 minutes at 95°C, 40 cycles of: 30 seconds at 94°C,

30 seconds at 49°C, 30 seconds at 72°C, and a final elongation step for 10 minutes at 72°C.

Rnase-free water negative controls and known positive norovirus controls were done concurrently with the unknown samples. The semi-nested PCR product was visualized using ethidium bromide stained 2% agarose gel run in 0.5X TBE buffer. An Alpha Imager 2000 (Alpha Innotech Company, San Leandro, CA) was used to visualize the resulting product bands.

Sequences of norovirus positive samples were compared to known sequences in the National Center for Biotechnology Information's nucleotide-nucleotide BLAST database for confirmation of positive samples as human norovirus. All amplicons reported as norovirus positive were confirmed as human noroviruses by sequencing.

IV. RESULTS

Epidemiological Findings

From December 1 to December 4, the illness affected a total of 30 individuals (75% attack rate), figure 1 below shows the epidemiological curve. There were 40 participants at the banquet but two were excluded because they could not be located for the interview; 38 individuals were interviewed for a response rate of

95%. Figure 2 shows the flow chart of study participation. The median age of the outbreak participants was 51 years (range 13-64 years) and 55% were male.

Figure 1: Epidemiological curve of the outbreak

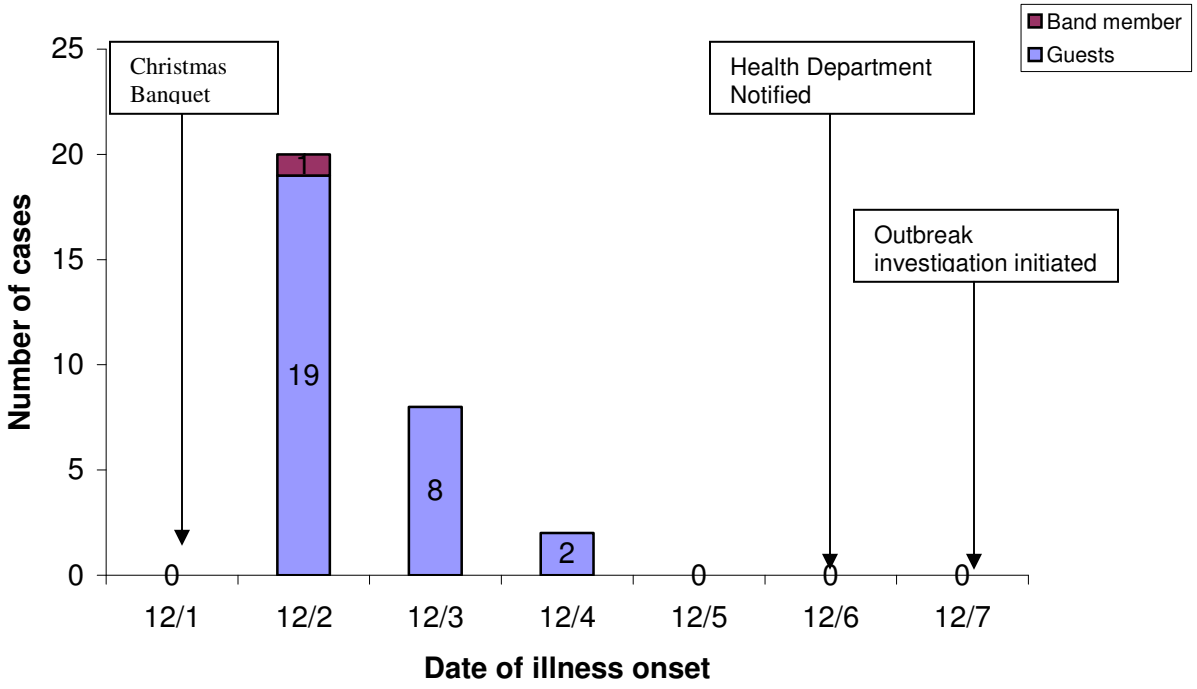
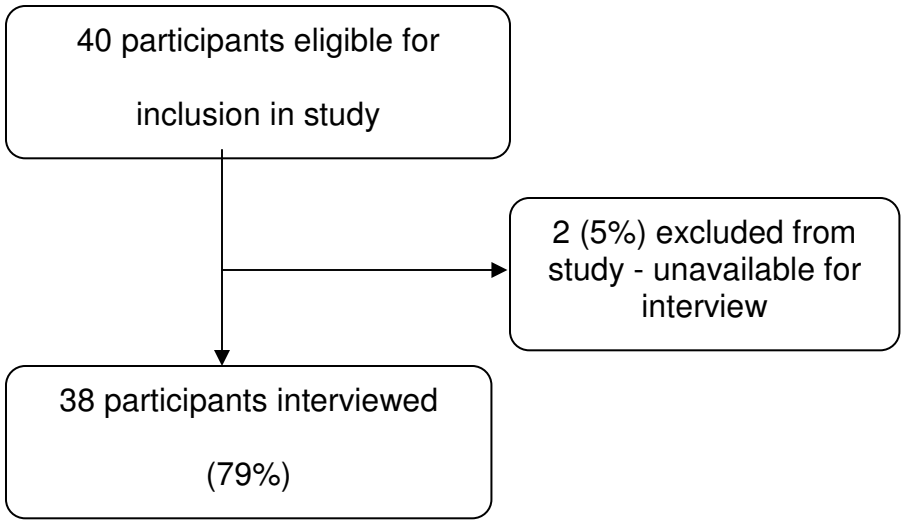


Figure 2: Flow chart of study participation



The median time from the Christmas dinner to onset of symptoms was 33 hours (range: 8-55.5 hours). Out of the 30 cases reported, 10 (33%) were women and 20 (67%) were men. The median age for the cases was 50 years (range 4-64 years). Most cases reported diarrhea (80%), vomiting (73%), nausea (67%), and abdominal pains (63%) (Table 1). Of the 30 cases, 23 (77%) had recovered at the time of completing the questionnaire. Although one case required medical attention, no fatalities occurred during the outbreak.

Table 1: Demographics of individuals reporting illness

Gender	**Total (%)**
Male	20 (67)
Female	10 (33)
Symptoms	
Diarrhea	24 (80)
Vomiting	22 (73)
Nausea	20 (67)
Abdominal pains or cramps	19 (63)
Sweats/Chills	18 (60)
Muscle aches/malaise	17 (57)
Headache	14 (47)
Weakness	13 (43)
Backache	11 (37)
Fever	10 (33)

Based on 30 participants reporting illness

When the questionnaires were completed, preliminary results seemed to implicate the chicken piccata and the mashed potatoes as possible causes of the outbreak. Univariate analysis of all the surveys showed no statistically significant (*P*-value < 0.05) association of illness with the consumption of food items, including the chicken piccata (OR = 4.0, *P*-value =0.09, 95% CI: 0.55-27.89) and the mashed potatoes (Table 2). This suggests a fomite driven infection route for

this norovirus outbreak. The wide 95% confidence interval observed with the chicken piccata might be due to a small sample size.

Table 2: Food consumed during the banquet and attack rates

	Ate/drank				Did not eat/drink						
	Ill (a)	Well (b)	Total (a+b)	Attack rate %	Ill (c)	Well (d)	Total (c+d)	Attack rate %	Attack rate difference	Odds Ratio	P-value
Fresh vegetables	15	4	19	79	15	4	19	79	0	1.00	1.00
Fresh fruits	11	5	16	69	19	3	22	86	-18	0.35	0.18
New York strip	22	7	29	76	8	1	9	89	-13	0.39	0.40
Chicken piccata	24	4	28	86	6	4	10	60	26	4.00	0.09
Orange roughy	16	5	21	76	14	3	17	82	-6	0.69	0.64
Potatoes	27	6	33	82	3	2	5	60	22	3.00	0.26
Steamed vegetables	18	5	23	78	12	3	15	80	-2	0.90	0.90
Roll & butter	15	4	19	79	15	4	19	79	0	1.00	1.00
Chocolate cake	12	0	12	100	18	8	26	69	31		
Cheese cake	18	3	21	86	12	5	17	71	15	2.50	0.26
Pumpkin pie	9	1	10	90	21	7	28	75	15	3.00	0.32
Ranch dressing	14	5	19	74	16	3	19	84	-11	0.53	0.43
Pesto dressing	2	0	2	100	28	8	36	78	22		
Fresh greens	20	5	25	80	10	3	13	77	3	1.20	0.83

Environmental Findings

Of the fomite samples collected on December 20, norovirus was detected on 12% (n = 4) of the samples. The surfaces that tested positive included the public banquet room door handles, the ice machine handles in the kitchen, the main walk-in cooler door handle, and the men's restroom lavatory handles. We did not conduct genotyping for norovirus positive fomite samples.

Three out of the four (75%) stool samples of ill persons were positive for norovirus. All three cases whose samples were sequenced were infected with the same genogroup I (GI.4) strain of norovirus.

V. DISCUSSION

Noroviruses represent a major cause of acute non-bacterial gastroenteritis outbreaks (18-21). This investigation describes an outbreak of norovirus illness among attendees of a Christmas banquet at a restaurant. Illness was not associated with the consumption of any food item served during the banquet, although the specific items could not be tested. A single sequence type of norovirus (GI.4) was identified in the stool specimen of three attendees. No statistically significant association (*P*-value < 0.05) was observed between illness symptoms and consumption of the various food items served during the banquet.

Fomites samples taken from the banquet service areas proved positive for norovirus. The fomites that tested positive were the banquet public doors, the kitchen ice machine handles, the main kitchen walk in handle, and the employee men's restroom lavatory handles.

We speculate that norovirus was spread by a non-symptomatic food handler, more specifically a male server. Despite the verbal reports that no employees reported any illnesses before, during, or after the event, we hypothesize that an asymptomatic male server contaminated serving items such as spoons, forks and knives that, in turn, contaminated a wide range of banquet food items.

This outbreak of norovirus during a banquet at a restaurant shows how easily contaminated food handlers can cause large outbreaks of gastroenteritis due to lack of knowledge. Although we could not pinpoint the ill server, we are fairly certain that the outbreak was due to an asymptomatic ill food handler. Based upon those interviewed, the Christmas party dinner on the evening of December 1, 2006, was the only common exposure for those who were ill. During the environmental investigation, the preparation procedure of all the buffet food was discussed as well as food temperatures, staff hygiene, and sanitizing procedures. The investigation revealed no apparent problems.

Our study has some limitations. A major limitation is the fact that environmental samples were collected on December 20, 19 days after the Christmas banquet, which might explain the low number of norovirus positive surfaces sampled (12%). We were also unable to test the food items served during the banquet for norovirus. Because the employees did not mention being ill before, during, or after the event, we neglected to collect stools specimen from them. Collecting and analyzing stool specimen from the employees might have helped us identify the asymptomatic shedder. The banquet took place on December 1, and the banquet attendees completed the questionnaires on December 6, which may introduce recall bias; participants who experienced an episode of illness during the outbreak period would be more likely, than those who did not get sick, to correctly recall the type of food they ate (differential recall). In turn, this might affect the internal validity of our study by inflating risk estimates, and may lead to a spurious inference of an association between a food item and norovirus illness; this might be the case of the chicken piccata (OR=4.0, *P*-value=0.09).

The small sample size (n = 38) in this study brings forward a power issue. The odds ratio (OR = 4.00, *P*-value = 0.09, 95% CI: 0.55-27.89) observed for the chicken piccata might be due to our small sample size. The probability of exposure (eating chicken piccata) among participants who became ill (P1) is 0.8 (24/30), and the probability of exposure (eating chicken piccata) among those who did not become ill (P2) is 0.5 (4/8). If we assume a 1:1 ratio of ill participants

to non ill participants, in order to have an OR = 4 be statistically significant, we would need a total sample size of 74 participants to observe an 80% power. In fact we might be observing a type II error; incorrectly stating that eating chicken piccata is not associated with norovirus illness.

The occurrence of this outbreak underscores the importance of preventing norovirus outbreaks in restaurant settings. On February 2, 2007, the Food and Beverage Director at the restaurant that hosted the Christmas banquet was notified of the swab sample results. The local health department advised the restaurant management on proper cleaning procedure to be adopted in order to avoid future norovirus illness outbreaks; 1) Disinfect lavatory handles, food equipment handles and door knobs with a chlorine solution containing 1 part bleach to 8 parts water (33); 2) emphasize the importance to food staff of reporting all illnesses and adequate and proper hand washing; 3) remind food handlers to wear disposable gloves or use utensils when handling ready-to-eat food. Employees should also be advised that they might be asymptomatic carrier of norovirus, in which case they can unknowingly shed and spread norovirus. Our results call attention to the ease with which asymptomatic persons can unwillingly infect several other people, especially in a restaurant setting.

DECLARATION OF INTEREST

None.

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APPENDIX D: HUMAN SUBJECTS APPROVAL

THE UNIVERSITY OF ARIZONA.

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Protection Program

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16 April 2008

Sonia Fankem, M.P.H. Student
Advisor: Charles Gerba, Ph.D.
Mel and Enid Zuckerman
College of Public Health
Drachman Hall, Room 206CC
PO Box 245033

RE: QUANTITATIVE MICROBIAL RISK ASSESSMENT: THE ROLE OF FOMITES IN THE TRANSMISSION OF NOROVIRUS

Dear Ms. Fankem:

We received documents concerning your above cited project. Regulations published by the U.S. Department of Health and Human Services [45 CFR Part 46.101(b) (4)] exempt this type of research from review by our Institutional Review Board.

Exempt status is granted with the understanding that no further changes or additions will be made to the procedures followed (copies of which we have on file) without the review and approval of the Institutional Review Board. Any research related physical or psychological harm to any subject must also be reported to the appropriate committee.

Thank you for informing us of your work. If you have any questions concerning the above, please contact this office.

Sincerely,

Rebecca Dahl, R.N., Ph.D.
Director
Human Subjects Protection Program

cc: Departmental/College Review Committee

APPENDIX E: STUDY QUESTIONNAIRES

Confidential Illness Report Form

Name: _____ DOB: _____ Date Illness Onset: __/___/___

Address: _____ □ Male □ Female Time Illness Onset: ___:00 □ AM □ PM

Phone: _____ □ Resident □ Staff □ Other Location Illness Onset: _____

Date Recovered by: ___/__/__ Time: _____:00 □ AM □ PM

Which of the following symptoms did the individual experience?

Y N

□ □ Nausea

□ □ Vomiting

...If YES, check the appropriate number of vomiting episodes per day (at maximum):

□ 1-2 □3-5 □ ≥ 6

□ □ Diarrhea (loose or unformed bowel movement)

...If YES, diarrhea lasted ____ days

...If YES, check appropriate number of diarrhea stools per day (at maximum):

□ 1-2 □3-5 □ ≥ 6

...If YES, was there any blood in the stool?

Y N

□ □ Fever

□ □ ...If YES, was temperature taken? ___ °F

□ □ Sweats/Chills

□ □ Abdominal Pain or Cramps

□ □ Severe weakness

□ □ Headache

□ □ Backache

□ □ Muscle aches

□ □ Other (specify) _____

□ □ Does this individual have any chronic medical conditions that could mimic the symptoms experienced (e.g., irritable bowel syndrome, inflammatory bowel disease, or stomach ulcers?)

Does the individual attribute alcohol ingestion as a likely explanation for his/her symptoms?

Did the individual take any medications for the illness (e.g., antibiotics, anti-diarrheal medications)?

Food History: _____

Were you seen by a physician? YES □ NO □ Name: _____ Number: _____

Did a physician take samples? YES □ NO □ If so, what? _____

Agency: _____ Form Completed by: _____

HOUSEBOAT INTERVIEW FORM

Name: _____Date of Interview: _____

Age: _____ Sex: ____ M _____ F Number of people on boat: _____

Trip: _____ 5/9/04 _____ 5/16/04 _____ 5/23/04 Ill: _____ Yes _____No

Symptoms: _____ Vomiting
_____ Diarrhea # stools/24 hour period _____
_____ Nausea
_____ Other _____
_____ Other _____
_____ Other _____

Onset: _____
Duration: _____
Have you seen a doctor: ____Y _____N

Exposures:
_____ Other people ill _____
_____ Food Preparation _____
_____ Cleaning _____
_____ Lake Water _____

Other tasks done:

Comments:

APPENDIX F: LIST OF ACRONYMS

AGE = Acute Gastrointestinal Enteritis

BLAST = Basic Local Alignment Search Tool

cDNA = Complementary DNA

CITI = Collaborative Institutional Training Initiative

CPE = Cytopathic effect

CRFK = Crandell Feline Kidney cells

C*t* = Concentration and time of exposure

DNA = Deoxyribonucleic acid

dNTP = Deoxynucleotide triphosphate

EPA = Environmental Protection Agency

FCV = Feline calicivirus

HSPP = Human Subjects Protection Program

mRNA = Messenger RNA

NCBI = National Center for Biotechnology Information

NLVs = Norwalk like viruses

ORF = Open reading frame

PCR = Polymerase chain reaction

pH = Hydrogen potential

PPM = Part per million

PRC = Plate recovery control

QAC = Quaternary ammonium compound

QMRA = Quantitative microbial risk assessment

RNA = Ribonucleic acid

Room Temp = Room temperature

RT-PCR = Reverse transcriptase polymerase chain reaction

SRSVs = Small round structured viruses

VP = Viral protein

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