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The Indicators of Norovirus and Hepatitis A Virus
In Six Environmental Samples Types:
A Systematic Review

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Master of Public Health

Global Health

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University of California, Los Angeles
2010

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An abstract of
A Thesis Submitted to the Faculty of the
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Abstract

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Norovirus and Hepatitis A Virus (HAV) are the two most prevalent enteric viruses that cause gastroenteritis in the US and globally. To test for these enteric viruses indicators, organisms developed assess fecal contamination, and are used in place of directly testing for the viruses themselves. The goal of this study was to investigate which indicators were most prevalent in each environmental sample of the agriculture environment, including water, faeces, food, soil, hands, and surfaces. This systematic review examines, from US and global sources, a comprehensive list of indicators used to test for enteric viruses. The results were organized based on environmental sample and indicator group, including bacterial and viral. A total of 297 articles were included in the database of results. Out of these, 67 were included for final review. 98 viral indicators and 92 bacterial indicators were found in at least 49 articles regarding Norovirus. 37 viral indicators and 27 bacterial indicators were found in at least six articles discussing HAV. The most prevalent indicator for Norovirus in water was *E. coli* and the most prevalent indicator of HAV in food was also *E. coli*. *E. coli* and Adenovirus were the most strongly connected with Norovirus and HAV. This systematic review demonstrates which indicators would be the most prevalent for assisting with identifying enteric viruses in the agricultural environment.

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CHAPTER I: LITERATURE REVIEW

Introduction

US and Global Burden of Norovirus and Hepatitis A Virus

Human Norovirus and Hepatitis A Virus (HAV) heavily burden both developed and developing nations. Norovirus is the most common cause of gastroenteritis in the world and the United States (Ahmed et al., 2014). Global norovirus prevalence in worldwide cases of acute gastroenteritis is estimated at 18%. Prevalence is higher in cases of acute gastroenteritis in community settings by 24%, outpatient settings by 20%, and inpatient settings by 17% (Ahmed et al., 2014). Norovirus has the most sporadic cases and outbreaks of acute gastroenteritis across all age groups (Ahmed et al., 2014).

Hepatitis A, caused by the Hepatitis A Virus (HAV), is the most common form of acute viral hepatitis (Franco, Meleleo, Serino, Sorbara, & Zaratti, 2012). It was the most frequently reported type of hepatitis in the US until 2004 (Centers for Disease & Prevention, 2011). Whereas after 2004, the US incidence of acute viral Hepatitis A decreased from 3.77 cases/100,00 in 2001 to 0.45 cases/100,000 in 2011 (Collier, Tong, & Xu, 2015). Globally, at least 1.5 million cases of Hepatitis A occur annually (Franco et al., 2012). The goal of this literature review is to detail the epidemiology of Norovirus and HAV, describe six transmission vehicles (stool, water, hands, produce, soil, and equipment surfaces) and ideal indicator, and advocate for new ways of detecting Noroviruses and HAV. Below is further information on both the clinical disease and epidemiology of Norovirus and HAV.

Clinical Disease and Epidemiology of Norovirus and HAV

Clinical Disease

Norovirus takes 24-48 hours for incubation and 12-72 hours for occurrence of symptoms. Infection with the disease causes a serious bout of gastroenteritis, where symptoms usually resolve within a few days (Karst, 2010). Symptoms include vomiting and diarrhea and possible co-occurrence of nausea and abdominal cramps. More severe symptoms may occur to patients who are young, elderly, or with existing co-morbidities (reviewed in Robilotti, Deresinski, & Pinsky, 2015).

On the other hand, HAV takes approximately 28 days for incubation and usually has an abrupt onset of fever, malaise, anorexia, nausea, abdominal discomfort, dark urine, and jaundice (Centers for Disease & Prevention, 2011). The disease ranges from a mild illness lasting 1-2 weeks, to a severely disabling disease lasting several months (reviewed in Heyman, 2004).

Epidemiology

Norovirus has multiple transmission routes and outbreak settings. It is transmitted through exposure to contaminated food or water, person-to-person contact, aerosolized vomitus particles, and fomites. 63%-73% of all associated events occur during winter months (reviewed in Aron J. Hall et al., 2013). Outbreaks occur in semi-closed settings including: nursing homes, schools, hospitals, cruise ships, disaster relief and evacuation sites, and military areas (Karst, 2010). The transmission of Norovirus takes a low infectious dose of 18 virus particles to infect a person (Teunis et al., 2008). With each additional dose, a higher probability of acute gastroenteritis occurs.

Similarly, HAV also has multiple transmission routes that can cause sporadic events. HAV is transmitted through the fecal-oral route by direct contact with an HAV-infected person or by ingestion of contaminated food or water (reviewed in Acheson & Fiore, 2004). Transmission occurs mostly during the high HAV infectivity period after the incubation period half-point, which then continues for a few days after onset of jaundice. In industrialized countries, transmission is most common among household and sexual contacts of acute cases (reviewed in Heyman, 2004). Transmission also occurs sporadically in daycare centers with diapered children and among travelers who travel to endemic countries, drug injection users, and men who have sex with men. Outbreaks occur in community-wide settings with the contamination of food due to food handlers and contaminated produce (reviewed in Heyman, 2004). In less developed countries, HAV infection is very common in the first years of life, with seroprevalence rates approaching 100% (Franco et al., 2012). Transmission also occurs in large community-wide epidemics. Lastly, the minimum infectious dose for HAV in humans is unknown (reviewed in Acheson & Fiore, 2004).

Mortality and Morbidity Associated with Norovirus and HAV

Norovirus and HAV Mortality and Morbidity in the US and the World

Each year in the US, Norovirus is estimated to cause an average of 570-800 deaths, 56,000-71,000 hospitalizations, 400,000 emergency department visits, 1.7-1.9 million outpatient visits, and 19-21 million total illnesses (reviewed in Aron J. Hall et al., 2013). Norovirus-associated deaths affect older populations (at least 65 years of age) with 90% of deaths per year. The US had increases of Norovirus-associated events by 50% or less during 2002-2003 and 2006-2007 when pandemic strains emerged (reviewed in Aron J. Hall et al., 2013). Norovirus is increasingly recognized as an important cause of gastroenteritis in the world. It makes up 14% of acute gastroenteritis cases in high-mortality countries (Ahmed et al., 2014). Noroviruses could cause up to 1.1 million hospitalizations and 218,00 deaths each year in children in developing countries (Patel et al., 2008).

Equally important is HAV, though its prevalence has slowly decreased. HAV infections have declined 87% from 2001 to 2011 (Klevens, Liu, Roberts, Jiles, & Holmberg, 2014). During 1999, 44% of Hepatitis A deaths were related to chronic liver disease, increasing to 67% during 2004 (Vogt, Wise, Bell, & Finelli, 2008). 33% of the general US population has serological evidence of prior HAV infection (reviewed in Heyman, 2004). Given that the US and other developed countries have low endemicity, infection rates are very low but Hepatitis A outbreaks associated with contaminated food or water remain a major public health problem in these industrialized countries (Franco et al., 2012). In the developing world, poor sanitation and hygiene contributes to endemic HAV infection where most people become infected in early childhood. Asymptomatic infection occurs at early age, causing the reported rates of the disease to be relatively low and outbreaks to be rare. However, the seroprevalence of HAV antibodies is high in such countries (Franco et al., 2012). In short, developing countries lowered the incidence and burden of HAV but differences of prevalence occur between lower and upper socio-economic classes. Endemicity also has reduced from high to intermediate or low levels, depending on sanitation and socio-economic conditions of the country (Hendrickx et al., 2008). HAV infection causes acute liver failure and death from a rare 0.2% of clinical cases, where the risk increases with age and presence of chronic liver disease (Franco et al., 2012).

Serotypes and Geographic Distribution

Of the five genogroups of Noroviruses, three (GI, GII, GIV) are primarily found in humans. The genogroups are further divided into clusters or genotypes, and then strains are named after the locations where they were first identified. For example, the Farmington Hills strain from Michigan was typed as GII.4. This strain and its variants have caused many pandemics in the US and possibly account for 70-80% of all Norovirus outbreaks at least since 2002 (reviewed in Karst, 2010). Any GII.4 strains are more likely to be associated with person-to-person transmission, especially in long-term-care facilities and hospital settings (reviewed in Robilotti et al., 2015).

Only a single serotype or variation of HAV is in humans. This is because of structural constraints in the capsid that prevents an emergence of a new serotype (Pérez-Sautu et al., 2011). Of the six strains of HAV identified in their genetic material or genotypes, three (I, II, III) have been associated with human disease. The three human genotypes are divided into subtypes A and B. Worldwide, genotype I is most prevalent and IA is reported more frequently than IB. Subgenotype IIIA was prevalent in Central Asia and subgenotype IA was reported in US and Western Europe more often, although all types have been reported in both developed areas. For example, subgenotype IIA strands isolated in France were imported from travelers who had been to West Africa (Desbois et al., 2010).

The Six Transmission Vehicles of Norovirus and HAV

Norovirus and HAV, referred in this section together as enteric viruses, are found in the agricultural environment through a hypothesized, by the author, six various environmental samples. These environmental samples are chosen because of evidence that the enteric viruses are primarily transmitted through them (Calder et al., 2003; Croci, De Medici, Scalfaro, Fiore, & Toti, 2002; D'Souza et al., 2006; Fallahi & Mattison, 2011; reviewed in /Aron J Hall et al., 2012; León-Félix, Martínez-Bustillos, Báez-Sañudo, Peraza-Garay, & Chaidez, 2010). The enteric viruses are transmitted primarily through 1) stool and secondarily through 2) water, 3) hands, 4) soil, 5) produce, and 6) equipment surfaces. This section will hone in on those six transmission vehicles. The main transmission vehicle is stool and transmission occurs through the fecal-oral route or when infected stool contaminates the other transmission vehicles (Franco et al., 2012; reviewed in /Aron J. Hall et al., 2013). Waterborne transmission of enteric viruses occurs when contaminated water was used for irrigation or washing produce (Brassard, Gagné, Généreux, & Côté, 2012; DiCaprio, Ma, Purgianto, Hughes, & Li, 2012). Hand transmission can occur when hands or fingertips are pressed against a surface or food and transfer the viruses (S. Bidawid, Farber, & Sattar, 2000; León-Félix et al., 2010; M. C. Mattioli, Davis, Mrisho, & Boehm, 2015; Mia Catharine Mattioli, Pickering, Gilsdorf, Davis, & Boehm, 2012; Mbithi, Springthorpe, Boulet, & Sattar, 1992). Soil transmission of viruses occurs when any type of virally contaminated soil transmits viruses to produce or other transmission vehicles (Parashar, Khalkar, & Arankalle, 2011; Pickering et al., 2012). For the purposes of this literature review, soil is defined as any unconsolidated mineral or organic material on the ground that serves to encourage produce growth (Soils). This may include natural soil along riverbeds and biosolids, which are organic material produced from leftover wastewater treatment (Agency, 2016a). Produce also operates as a transmission vehicle, occurring at any point from farm-to-table when produce contaminates hands, soil, water, and equipment surfaces (Aron J Hall et al., 2012). And finally, fomites or infected surfaces such as steel or wood can transfer viruses to other transmission vehicles (Bae, Park, Kim, Oh, & Ha, 2014; Croci et al., 2002; Kim, Park, Bae, Oh, & Ha, 2014).

Stool

Enteric viruses are primarily transmitted through stool that ends up in water, on hands, and in soil. Various strains of Norovirus can be shed or excreted in stool from 5 to 17 days or more during clinical illness. Thus, the virus has many opportunities for direct or indirect contamination through the six transmission vehicles (Kirby, Shi, Montes, Lichtenstein, & Moe, 2014). Similarly, HAV can be shed or excreted in the stool during clinical illness or for as long as 3 to 11 months, where it has many opportunities to be transferred to other transmission vehicles (reviewed in Cuthbert, 2001). Faeces are the primary transmission vehicle as it spreads easily to other vehicles or direct person-to-person contact. Focusing on the secondary transmission passages, the next paragraphs review water, hands, soil, produce and equipment surfaces transmission vehicles.

Water

Contamination of water, then spread to other vehicles or direct human contamination, by Norovirus and HAV may occur in irrigation waters used for agriculture purposes. In Silverman‘s article, 16 out of 20 water samples were positive for Norovirus. The irrigation water, from rivers and streams contaminated by effluent from treatment plants, was used for agricultural purposes without prior treatment. The author suggests treatment of the irrigation water before use for agriculture. In a critical review by Blumenthal and Peasey, three studies mentioned water capable of spreading diseases to the community (Blumenthal & Peasey, 2002). The first study suggested that increases in infectious hepatitis and other diseases of the kibbutz population were due to living near a sprinkler-irrigated field. The second study found double excess risk of clinical 'enteric' disease in children who live within 600-1000m of sprinkler-irrigated fields. However, the third study found that there was no effect of exposure to the sprinkler irrigation. These studies suggest that contaminated irrigation water can be a transmission vehicle for enteric viruses.

For the enteric viruses in water to transfer to other vehicles, they must be able to survive in water until the time of transfer. In a study by Seitz, well water was purposely inoculated with Norovirus. Human Norovirus may be infectious for at least 61 days in groundwater and remains detectable in groundwater for over 3 years (Seitz et al., 2011). HAV can persist in water for 3 to 10 months (Tallon, Love, Moore, & Sobsey, 2008). The long survival rates of enteric viruses mean that the viruses have many opportunities to contaminate several transmission vehicles or directly infect from person-to-person. Such transmission can occur when handling produce as well.

Hands

Contamination of enteric viruses by hand transmission can occur when humans touch, pick, or pack food. For example, in one study, Schmid et al. reported that a grocery butcher, the hypothesized first case of an outbreak, had poor hand hygiene while handling raw meats and other produce. He did not wear gloves while preparing food for the 11-15 days he had infectious HAV. The butcher also did not wash his hands between serving food items and working on raw meat (Schmid et al., 2009). In another study, Leon-Felix swabbed workers', including pickers, classifiers, and packers, hands for viruses before beginning field activities. Fruit classifiers and packers already had Norovirus present on their hands. After the activities were done, hands were swabbed again and Norovirus was present for all workers (León-Félix, Martínez-Bustillos, Báez-Sañudo, Peraza-Garay, & Chaidez, 2010).

Hands can transfer enteric viruses to other hands, specifically the fingerpads, and to surfaces or foods and vice versa. For example, Bidawid's 2004 study evaluated, a surrogate for Norovirus, Feline Calicivirus (FCV) transfer from inoculated fingerpads to foods and steel, and then vice versa (S Bidawid, Malik, Adegbunrin, Sattar, & Farber, 2004). In another example, the transfer of HAV on fingerpads to pieces of fresh lettuce can be recovered from fingerpads purposely infected with the virus (S. Bidawid, Farber, & Sattar, 2000). HAV transfer with added friction, pressure, can transfer viruses between fingerpads and steel disks and vice versa, and can transfer viruses between different fingerpads (Mbithi, Springthorpe, Boulet, & Sattar, 1992). These lab studies demonstrate that the transmission of HAV was possible through hand contact with different surfaces, surfaces to hands, and fingers to fingers where transfer efficiencies are influenced by surface type, pressure, and friction. In the agricultural setting, such transmission of HAV can lead to contamination of produce or equipment surfaces.

Soil

Soil can be contaminated and become a vehicle of enteric viruses if the water was not treated. No articles were found with soil contaminated with Norovirus. One article was found with soil contaminated with HAV. Parashar's study tested both lab setting and environmental setting soils. 403 soil samples were collected at Mutha River, Pune, India and control samples were collected at a well-protected Pashan Lake with no sewage contamination (Parashar, Khalkar, & Arankalle, 2011). 77 out of 403 (19.1%) river samples collected were positive for HAV and all control samples tested negative for HAV. This suggests that sewage discharge was causing a high prevalence of HAV. Similarly, wastewater or untreated water use for irrigation could contaminate soil used for agriculture, leading to produce absorption of viruses via soil.

Produce

Produce can become a transmission vehicle when infected with enteric viruses. Calder et al investigated raw blueberries that caused an HAV outbreak in New Zealand. Cases' stool were collected and tested for HAV, where 5 out of 9 stool specimens were positive for the virus (Calder et al., 2003). HAV was also detected on 3 out of 6 samples of stored frozen blueberries from the coolstore. In a farm to fork risk assessment in Ghana, none of the produce samples from farms and markets were positive for Norovirus (Antwi-Agyei et al., 2015).

Surfaces

While a direct and conclusive investigation of equipment contaminated with Norovirus and HAV could not be found, the following article suggests that there were such occurrences with HAV. Frank et al. investigated a HAV outbreak occurring with tourists who were traveling in Egypt (Frank et al., 2007). The investigation showed a significant association of consuming local orange juice to those who developed Hepatitis A. The authors surmised that the juice was contaminated during the manufacturing process, either by an infected worker or contact of fruit or machinery with sewage-contaminated water. These examples suggest that transfer of viruses was possible by equipment surfaces though it was difficult to determine the contamination source or the specific genotype.

Difficulties in Detecting Enteric Viruses

Detecting the exact source or specific genotype of enteric viruses was difficult in environmental settings. For example, Brassard detected high concentrations of Norovirus on strawberries that were irrigated by river water (Brassard et al., 2012). However, the tested river water had low or no detected concentration of the virus. In another example, Makaray tested all kitchen objects that may have transferred Norovirus during an outbreak in a Finnish canteen (Makary et al., 2009). However, no virus was revealed in all of the tested objects. Makaray surmised that the lack of positive results were due to less optimal sampling, issues with prolonged food storage, and delayed laboratory investigations. The supported hypothesis was that the outbreak was caused by food previously contaminated before arrival at the canteens. There was also difficulty in identifying the exact genotype of a virus. In conclusion, the findings in environmental section suggest that alternative ways must be developed for detecting enteric viruses contamination through the various transmission vehicles before produce enters the consumer market.

Indicators and the Need for Standardized Proxy Indicators

Shortcomings of Laboratory and Testing Methods

The previous section described that enteric viruses were difficult to detect especially from the environmental settings. Indicators are another way to test for microbial and viral presence and could be an alternative to direct testing. Indicators can be used in all six transmission vehicles (faeces, water, produce, hands, soil, and surfaces); however, current literature focuses on indicators used on water or hands and hand rinses for the most part (Mia Catharine Mattioli, Pickering, Gilsdorf, Davis, & Boehm, 2012).

There are several difficulties in testing for Norovirus because the virus is not cultivable in lab settings, has no cell culture system, and no animal model (reviewed in Aron J Hall et al., 2011). Instead, surrogate viruses such as Murine Norovirus-1 (MNV-1) or Feline Norovirus have been proposed. Caleb notes the issues with surrogate viruses and highlighted the "need for a concerted effort towards the development of rapid, simple and reproducible techniques for the detection of foodborne viral particles" (Caleb, Mahajan, Al-Said, & Opara, 2013). As for using MNV-1, Bidawid noted Seitz's 2011 study where "MNV genomes were not significantly reduced for up to 42 days, suggesting that genomic detection is not a reliable indicator of viability" (S. Bidawid, 2013). For testing HAV, Croci observed that RT-nested PCR could determine if the virus was on produce but the HAV detected by the test was not necessarily infectious. Croci recommended using cell cultures for detecting infectious HAV and the consequent risk to consumers (Croci, De Medici, Scalfaro, Fiore, & Toti, 2002). Therefore indicators are another option and are needed to investigate viruses in environmental settings.

Overview of Ideal Indicators for Detecting Enteric Viruses

Indicator organisms, such as bacteria or viruses, were developed to assess fecal contamination in water (Gerba, 2015). Water indicators are used to indicate the possibility of other present pathogenic microorganisms in water (Gerba, 2015). Criteria for an ideal indicator organism for indicating water quality are described in *Table 1*. However, no such indicator fits all the criteria. Criteria and guidelines are used as guidance indicating that a potential water quality issue exists.

With an ideal indicator organism identified, the methods of testing for indicators should be specific, where the ability to measure the target indicator organism is done in an unbiased manner. The concern is for false positives, where a confounding organism reacts similarly in the test and gives an incorrect result (Pathogens, 2004). *Table 2* describes the desirable attributes of methods for testing for indicators.

Ashbolt identified three groups of microbial indicators: 1) general (process) microbial indicators, 2) faecal indicators such as *E. coli*, 3) index organisms and model organisms (Ashbolt, Grabow, & Snozzi, 2001). Process indicators are a group of organisms that demonstrates the efficacy of a process. Faecal indicators are a group of organisms that indicate or infer the presence of faecal contamination. Index and model organisms are a group or species indicative of pathogen presence and behavior, respectively. Given the multiple types of indicators, it was important to know which types exist for potential usage. The next section details viral, total, coliform, coliform bacteria, fecal coliform, phages, and other indicators.

Types of Indicators and Current Use with Enteric Viruses

Currently, there are many types of indicators used for detecting enteric viruses. Below is a list and description of the different groups including viral, coliform, fecal streptococci, bacteriophage, and other indicators.

Viral Indicators

Viral indicators can be other viruses used to indicate the presence of Norovirus and HAV. An example would be adenovirus, with its stability and commonality, mostly human origins, and usefulness as a molecular index of the presence of human viruses (Pina, Puig, Lucena, Jofre, & Girones, 1998).

Total Coliforms, Coliform Bacteria, and Fecal Coliforms

The coliform group included *Escherichia*, *Citrobacter*, *Enterobacter*, and *Klebsiella* species (Gerba, 2015). Coliform bacteria normally occur in intestines of all warm-blooded species, becoming present in stool, where it could contaminate other transmission vehicles, such as water. Coliform bacteria are proportional to the degree of fecal pollution in untreated wastewater. Coliform group presence indicates that the water is unsafe to drink and that other microorganisms may also be present. Absence of coliforms in 100 ml of drinking water means there should not be bacterial waterborne disease outbreaks. Unfortunately, coliform bacteria have several deficiencies. Coliforms can re-grow in aquatic environments and distribution systems, can be suppressed by high background bacterial growth, is not indicative of a health threat, and has no relationship between enteric protozoan and viral concentration. Fecal coliforms specifically include the genera Escherichia and Klebsiella and have some of the same limitations as coliform bacteria.

Fecal Streptococci

Fecal Streptococci have been suggested as possible indicators for the presence of enteric viruses (Gerba, 2015). They include the genera Enterococcus and Streptococcus and have some advantages over coliform and fecal coliform bacteria. Fecal streptococci rarely multiply on water, are more resistant to environmental stress and chlorination than coliforms, and usually persist longer in the environment. They are also relatively high in numbers of excreta of humans and other warm-blooded animals and are present in wastewaters and known polluted waters (Ashbolt et al., 2001). Fecal streptococci and enterocci are absent in pure waters, virgin soils, and environments having no contact with human and animal life.

Bacteriophage

Bacteriophage or bacterial viruses are constantly present in sewage and polluted waters and have been suggested as indicators of viral pollution (Gerba, 2015). The structure, morphology, size, and aquatic behavior of bacteriophage resemble those of enteric viruses. Somatic coliphage, and F-specific RNA coliphage are two groups of useful indicators. Bacteriophage that infect *B. fragilis* appear to be of human origin only but the host anaerobic bacterium is tedious and complicated to detect. Coliphages are phages to coliforms and were used to model human enteric viruses (Ashbolt et al., 2001). Use of phages in water environments however, has been inconsistent because of the survival and behavior of phages in different water environments, and inconsistency of techniques used to recover and detect phages. Negative results of phages have occurred even when enteric viruses were detected on 500 ml samples of water.

Other Indicators

This section includes other possible indicators to test for enteric viruses. Clostridium perfringens is a sulfite-reducing anaerobic spore former; it has been suggested as an indicator of removal of viruses during drinking water and wastewater treatment (Gerba, 2015). Foodborne outbreaks have occurred with *Aeromonas hydrophilia* and it is considered an opportunistic pathogen in humans. Another set of possible indicators is aerobic and facultatively anaerobic bacteria. The two types of bacteria exist in water and derive their carbon and energy from organic compounds and include *Pseudomonas*, *Aeromonas*, *Klebsiella*, *Flavobacterium*, *Enterobacter*, *Citrobacter*, *Serratia*, *Acinetobacter*, *Proteus*, *Alcaligenes*, *Enterobacter* and *Moraxella*. These are assessed through a heterotrophic plate count (HPC) of untreated drinking water and chlorinated distribution water. These bacteria are commonly isolated from surface and groundwater and are widespread in soil and vegetation, including raw vegetables (Gerba, 2015). Given the number of possible indicators used to detect enteric viruses, there is a need to standardize testing for these indicators across the six transmission vehicles (stool, water, hands, produce, soil, and surfaces). Doing so would help prevent transmission and alert the public from gastroenteritis illnesses before consumption.

Goals and Aims

Given the challenges discussed about testing directly for enteric viruses, it is important to move towards using indicators as a standard of testing. The purpose of this thesis is to assess which type of indicators of Norovirus and HAV are most prevalent and significantly associated in stool, water, hands, soil, produce, and equipment surfaces found in environmental settings. Three aims are implemented to help determine the best indicators. First, is to determine the appropriate proxy indicators of the enteric viruses in environmental settings. Second, with the appropriate indicators, is to describe the associations, presence or statistical measure, of each indicator on the environmental sample. Third, with the information concluded, is to determine which indicators are most relevant and frequently used in the six transmission vehicles found in the six environmental settings.

Conclusion

From this literature review, enteric viruses are present in environmental settings and it is important to test for the viruses before agricultural products enter into the public.

Prevention of enteric diseases should be a priority instead of having a passive monitoring of the diseases, thereby achieving consumer protection and preventing losses from agricultural companies and employees. Being able to test for indicators in each environmental setting is important for prevention of Norovirus and HAV. Preventing these enteric viruses from contaminating the six transmission vehicles can reduce the incidence and morbidity, prevent outbreaks, and save agricultural expenses.

CHAPTER II: METHODS

Protocol and Registration

There is currently no systematic review on proxy indicators of Norovirus and HAV. The most similar ones found were related to waterborne microbial indicators. For example, Wade et al.'s "Do U.S. Environmental Protection Agency Water Quality Guidelines for Recreational Waters Prevent Gastrointestinal Illness? A Systematic Review and Meta-analysis" quantified the association between microbial indicators of recreational water quality and gastrointestinal (GI) illness (Wade, Pai, Eisenberg, & Colford Jr, 2003). The review also evaluated the likelihood for GI illness below current guidelines and looked at 976 potentially relevant studies, accepting only 27 (epidemiologic) studies. This systematic review study did not require review by the Institutional Review Board (IRB) as the research did not involve human subjects and did not use any identifiable personal data.

Criteria for Norovirus and HAV Indicators Database

Research Protocol Objective

The objective for the research protocol was to create a database of indicators of Norovirus and HAV using the best available practices for literature searching and data inclusion. The data collected was analyzed to identify indicators that have or do not have a relationship with Norovirus, HAV, or both. The end results would be a flow diagram, table of results, discussion of findings and conclusion.

Possible Outcomes and Additional Information

Possible outcomes include whether there a relationship or no relationship exists between the indicator and Norovirus, HAV, or both. Additional information included which virus or viruses corresponded with the indicator, such as is it absent or present when the indicator itself is absent or detected. Additionally, the transmission vehicle that the indicator is tested in was included.

Search Criteria for Systematic Review

The search for articles documenting relationships between indicators, Norovirus and HAV had several criteria. There were no limits on publication year, limits set on the English language, and an initial search through title and abstract, then followed by a full text search. Key search terms included Norovirus, Hepatitis A, a list of indicators, and the six transmission vehicles: virus OR viral OR Adenovirus OR "bacterial indicator" OR Coliforms OR Escherichia* OR Citrobacter* OR Enterobacter* OR Klebsiella* OR "Fecal Streptococci" OR coliphage*$ OR bacteriophage*$ OR "Somatic coliphage" OR "F-specific RNA coliphage" OR "Clostridium perfringens" OR "Aeromonas hydrophila" OR "Aerobic bacteria" OR HPC OR SPC OR "Standard Plate counts" OR APC OR "Aerobic Plate Counts" OR Faeces* OR water OR Hands* OR Soil* OR Food* OR Produce* OR Surfaces* (indicator$ (norovirus* OR hepatitis a)). The article search was repeated once to make sure relatively same results showed up during each search.

Article Inclusion and Exclusion Criteria

Articles included the words Norovirus, HAV or both, and an indicator in the title, abstract, or text. Noroviruses are a diverse group of non-enveloped, single strand RNA virus classified as a calicivirus, Norwalk-like, or small round structured virus where genotypes II and I are responsible for the majority of gastroenteritis in humans. HAV is a small non-enveloped, single strand RNA virus classified as a member of the Hepatovirus genus of the family Picornaviridae where humans are the only natural host. An indicator is an organism that indicates the presence of fecal contamination or the presence of a pathogen. Inclusion and exclusion criteria are listed in *Figure 1*.

Information Extracted from Articles into Database

Information on the following was recorded when available: Citation information, type of indicator, type of virus, presence or absence of a relationship between indicator and virus, assessment of the relationship by statistical means or simple presence, transmission vehicle, and other comments.

Study Selection

As shown in *Figure 1*, 297 abstracts or titles were reviewed for relevance. 68 of the total 297 seemed relevant and were selected for further review. Of these, 67 were included in the final review. Of the 230 excluded studies, they were removed based on the following criteria. Studies were not on topic because they only used surrogate Noroviruses or Hepatitis viruses. The title or abstract did not mention indicators. The title or abstract talks about laboratory methods only. The transmission vehicle was artificially inoculated. The samples in the article were beach water instead of agricultural water.

CHAPTER III: RESULTS

Description of Dataset

The articles found mostly ranged in the recent years of 2011 to the present, followed by the years 2000-2005, and then 2006-2010. Most of the articles found were studied in Europe, followed by North America, Asia and Pacific, Africa and the Middle East, and South America. Most articles were about Norovirus, then both Norovirus and HAV, and finally about HAV. Of the types of relationships between enteric viruses and indicators, there was either a relationship measured by simple presence, by a statistical measure, or no relationship between the virus and indicators as shown in *Tables 3* and *4*. These types of relationships can be measured by simple presence, which is just the identified enteric viruses and indicators together, or, by a statistical measure, such as Pearson's correlation. Overall, Norovirus had 54 existing relationships with indicators, of which 37 are measured by simple presence and 17 measured by statistical measure (Data not shown). Norovirus also had ten non-existent relationships with indicators, of which nine were measured by simple presence and one was measured by statistical measure. HAV had 12 total existing relationships with indicators, of which eight were measured by simple presence and four were measured by statistical measures. HAV also had eleven non-existent relationships with indicators, of which ten where measured by simple presence and one measured by statistical measure. The categories of transmission vehicles and their frequencies (number of articles) were as follows: faeces (n=9), food (n=18), hand rinse sample (n=1), hands(n=1), soil (n=3), surfaces (n=2), and water (n= 41) (Data not shown) .

Indicators of Norovirus in each Environmental Sample and Bacterial or Viral Group

It was important to assess which indicators of Norovirus were most prevalent and significantly associated in each environmental sample and each indicator group. Doing so would demonstrate which indicators of Norovirus were the most researched and how they are distributed in the literature**.** From the database of results, each indicator was separated by whether a relationship exists between Norovirus and the indicator. Then the indicator was grouped by its environmental sample according to each article. The indicators were also grouped in either viral or bacterial categories. The systematic review produced a total of 26 different indicators. For simplicity purposes, that number was reduced to the top ten most frequent indicators found in the literature. The results in *Table 3* suggested that *E. coli* was the most frequent indicator of all the indicators and the most frequent that showed up in water. Also, *E. coli* was the most prevalent bacteria and Adenovirus was the most prevalent virus. In conclusion, *E. coli* and Adenovirus were the most frequently discussed indicators of Norovirus in the systematic review.

Indicators of HAV in each Environmental Sample and Bacterial or Viral Group

Additionally, it was also important to see which indicators of HAV were the most prevalent and significantly associated in each environmental sample group and each indicator group. Doing so would demonstrate which indicators of HAV were the most researched and how they are distributed in the literature. From the database of results, each indicator was grouped with its environmental sample according to each article, and further separated by whether a relationship existed between HAV and the indicator. The indicators were also grouped in either viral or bacterial categories. The total number of indicators for HAV was also reduced to the top ten most frequently found in the literature. The results in *Table 4* suggested Adenovirus as the most frequent indicator, which was in the environmental sample of water. Adenovirus was the most common viral indicators and *E. coli* was the most common bacterial indicator.

In conclusion, Adenovirus, and *E. coli* were the most frequently discussed indicators of HAV in the literature.

Indicator Relationships Found for The Environmental Samples

In *Table 5 and 6*, indicator relationships with statistical measures were found in some of the articles regarding the environmental samples of food and water, hands, soil, and surfaces. *Table 5* shows that the most frequent statistical measures were found in food and water articles about Norovirus. Most articles on water had statistical measures that were either tables showing relationships between indicator and virus levels, Spearman rank order correlations, or Pearson correlation. The effect sizes of water and food articles were mostly from the Spearman rank correlations and Pearson correlations The 10 most commonly used indicators in water articles were Adenovirus, F+ coliphages, *E. coli*, Fecal coliforms, or Somatic coliphages (Table 3, 6). Several food articles had statistical measures of logistic regression, Pearson chi-squared test, multiple logistic regression, or figures of indicator versus virus levels. The most used indicators in food articles were Adenovirus, *E. coli*, or F+ coliphages (Table 3, 6). There were 11 articles regarding Norovirus, two regarding HAV, and two regarding Norovirus and HAV (Table 6).

No Indicator Relationships Found for the Environmental Samples

In *Table 7*, no indicator relationships were described in the articles regarding the environmental samples of water, food, and hand rinse sample. Most articles on water had used either simple presence or statistical measures of correlation co-efficient, Spearman rank order correlation, or Pearson correlation coefficient to find that there was no relationship between the indicator and viruses. The most used indicators in water articles were *E. coli*, F+ coliphages, rotavirus, or enteroviruses. All the articles mostly used effect sizes of correlations. Most articles on food had multiple logistic regression, X^2 test, Pearson chi-square test, or Pearson correlation co-efficient. The most used indicators in food articles were *E. coli*, F+ coliphages, and Adenovirus. The single hand rinse article used Pearson correlation coefficient for its statistical measure. The indicators used by the hand rinse article were *E. coli* and enterococci. There were 5 articles about Norovirus, 1 article about HAV, and 1 article about Norovirus and HAV.

Articles with Simple Presence and Statistical Measures and or Effect Sizes

It was important to see which indicators of Norovirus and HAV had simple presence versus statistical measures and effect sizes. Doing so would show how many viral indicators were in each category for reference. Indicators were split up into categories or groups. Then they were filtered in either presence or statistical measure categories. In *Table 5*, Norovirus wass in water with 26 articles using simple presence and nine articles using statistical measures as a measurement. HAV was in food with four articles using simple presence and two articles using statistical measures. The most frequent articles with statistical measures were food and water articles about Norovirus.

Description of the Associations

Norovirus and the indicator

While most articles found in the systematic review demonstrated a simple presence of Norovirus and indicators, below were some examples of associations between Norovirus and the indicators found in the systematic review. Some examples were found below and in *Table 6 and 7.* Haramoto used the correlation co-efficient to look at Norovirus and indicator bacteria (E. Haramoto et al., 2006). Several articles used the Pearson correlation coefficient or Pearson chi-squared two-sided test of presence of Norovirus and indicators (J. Y. Kim, H. Lee, J. E. Lee, M. S. Chung, & G. P. Ko, 2013; Lee et al., 2011; M. C. Mattioli et al., 2015). Horman used proportions of surface water samples positive for indicators and pathogens at various levels of *E. coli* or thermotolerant coliforms in samples and a Spearman rank order correlation between indicators and pathogens (Horman et al., 2004). Several articles used the Spearman rank order correlation coefficient matrix between Norovirus and indicators (Kishida et al., 2012; Poma et al., 2012). Some articles showed a relationship between the indicators and Norovirus, with one using frequency distribution (M. Myrmel et al., 2004; Masoud Yavarmanesh, Absar Alum, & Morteza Abbaszadegan, 2015). Few articles included figures showing indicator concentration compared with presence of viruses or showing positive samples of indicators and viruses (Philip & Pay, 2013; Silverman, Akrong, Amoah, Drechsel, & Nelson, 2013; Tryland et al., 2014). An article used linear regression test to analyze the relationship between levels of viruses and densities of bacterial indicators (He, Wei, Cheng, Zhang, & Wang, 2012). Two articles used a logistic regression of indicators to enteric viruses or of indicators to the presence of positive virus samples (Formiga-Cruz et al., 2003; Rodriguez-Manzano et al., 2013). An article used the multiple logistic regression analysis to describe a relationship between Norovirus and indicators (Hernroth, Conden-Hansson, Rehnstam-Holm, Girones, & Allard, 2002). Some articles had tables showing indicator levels with respect to the presence of Norovirus or indicators predicting the absence or presence of Norovirus (Ebdon, Sellwood, Shore, & Taylor, 2012; Locas, Barthe, Barbeau, Carriere, & Payment, 2007). One article used the X^2 test for presence or absence at detectable levels of Norovirus and indicator organism and tested for independence (Lowther et al., 2008).

HAV and the Indicator

Compared to the Norovirus results, HAV had less results and variety of statistical measures used because of a smaller sample size. Baggi compared the Somatic and F+ coliphages load with measurements of HAV by RT-PCR and nPCR (Baggi, Demarta, & Peduzzi, 2001). Two articles used logistic regression models of indicators to HAV (Formiga-Cruz et al., 2003; Muniain-Mujika, Calvo, Lucena, & Girones, 2003). Most articles found in the systematic review demonstrated a simple presence of HAV and indicators.

Approvals and Disapprovals of Using Indicators

It was important to quantify which articles demonstrated approvals and disapprovals of using indicators with enteric viruses, as doing so would show how many articles fall into each category. An approval meant that the authors agreed that using indicators was a benefit. A disapproval meant that the authors did not agree on using indicators as it provided no benefit. All articles were sorted into three categories and the results were as such. 10 (15%) articles mentioned approvals, 10 (15%) articles mentioned disapprovals, and 47 (70%) mentioned neither approvals nor disapprovals.

Several articles suggested using direct testing of the viruses or multiple indicators instead of one indicator. One example of combining direct testing with the use of multiple indicators was to test shellfish on a routine basis by direct testing of Norovirus or a combination of F+ coliphages and human adenovirus (Myrmel, Berg, Rimstad, & Grinde, 2004). Another example of direct testing was in Jurzik et al.'s findings, in which they concluded that the ideal indication was provided by the viral pathogen itself (Jurzik, Hamza, Puchert, Uberla, & Wilhelm, 2010). Another example of using multiple indicators to test was Norman et al. who recommend using more than one indicator to monitor pathogen presence (Norman et al., 2013). Montazeri concluded that direct monitoring of infectious pathogens was still better than monitoring of bacterial indicators (Montazeri et al., 2015).

Several studies concluded that more research needed to be done in order to see the effectiveness of using indicators to detect enteric viruses. One example of further research was in an article where the simple presence of adenovirus and Norovirus were both found in biosolids (Wong, Onan, & Xagoraraki, 2010). The authors surmised that more studies needed to be done because of the occurrence of Norovirus in various types of biosolids (Wong et al., 2010). Another example of further research needed was Gentry-Shields et al. who noted that enteric viruses were rarely detected, signifying a need for improving methods for concentration of viruses from environmental samples (Gentry-Shields, Rowny, & Stewart, 2012). Another example of further research was Haramoto et al. who found that more studies were needed to see if F+ coliphages could be used as an indicator of virus reduction at different wastewater treatment plants (Haramoto, Fujino, & Otagiri, 2015). In another example, further study needed before validating possible correlation between viral indicator at high concentration and human noroviruses (Yavarmanesh, Alum, & Abbaszadegan, 2015). In another example, even though Lowther et al. found that F+ coliphages to be an effective risk management tool for norovirus, they underscored their finding that Norovirus was still detected even with low levels of F+ coliphages at <30 PFU/100 g flesh (Lowther et al., 2008). In the last example, Muniain-Mujika et al. concluded that while HAV, adenovirus, and enterovirus were founded together, more data needed to be collected in geographically diverse areas (Muniain-Mujika et al., 2003).

Chapter IV: Discussion

The goal of this study was to see which indicators of Norovirus and HAV were the most prevalent and significantly associated in each environmental sample and indicator group. The systematic review found twenty-six indicators of enteric viruses that were reduced to ten for simplicity purposes. Included information was the type of association between the indicator and the enteric viruses. *E. coli* is the most prevalent bacterial indicator for both Norovirus and HAV. Adenovirus is the most prevalent viral indicator for both Norovirus and HAV.

E. coli and Adenovirus were the most prevalent bacterial and viral indicators for Norovirus and HAV. One hypothesis to explain this result is that *E. coli* is one of the oldest bacteria used as an indicator (Ashbolt et al., 2001). The evidence to support this hypothesis is that *E. coli* has been selected as an indicator for water since the 1890s (Edberg, Rice, Karlin, & Allen, 2000). Since it is the oldest bacteria used as an indicator, the EPA also used *E. coli* as an indicator of water quality and that may also influence the usage of indicators and therefore influence the literature results (Agency, 2016b). A second hypothesis is that *E. coli* is a popular choice as an indicator. The evidence to support is that that biologically, *E. coli* is shed from the intestines of both humans and animals; thus its presence co-occurs with Norovirus and HAV and that makes it a popular indicator (Russell & Jarvis, 2001).

A hypothesis to support the results that Adenovirus is a prevalent indicator is that Adenovirus is ubiquitous in the environment (Osuolale & Okoh, 2015). The evidence to support this hypothesis is that the virus is highly specific to humans and can persist wherever the environment is polluted by human faeces or sewage (Osuolale & Okoh, 2015). Adenovirus was detected in 64% of samples in Osuolale's study, similar to other studies that reported Adenovirus in equal or greater than 50% of wastewater and environmental water samples. Adenovirus is recommended to use by Osuolale and Okoh as an indicator of viral contamination because it is reportedly more stable in the environment and occurs more often than other enteric viruses (Osuolale & Okoh, 2015).

Limitations and Strengths

This systematic review has a number of limitations. The literature search was conducted on only one database, PubMed. Searches on other databases such as Web of Science, WorldCat, or Google Scholar provided too many results to sift through. Additionally, the database search was conducted by the thesis author alone and was not checked by others.

Despite these limitations, several strengths were found in this systematic review. The inclusion and exclusion criteria were very broad, allowing a range of studies to be included. With the included articles, this systematic review reported the types of indicators and statistical measures used to validate the relationships between indicator and enteric viruses. The findings show the viewpoints of many authors and their results regarding the usage of appropriate indicators to identify the presence of enteric viruses.

Implications

It was shown that *E. coli* was a strongly connected with Norovirus and HAV and a popular indicator and so continued usage of *E. coli* as an indicator in the future is most likely to follow suit in all the environmental samples. Adenovirus was the most common indicator for HAV. It causes gastroenteritis, conjunctivitis, and respiratory diseases

(Osuolale & Okoh, 2015). Given that adenovirus co-occurred the most frequently with HAV, using it as an indicator could be useful in the future on the six environmental samples. Given that there was little to no pattern in the type of indicators used in the six environmental samples, future research should include additional research on indicators and environmental samples and on any type of relations between the two topics. The lack of results on HAV and indicators shows a possible publication bias or inconclusive results. Future research, such as another systematic review, on the relationship between HAV and indicators is needed to demonstrate if there is more articles on the topic, a possible publication bias, or inconclusive results.

Conclusions

The systematic review of indicators of Norovirus and HAV demonstrated varying usage of bacteria and viruses as indicators in the six environmental samples. The most used environmental sample was water, followed by food, faeces, soil, surfaces, and then hands. The most prevalent indicator of Norovirus seemed to be *E. coli* and the most prevalent indicator of HAV seemed to be Adenovirus. The most discussed indicators with statistical measures were Adenovirus and *E. coli*. As this systematic review relied on only one database for its literature collection, future studies should use multiple sources of information for their research. Future studies should also further the research on whether the identified indicators can help in reducing transmission of enteric viruses to environmental samples in the agricultural field.

PUBLIC HEALTH IMPLICATIONS

- *E. coli* is a strongly connected with Norovirus and HAV and is a popular indicator
  - Continued usage of *E. coli* as an indicator in the future is most likely to follow suit in all the environmental samples.
- Adenovirus was the most common indicator for HAV
  - Causes gastroenteritis, conjunctivitis, and respiratory diseases (Osuolale & Okoh, 2015).
  - Co-occurred the most with HAV:
    - Using adenovirus as an indicator could be useful in the future on the six environmental samples.
- Little to no pattern in the type of indicators used in the six environmental samples
  - Future research should include additional research on indicators and environmental samples regarding any types of patterns between the two
- The lack of results on HAV and indicators shows a possible publication bias or inconclusive results
  - Future research, such as another systematic review, on the relationship between HAV and indicators is needed to demonstrate if there is more articles on the topic, a possible publication bias, or inconclusive results

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Tables and Figures

Table 1: Criteria for an Ideal Indicator Organism

- The organism should be useful for all types of water
- The organism should be present whenever enteric pathogens are present
- The organism should have a reasonably longer survival time than the hardiest enteric pathogen
- The organism should not grow in water
- The testing method should be easy to perform
- The density of the indicator organism should have some direct relationship to the degree of fecal pollution
- The organism should be a member of the intestinal microflora of warm-blooded animals (Gerba, 2015)

Table 2: Criteria for Desirable Attributes of Methods for Testing for Indicators

- Specificity to desired target organism
  - Independent of matrix effects
- Broad applicability
- Precision
- Adequate sensitivity
- Rapidity of results
- Quantifiable
- Measures viability or infectivity
- Logistical feasibility
  - Training and personnel requirements
  - Utility in field
  - Cost
  - Volume requirements

Figure 1: Flow Chart of Systematic Review

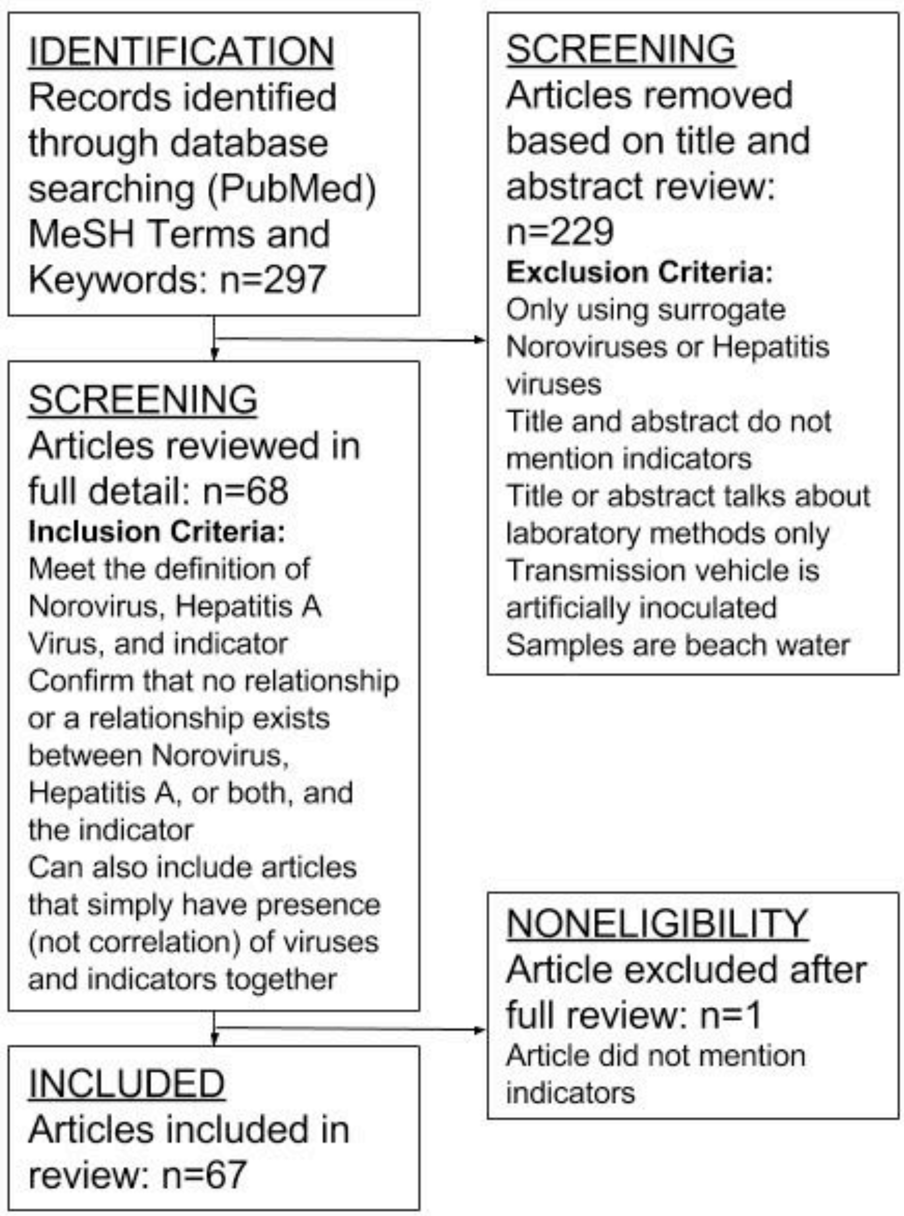

Table 3: Indicators of Norovirus in Six Transmission Vehicles and Indicator Groups (Number of articles found in systematic review)

	Adenovirus		Enterovirus		F+ coliphages		Rotavirus		Somatic Coliphages		**Viral Total**	*E. coli*		Enterococci		Fecal Coliform		Salmonella		Total Coliform		**Bacterial Total**
	R	NR	R	NR	R	NR	R	NR	R	NR		R	NR	R	NR	R	NR	R	NR	R	NR	
Faeces	5	0	0	0	0	0	7	1	0	0	**13**	2	0	0	0	0	0	3	1	0	0	**6**
Food	6	0	3	0	7	0	0	0	2	0	**18**	8	1	1	0	1	0	2	0	0	0	**13**
Hand rinse sample	0	0	0	0	0	0	0	0	0	0	**0**	1	0	1	0	0	0	0	0	0	0	**2**
Hands	1	0	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	**0**
Soil	1	0	1	1	0	0	0	1	1	0	**5**	1	1	1	2	0	0	0	1	0	0	**6**
Surfaces	1	0	0	1	0	0	0	1	0	0	**3**	0	1	0	1	0	0	0	0	0	0	**2**
Water	15	2	16	1	12	1	12	2	10	0	**71**	23	1	6	0	4	1	4	0	11	0	**50**
Total	**29**	**2**	**20**	**3**	**19**	**1**	**19**	**5**	**13**	**0**	**111**	**35**	**4**	**9**	**3**	**5**	**1**	**9**	**2**	**11**	**0**	**79**

Table 4: Indicators of HAV in Six Transmission Vehicles and Indicator Groups (Number of articles found in systematic review)

	Adenovirus		Enterovirus		F+ coliphages		Rotavirus		Somatic Coliphage		**Viral Total**	*E. coli*		Enterococci		Fecal Coliform		Salmonella		Total Coliform		**Bacterial Total**
	R	NR	R	NR	R	NR	R	NR	R	NR		R	NR	R	NR	R	NR	R	NR	R	NR	
Faeces	0	0	0	0	0	0	0	0	0	0	**0**	0	0	0	0	0	0	0	0	0	0	**0**
Food	3	1	3	0	4	0	0	0	3	0	**14**	4	0	0	0	1	0	2	0	1	0	**8**
Hand rinse sample	0	0	0	0	0	0	0	0	0	0	**0**	0	0	0	0	0	0	0	0	0	0	**0**
Hands	1	0	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	**0**
Soil	0	1	0	0	0	0	0	0	0	1	**2**	0	1	0	1	0	0	0	0	0	0	**2**
Surfaces	0	0	0	0	0	0	0	0	0	0	**0**	0	0	0	0	0	0	0	0	0	0	**0**
Water	3	4	2	4	3	2	1	5	3	1	**28**	1	2	1	1	1	1	0	0	1	1	**9**
Total	**7**	**6**	**5**	**4**	**7**	**2**	**1**	**5**	**6**	**2**	**45**	**5**	**3**	**1**	**2**	**2**	**1**	**2**	**0**	**2**	**1**	**19**

Table 5: Number of Articles with Simple Presence and Statistical Measures of All Indicators of Norovirus and HAV

	Faeces		Food		Hands		Hand Rinse Sample		Soil		Surfaces		Water		Bacterial		Viral	
	NoV	HAV	NoV	HAV	NoV	HAV	NoV	HAV	NoV	HAV	NoV	HAV	NoV	HAV	NoV	HAV	NoV	HAV
Presence	7	0	5	4	1	1	1	0	3	0	1	1	26	3	86	11	110	16
Statistical Measures	0	0	7	2	0	0	1	0	0	0	0	0	9	2	22	2	27	11
Total	7	0	12	6	1	1	2	0	3	0	1	1	35	5	108	13	137	27

Table 6: Indicator Relationships Found for the Environmental Samples

Environmental Sample	Statistical Measure	Effect Size	Indicators	Virus	Author
Water	Somatic and F-specific phages load (log10 (PFU/mL)) (logarithim) compared with measurements of viruses by RT-PCR and nPCR	None	F+ specific coliphages, Somatic coliphages	HAV	(Baggi et al., 2001)
Water	Table showing relationship between Phage and FIB Levels with Respect to Presence of HAdV and/or NoV	None	Adenovirus, Enterococci, Fecal Coliforms	Norovirus	(Ebdon, Sellwood, Shore, & Taylor, 2012)

Water	Figure 1 of *E. coli* concentation (CFU/100 ml) compared with presence of Norovirus, Adenovirus, and Bacteroidales. Figure 2 of positive samples of Adenovirus and Norovirus. Table 1 of Adenovirus, Norovirus and Bacteroidales listed in order of decreasing E. coli concentration within each category	None	Adenovirus, *E. coli*	Norovirus	(Silverman, Akrong, Amoah, Drechsel, & Nelson, 2013)
Water	Spearman rank order correlation coefficient	r = 0.20	*E. coli*	Norovirus	(Horman et al., 2004)

Water	Pearson correlation	F+ coliphages r = 0.58	F+ coliphages, Fecal coliphages, Total coliphages	Norovirus	(J. Y. Kim, H. Lee, J. E. Lee, M. S. Chung, & G. P. Ko, 2013)
Water	Spearman's rank order correlation	NoV GI: $r_s = 0.73$ NoV GII: $r_s = 0.47$	Adenovirus	Norovirus	(Kishida, Morita, Haramoto, Asami, & Akiba, 2012)

Water	1) Table comparison of indicators used to predict absence or presence of enteric viruses, 2) Table showing sensitivity, specificity, and positive and negative predictive values of the microbiologic parameters measured	None	*E. coli*, Enterococci, F+ coliphages, Somatic coliphages, Total coliforms	Norovirus	(Locas, Barthe, Barbeau, Carriere, & Payment, 2007)

Water	Significant Spearman rank correlations	Adenovirus: dry and wet seasons r_s = 0.65 and 0.69 Enterovirus: dry and wet seasons r_s = 0.84 and 0.78 total and fecal coliforms: r_s = 0.58 and 0.61 in wet season	Adenovirus, Enterovirus, Fecal coliforms, Total coliforms,	Norovirus	(Poma, Gutierrez Cacciabue, Garce, Gonzo, & Rajal, 2012)
Food	X^2 test	None	*E. coli*, F+ coliphages	Norovirus	(Lowther, Henshilwood, & Lees, 2008)
Food	Logistic regression	*E. coli* sample size 33: p = 0.1998 Total coliforms sample size 33: p = 0.1988, sample size 39: p = 0.1985	Adenovirus, *E. coli*, Enterovirus, F+ coliphages, Somatic coliforms, Total coliforms	HAV	(Muniain-Mujika et al., 2003)
Food	Pearson chi-squared (two-sided) test	F+ coliphages: P = <0.01	Adenovirus, F+ coliphages	Norovirus	(Myrmel et al., 2004)

Food	Logistic regression	None	Adenovirus, Enterovirus, F+ coliphages, somiatic coliphages	Norovirus and HAV	(Formiga-Cruz et al., 2003)
Food	Multiple logistic regression analysis	None	E. coli, F+ coliphages, Somatic coliphages	Norovirus and HAV	(Hernroth et al., 2002)
Food	Logistic regression	Fisher exact test, Adenovirus P = 0.007	Adenovirus, E. coli	Norovirus	(Rodriguez-Manzano et al., 2013)
Food	Figure of E. coli vs Norovirus	*E. coli*: $R^2 = 0.65$	E. coli, Enterococci	Norovirus	(Tryland, Myrmel, Ostensvik, Wennberg, & Robertson, 2014)

Table 7: No Indicator Relationships Found for the Environmental Samples

Environmental Sample	Statistical Measure	Effect Size	Indicators	Virus	Author
Water	Correlation Co-efficient	NoV GI: Total coliform r = -0.04, *E. coli* r = 0.08, F+ coliphages r = 0.44 NoV GII: Total coliform r = -0.29, E. coli r = 0.06, F+ coliphages r = 0.23	*E. coli,* F+ coliphages, total coliform	Norovirus	(Haramoto et al., 2015)
Water	Presence	None	F+ coliphages, Rotavirus	Norovirus	(Wilkes et al., 2013)

Water	1) Proportions of surface water samples positive for indicators and pathogens at various levels of *E. coli* in samples, 2) Proportions of surface water samples positive for indicators and pathogens at various levels of thermotolerant coliforms in samples, 3) Analysis of significant ($P < 0.05$) bivariate Spearman rank order correlation coefficients with two-tailed P values and ORs with 95% confidence intervals for correlation between various indicator parameters and pathogens	None	*E. coli*, Fecal Coliforms, F+ coliphages	Norovirus	(Horman et al., 2004)

Water	Presence	None	Adenovirus, Enterovirus, Fecal coliforms, F+ coliphages, Somatic coliphages	HAV	(Jiang & Chu, 2004)
Water	Presence	None	Enterovirus, F+ coliphages, Rotavirus, Somatic coliphages, Total coliforms	Norovirus	(Jung et al., 2011)
Water	Pearson Correlation Co-efficient	*E. coli*: r = 0.048, F+ coliphages: r = 0.073, Somatic coliphages: r = 0.046, Total coliforms: r = 0..043,	*E. coli*, Enterovirus, F+ coliphages, Somatic coliphages, Total Coliform	Norovirus	(Lee et al., 2011)
Food	Multiple Logistic Regression	None	Enteroviruses, *E. coli*, F+ coliphages, Somatic Coliphages	Norovirus and HAV	(Hernroth et al., 2002)

Food	X^2 test	None	*E. coli*, F+ coliphages	Norovirus	(Lowther et al., 2008)
Food	Pearson chi-squared (two-sided) test	Adenovirus: P = 0.076	Adenovirus, F+ coliphages	Norovirus	(Myrmel et al., 2004)
Food	Presence	None	Adenovirus, E. coli, Salmonella	Norovirus	(Serracca et al., 2010)
Food	Correlation between *E. coli* and Norovirus using frequency distribution; frequency analysis using different concentrations of F+ coliphage	None	*E. coli,* Enterovirus, F+ colliphages	Norovirus	(Yavarmanesh et al., 2015)
Hand rinse sample	Pearson Correlation Coefficient	None	*E. coli* and Enterococci	Norovirus	(M. C. Mattioli, Davis, Mrisho, & Boehm, 2015)

Appendix

More Information on Indicator Relationships Found

Most articles (47), found no mention of the relationship between Norovirus, HAV, or both and the indicator. These articles looked at other issues that went over testing of the virus and indicator but did not discuss the relationship between the two. Referring to *Table 6*, several articles (11) found that a relationship existed between enteric viruses and different kinds of indicators. Some results were found regarding adenovirus. For example, Rodriguez-Manzano concluded that adenovirus might be used as an indicator of viral pollution of shellfish. While the logistic regression did not show a direct quantitative correlation of adenovirus and Norovirus, an absent or low level of adenovirus may predict a low risk for pathogens like Norovirus (Rodriguez-Manzano et al., 2013). Kishida found a strong positive Spearman's rank order correlation between adenovirus and Norovirus GI (Kishida et al., 2012). With Norovirus GII, the r_s was at a weaker positive value. Poma et al. found several results regarding adenovirus, total coliforms, fecal coliforms, and enterovirus in river water (Poma, Gutierrez Cacciabue, Garce, Gonzo, & Rajal, 2012). Many results were found regarding F+ coliphages. Henroth et al. concluded that while F+ coliphages could be a reliable indicator for enteroviruses, E. coli was a better organism to use, as it was uncertain if F+ coliphages would be safer (Hernroth et al., 2002). Using a logistic regression of F+ coliphages to Norovirus, Formiga-Cruz et al. found that F+ coliphages where significantly related to Norovirus, providing a strong predictive capability for the virus (Formiga-Cruz et al., 2003). Yet in the same study, it was found that F+ coliphages had very weak predictive capability for HAV. Using a Pearson correlation, Norovirus was positively correlated with F+ coliphages (J. Y. Kim, H. Lee, J. E. Lee, M.-S. Chung, & G. P. Ko, 2013). Additionally, total coliform, fecal coliform and F+ coliphages were positively correlated with levels of other enteric viruses. Increasing levels of F+ coliphages corresponded with increasing levels of Norovirus. E. coli does occur with Norovirus, but it doesn't have the same corresponding increasing level relationship as F+ coliphages with Norovirus (Lowther, Henshilwood, & Lees, 2008). Lowther et al. found a strong association of correlation between F+coliphages and Norovirus. Myrmel et al. found a significant correlation between Norovirus and F+ coliphages (M. Myrmel, E. M. Berg, E. Rimstad, & B. Grinde, 2004).

E. coli was also found to be an appropriate indicator for Norovirus. Tryland et al. found that correlation between E. coli and Norovirus was a moderately strong positive correlation. The correlation was also dependent on water temperature because depuration levels were lower in the winter for Norovirus (Tryland et al., 2014). In Baggi et al.'s research, no correlation was observed between burden of bacterial indicator organisms and actual bacteriophage or viral contamination. Instead, the presence of bacteriophages correlated better with the presence of enteric viruses detected by RT-PCR and nPCR, This confirms bacteriophages has a potential role as indicators of viral contamination (Baggi et al., 2001). Horman et al. found a weak monotonic relationship between Norovirus and E. coli using a Spearman rank order correlation coefficient (Horman et al., 2004).

More Information on No Indicator Relationships Found

Referring to *Table 7*, some articles (11) found that no correlation between the enteric viruses and indicators existed. With a Pearson correlation coefficient, fecal indicator bacteria (FIB) levels were not associated with Norovirus hand contamination (M. C. Mattioli et al., 2015). Additionally*, E. coli* and enterococci were not significantly different when Norovirus was detected versus not detected. Using a correlation coefficient to test for a Norovirus relationship, none of the tested bacteria were deemed reliable as an indicator (E. Haramoto et al., 2006). The relationships ranged from a weak negative relationship to a moderate positive relationship. Serracca's results do not support the hypothesis that human adenoviruses could be used as a valid indicator of viral pollution (Serracca et al., 2010). None of the fecal indicators in Lee et al.'s study were statistically correlated with Norovirus occurrence in groundwater based on a Person correlation coefficient (Lee et al., 2011). There was no apparent relationship between the occurrence of human viruses and the microbial quality of the water based on indicators even though there was presence of HAV (Jiang & Chu, 2004). With E. coli, Lowther et al.'s results showed the bacteria had no correlation with norovirus when both were present (Lowther et al., 2008). Myrmel et al. found no significant relationship between Norovirus and adenovirus (Myrmel et al., 2004).

The systematic review produced contrary evidence of using F+coliphages as an indicator. Even though Norovirus and rotavirus were found together at monitoring sites, there were no statistically significant associations found with indicator bacteria or F+ coliphages (Wilkes et al., 2013). Horman et al. looked at the proportions of water samples positive for indicators and viruses at various levels of E. coli samples and of thermotolerant coliform samples. They could not link the presence of F+ coliphages with the presence of Norovirus or other enteric pathogens, concluding that the phages were not a reliable indicator (Horman et al., 2004). In Jung's article regarding Norovirus, no relationship exists with indicator bacteria nor somatic and F+ coliphages (Jung et al., 2011). Henroth found that enteroviruses coexisted with F+ coliphages, but had no other significant results (Hernroth et al., 2002).