THE TRANSMISSION, DETECTION AND OCCURRENCE OF VIRUSES ON INDOOR ENVIROMENTAL FOMITES

by

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DEDICATION

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ABSTRACT

Viruses cause 60% of human infections and are probably the most common cause of infectious disease acquired indoors. Rapid spread of viral illness in indoor establishments facilitates disease morbidity and mortality. The goal of this dissertation is to clarify the role of fomites in the viral infection cycle. Research methods include investigation of published literature, and the use of polymerase chain reaction (PCR) for viral detection.

The Appendix A study reviewed published literature to assess the significance of fomites in the transmission of ten common respiratory and enteric viruses (rhinovirus, respiratory syncytial virus (RSV), influenza A, parainfluenza 1 (HPIV1), coronavirus, rotavirus, calicivirus, hepatitis A virus (HAV), astrovirus and adenovirus). Results suggest that fomites play an important role in the transmission of common viral pathogens, and the use of disinfectants may limit the spread of viral disease. The Appendix B study examined PCR primer detection limits by determining the time length viruses can be isolated on fomites. Results indicated that poliovirus 1 and hepatitis A virus could be detected for up to 60 days. Parainfluenza 1 virus isolation yielded detection at 30 days and 50 days. Norovirus isolation yielded detection at 20 days and 30 days. Influenza virus isolation results were inconsistent, yielding no initial detection and detection up to 20 days. Appendix C assessed the occurrence of human parainfluenza 1 virus (HPIV1) on surfaces in office settings. HPIV1 was detected on 37% of fomites. HPIV1 was detected most on desktops (47%), and least on light switches (19%). Study results indicated a statistically significant difference between positive fomites in different buildings (Chi-square $p < 0.011$), and between building cubicles and conference room

fomites (Chi-square $p < 0.011$). Appendix D evaluated the prevalence of influenza A virus on surfaces in day care and home settings. Influenza A was isolated on 23% of fall day care fomites and 53% of spring day care fomites. Influenza was isolated on 59% of home fomites sampled during March, and no influenza was detected on home fomites sampled during the summer. Overall, Influenza A virus was isolated on over 50% of fomites in homes and day care centers.

INTRODUCTION

Problem Definition

For centuries it was assumed that infectious diseases were primarily transmitted by the airborne route or through direct patient contact, and the surrounding environment played no role in disease transmission.[1,2] Ninety percent of global mortality is caused by infectious disease and viruses account for at least 60% of human infections.[3-5] Viruses are the most common cause of infectious disease acquired indoors.[5,6] The swift spread of viral disease in crowded indoor establishments including; schools, daycare facilities, nursing homes, business offices, and hospitals consistently facilitates disease morbidity and mortality.[6] Growing research evidence indicates that contaminated surfaces or fomites play a key role in the spread of viral infections.[5,6] Present laboratory, epidemiological and disinfection intervention studies are changing the perspective of viral transmission to include a more complex multifactorial model of disease spread.[1] However, the role of surfaces and inanimate objects in viral disease transmission is a debatable subject and has not been thoroughly investigated.[7]

Dissertation Format

This dissertation consists of 2 manuscripts for publication and 2 published papers as appendices. Appendix A is a review article manuscript that will also serve the function as the literature review for this dissertation. Appendix A discusses the issue of viral transmission via contaminated fomite by reviewing current published literature focusing on 10 common respiratory and gastrointestinal viruses. Both appendices A and B will be

submitted for publication during the following month in a journal yet to be determined. Appendix B investigates the detection of viruses on fomites using five different polymerase chain reaction primers and two different types of PCR reactions (semi-nested and non-nested RT-PCR). Appendices C and D are currently published in the Journal of Infection. Appendix C investigates the prevalence of human parainfluenza virus 1 on offices fomites. Appendix D investigates the occurrence of human influenza A virus on fomites in homes of infected children and in child day care facilities. Samples in all of the studies were collected by various trained individuals. However all sample processing, molecular methods, statistics and writing of manuscript was completed by me.

PRESENT STUDY

The methods, results and conclusions of this study are presented in four manuscripts appended to this dissertation. The following is a summary of the most important findings in this document.

The first manuscript or appendix A examines laboratory, epidemiology and disinfection studies for indirect evidence and data supporting the transmission of enteric (calicivirus, rotavirus, adenovirus, astrovirus, hepatitis A virus) and respiratory (influenza, parainfluenza, respiratory syncytial virus, coronavirus, rhinovirus) viruses via fomite. The review examined viral survival on fomites and hands, viral contamination of fomites and hands, hand to fomite spread of viruses and vice versa, contact of hands with portals of entry, viral disease outbreaks associated with fomite contamination, and chemical disinfection affects on viral illness. Review findings indicate that scientific data has established that all 10 common respiratory and enteric viruses can survive on fomites for extensive periods of time and that these viruses can be detected on biologically contaminated environmental surfaces, or hands of infected individuals. In addition, viral transfer from hands to surrounding surfaces is possible in 7 out of 10 of the viruses investigated. Epidemiology studies document that fomites are a potential vehicle for disease transmission in 8 viruses (HAV, RSV, norovirus, rotavirus, influenza, coronavirus, astroviruses, adenoviruses). Hygiene and disinfection intervention studies demonstrate that cleaning hands decreases respiratory and gastrointestinal illness, and the disinfection of contaminated surfaced interrupts disease spread. Generally, viral disease transmission via contaminated fomite has been established or is suspected in all 10 of the

enteric and respiratory viruses reviewed, and interrupting viral spread via contaminated fomites could be the most effective way to limit viral disease increases.

The second manuscript or appendix B investigates the sensitivity of reverse transcriptase -polymerase chain reaction (RT-PCR) as a tool in the detection of viruses on fomites. Five different primer sets where tested in semi-nested RT-PCR and non-nested RT-PCR assays. Results revealed that both Poliovirus 1 and Hepatitis A virus were detected on the nonporous surface for up to 60 days in duplicate using both semi-nested PCR and RT-PCR. Parainfluenza yielded detection at 30 days and 50 days. Norovirus also varied yielding detection at 20 days and 30 days using semi-nested PCR. There were no PCR product bands detected when using RT-PCR only for norovirus. Influenza virus results were inconsistent with no detection and detection up to 20 days during the assay replicate. In general, virus detection on fomites appeared to be dependent on the primer specificity and sensitivity.

Appendix C investigates the prevalence of human parainfluenza virus on office fomites. Three hundred twenty-eight samples including seven different fomites from 12 different office buildings in five different cities were assayed for HPIV1. Overall, 37 % of the samples tested were positive for HPIV1. The city with the highest number of positive fomites was New York with 50% and the lowest was the city of Tucson at 27%. The quantity of positive fomites per city varied within object category from 20 % (New York phone) to 66% (Atlanta phone). There was a statistically significant difference found between the occurrence of HIPV1 in Arizona buildings 1 (86%) and building 5 (14%), Chi-square analysis $p< 0.003$ and Fisher's Exact test $p = 0.0017$. This variation in

HPIV1 occurrence may reflect the pattern of disease incidence in the offices assessed. Additionally, data indicated a statistical difference between the total quantity of positive fomites found in cubicles and conference rooms, Chi-square analysis $p< 0.011$ and Fisher's Exact test $p = 0.054$. HPIV1 was detected more often on the desktops (47%), the computer mouse (46%) and the phone (45%). Virus was isolated least often on door handles (26%) and light switches (19%).

Appendix D investigated the occurrence of influenza A virus on fomites in homes and daycare centers during influenza season. Influenza A was detected on 23% of day care fomites sampled during the fall and 53% of fomites sampled during the spring. Spring and fall sample data was determined to be statistically different at the 0.05 α-level by Chi-square analysis $p< 0$ and Fisher's Exact test $p = 0.00002$. There was no statistical difference found between moist and dry fomites (Chi square $p = 0.13998$). No influenza was detected on home fomites sampled during the summer. In contrast, influenza was detected on 59% of home fomites sampled during March. Influenza A virus was detected on over 50% of the fomites tested in homes and day care centers during influenza season.

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APPENDIX A

LITERATURE REVIEW

THE SIGNIFICANCE OF FOMITES IN THE SPREAD OF RESPIRATORY AND GASTROINTESTINAL VIRAL DISEASE.

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Abstract

Viruses account for at least 60% of human infections.[5] Specifically, viruses causing respiratory and gastrointestinal infections which are the most prevalent.[5] Scientific and clinical evidence has suggested that contaminated indoor surfaces may play a role in the spread of viral infections.[5] However, viral disease transmission via surfaces and objects (fomites) has not been extensively investigated.[7] The goal of this review is to assess the significance of fomites in the transmission of viral disease. Ten viruses were evaluated for potential transmission via fomites; rhinovirus, respiratory syncytial virus (RSV), influenza A, parainfluenza 1 (HPIV1), coronavirus, rotavirus, calicivirus, hepatitis A virus (HAV), astrovirus and adenovirus. Study results indicate that disease transmission via contaminated fomites has been established or is suspected in all 10 of the most common enteric and respiratory viruses. Consequently, fomites may play a greater role in the transmission of common viral pathogens than previously understood, and consistent disinfection of indoor surfaces could limit the spread of viral illness in these environments.

1. Introduction

Increases in population mobility and concentration have enhanced pathogen spread, and amplified the complexity of interrupting disease transmisson.[8] Ninety percent of global mortality is caused by infectious disease.[3,4] Worldwide in 2002, respiratory infections resulted in 3.9 million deaths and gastrointestinal infections resulted in 1.8 million deaths.[3,4] Viruses account for at least 60 % of human infections and most common illnesses are caused by respiratory and enteric viruses.[5,6] Unlike some bacterial diseases, viral infection can not be resolved with the use of antibiotics. To date vaccines and antiviral drugs are heavily relied upon for prevention of viral illness.[9] However, both vaccines and antiviral drugs are only 60% effective.[10, 9] Presently, there are no vaccines or antiviral drugs for the most common enteric and respiratory viruses with the exception of influenza.[6] Consequently the spread of viral illness is most effectively deterred by limiting viral transmission.

Control of viral disease outbreaks requires a clear understanding of how viruses are transmitted in the environment.[1] For centuries it was assumed that infectious diseases were spread primarily by the airborne route or through direct patient contact.[1,2] Until 1987 the Center for Disease Control (CDC) and the American Hospital Association concentrated on patient diagnosis and limited patient contact due to the belief that nosocomial infections were not related to microbial contamination of surfaces.[2] Laboratory, epidemiological and disinfection intervention studies are changing the perspective of viral transmission to include a more complex multifactorial model of disease spread.[1]

Viruses are probably the most common cause of infectious disease acquired indoors.[5,6] The rapid spread of viral disease in crowded indoor establishments including; schools, daycare facilities, nursing homes, business offices, and hospitals consistently facilitates disease morbidity and mortality.[6] Transmission of viruses are dependent on interaction with the host as well as virus interaction with the environment.[7] Research evidence implicating contaminated fomites or surfaces in the spread of viral disease has been increasing.[5,6] However, the role of surfaces and objects (fomites) in viral disease transmission has not been completely investigated.[7] The goal of this review is to investigate the significance of fomites in the transmission of viral disease by clarifying the role of fomites in the spread of the most common respiratory and enteric viruses.

2. Role of fomites in viral disease transmission

Fomites consist of both porous and nonporous inanimate surfaces or objects that can become contaminated with pathogenic microorganisms and serve as vehicles in disease transmission (Figure 1).[11,13] During and after infection, viruses are shed in large numbers in body secretions including; blood, feces, urine, saliva and nasal fluid.[12] Fomites become contaminated with virus by direct contact with body secretions or fluids, contact with soiled hands, contact with aerosolized virus (large droplet spread) generated via talking, sneezing, coughing, vomiting, or airborne virus that settles after disturbance of a contaminated fomite (i.e. shaking a contaminated blanket).[1,11,13,14] Once the fomite is contaminated the transfer of infectious virus can readily occur between inanimate and animate objects and vice versa, or between two separate fomites (if brought together).[1,13] The nature and the frequency of contact with contaminated fomites varies for each person

depending on age, personal habits, type of activities, personal mobility and the level of cleanliness in the surroundings.[13] Viral transfer and disease transmission is complicated by variations in virus survival on different fomites and the release of viruses from surfaces upon causal contact.[11,13] Virus survival on fomites is influenced by temperature, humidity, pH, exposure to ultraviolet light and the number of microbes present in the surroundings (Figure 2 and Table 1).[11,13] If the virus survives on the fomite long enough to come in contact with a host, the virus must be present in sufficient numbers to infect the host.[6,12,13] The number of viruses needed to infect a host varies with viral type and host's susceptibility. During host contact viruses can gain entry into host systems through portals of entry (i.e. mouth, nasopharynx, and eyes).[11,12,13] Pathogenic viruses vary in their ability to cause and transmit infection.[15] Host virus susceptibility is influenced by previous viral contact and current status of the immune system.

Many complex variables influence virus survival on fomites, transfer from fomites and infection of the host.[5,11-13] Consequently, direct laboratory evidence of viral transmission by fomite has been very difficult to generate. The lack of direct scientific evidence in this area is due to the variety of uncontrollable variables, and the unpredictability of human infection.[5,13] Epidemiological data indicating transmission via fomite is problematic.[2] Epidemiological studies experience difficulties when distinguishing between different routes of transmission such as person to person transmission, or autoinculation.[2] Laboratory research, epidemiological evidence, and disinfection intervention studies have generated strong indirect and circumstantial data that supports fomite involvement as a vehicle in respiratory and enteric virus transmission.

Studies investigating viruses clearly support the following:(1) fomites and hands can become contaminated with viruses from both organic and laboratory sources; (2) viruses can survive on fomites and hands; (3) viral transfer from fomites to hand is possible; (4) hands come in contact with portals of entry for viral infection and; (5) chemical disinfection of fomites interrupts viral transmission.[5,11,13]

2.1 Laboratory evidence of respiratory virus transmission via fomites

Infections resulting in respiratory disease are the number one cause of mortality and morbidity in the world.[10,9] Viral respiratory infections are caused by several different pathogenic viruses including respiratory syncytial virus (RSV), human parainfluenza virus (HPIV 1,2,3, and 4), influenza (A and B), human coronavirus (SARS, OC43 and 229E), rhinovirus and adenovirus especially serotypes 4 and 7.[16] It is generally accepted that respiratory viruses are spread person to person via aerosol transmission.[1,5] Nevertheless, current scientific evidence suggests that fomites can be an important vehicle in the spread of respiratory viruses.[5] Respiratory viruses cause sneezing and coughing which expel an estimated 107 infectious viral units/ml. of nasal secretions.[16] During sneezing or coughing nasal fluid can travel 103 miles/hr. over a distance of up to 6 ft. to contaminate surrounding fomites.[17-19] Nasal secretions can also be transferred to fomites via hands. Research studies have demonstrated that RSV, HPIV, influenza, coronavirus and rhinovirus can all survive on porous and nonporous fomites for several minutes to several hours as seen in Figure 3. [5,20-22] Coronavirus appears to be among the longest surviving respiratory virus, remaining viable 72 hours on nonporous Formica surfaces.[20] Viruses have been isolated from organically contaminated fomites in day care

centers and homes (influenza A)[23], offices (parainfluenza)[24], and hospitals (coronavirus, parainfluenza, RSV)[25] using polymerase chain reaction (PCR). A hospital in Taiwan used reverse transcriptase polymerase chain reaction (RT- PCR) to detect coronavirus on hospital phones, doorknobs, computer mouse, and toilet handles during an outbreak of severe acute respiratory syndrome (SARS).[25] Laboratory studies have verified that RSV, influenza, parainfluenza, and rhinovirus can survive on hands for several minutes to hours and that these viruses can be transferred from hands and fingers to fomites, and back again (Table 1).[5,22,26,27] In a study by Gwaltney et al., subjects with cold symptoms had rhinovirus on their hands and the virus was recovered from 43% of the plastic tiles they touched.[28] Contaminated hands frequently come into contact with portals of viral entry, so the potential for viral infection from contaminated fomites and hands exists. A study by Hendley et al., found that one in 2.7 hospital grand round attendees rubbed their eyes and 33% made hand contact with their nasal cavity within the one hour observation period.[29] Overall, indirect evidence from clinical and laboratory studies clearly supports the involvement fomites in respiratory infection transmission.

2.2 Laboratory evidence of enteric virus transmission via fomites

Each year gastrointestinal illness caused by enteric viruses results in 2 to 4 million deaths worldwide.[30] Viruses causing gastroenteritis include; rotavirus, astrovirus, hepatitis A virus (HAV), calicivirus (norovirus, small round viruses) and adenovirus (serotypes 12, 40 and 41).[31,32] Enteric viruses are spread by the fecal-oral route and in many disease outbreaks transmission occurs via contaminated surfaces.[30, 33] It has been estimated that one single vomiting incident may produce an estimated 10^7 viral

particles.[5,7] In addition, enteric viruses are excreted in the stool of an infected individual at a rate of more than 10^{11} viral particles per gram of feces.[5,7,33-36] Contamination of fomites from enteric viruses can originate from aerosolized vomit or the transfer of vomit and fecal matter from hands to surfaces.[5,7,34] A study by Gerba et al, found that viruses aerosolized from flushing the toilet can remain airborne long enough to contaminate surfaces throughout the bathroom.[37] Contamination of surfaces (i.e. knives, sinks) during food preparation by virus-infected (norovirus, HAV, rotavirus, astrovirus) individuals occurs often, and has been documented to be the source of several foodborne outbreaks.[38]

Research investigating virus survival has indicated that enteric viruses are viable for at least 30 days on nonporous fomites, with astrovirus remaining infective for up to 90 days (Figure 4). A study by Fischer et al., found that rotavirus stored in feces would remain viable for 2½ months at 30°C and remained infective 32 months at 10°C.[39] In addition, norovirus, adenovirus and rotavirus have all been isolated from biologically contaminated surface samples. Norovirus has been detected in hotels, hospital wards, and cruise ships during outbreaks of gastroenteritis.[5,7] Adenovirus has been isolated on drinking glasses from bars and coffee shops, and rotavirus was detected on 16 -30% of fomites in daycare centers.[5,11,40] Very small amounts of enteric virus (norovirus estimated at 10 to100 virions) can cause infection, with many viral infections being largely asymptomatic in healthy adults.[5,7] Consequently, viral shedding onto surfaces or the spreading of virions into the environment by infected individuals can go on undetected.[5,36, 41]

The spread of viable HAV, rotavirus, and astrovirus from hands to fomites and vice versa has been well documented in several studies as seen in Table 1. A study by Paulson demonstrated that gloved hands could transfer calicivirus to spatulas, lettuce, forks, door knobs and cutting boards.[38] Baker et al., found that norovirus transferred from contaminated surfaces to clean hands could subsequently be transferred to a secondary surface such as a phone, door handle or water tap handle.[41] Baker also found that contaminated hands could transfer norovirus to a series of 7 clean surfaces.[41] When fomites and hands become contaminated viruses can easily be spread to the mouth. A small child puts their fingers in their mouth once every three minutes, and children up to age 6 average a hand to mouth frequency of 9.5 contacts per hour.[42,43]

Laboratory studies directly supporting enteric and respiratory virus transmission via surfaces is deficient. Research by Ward et al. is one of a few studies that have demonstrated direct evidence of enteric virus disease transmission via fomites. Volunteers in the Ward study licked a rotavirus contaminated plate, subsequently all of the volunteers became infected.[35] In the same study, only half of the volunteers who touched the contaminated plate and then licked contaminated fingers became infected.[35] The study by Gwaltney and Hendley appears to be the only laboratory study using a respiratory virus to verify that an inanimate object can lead to disease transmission or infection. In 1982 Hendley et al., observed that 50% of subjects developed infections after handling a coffee cup contaminated with rhinovirus.[28] The study also demonstrated that self-inoculation with rhinovirus as a result of rubbing the nasal mucosa with contaminated fingers could lead to infection.[28] In general, laboratory evidence supporting

virus transmission via fomites is considered indirect and circumstantial, but it represents an important component in understanding potential virus transmission and infection.[5,13]

3. Epidemiology of virus transmission by fomites

The involvement of fomites in viral disease transmission was first recognized long before the identification of pathogenic organisms, when smallpox outbreaks were traced to imported cotton in 1908.[11] Most early epidemiology studies of viral disease lacked the scientific methods to detect and distinguish between a variety of bacterial and viral illnesses. Consequently, most epidemiology studies do not identify the microbial cause of a disease, and outbreaks are characterized by disease symptoms only. For example, in 1929 an epidemic of non-bacterial gastroenteritis was described as the winter vomiting disease by epidemiologist.[32] Lately, epidemiological studies have developed stronger scientific links to gastrointestinal and respiratory viral diseases by using antibody assays, viral cultures, polymerase chain reaction and other molecular methods to identify viral pathogens in the host and environment.

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 (i.e. towels, medical cart items etc.).[22] An outbreak of coronavirus (SARS) in a Hong Kong apartment complex may have resulted from fecal-oral transmission combined with environmental contamination.[44] Epidemiological studies in daycare centers have detected rotavirus on various surfaces including toys, phones, toilet handles, sinks and water fountains.[45] Also, the transmission of HAV by contaminated drinking glasses was

associated with an outbreak of gastroenteritis in a public house when an ill barman with HAV served drinks.[46] In the Hall study of 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.[1,47] An HPIV1 air sampling study showed that only 2 of 40 children could be infected at a distance of 60 cm.[48] Therefore, HPIV transmission by small particle spread was unlikely and viral transmission most likely took place via surface contamination or close contact.[48]

Epidemiology studies also provide additional information by using statistical tools such as risk assessments, and attack rates to illuminate viral transmission routes. The potential for norovirus transmission via fomites was demonstrated during a wedding reception where the guests suffered a 50% attack rate of gastroenteritis after a kitchen assistant vomited in a sink subsequently used for salad preparation.[5] A risk exposure analysis completed after an outbreak of gastroenteritis on a hospital elderly care ward found that areas where patients vomited were the most significant factor in the spread of norovirus.[5] When naturally acquired colds were studied by Hendley et al, rhinovirus was found on 39% of symptomatic individuals hands.[29] Additionally, there was a higher attack rate of colds if volunteers inoculated their own eyes or nose after touching virally contaminated objects and, or the fingers of symptomatic individuals.[29] In a hospital pediatric ward study by Soule et al., a positive correlation was found between an increase in rotavirus contaminated surfaces an increase in the number of ill children.[49]

4. Disinfection and hygiene intervention studies

Like epidemiological studies many disinfection and hygiene intervention studies do not specify microbial origins and identify diseases by symptoms (gastrointestinal, respiratory, cold symptoms). For example, research by Krilov et al., demonstrated that when environmental surfaces (school bus, toys etc.) were regularly cleaned or disinfected there was a reduction in enteric and respiratory illness among attending children.[5] A study in 1980 by Carter et al., found that families using an iodine - based hand wash had lower rates of respiratory disease.[50] In addition, a review article by Barker et al., cited over 15 research studies that indicated a decrease in viral contamination and viral infection when hand washing was used regularly as an intervention.[5] Consequently, disinfection and hygiene intervention studies which cite a reduction in non-specific illnesses are only supportive of the interruption of disease transmission.

Recently, more antibody specific, molecular, and cultural methods have been used to detect and identify viral presence in the environment before an after disinfection and cleaning. In 2002 norovirus caused consecutive outbreaks of gastroenteritis on various cruise ships.[20] Three out of five of the cruise ships required discontinuation of service and aggressive environmental disinfection to halt further infection.[20] In a study by Baker et al., surfaces cleaned with a detergent solution spread norovirus to uncontaminated surfaces.[41] As a result, the contaminated surface, the cleaning cloth and the cross-contaminated surface all tested positive for norovirus after testing.[41] However, cleaning with a 5000 mg/L chlorine solution was effective in preventing cross-contamination and eliminating norovirus from environmental surfaces.[41] A Taiwan hospital reported that following an

outbreak environmental samples which tested positive for coronavirus were negative after resampling the cleaned emergency department, and isolating the infected patients.[20] A study by Ward et al, demonstrated that spraying rotavirus contaminated surfaces with disinfectant prevented infection.[35] Infection occurred in 63% to 100% of volunteers who touched rotavirus contaminated surfaces then licked fingers.[35] No volunteers became infected after licking contaminated surfaces that had been disinfection.[35] When disinfection intervention studies specify the microbial cause of disease and details on environmental decontamination, the study relays more practical information about interruption of viral spread.

5. Discussion

Infectious disease causes 90% of global morality with respiratory and gastrointestinal disease being the 1st and 3rd leading cause of death worldwide.[11,4] Viruses account for approximately 60% of human infection and are effortlessly spread through indoor environments.[5] Since viral infections are not easily treated, prevention of infection is still the best method of control.[5] To limit or prevent viral infection, transmission of pathogens need to be fully understood.[1] Viruses usually have more than one route of transmission.[42] Respiratory viruses are known to be spread by person to person contact, the airborne route and contaminated surfaces (fomites).[5,6] Enteric viruses are spread by the fecal-oral route through contaminated water, surfaces, food and person to person contact.[7,5,35] Respiratory viruses appear to be more efficient in spreading disease (via airborne route) when compared to enteric viruses. Respiratory viruses have the highest worldwide mortality, spread faster (a sneeze or aerosolized virus can travel 6 feet at 103

mph),[17-19] have relatively short incubation times (1 to 8 days)[25], and may have a high infectivity rate (see Figure 1, 3 and Table 1).[4,42] Generally, there is a higher probability of infection from exposure to smaller numbers of infectious respiratory virus.[42] On the other hand, enteric viruses have the third highest worldwide mortality, spread slower (environmental contamination, water, food),[33,34] have longer incubation times (1 to 60 days)[34], and may have a lower rate of infectivity.[4,23,42] Current disease transmission data indicates that the airborne route may result in superior viral spread when compared to the fecal-oral route. In contrast, common disease transmission routes for both respiratory and enteric viruses are person to person contact and environmental contamination via fomites. Virus spread by person to person contact can be interrupted with isolation, or may prove to be impractical and difficult if there are many people or the source of infection is unknown.[6] Therefore, interrupting viral spread via contaminated fomites could be the most effective way to limit increases in both gastrointestinal and respiratory disease.

This review article focused on ten of the most common respiratory (rhinovirus, influenza, parainfluenza, RSV, coronavirus) and enteric viral pathogens (rotavirus, norovirus, HAV, astrovirus, adenovirus) to evaluate the role of contaminated surfaces in disease transmission. Laboratory and clinical research has established that all 10 of the respiratory and enteric viruses can survive on fomites for extensive periods of time (see Figures 3 and 4). Generally, the virus survival studies in Figures 3 and 4, and Table 1 used different initial viral titers, inoculum media, humidity and fomites, so virus survival comparison is difficult. Nonetheless, the majority of common respiratory viruses are enveloped viruses (parainfluenza, influenza, RSV, coronavirus) and can survive on

surfaces for hours or several days. In contrast, enteric viruses are non-enveloped and display an ability to survive on fomites for weeks to months (average 2 months). All 10 viruses can be detected on biologically contaminated environmental surfaces, or hands of infected individuals. Viruses have been isolated on surfaces in day care centers (influenza, rotavirus, coronavirus, astrovirus), restaurants or bars (adenovirus, HAV), hospitals (coronavirus, RSV, influenza, parainfluenza, norovirus), and on hands (rhinovirus, rotavirus).[5,20,23,35,24,51] Other studies have further demonstrated that viral transfer from hands to surrounding surfaces is possible in 7 out of 10 of the viruses as seen in Table 1. Additionally, the transfer of coronavirus, adenovirus and astrovirus from hands to surfaces is potentially possible, but the research to verify this data was not found (see Table 1.).

Essentially, the majority of research evidence involving viral transmission via fomites is considered circumstantial or indirect except for the data gathered in studies by Ward (rotaviruses) and Hendley (rhinoviruses). Research evidence is considered circumstantial because viral illness is difficult to produce from virally contaminated hands or surfaces.[5,13] Complications inducing viral illness stem from virus pathogencity variations and host immune system disimilarities.[5] An example of the difficulty in producing disease in a host after exposure is indicated in the Gwaltney study using rhinovirus. Over a ten-year period Dr. Gwaltney intranasally challenged 343 adults with no rhinovirus antibodies, and infected 95% of the participates.[8] However, only 30% of the individuals who became infected displayed disease symptoms.[8]

Overall, laboratory and clinical evidence is considered indirect but it is supported by both epidemiology and interventions studies. It is documented that environmental surfaces act as reservoirs for pathogenic viruses with the potential for transmission of infectious disease in both epidemiological outbreak studies and disinfection intervention studies.[2] Epidemiology studies have verified disease outbreaks in 8 of 10 the viruses (HAV, RSV, norovirus, rotavirus, influenza, coronavirus, astroviruses, adenoviruses) where fomites were indicated as a potential vehicle for transmission. Hygiene and disinfection intervention studies have demonstrated two concepts which support transmission of viral illness via fomites. One is the cleaning of hands decreases respiratory and gastrointestinal illness. The second is disinfection of fomites decreases surface contamination, and interrupts disease spread (noroviruses, coronaviruses, and rotavirus). Overall, disease transmission via contaminated fomites is has been established or is suspected in all 10 of the enteric and respiratory viruses reviewed (Table 1).

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Figure 1. Virus transmission by fomite.

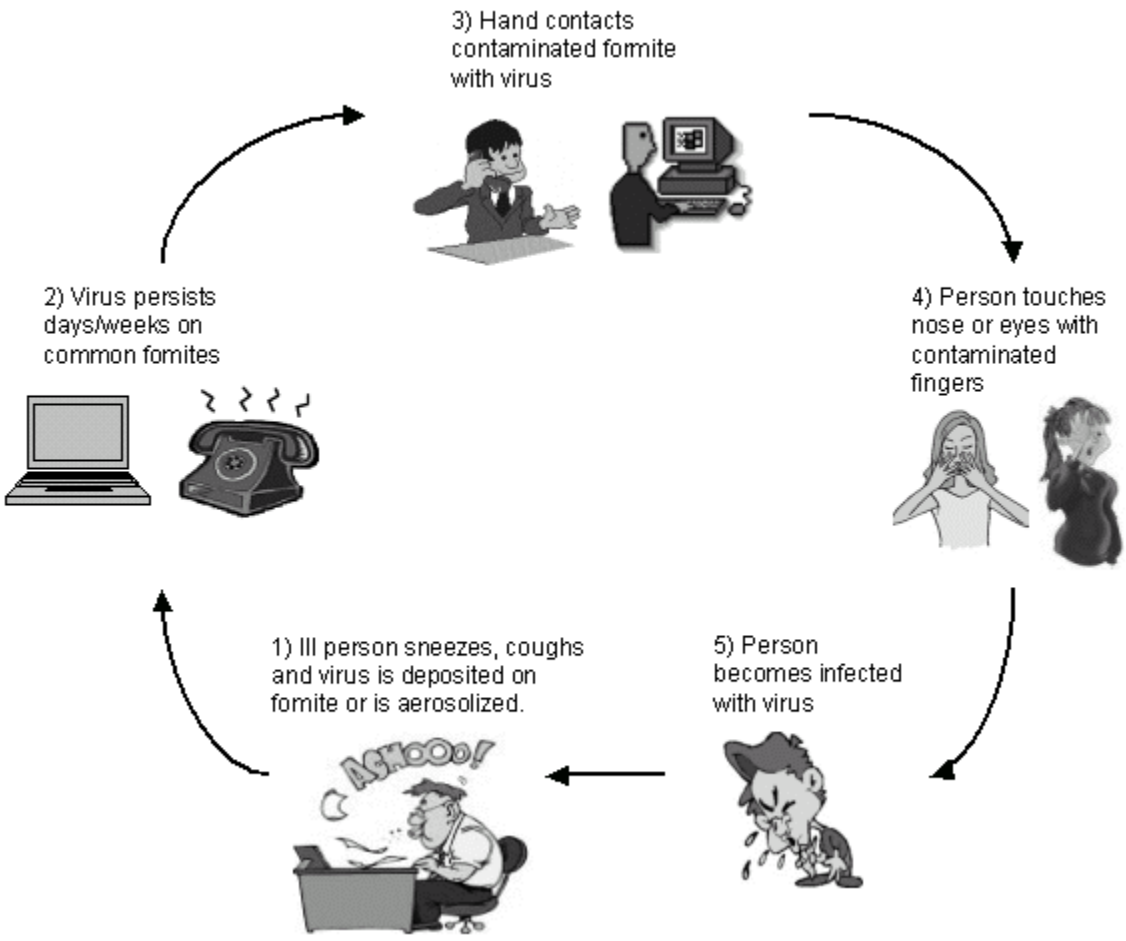


Figure 2. Factors influencing virus survival on fomites.

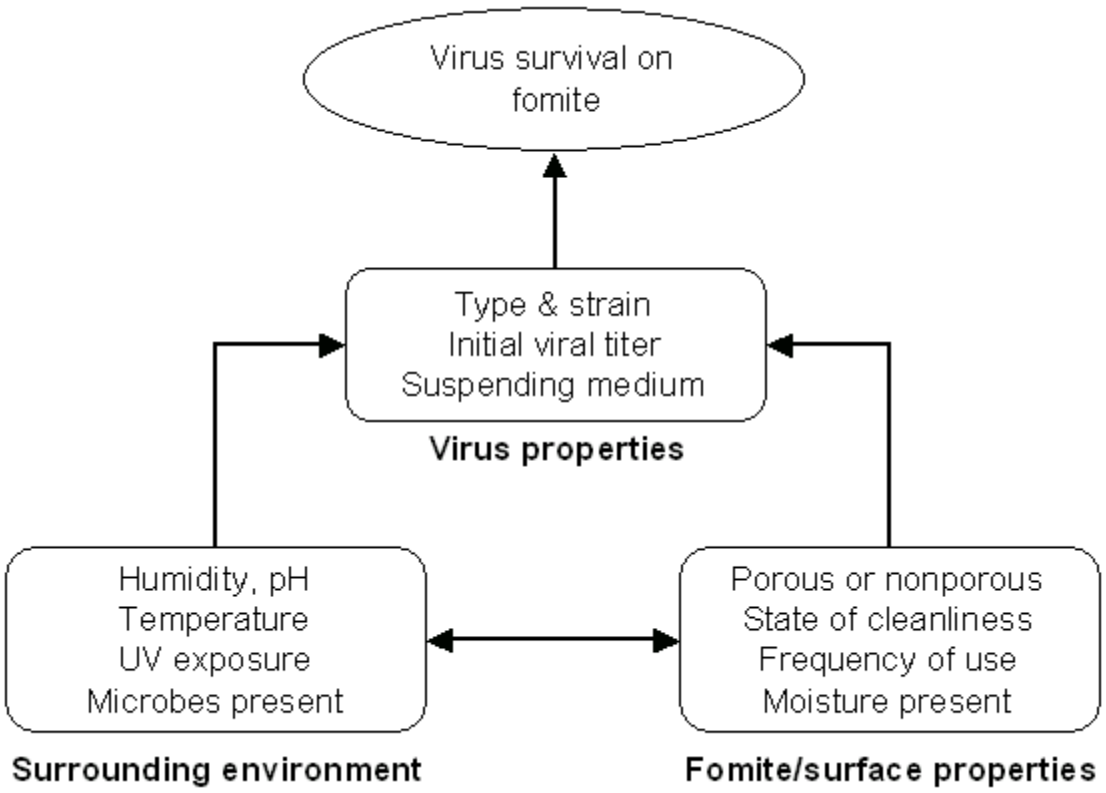


Table 1. Gastrointestinal and respiratory virus.

Virus	Optimal environmental conditions for survival	Hand to fomites transfer	$TCID_{50}$ Infectious dosage of virus	Evidence of transmission by fomite
Respiratory Syncytial Virus	Composition of surface more important than humidity and temperature. [11,27]	Transfer from porous (tissues, gloves) and non-porous fomites (countertops) to hands.[27]	Intranasal inoculation humans – 100 to 640 $TCID_{50}$. [52,53]	Established.[14,54]
Rhinovirus	Survived well at high humilities but poorly in dry conditions. [55]	Clean hands pick-up virus when handling contaminated fomites.[26] 70% of virus on hands transferred to recipients fingers.[28]	Intranasal inoculation Humans - .032 to .4 TCID [52] Also listed as 1 to 10 $TCID_{50}$.[5,56]	Established but considered minor.[14]
Influenza	Survival at lab temp. 28ºC and humidity 40% for 48 hours dry surface.[23] 72 hours on wet surface.[43]	Virus transferred from contaminated surface to hands for up to 24 hours after inoculation.[21]	Intranasal inoculation Humans – 2 to 790 $TCID^{50}$. [52,53]	Established but considered secondary or minor.[20]
Parainfluenza	Survival decreases above 37°C. Greatest stability at 4°C, pH 7.4 to 8.0 and low humidity. Viable virus recovered after freezing tweny-six years.[48]	Virus transfer from stainless steel surfaces to clean fingers.[26]	Intranasal inoculation humans – 1.5 to 80 $TCID^{50}$ (parainfluenza 1).[5,53]	Not proven, indirect evidence supports.[14]
Coronavirus	Humidity 55-77% and temperature 21°C remained infective up to six days in PBS.50 Remain infective 1-2 days in feces. [20]	Theoretically possible but not studied. [58]	Not found	Not proven but suspected. [20]
Calicivirus	Survival at 4°C when dried on cover slip for 56 days. Survival decreased with temperature.[14] Sensitive to humidity in 30-70% range. [7,55]	Transfer from gloved hands to kitchen utensils and doorknob and visa versa.[38] Transfer from contaminated surface to clean hands to phone, door handle or water tap handle. [41]	Estimated to be as few as 10 particles. [5, 41,59]	Not proven, indirect evidence supports, CDC lists surface contamination.[32, 59]
Rotavirus	Remained infective for 32 months at 10°C and 2½ months at 30°C when stored in feces.[39]	Contaminated fingertips contacted steel disc with 16% viral transfer after 20 min.[60]	Not found – estimated at 10 to 100 $TCID_{50}$.[5,52]	Established- [5,14]
Hepatitis A Virus	Survival inversely proportional to relative humidity and temperature, 5ºC optimal temp. 22,[61]	Mbithi et al. - 25% of virus was transferred from fingers to disc and visa versa. Moisture facilitated transfer.[62]	Estimated 10 to 100 $TCID_{50}$.[5]	Established, (food and fecally contaminated surface). [30,33]
Adenovirus	Survived shorter periods in the presence of feces and at lower humidity. [7,30,33]	Not found	Intranasal 150 $TCID_{50}$/ oral 1000 $TCID_{50}$ (capsule form of Serotype 4 & 7. [53]	Established, contaminated surfaces.[33]
Astrovirus	Survived 4 °C on china	Not found	Not found	May play an

	for 60 days and paper for 90 days. Faster decay at higher temperature. [7,30]			important role in secondary transmission.[7,30]

Figure 3. Survival of respiratory viruses on fomites.

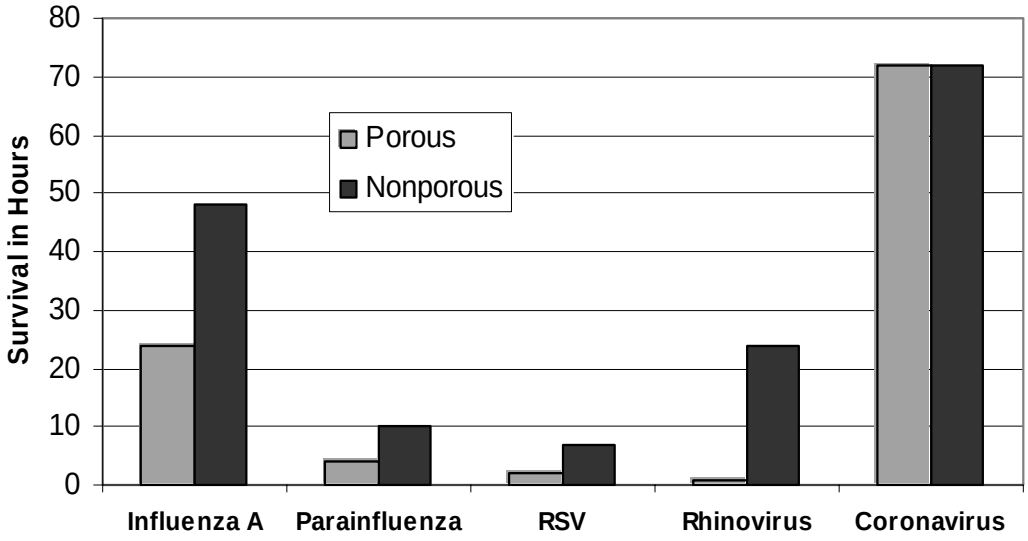
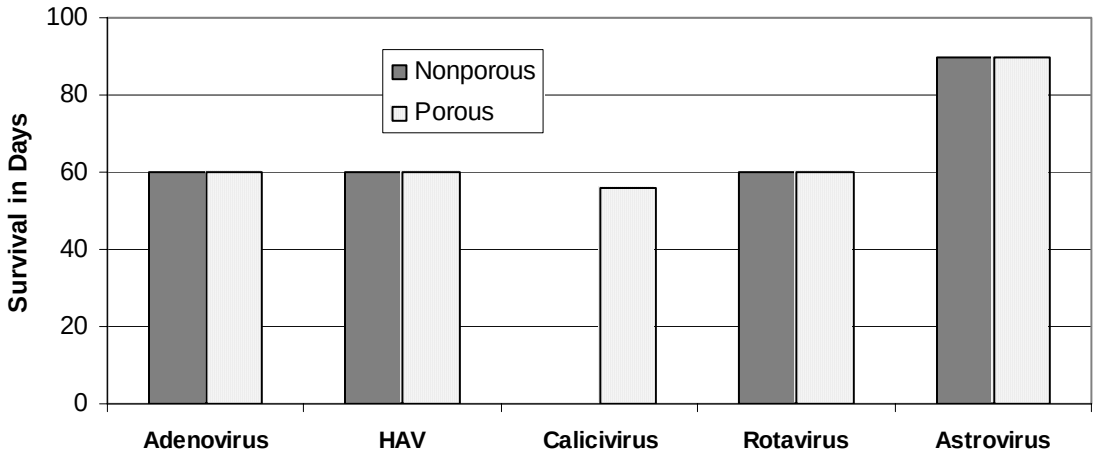


Figure 4. Survival of enteric viruses on fomites.



APPENDIX B:

THE DETECTION OF VIRUSES ON NONPOROUS INANIMATE SURFACES USING RT-PCR AND NESTED RT-PCR.

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Abstract

Viruses have been detected using PCR on fomites in day care centers and homes (influenza A),[1] offices (parainfluenza),[2] hospitals (coronavirus, parainfluenza, RSV)[3] cruise ships (norovirus)[4] and restaurants or bars (adenovirus, HAV).[5,6] Various studies isolated pathogens on fomites using PCR under the hypothesis that DNA and RNA can be detected only for short periods of time due to degradation by Rnases, Dnases, disinfectants and UV light. There have been very few studies investigating how long viral nucleic acids remain on inanimate objects. The goal of this study is to examine the detection limit of several PCR viral primers by determining the length of time viruses can be detected on an inanimate nonporous surface. Both Poliovirus 1 and Hepatitis A virus were detected for up to 60 days using both semi-nested and RT-PCR. Parainfluenza 1 virus isolation varied yielding detection at 30 days and 50 days. Norovirus isolation also varied yielding detection at 20 days and 30 days. Influenza virus results were inconsistent with no detection initially and detection up to 20 days.

1. Introduction

Polymerase chain reaction (PCR) and other molecular methods have proven readily adaptable for use in clinical diagnostic laboratories, environmental laboratories, epidemiologic investigations and infection control studies.[7,8] Currently, the most practical use for PCR is detecting and identifying both non-culturable and culturable infectious pathogens.[9,10] There are several advantages to using PCR compared to techniques such as cell culture for the detection of viruses. The time required for the assay can be reduced from days or weeks to hours. Both the initial and recurring costs of PCR are less than the costs of cell culture techniques, and PCR is easily performed. Clinical laboratories have used PCR to evaluate fecal, vomit, blood, saliva and nasal secretion samples for both bacteria and viral pathogens. Environmental labs use PCR to isolate pathogens in air, soil, water and biosolids. During outbreaks epidemiology studies have used PCR to track genes, evaluate genetic activity and detect pathogen nucleic acid in the environment.

Various clinical, environmental and epidemiology studies have used PCR to identify pathogens on common indoor fomites. Viruses commonly occur in the environment and have been isolated on fomites in day care centers and homes (influenza A),[1] offices (parainfluenza),[2] hospitals (coronavirus, parainfluenza, RSV)[3] cruise ships (norovirus)[4] and restaurants or bars (adenovirus, HAV).[5,6] A hospital in Taiwan used reverse transcriptase – polymerase chain reaction (RT- PCR) to isolate coronavirus on hospital phones, doorknobs, computer mouse, and toilet handles during an outbreak of severe acute respiratory syndrome (SARS).[3] Day care center research studies have used PCR to

detect rotavirus and influenza on various surfaces including toys, phones, toilet handles, and sinks.[2,11]

Various studies detect pathogens on fomites using PCR under the hypothesis that DNA and RNA can be isolated only for short periods of time due to degradation by Rnases, Dnases, disinfectants and UV. However, a study by Baker found that PCR detected norovirus RNA 12 days after it was dried on a plastic surface.[12] There are very few studies investigating how long viral nucleic acids remain on inanimate objects. The goal of this study is to examine the detection limit of several PCR viral primers by determining the length of time viruses can be detected on inanimate surfaces.

2. Methods

2.1 Sample Plan

Influenza A Kong/8/68 (initial virus concentration 10^7) and Parainfluenza 1 Human paramyxovirus (initial virus concentration 10^6) were obtained from American Type Culture Collection (ATCC, Manassas, VA). Norovirus was obtained from a mixture of 3 cruise ship outbreak positive stool samples suspended in phosphate buffer solution (PBS) (initial virus concentration unknown). Poliovirus 1 (LSc strain initial concentration 10^8) was propagated in Buffalo Green Monkey kidney cells (BGM) and hepatitis A virus was propagated using RK-13 cells. All viruses with known concentrations were diluted to 10^6 by adding appropriate amounts of PBS. A sterile pipette was used to inoculate 100 ul of each virus on to a 2 inch square space of a nonporous surface and allowed to dry and sit at room temperature. The nonporous surfaces used in the assay consisted of multipurpose shelving boards constructed by Rubbermaid (Newell Rubbermaid company, Fairlawn

OH.). Samples were placed in a closed chamber after inoculation to prevent tampering and regulate humidity. Surface samples were collected at the following times; 60 minutes, day 1, 3, 6, 10, 15, 20, 25, 30, 40, 50, 60. A sterile polyester fiber-tipped applicator swab (Becton Dickinson and Company) moistened in sterile PBS was used to collect each sample. Samples were collected in two separate sets. The first samples were collected from May 1st to June 30th, and the second samples were collected from July 1st to August 30th. All samples were frozen immediately after collection at -20°C until assayed. Samples were homogenized by vortex and then viral RNA was extracted.

2.2 RNA Extraction and reverse transcription

No positive controls were used during RNA extraction because initial samples were seeded, and to prevent contamination of samples. Negative controls were used that consisted of 140 µl PBS. RNA extraction, and RT-PCR procedures were all performed in separate rooms to prevent sample contamination. Samples and reagents were also kept in separate rooms and separate freezers to further protect from contamination. QIAmp Viral RNA Mini kits and procedure from Qiagen Inc. (Valencia, CA) were used as recommended by the manufacturer to extract and concentrate viral RNA from fomite and negative control samples. An initial volume of 560 µL was used in the RNA extraction process to produce a final volume of 80µL per sample.

All reagents used for reverse transcriptase and polymerase chain reaction were obtained from Applied Biosystems (Roche Molecular Systems Inc. Branchburg, NJ). The reverse transcriptase reaction (RT) mixture a contained 10µL of sample RNA extract, 3.5 µL of 25 mM $MgCl_2$ solution, 1.5 µL of Amplitaq Gold GeneAmp 10 × PCR

buffer, 4.0 µL of 2.5 mM of dNTP mix, 0.5 µL of 50 mM Random Hexamers, 0.5 µL of 20 U/µL RNase Inhibitor and 0.5 µL of 50 U/µ MuLV Reverse Transcriptase to yield reverse-transcriptase mixture totaling 20.5 µL per sample. The reaction mixture was then placed in an Applied Biosystems Gene Amp PCR System 9700 thermocycler for 10 minutes at 24ºC, 60 minutes at 44°C, 5 minutes at 99°C and 5 minutes at 5°C. The above RT procedure was used for all viruses (HAV, parainfluenza 1, influenza A, HAV, polio) except norovirus. A one step RT-PCR reagent kit from Qiagen Inc. (Valencia, CA.) was used for norovirus.

2.3 PCR and nested PCR

The norovirus RT-PCR procedure was as follows: Norovirus PCR was preformed using all reagents from the Qiagen one step kit. (Valencia CA.). Ten microliters of sterile H_2O, 5 X qiagen Onestep RT-PCR buffer containing 12.5mM MgCl, 2.0µL of 10 mM dNTPs mix, Onestep RT-PCR enzyme mix, 1µL upstream and downstream primers, 1µL of 20 U/µL RNase Inhibitor from Applied Biosystems (Roche Molecular Systems Inc. Branchburg, NJ). Ten microliters of sample was added to the 40µL mixture to totaling 50 µL per sample. The RT-PCR mixture was placed in a Applied Biosystems Gene Amp PCR System 9700 thermocycler, times and temperature listed in Table 2. Semi-nested norovirus PCR was performed using 32..75 µL of Rnase free water (Promega Madison, WI), 5.0 µL of 25 mM $MgCl_2$ solution, 5.0 µL of Amplitaq Gold with GeneAmp 10 X PCR buffer, 4.0 µl of 2.5 mM of dNTP mix, 0.5 µL of 200 µM (upstream and downstream) primers and 0.25 µL of 5 U/µL AmpliTaq Gold DNA Polymerase. Two

microliters of the cDNA product from the reverse transcriptase reaction was added to the PCR master mix and resulted in a final mixture volume of 50 µL per sample.

Influenza A primers used were based on a nucleoprotien gene segment which is highly conserved among the subtypes of type A influenza virus. PCR was performed using 14.75 µL of Rnase free water (Promega Madison, WI), 5.0 µL of 25 mM $MgCl_2$ solution, 5.0 µL of Amplitaq Gold with GeneAmp 10 × PCR buffer, 4.0 µl of 2.5 mM of dNTP mix, 0.5 µL of 200 µM (upstream and downstream) primers and 0.25 µL of 5 U/µL AmpliTaq Gold DNA Polymerase. Twenty microliters of the cDNA product from the reverse transcriptase reaction was added to the PCR master mix and resulted in a final mixture volume of 50 µL per sample. Parainfluenza 1 primers were based on the protein segment gene, which is highly conserved among HPIV-1. PCR was performed using 20.30 µL of Rnase free water (Promega Madison, WI), 3.0 µL of 25 mM $MgCl_2$ solution, 4.0 µL of Amplitaq with GeneAmp 10 × PCR buffer, 3.0 µl of 2.5 mM of dNTP mix, 0.4 µL of 200 µM (upstream and downstream) primers and 0.50 µL of 5 U/µL AmpliTaq DNA Polymerase per sample..

The enterovirus, and hepatitis A virus the PCR reaction consisted of 20.5 µL of RT product and 29.5 µL of PCR reaction. The reaction solution consist of: 1 X reaction buffer (described previously), 2.9 mM of MgCl, , 0.4 µM of each primers, 1.25 units of Taq polymerase(Applied Biosystems, Roche molecular systems Inc. Branchburg, New Jersey). The second round semi- nested PCR mixture consist of: 2.9 mM of MgCl, 200 µM of each dNTP, 0.4 µM of each primer, 1.25 units of Taq polymerase (Applied Biosystems, Roche molecular systems Inc. Branchburg, New Jersey) and 2 µL of the first

round PCR products for a final reaction volume of 50 μL. All PCR mixtures were placed in an Applied Biosystems Gene Amp PCR System 9700 thermocycler, amplification times and temperatures are listed in Table 2.

2.4 PCR product detection

Ten microliters of PCR product was detected using agarose gel-eletrophoresis. The two percent agarose gel was stained by adding 5 to 8 microliters of molecular grade ethidium bromide solution 10 mg/mL (Promega, Madison, WI) to the liquid gel buffer mixture. An Alpha Imager 2000 Documentation Analysis System (Alpha Innotech, San Leandro, California) was used to visualize the resulting PCR product bands. 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.

3. Results

Twenty-four samples per virus were assayed making an overall sample total of 120. Both Poliovirus 1 and Hepatitis A virus were detected on the nonporous surface for up to 60 days in duplicate using both semi-nested PCR and RT-PCR. Parainfluenza yielded detection at 30 days and 50 days. Norovirus also varied yielded detection at 20 days and 30 days using semi-nested PCR and there were no PCR product bands detected when using RT-PCR only. Influenza virus results were inconsistent with no detection and detection up to 20 days during the assay replicate.

4. Discussion

Virus was consistently detected in four out of five of the PCR primers sets used. HAV and enterovirus semi-nested PCR and RT-PCR appeared to be the most sensitive primers with isolation of RNA up to 60 days. Beyond 60 days was not tested. Norovirus primers were most sensitive when semi-nested PCR was used, RT-PCR was not as sensitive. Parainfluenza virus RNA was isolated up to 50 days. There appeared to be little or no difference between PCR and semi-nested PCR detection. The primers used for PCR appeared to be the most significant factor in surface detection. The inconsistencies found in influenza A virus may have been due to viral titer irregularities, primer variation, or humidity variations. More investigation is needed to determine the exact problem. Increases in detection from the first sampling to the second sampling found in norovirus and parainfluenza may be attributed to humidity changes, or viral titer inconsistencies. However, further in this area is also needed to verify these trends.

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Table 1. PCR primers used for isolation**.**

Target virus	Primer	Product size	Sequence	Reference
Hepatitis A virus	HAV 1		5'- CAG CAC ATC AGA AAG GTG AG- 3'	(Schwab et al 1991)
	HAV 2	HAV1/ HAV2 200pb	5'-CTC CAG AAT CAT CTC CAA C-3	(Schwab et al 1991.)
	HAV 3	HAV2 / HAV3 100 pb	5'-GCT TCC CAT GTC AGA GTG-3'	(Reynolds et al. 2001)
Entero virus	P1		5'-CCT CCG GCC CCT GAA TG-3'	
	P2	P1/P2 -196pb	5'-ACC GGA TGG CCA ATC CAA-3'	
	P33	P33/P1- 105 pb	5'-CCC AAA GTA GTC GGT TCC GC-5'	
Influenza A	Anp1		5’-ATC-ACT-CAC-TGA-GTG-ACA-TC-3’	(Wright et al., 1995)
	Anp2	Anp1/Anp2 306 pb	5’-CCT-CCA-GTT-TTC-TTA-GGA-TC-3’	(Wright et al., 1995)
Parainfluenza 1	HPIV1a		5’ATT TCT GGA GAT GTC CCG TAG GAG AAC -3’	(Fan et al., 1996)
	HPIV1b	HPIV1a /HPIV1b 180 bp	5’-CAC ATC CTT GAG TGA TTA AGT TTG ATG A -3’	(Fan et al., 1996)
Norovirus	Mjv12		5’TAY CAY TAT GAT GCH GAY 3’	
	Reg A	Mjv12/RegA 306bp	5’CTC RTC ATC ICC ATA RAA IGA 3’	
	MP290	MP290/RegA 306bp	5’GAY TAC TCY CS/ideoxi/ TGG GAY TC 3’	

Table 2. Reaction and thermocyler temperatures.

Virus	Reaction	Thermocycler temperatures
Influenza A	PCR	initial step 95°C - 10 min., 40 cycles (denaturation 94°C, 30 sec.; annealing 55°C, 30 sec.; elongation 72°C, 30 sec.), final elongation 72°C, 10 min.
Parainfluenza 1	PCR	initial 94°C, 3 min. 3 cycles (denaturation 94°C , 30 sec.; annealing 53°C, 30 sec.; elongation 72°C, 30 sec.), 37 cycles (denaturation 94°C, 30 sec.; annealing 60°C, 30 sec.; elongation 72°C, 30 sec.) and final elongation 72°C, 7 min.
Hepatitis A virus and Enterovirus	PCR	initial 95°C, 7 min. 1 cycles (denaturation 95°C , 45 sec.; annealing 50°C, 45 sec.; elongation 72°C, 1 min.), 35 cycles (denaturation 95°C, 45 sec.; annealing 55°C, 45 sec.; elongation 72°C, 1 min.) and final elongation 72°C, 7 min.
	Semi-nested PCR	initial 95°C, 7 min. 1 cycles (denaturation 95°C , 45 sec.; annealing 55°C, 45 sec.; elongation 72°C, 1 min.), 35 cycles (denaturation 95°C, 45 sec.; annealing 60°C, 45 sec.; elongation 72°C, 1min.) and final elongation 72°C, 7 min.
Norovirus	PCR	RT step 42°C, 60 min., initial step 95°C - 15 min., 40 cycles (denaturation 94°C, 30 sec.; annealing 50°C, 30 sec.; elongation 72°C, 30 sec.), final elongation 72°C, 10 min.
	Semi-nested PCR	initial step 95°C - 10 min., 30 cycles (denaturation 94°C, 30 sec.; annealing 50°C, 30 sec.; elongation 72°C, 30 sec.), final elongation 72°C, 10 min.

APPENDIX C:

THE PREVALENCE OF HUMAN PARAINFLUENZA VIRUS 1 ON INDOOR OFFICE FOMITES.

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Abstract

The goal of this study was to assess the occurrence of human parainfluenza 1 virus (HPIV1) on surfaces in office settings, and to evaluate the potential role of fomites in HPIV1 transmission. During September and October of 2004, 328 fomites were tested from 15 different office buildings in 5 different cities. The seven different fomites from cubicles, offices, and conference rooms included telephones, the computer mouse, desktops, tabletops, chair arms, door handles, and light switches. HPIV1 viral RNA was isolated using reverse transcriptase-polymerase chain reaction (RT-PCR). HPIV1 RNA was detected on 37% of all fomites tested. HPIV1 was most frequently isolated on desktops (47%), and isolated the least on light switches (19%). Study data revealed a statistically significant difference between the percentage of HPIV1 positive fomites in different buildings (Chi-square $p< 0.011$, Fisher's Exact $p = 0.054$), and significant difference between positive fomites in cubicles and conference rooms (Chi-square $p< 0.011$, Fisher's Exact $p = 0.054$). There was no statistical difference found between office and cubicle fomites (Chi square $p = 0.242$). HPIV1 was consistently isolated on fomites in various office buildings from different geographical locations during the 6-week testing period.

Author Keywords: Parainfluenza 1; virus; fomites; offices, PCR

1. Introduction

Acute respiratory tract infection is the most common illness in humans regardless of age or gender.[1] Human parainfluenza viruses (HPIV) are medically important respiratory pathogens and are second only to respiratory syncytial virus (RSV) as a major cause of lower respiratory tract illness in infants and young children.[2,3] HPIV are common community acquired pathogens without ethnic, gender, age or geographic boundaries.[3] Immunity to HPIV is incomplete and infection occurs throughout life.[3] Several studies have shown that HPIV causes yearly hospitalizations in healthy adults.[3] The virus may also play a role in bacterial pneumonia and the death of nursing home residents.[3] At this time only limited data on the mortality and morbidity of HPIV in adults is available due to the lack of laboratory diagnosis.[4] As a result, the true prevalence and incidence of HPIV related respiratory disease in adults is unknown.

Rational infection control of any virus requires a clear understanding of pathogen survival and environmental transmission.[5] Studies involving HPIV1 document low incidence levels most months of the year, however its prevalence is highest biennially from September to November.[3,7,- 10] HPIV environmental viability and infectivity is influenced by temperature, humidity and pH. HPIV environmental stability is greatest at 4°C and at physiological pH (7.4 - 8.0) with viral viability decreasing significantly at 37°C and low humidity.[7,11] When HPIV is held at room temperature its viability may vary from 2 hours to 1 week depending environmental conditions.[3] Average

viability of HPIV on nonporous surfaces is 10 hours, and 4 hours on porous surfaces.[6,7,12,13]

Studies further indicate that HPIV transmission by small particle aerosol spread is unlikely.[14] Aerosol sampling studies by Mclean et al, documented that only 2 of 40 children were infected with HPIV1 when exposed at a distance of 60 cm.[14] In 1991 Ansari et al. demonstrated that parainfluenza virus (PIV) could be transferred from stainless steel surfaces to clean fingers, and that HPIV3 remained viable on hands after one hour.[7] The Ansari and the Mclean studies suggest that fomites may play a role in the spread of HPIV.[7] To date, several studies have documented pathogenic viruses occurring in the surrounding environment. Rotavirus was detected in hospitals, astrovirus, adenovirus, influenza A and rotavirus have been isolated in day care facilities and enterovirus and influenza A virus RNA has been detected in homes.[5,6,15,16] Presently, there are only limited studies investigating HPIV transmission. [4,6,7] In addition, there appear to be no studies documenting the prevalence of HPIV or other viruses on surfaces in office settings. The goal of this study was to evaluate the prevalence of HPIV1 on indoor surfaces in office settings during HPIV1 season to better assess the potential role of fomites in its transmission.

2. Methods

2.1 Sample plan

Selected fomites were sampled in office buildings located in Atlanta, Ga., Chicago, IL., San Francisco, CA., New York, NY., and Tucson, AZ. A total of 328 samples from 15 different office buildings were evaluated over a six-week period

from September to October 2004 during peak HPIV1 season. Surfaces sampled included telephone receivers, the computer mouse, office or cubical desktops, conference room tabletops, chair armrests, doorknobs or door handles, light switches, and audiovisual remote controls. The 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, Maryland).

All samples were transported to the laboratory on ice and frozen at -80°C until assayed. Samples were homogenized using a vortex mixer followed by viral RNA extraction. HPIV1 was detected using reverse transcriptase-polymerase chain reaction (RT-PCR). Gel-electrophoresis was used to concentrate the RT- PCR product and photographs of PCR product bands were taken using an Alpha Imager 2000 Documentation Analysis System (Alpha Innotech, San Leandro, California). Sample PCR product was then sent to the University of Arizona Genomic Analysis Technical Center for verification by genomic sequencing.

2.2 RNA extraction and reverse transcription

Parainfluenza 1 human paramyxovirus was obtained from American Type Culture Collection (ATCC, Manassas, VA) and used as a HPIV1 positive control throughout the procedure. RNA extraction and RT-PCR procedures were performed in separate rooms and in separate PCR hoods to prevent sample contamination. Also, samples and reagents were kept in separate rooms and separate freezers to further protect from contamination. QIAmp Viral RNA Mini kits and procedure from Qiagen Inc.

(Valencia, CA) were used as recommended by the manufacturer to extract and concentrate viral RNA from fomite and control samples. An initial volume of 420 µL was used in the RNA extraction process to produce a final volume of 80µL.

All reagents used for reverse transcriptase and polymerase chain reaction were obtained from Applied Biosystems (Roche Molecular Systems Inc. Branchburg, NJ). The reverse transcriptase reaction mixture contained 10µL of sample RNA extract, 3.5 µL of 25 mM $MgCl_2$ solution, 1.5 µL of Amplitaq GeneAmp 10 × PCR buffer, 4.0 µL of 2.5 mM of dNTP mix, 0.5 µL of 50 mM Random Hexamers, 0.5 µL of 20 U/µL RNase Inhibitor and 0.5 µL of 50 U/µ MuLV Reverse Transcriptase to yield reverse-transcriptase mixture totaling 20.5 µL per sample. The reaction mixture was then placed in an Applied Biosystems Gene Amp PCR System 9700 (Roche Molecular Systems Inc. Branchburg, New Jersey) thermocycler for 10 minutes at 24ºC, 60 minutes at 44°C, 5 minutes at 99°C and 5 minutes at 5°C.

2.3 PCR and product detection

The primers used were based on a surface protein gene segment that is highly conserved among the subtypes of type parainfluenza 1 virus.[17] The viral primers amplify a 179 base pair product with the following nucleotide sequences: 5'ATT TCT GGA GAT GTC CCG TAG GAG AAC -3' (upstream), and 5'-CAC ATC CTT GAG TGA TTA AGT TTG ATG A -3' (downstream).[17] PCR was performed using 20.30 µL of Rnase free water (Promega Madison, WI), 3.0 µL of 25 mM $MgCl_2$ solution, 4.0 µL of Amplitaq with GeneAmp 10 × PCR buffer, 3.0 µl of 2.5 mM of dNTP mix, 0.4 µL of 200 µM (upstream and downstream) primers and 0.50 µL of 5 U/µL

Amplitaq DNA Polymerase per sample. Twenty microliters of the cDNA product from the reverse transcriptase reaction was added to the PCR master mix and resulted in a final mixture volume of 50 μL. The PCR mixture was placed in an Applied Biosystems Gene Amp PCR System 9700 thermocycler Roche Molecular Systems Inc. Branchburg, New Jersey) for amplification at following times and temperatures: initial step 94°C for 3 minutes then 3 cycles of each; denaturation 94°C for 30 seconds, annealing 53°C for 30 seconds, elongation 72°C for 30 seconds, and 37 cycles of each; denaturation 94°C for 30 seconds, annealing 60°C for 30 seconds, elongation 72°C for 30 seconds and final elongation 72°C for 7 minutes.

Ten microliters of PCR product was detected using agarose gel-eletrophoresis. The 2 % agarose gel was stained by adding 5 μL of molecular grade ethidium bromide solution 10mg/mL (Promega, Madison, WI) to the liquid gel buffer mixture. An Alpha Imager 2000 (Alpha Innotech Company, San Leandro, CA) was used to visualize the resulting 179 base pair parainfluenza 1 product bands. 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 positive PCR product. Sample genomic sequencing was conducted at the University of Arizona Genomic Analysis Technical Center using a 377 ABI sequencer from Applied Biosystems (Roche Molecular Systems Inc. Branchburg, NJ). Systat 9.0 Chi-square analysis and the Fisher's Exact test were used for statistical analysis of the sample test results.

3. Results

Three hundred twenty-eight samples including seven different fomites from 12 different office buildings in five different cities were assayed for HPIV1. Overall, 37 % of the samples tested were positive for HPIV1 (Figure 1). The city with the highest number of positive fomites was New York with 50%. The lowest quantity of positive fomites was obtained in the city of Tucson at 27% (Figure 1). The quantity of positive fomites per city varied within object category from 20 % (New York phone) to 66% (Atlanta phone) as seen in Figure 2. Data indicated a statistically significant difference within the computer mouse category between the cities of Atlanta (0%) and New York (100%), Chi-square $p < 0.0000$ and Fishers Exact test $p = 0.000005$ (Figure 2). Differences between the total quantities of positive fomites per building were also assessed (Figure 3). There was a statistically significant difference found between the occurrence of HIPV1 in Arizona buildings 1 (86%) and building 5 (14%), Chi-square analysis $p< 0.003$ and Fisher's Exact test $p = 0.0017$. This variation in HPIV1 occurrence may reflect the pattern of disease incidence in the offices assessed (Figure 3). Additionally, results revealed a variation in the quantity of positive fomites detected in cubicles, offices and conference rooms (Figure 4). Data indicated a statistical difference between the total quantity of positive fomites found in cubicles and conference rooms, Chi-square analysis $p< 0.011$ and Fisher's Exact test $p = 0.054$ as seen in Figure 4. There was no statistical difference found between offices and cubicles (Chi square $p = 0.242$), although more positive fomites occurred in cubicles. HPIV1 was detected more often on the desktops (47%), the

computer mouse (46%) and the phone (45%) (Figure 5). Virus was isolated least often on door handles (26%) and light switches (19%) as seen in Figure 5.

4. Discussion

This study is the first to investigate the occurrence of HPIV1 or any virus on indoor office surfaces. Results from this study clearly demonstrate that HPIV1 was consistently present on surfaces in various office buildings from different geographical locations. The United States experiences its highest seasonal incidence of HPIV1 in the fall months of September to November biennially. [4,7-10] This study collected fomite samples during peak HPIV1 under the presumption that infectious viruses are spread easily through closed indoor environments.[5] Concurrently, the data in this study revealed an occurrence of HPIV1 in all of the office buildings tested.

The shed and spread of HPIV1 by adult individuals with active infections is indicated by the occurrence of positive office surfaces. Viruses are shed in high numbers in respiratory secretions, and shedding may occur before symptom onset and continue for several days or weeks after symptoms have ceased.[6] Nasal secretion droplets containing infectious virus particles are generated via coughing, sneezing, and talking and are easily transmitted over considerable distances.[6] Research by Koeniger et al., documented that dissemination of bacteria from the mouth during speaking, coughing and sneezing can reach a distance of up to 12.5 meters.[18] Studies also show that an average sneeze can carry pathogens up to 6 feet traveling 103 miles per hour.[19,20] Other studies have shown that viruses can be transferred from surfaces to hands, and vice versa.[5,6, 7,13,16] Parainfluenza virus (PIV) can be transferred from

stainless steel surfaces to clean fingers and HPIV3 remained viable on hands after one hour.[6,7] Consequently, the majority of positive fomites in this study appears to have resulted from the transfer of contaminated nasal secretions to fomites by touching (hand to nose or mouth and surfaces etc.), sneezing, coughing and talking.

HPIV1 presence on fomites in offices can indicate several extraneous factors. Variations in viral occurrence on office fomites may reflect the HPIV1 disease pattern experienced by office workers or incoming patrons. Studies have demonstrated that HPIV at room temperature has an average survival of 10 hours on nonporous surfaces.[6,7,12,13] Offices with more ill individuals may have higher quantities of positive fomites as a result of increasing viral shedding or higher viral titers. The HPIV1 positive fomite samples in this study are reflective of both infective and non-infective viruses due to the detection method used (PCR). Additional research is needed to assess the infectivity of the virus when transferred from fomites. Office cleaning practices can also influence viral occurrence in the surrounding environment. Office surfaces like the telephone receiver, mouse and desktops are frequently used, but infrequently cleaned or disinfected. General cleaning with disinfectants that degrade viral RNA can reduce the possibility of viral transfer, transmission and possible infection. [6,12,13,21] Lower occurrence of HPIV1 on surfaces such as the light switch and doorknobs may reflect infrequent use. The lower occurrence of HPIV1 in conference rooms as compared to cubicles may reflect infrequent use and as well as cleaning practices.

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Figure 1. The percentage of fomites positive for HPIV1 in each city.

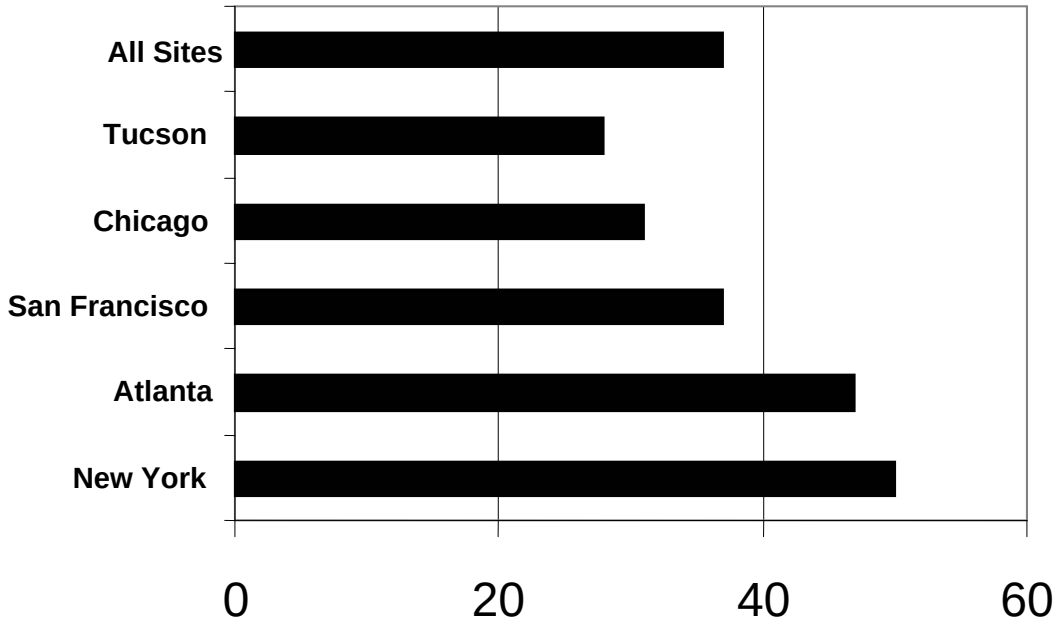


Figure 2. Fomites positive for HPIV1 by city.

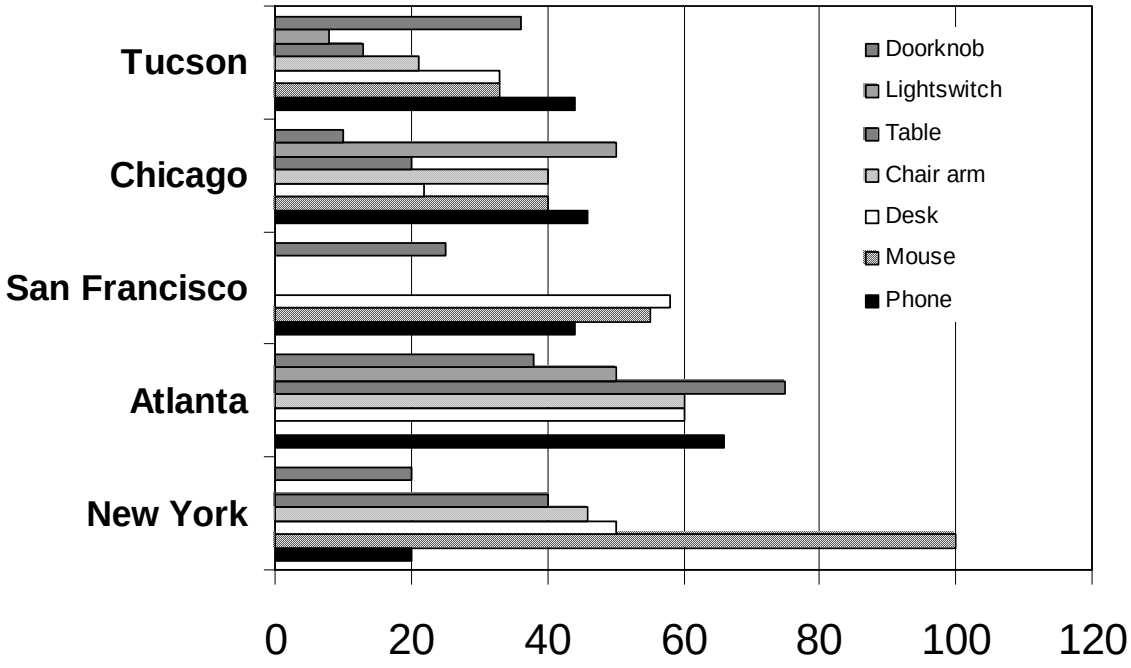


Figure 3. The percentage of fomites positive for HPIV1 by building.

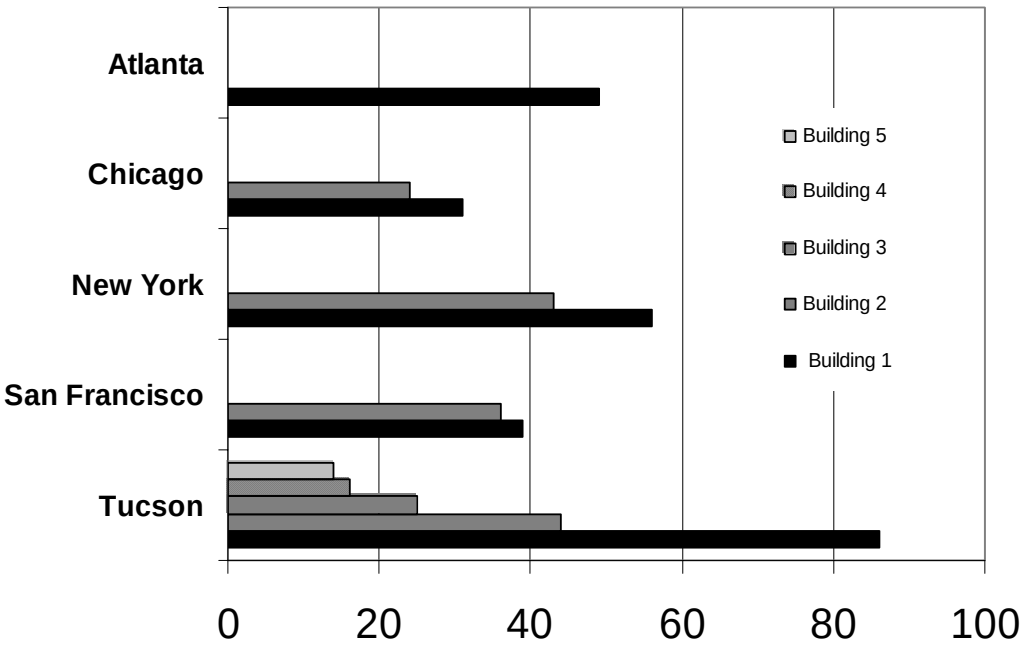


Figure 4. The percentage of fomites positive for HPIV1 by work area.

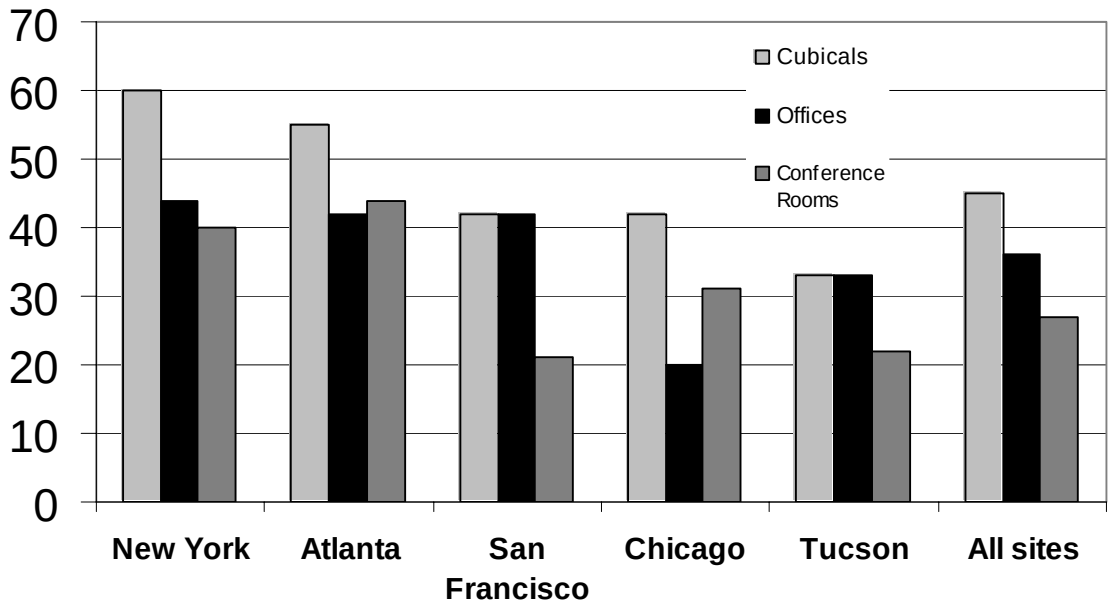
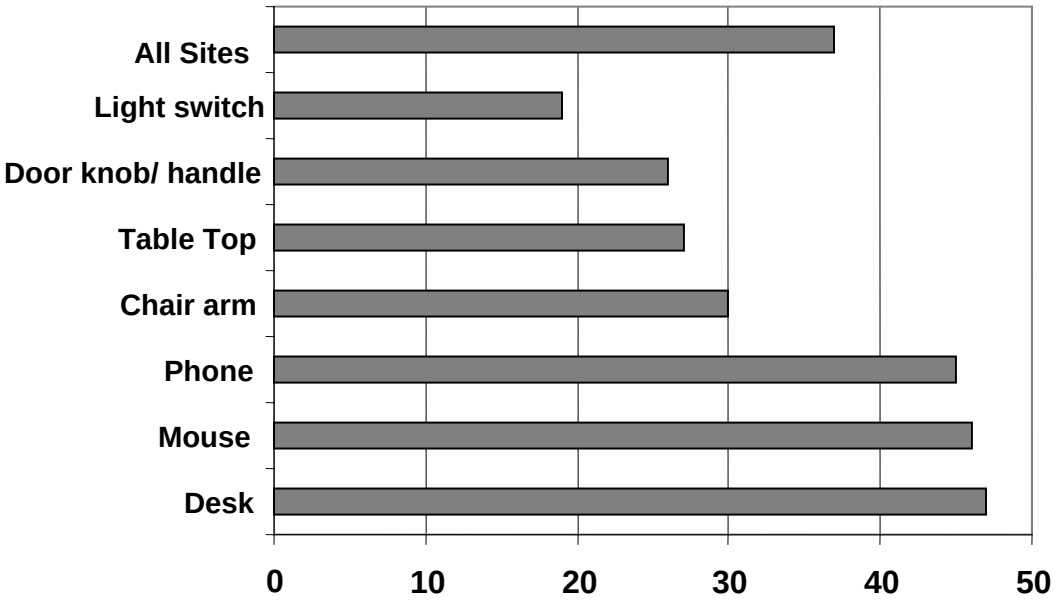


Figure 5. Total percentage of each fomite positive for HPIV 1.



APPENDIX D:

THE OCCURRENCE OF INFLUENZA A VIRUS ON HOUSEHOLD AND DAY CARE CENTER FOMITES.

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Abstract

The goal of this study was to evaluate the prevalence of influenza A virus on surfaces in day care and home settings to better assess the potential role of fomites in the transmission of influenza. During 2 ½ years, 218 fomites were tested from 14 different day care centers. Ten different fomites from bathrooms, kitchens, and play areas were sampled. In addition, 92 fomites from 8 different homes with children were tested over 6 months. Fourteen different household fomites from bathrooms, kitchens and living areas were sampled. Influenza A viral RNA was detected using reverse transcriptase-polymerase chain reaction. Influenza was detected on 23% of day care fomites sampled during the fall and 53% of fomites sampled during the spring. Spring and fall sample data was determined to be statistically different at the 0.05 α-level by Chi-square analysis $p< 0$ and Fisher's Exact test $p = 0.00002$. There was no statistical difference found between moist and dry fomites (Chi square $p = 0.13998$). No influenza was detected on home fomites sampled during the summer. In contrast, influenza was detected on 59% of home fomites sampled during March. Influenza A virus was detected on over 50% of the fomites tested in homes and day care centers during influenza season.

1. Introduction

Every year in the United States influenza causes illness in over 10% of the population, an estimated 114,000 hospitalizations, and 36,000 deaths. [1] The Center for Disease Control (CDC) and various studies suggest that influenza is transmitted by direct contact with infected birds or influenza contaminated fomites. [2, 3] Vaccine and antiviral drug usage are not enough to counter future pandemics or widespread outbreaks of influenza.[4] Rational infection control requires a clear understanding of how pathogens are transmitted.[5] Despite the major health burden caused by influenza there are few studies that clarify the disease transmission, infection or spread of this virus.

Early (1930 - 1940) influenza disease transmission research investigated the aerosol inoculation of humans and animals. These studies used large doses of virus and lacked information on the physical properties of aerosols thus the information gained was minimal. [6] In 1941 Edwards et al showed that a sneezing ferret could spread aerosolized influenza virus to fomites within a 3-inch distance. Other research by Edwards demonstrated that a vigorously shaken contaminated blanket could spread influenza particles to the surrounding environment, and that virus recovered from the environment could infect mice.[7] In 1962 Schulman and Kilbourne varied the infection rate of mice by controlling the airflow rate and humidity.[8] A study by Alford et al. in 1966 dispersed an influenza liquid suspension of 1 - 3μm diameter aerosol particles through a 7 ft. copper tube to infect volunteers. Study results determined that an aerosolized infectious dose as little as 1 $TCID_{50}$ could infect volunteers.[6] While early studies clarified variables that

affect the aerosol spread of influenza, alternative routes such as fomite disease transmission were not clearly investigated.

In a study by Rheinbaben et al., 14 people became contaminated with bacteriophage ØX174 by touching an experimentally contaminated door handle, the successive transmission could then be followed up to the sixth contact person.[9] In 1982 a study by Bean et al. found that influenza A and B viruses could survive on hard nonporous surfaces for 24 to 48 hours, and that transfer from environmental surfaces to hands was possible.[9, 10] Other studies showed that rotavirus, adenovirus, poliovirus, herpes simplex virus and hepatitis A virus could survive for significant periods of time on dry surfaces.[5, 9] Several different studies investigating fomites have recovered parainfluenza and rotavirus in hospitals, astrovirus, adenovirus and rotavirus in day care centers, and enterovirus RNA in homes.[5, 9] Research by Ansari et al. demonstrated that rotavirus could remain infective for several hours on skin allowing infectious virus to be transferred to other surfaces.[11, 12] In 1981 Hall et al. clearly documented that respiratory syncytial virus appears to be spread primarily by hands contaminated from contact with respiratory secretions.[9, 13]

Epidemiological evidence also supports laboratory data, in a study by Morens et al., where a nursing outbreak of influenza was thought to be spread by staff through contaminated hands or by touching contaminated fomites.[9, 14] However, there are no studies on the prevalence of influenza virus in homes or community settings, or its infectivity by direct contact with contaminated surfaces. The goal of this study was to

evaluate the prevalence of influenza A virus on indoor environmental surfaces in day care and private home settings to better assess their potential role in viral transmission.

2. Methods

2.1 Sample plan

Selected fomites were sampled in homes and day care centers located in Tucson, Arizona. A total of 92 samples from 8 different homes were evaluated for influenza A virus over a six-month period from March to September 2003. Five of the eight homes contained at least one ill child experiencing flu-like symptoms for three or more days. All homes containing ill children were referred from day care centers and sampled during March of the influenza season. Three of the eight homes were sampled during the summer months. These homes contained no ill children or adults and were chosen from volunteers. Nine to fourteen different fomites were sampled in each home. Surfaces sampled included kitchen and bathroom faucet handles, doorknobs, phone receivers and handles, computer keyboards, toilet handles, microwave handles, refrigerator handles, light switches, TV remote controls, and glass door handles. The home samples were obtained by swabbing each individual surface with a sterile polyester fiber-tipped applicator swab (Becton Dickinson and Company) moistened in 3 mL of 0.85% sterile saline solution.

A total of 218 samples from 14-day care centers were tested over 2 ½ years from April 2001 to November 2003. The 10 different fomites sampled in each day care center included toddler toys, infant toys, diaper changing areas, toilet seat tops, floor below toilets, kitchen counter tops, bathroom faucet handles, kitchen dishcloths, kitchen and

bathroom drains. The day care samples were collected using a sterile polyester fiber-tipped applicator swab (Becton Dickinson and Company) moistened in Letheen broth (Difco, Becton Dickinson, Sparks MD).

All samples were transported to the laboratory on ice and frozen at -20°C until assayed. Samples were homogenized by vortex then viral RNA was extracted. Influenza A virus was detected using reverse transcriptase-polymerase chain reaction (RT-PCR). Gel-electrophoresis was used to concentrate the RT- PCR product and photographs of PCR product bands were then taken and analyzed. Positive PCR product was then sequenced for further verification using an independent lab facility.

2.2 RNA Extraction and reverse transcription

Virus strains A/Hong Kong/8/68 and A/Victoria/3/75 human Orthomyxovirus were obtained from American Type Culture Collection (ATCC, Manassas, VA) and used as influenza A virus (H3N2) positive controls throughout the procedure. RNA extraction and RT-PCR procedures were performed in separate rooms to prevent sample contamination. Also, samples and reagents were kept in separate rooms and separate freezers to further protect from contamination. QIAmp Viral RNA Mini kits and procedure from Qiagen Inc. (Valencia, CA) were used as recommended by the manufacturer to extract and concentrate viral RNA from fomite and control samples. An initial volume of 280 µL was used in the RNA extraction process to produce a final volume of 80µL per sample.

All reagents used for reverse transcriptase and polymerase chain reaction were obtained from Applied Biosystems (Roche Molecular Systems Inc. Branchburg, NJ).

The reverse transcriptase reaction mixture contained 10μL of sample RNA extract, 3.5 μL of 25 mM $MgCl_2$ solution, 1.5 μL of Amplitaq Gold GeneAmp 10 × PCR buffer, 4.0 μL of 2.5 mM of dNTP mix, 0.5 μL of 50 mM Random Hexamers, 0.5 μL of 20 U/μL RNase Inhibitor and 0.5 μL of 50 U/μ MuLV Reverse Transcriptase to yield reverse-transcriptase mixture totaling 20.5 μL per sample. The reaction mixture was then placed in an Applied Biosystems Gene Amp PCR System 9700 thermocycler for 10 minutes at 24ºC, 60 minutes at 44°C, 5 minutes at 99°C and 5 minutes at 5°C.

2.3 PCR and product detection

The primers used were based on a nucleoprotien gene segment which is highly conserved among the subtypes of type A influenza virus. The viral primers consist of the following nucleotide sequences: 5'-ATC-ACT-CAC-TGA-GTG-ACA-TC-3' (upstream), and 5'-CCT-CCA-GTT-TTC-TTA-GGA-TC-3' (downstream), which amplify a 306 base pair product.[15, 16] This pair influenza A primers have a specificity of 100% and sensitivity of 70% when compared with other methods of detection.[16] PCR was performed using 29.75 μL of Rnase free water (Promega Madison, WI), 5.0 μL of 25 mM $MgCl_2$ solution, 5.0 μL of Amplitaq Gold with GeneAmp 10 × PCR buffer, 4.0 μl of 2.5 mM of dNTP mix, 0.5 μL of 200 μM (upstream and downstream) primers and 0.25 μL of 5 U/μL AmpliTaq Gold DNA Polymerase. Five microliters of the cDNA product from the reverse transcriptase reaction was added to the PCR master mix and resulted in a final mixture volume of 50 μL per sample. The PCR mixture was placed in an Applied Biosystems Gene Amp PCR System 9700 thermocycler for amplification at following times and temperatures: initial step 95°C for 10 minutes, then 40 cycles of each,

denaturation 94°C for 30 seconds, annealing 55°C for 30 seconds, elongation 72°C for 30 seconds, and final elongation 72°C for 10 minutes.

Ten microliters of PCR product was detected using agarose gel-eletrophoresis. The 2 % agarose gel was stained by adding 5 to 8 microliters of molecular grade ethidium bromide solution 10mg/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. 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. Systat 9.0 Chi-square analysis and the Fisher's Exact test were used for statistical analysis of the sample test results.

3. Results

3.1 Day care centers

Ten different fomites from 14 different day care facilities resulted in 218 samples assayed for influenza A virus. Results revealed a seasonal variation in the presence of influenza A virus on fomites in the spring and fall as seen in Figure 1. Fifty-three percent of the samples collected during spring months were positive for influenza A. However, only 23% of the samples collected during the fall months were positive for the virus. Data analysis using Chi-square test ($p = 0.00000$) and Fisher's Exact test ($p = 0.00002$) demonstrated significant p-values at the 0.05 α-level indicating a significant difference

between spring and fall results. The monthly variation in influenza A virus prevalence coincides with influenza season in Arizona (September to May) and may additionally reflect the pattern of disease incidence in the day care centers assessed (Figure 2). Results also indicated a variation in the number of positive samples collected from moist surfaces as compared with dry surfaces (Figure 3). However, the difference between positive moist and dry surfaces was not statistically significant at the 0.05 α-level (Chi-square $p = 0.13998$). Influenza A was detected more often in kitchen dishcloths (58%) and in diaper changing areas (57%) (Figure 4). The virus was detected least often on infant (33%) and toddler toys (30%) and bathroom surfaces (42% toilet seat tops, 41% toilet floor and 36% bathroom faucet).

3.2 Homes

Yearly variation in the occurrence of influenza A virus on fomites is also supported by data from the assessment of home surfaces. Influenza A virus was detected only in March in homes with children experiencing flu-like illness. Results reflect a higher presence of influenza A virus on home fomites during the spring, and an absence of influenza A virus on home fomites during the summer. Ninety-two samples from 8 different homes and 14 different surfaces were tested for the presence of influenza A virus. All five homes with ill children sampled during peak flu season in Arizona contained at least two fomites positive for influenza A virus (Figure 5). The occurrence of influenza A virus on surfaces in each home may reflect shedding of the virus or spread of the virus by infected individuals into the surrounding environment. In homes assessed during March, 59 % of the 59 samples assessed were positive for influenza A virus. None

of the 33 fomites tested in the 3 control homes during the summer months were positive for influenza A virus. Influenza A viral RNA was detected most frequently on phone receivers, and infrequently on computers (keyboards and mouse), as shown in Figure 6.

4. Discussion

This study is the first to investigate the seasonal occurrence of influenza A virus on indoor surfaces. Results from this study clearly demonstrate that influenza A virus was consistently present on fomites in various homes and day care centers during the influenza season. The state of Arizona experienced its highest seasonal incidence of influenza in the spring of 2001 and 2002.[17] Concurrently, the day care center data in this study revealed a statistically higher presence of influenza A virus on fomites during the spring 53%, as compared to fall 23% (Figure 1). Data from homes with ill children sampled during March 2003 are consistent with data from day care centers sampled during the influenza season (September 2001 to May 2002). Influenza A virus was present on 59% of surfaces in homes with ill children and not present in control homes tested during the summer. The shed and spread of influenza by individuals with active infections is indicated by the occurrence of positive household surfaces in homes with only ill children.

Viruses are shed in high numbers, and shedding may occur before symptom onset and continue for several days or weeks after symptoms have ceased.[9] Viral shedding has been detected in nasal secretions, up to 107 infectious influenza viral particles per mL.[18] Nasal secretion droplets containing infectious virus particles are generated via coughing, sneezing, and talking and are easily transmitted over considerable distances.[13] Research

by Koeniger documented the dissemination of bacteria from the mouth during coughing and sneezing at a distance of up to 12.5 meters.[19] Studies also show that an average sneeze can carry pathogens up to 6 feet traveling 103 miles per hour.[20, 21] Edwards et al. demonstrated that infectious aerosolized influenza virus could be recovered from petri dishes, glass, and fabrics present in the surrounding environment.[7] Other studies have shown that viruses can be transferred from surfaces to hands, and vice versa.[5, 7, 9, 13] Hall et al. clearly documented that primary spread of respiratory syncytial virus appears to be via hands contaminated from contact with respiratory secretions.[9, 13] Infectious viruses are spread easily through closed indoor environments.[5] Consequently, the majority of positive fomites in this study appears to have resulted from the transfer of contaminated nasal secretions to fomites by touching (hand to nose or mouth and surfaces etc.) and sneezing.

The throat is connected to the nasal cavity and the oral cavity; therefore nasal secretions are often swallowed and pass to the digestive tract. As a result there could be an increased concentration of influenza virus found in saliva and feces. Recent research has proven that birds shed avian influenza in feces, saliva and nasal secretions.[22] Influenza virus was recovered from 69% of the day care center diaper changing areas in this study. The high recovery rate of influenza from diaper changing areas could indicate a viral presence in infant feces, and if present an increased virus survival time. Additionally, influenza virus may have been transferred to non-toy objects via contact through hands, mouthing or talking. Studies demonstrate that children under 3 frequently mouth non-toys, household objects, phone receiver etc.[23, 24] The Koeniger study

documented the dispersal of bacteria from the mouth during speaking.[19] Influenza virus was recovered from 80% of the home phone receivers in this study. The high recovery rate of influenza from phone receivers may indicate transfer of virus via mouth, and viral shed in saliva. However, information on the shed of influenza in human feces and saliva has not been documented.

Various studies have demonstrated that influenza virus survives longer on hard nonporous surfaces, in low humidity and at cooler temperatures.[7, 9, 10] In this study all of the fomites tested in homes, and 9 out of 10 of the fomites tested in day care centers were hard nonporous surfaces. All of the fomite samples in our study were collected during seasons with cooler temperatures and low humidity. Ideally, the number of positive samples in this study should reflect the occurrence of influenza on hard nonporous surfaces under optimal environmental conditions, and support previous research. However, viral viability was not assessed due to the detection method (RT-PCR) used. Research has also indicated that viable influenza virus can survive on wet surfaces up to 72 hours and dry surfaces up to 48 hours.[9, 10, 25] In this day care study influenza A virus was detected on both moist and dry surfaces but no statistical difference was found. Additional research is needed to assess the infectivity of the virus when transferred from fomites.

Influenza viral presence on fomites in both day care centers and homes can indicate several extraneous factors. Variations in viral occurrence on day care center and home fomites may reflect the influenza disease pattern experienced by day care patrons or home occupants. Households and day care centers with more ill individuals could have a

higher number of positive fomites as a result of increases in viral shedding. Cleaning practices can also affect viral presence in the surrounding environment. Home surfaces like the telephone receiver and refrigerator handle are frequently used, but infrequently cleaned or disinfected. The lower occurrence of influenza virus on toys and floors may reflect the greater ease at which these surfaces can be cleaned. Generally, cleaning with disinfectants that degrade viral RNA in addition to disinfection can reduce the possibility of viral transfer, transmission and possible infection.

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Figure 1. Occurrence of influenza A virus on day care center surfaces during the spring and fall.

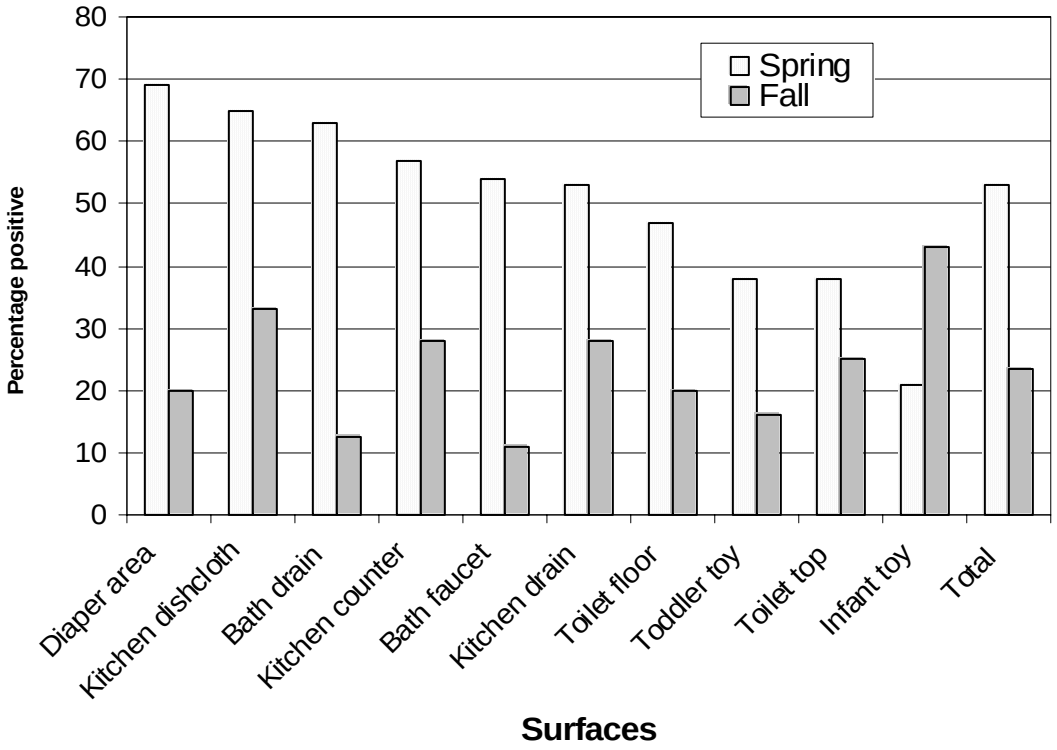

Figure 2. Occurrence of influenza A virus on surfaces in day care centers by month.

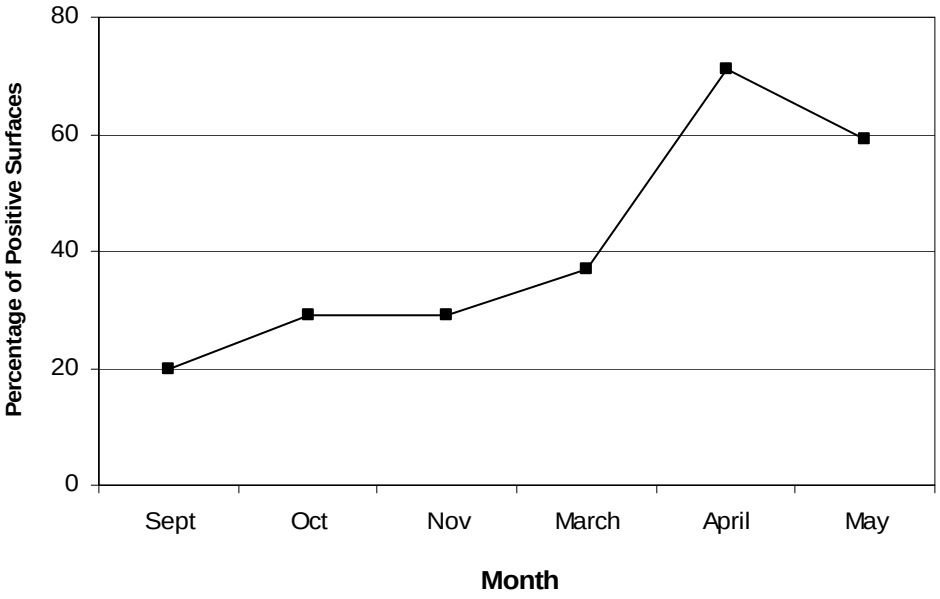

Figure 3. Occurrence of influenza A virus on moist and dry surfaces in day care centers.

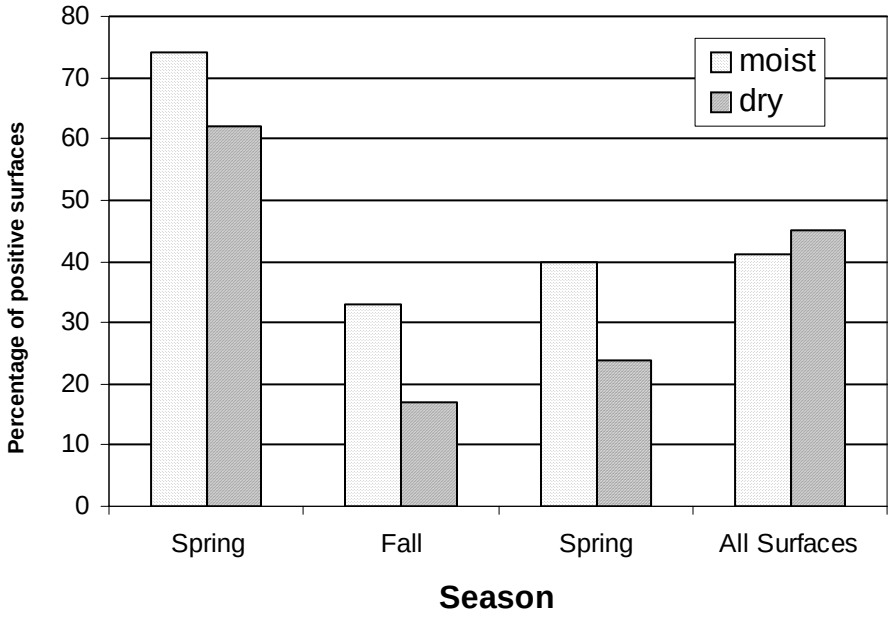

Figure 4. Occurrence of influenza A virus on surfaces in day care centers (fall and spring combined).

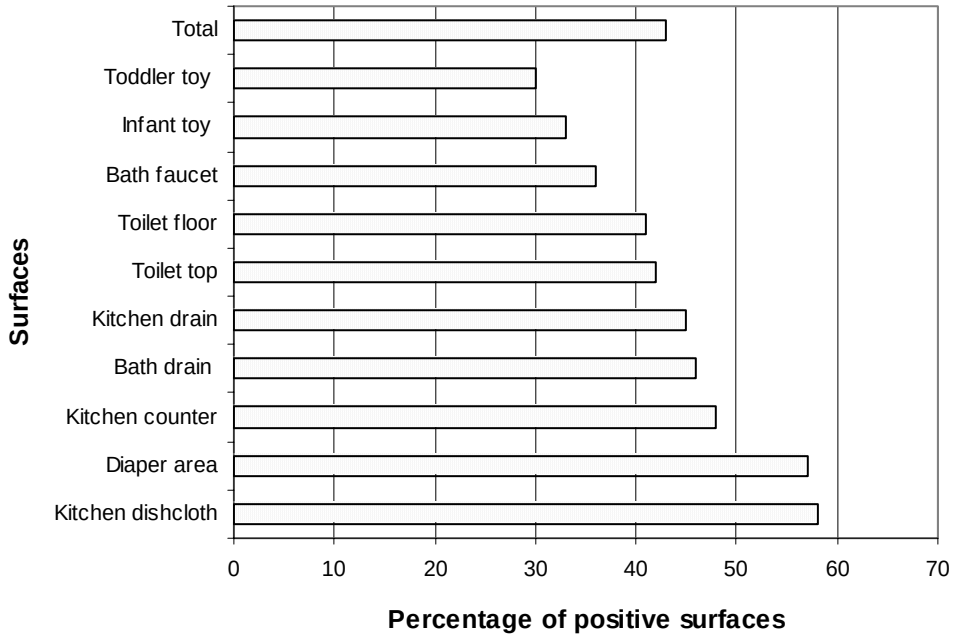

Figure 5. Occurrence of influenza A virus in homes with ill children.

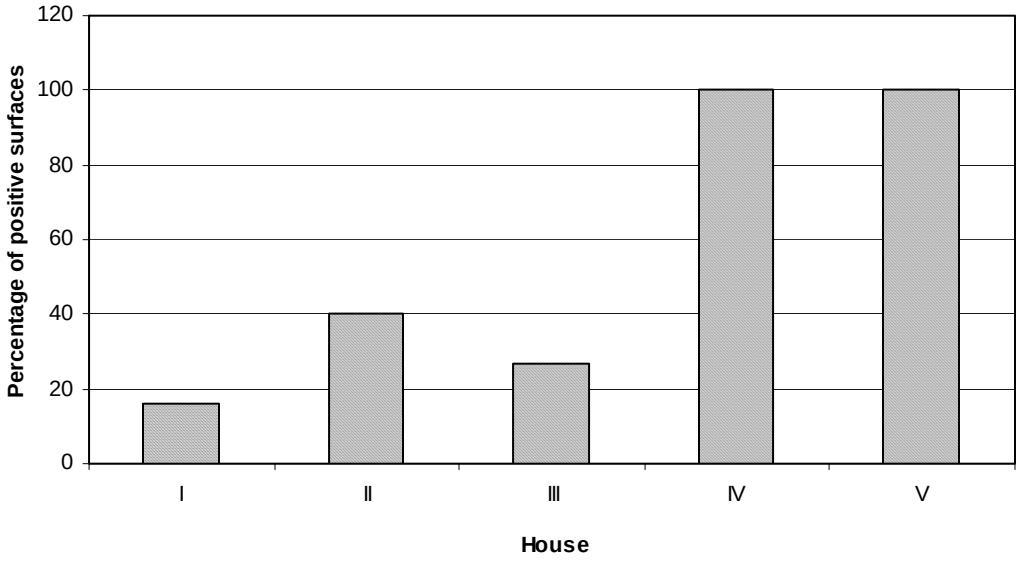

Figure 6. Occurrence influenza A virus on surfaces in homes with ill children.