Title: The prevalence of human parainfluenza virus 1 on indoor office fomites.

Running Title: Parainfluenza Prevalence on Indoor 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 an adult setting (office) 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 percentages of HPIV1 positive fomites in different buildings (Chi-square $p< 0.011$, Fisher's Exact $p = 0.054$) and 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.[1] Human parainfluenza viruses (HPIV) are medically important respiratory pathogens second only to respiratory syncytial virus in causing major lower respiratory tract disease in infants and young children.[2,3] HPIV are considered community acquired pathogens without ethnic, gender, age or geographic boundaries.[3] Thus far, HPIV are commonly associated with pediatric illness, but immunity is incomplete and infection occurs throughout life.[3] In contrast, there are very few studies which document HPIV as a cause of lower respiratory tract illness and hospitalization in adults.[3,22]

In 1999 an outbreak of lower respiratory tract illness among an international tourist group was diagnosed as HPIV1 after evaluation of nasopharyngeal and throat swabs. During the tourist outbreak, illness attack rates ranged from 100% (65 years and older) to 45% (less than 65 years).[22] A 2002 study conducted during influenza season at the San Francisco University Medical Center found HPIV1 in healthy adults with respiratory infections after using polymerase chain reaction (PCR) for diagnostic testing.[23] A 10 year study of adult lung transplant recipients identified HPIV infections as the leading cause of lower respiratory tract infections in patients with a median age of 48.[24] Adult respiratory infections are caused by several different viruses that often have clinically indistinguishable symptoms. In addition, 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 investigating HPIV1 prevalence document consistent levels of disease most months of the year with the highest incidence occurring during October of odds years.[3,7,- 10, 24-26] 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 investigating HPIV transmission indicate that 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 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**.(REF)** Astrovirus, adenovirus, influenza A and rotavirus have been isolated in day care facilities. **(REF)** 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] Additionally, 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 an adult setting (office) during a HPIV1 low incident year 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. 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 the 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 19.2 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. All sample cDNA product from the reverse transcriptase reaction was added to the PCR master mix, which 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 $p = 0.0017$. 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

184 detected more often on the desktops (47%), the computer mouse (46%) and the phone
185 (45%) (Figure 5). Virus was isolated least often on door handles (26%) and light
186 switches (19%) as seen in Figure 5.

187 **4. Discussion**

188 This study is the first to investigate the occurrence of HPIV1 or any virus on
189 indoor office surfaces. Results from this study clearly demonstrate that HPIV1 was
190 consistently present on surfaces in various office buildings from different
191 geographical locations. Study data revealed an occurrence of HPIV1 in all of the
192 office buildings tested during a year of low HPIV1 incidence. Positive fomite samples
193 obtained during a year with low HPIV1 incidence reinforces the theory that these
194 viruses can be spread easily through closed indoor environments.[5]

195 Viruses are shed in high numbers in respiratory secretions, and shedding may
196 occur before symptom onset and continue for several days or weeks after symptoms
197 have ceased.[6] Nasal secretion droplets containing infectious virus particles are
198 generated via coughing, sneezing, and talking are easily transmitted over considerable
199 distances.[6] Research by Koeniger et al., documented that dissemination of bacteria
200 from the mouth during speaking, and coughing can reach a distance of up to 12.5
201 meters.[18] Other research has indicated that an average sneeze can carry pathogens up
202 to 6 feet traveling 103 miles per hour.[19,20] Several studies have shown that viruses can
203 be transferred from surfaces to hands, and vice versa.[5,6, 7,13,16] Parainfluenza virus
204 (PIV) can be transferred from stainless steel surfaces to clean fingers, and HPIV3 has
205 remained viable on hands after one hour.[6,7] The majority of positive fomites in this
206 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. Consequently, the shed and spread of HPIV1 by adult individuals with active infections is indicated by the occurrence of HPIV1 RNA on office surfaces.

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, and Tucson as compared to New York may reflect infrequent surface use, cleaning practices or a variation in the HPIV infection rate.

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

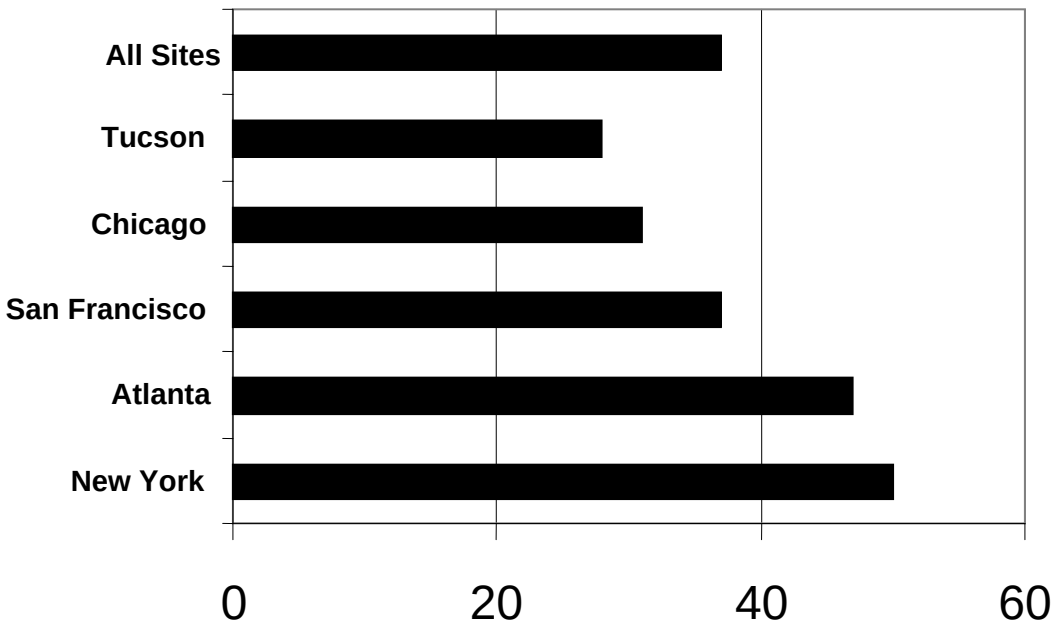

Figure 2. Fomites positive for HPIV1 by city.

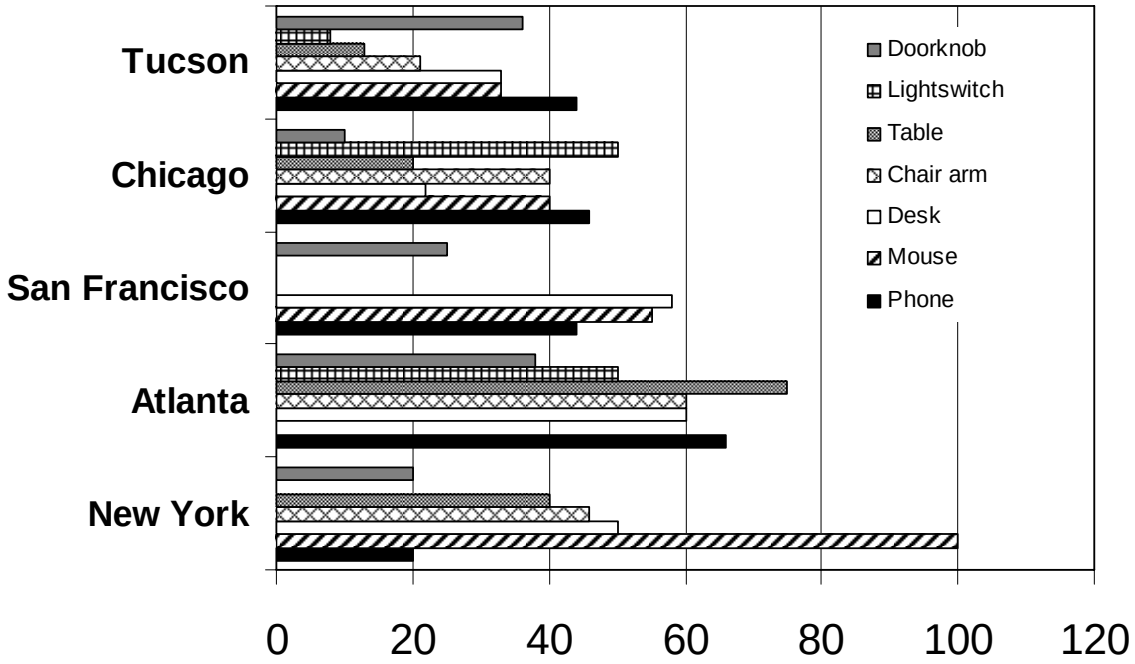

Figure 3. The percentage of fomites positive for HPIV1 by building.

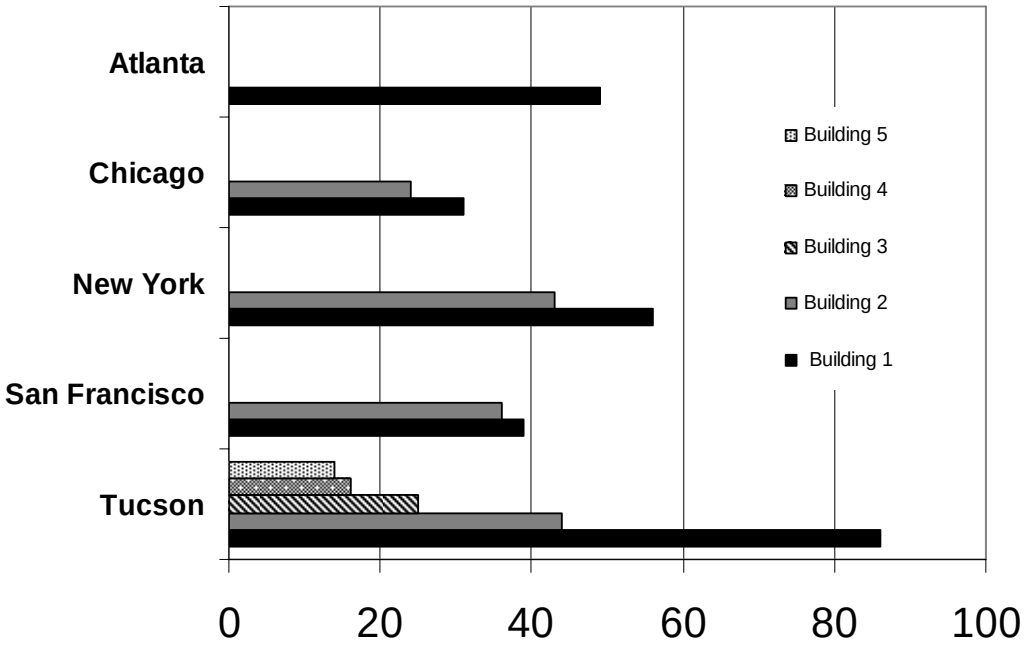

Figure 4. The percentage of fomites positive for HPIV1 by work area.

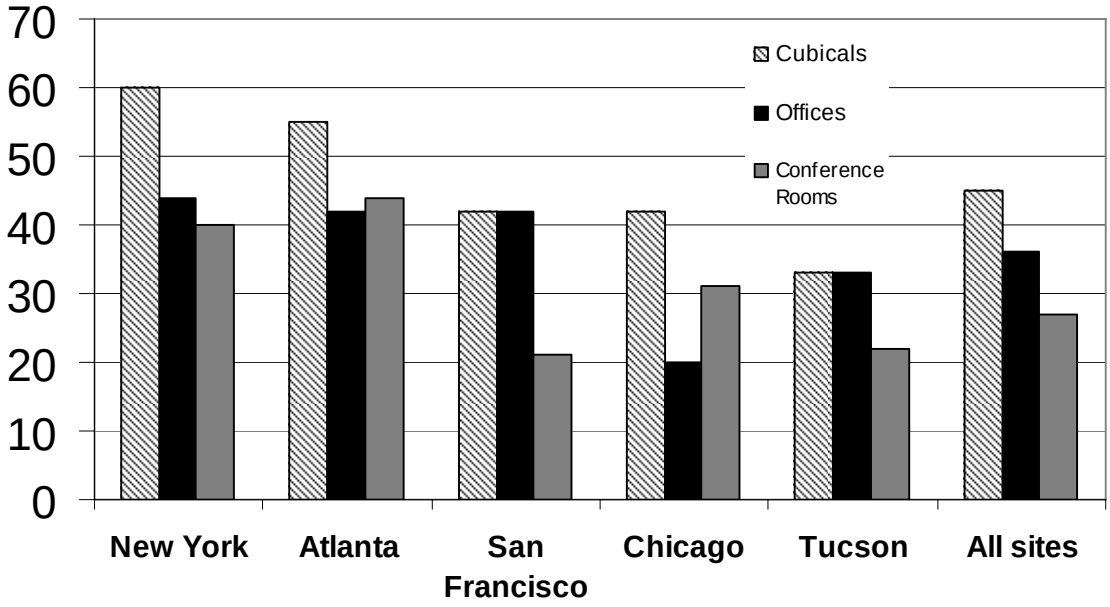

Figure 5. Total percentage of each fomite positive for HPIV 1.