Journal of Applied Microbiology

Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

Virus transfer between fingerpads and fomites

T.R. Julian, J.O. Leckie and A.B. Boehm

Department of Civil and Environmental Engineering, Environmental and Water Studies, Stanford University, Stanford, CA, USA

Keywords
bacteriophage, environmental hygiene, fomites, hand hygiene, quantitative microbial risk assessment, surfaces, virus transfer.

Correspondence
Alexandria B. Boehm, Department of Civil and Environmental Engineering, Environmental and Water Studies, Stanford University, Stanford, CA 94305-4020, USA.
E-mail: aboehm@stanford.edu

2010/0780: received 7 May 2010, revised 18 June 2010 and accepted 27 June 2010

doi:10.1111/j.1365-2672.2010.04814.x

Abstract

Aims: Virus transfer between individuals and fomites is an important route of transmission for both gastrointestinal and respiratory illness. The present study examines how direction of transfer, virus species, time since last handwashing, gender, and titre affect viral transfer between fingerpads and glass.
Methods and Results: Six hundred fifty-six total transfer events, performed by 20 volunteers using MS2, φX174, and fr indicated $0{\cdot}23 \pm 0{\cdot}22$ (mean and standard deviation) of virus is readily transferred on contact. Virus transfer is significantly influenced by virus species and time since last handwashing. Transfer of fr bacteriophage is significantly higher than both MS2 and φX174. Virus transfer between surfaces is reduced for recently washed hands.
Conclusions: Viruses are readily transferred between skin and surfaces on contact. The fraction of virus transferred is dependent on multiple factors including virus species, recently washing hands, and direction of transfer likely because of surface physicochemical interactions.
Significance and Impact of the Study: The study is the first to provide a large data set of virus transfer events describing the central tendency and distribution of fraction virus transferred between fingers and glass. The data set from the study, along with the quantified effect sizes of the factors explored, inform studies examining role of fomites in disease transmission.

Introduction

To better understand transmission routes for viral disease and develop more refined quantitative microbial risk assessment models (Atkinson and Wein 2008; Julian *et al.* 2009; Nicas and Jones 2009), additional information on the importance of fomites in the transmission of viruses is needed (Boone and Gerba 2007; Brankston *et al.* 2007). Insight into the role of fomites in the transmission of infectious disease can be obtained by studying the transfer of viruses between skin and surfaces.

Virus transfer between skin and surfaces can be described quantitatively by the fraction of virus on a contaminated (donor) surface that is transferred on contact to a recipient surface (Reed 1975; Gwaltney 1982; Ansari *et al.* 1988; Mbithi *et al.* 1992; Rusin *et al.* 2002). This fraction could be modulated by a number of factors including the donor/recipient surfaces and the virion surface.

Previous studies have reported a wide range of transfer fractions (0·0001–0·67) for transfer of a single bacteriophage (e.g. PRD-1) or pathogenic virus (e.g. rotavirus, hepatitis A, human parainfluenza virus-3, rhinovirus) between skin and various surfaces (Reed 1975; Ansari *et al.* 1988; Mbithi *et al.* 1992; Rusin *et al.* 2002; Bidawid *et al.* 2004). The range of transfer fractions is significantly influenced by the type of surface (porous or nonporous) contacted by the skin, with transfer between porous and food [cloth, lettuce, ham, beef, and carrots (Rusin *et al.* 2002; Bidawid *et al.* 2004)] surfaces generally lower than transfer to nonporous [stainless steel and plastic (Reed 1975; Rusin *et al.* 2002; Bidawid *et al.* 2004)] surfaces.

Only one published study has examined the transfer between skin and surface of more than one virus. In particular, Ansari *et al.* (1991) reported a difference in fraction transferred for rhinovirus and human parainfluenza virus-3 between fingers and metal disks. However,

the small sample size of the study presumably precluded statistical analysis.

This study explores how viral species and factors including inoculum size, direction of transfer, and skin condition affect virus transfer. We quantify the transfer of three different viruses, MS2, fr, and φX174, between fingerpads and a glass surface. Additionally, we applied experimental treatments to isolate the effects of the following on virus transfer: (i) inoculum size, (ii) direction of transfer, and (iii) skin condition defined by the gender and time since last hand washing. Inoculum size may influence fraction of virus transferred as the phenomenon was demonstrated in bacterial transfer by Montville and Schaffner (2003). Direction of transfer refers to the direction that virus is transferred, such as from skin-to-fomite versus from fomite-to-skin. Gender may influence virus transfer because men typically have a significantly lower skin pH (van de Vijver *et al.* 2003). Similarly, hand washing shifts the biological and chemical characteristics of the skin by decreasing organic and inorganic constituents (e.g. sebum, sweat, microflora), increasing pH, and decreasing hydrophobicity (Elkhyat *et al.* 2001; Kownatzki 2003; Barel *et al.* 2009). To our knowledge, this is the first study to examine the effects of virus species, inoculum size, and skin condition on virus transfer between skin and a surface.

Materials and methods

Volunteers

Permission of the Stanford University Research Compliance Office for Human Subjects Research was obtained prior to the study. Volunteers included 8 men and 12 women, with an age range of 20–32 years. To standardize unwashed state of volunteers' hands, volunteers washed their hands for 15 s using soap and water at least 1·5 h before the experiment, and avoided eating or going to the restroom within that time frame. No brand or type of soap was recommended or provided, and no effort was made to account for residual effects of soap products used before the experiment.

Virus and preparation of inoculum

This study quantifies transfer of three different bacteriophage (MS2, fr, and φX174) obtained from the American Type Culture Collection (ATCC). MS2 (ATCC #15597-B1), fr (ATCC #15767-B1), and φX174 (ATCC #13706-B1) strains were chosen because they have similar size (19–27 nm) and shape (icosahedral, no tail) to several human viruses, such as norovirus (Abbaszadegan *et al.* 2007). MS2 and fr bacteriophage are both +-sense RNA viruses of the *Leviviridae* family, with similar surface characteristics but different isoelectric points (3·9 and 8·9, respectively) (Gerba 1984; Liljas *et al.* 1994; Dowd *et al.* 1998; Herath *et al.* 1999). φX174 is a single-stranded DNA virus of the *Microviridae* family with an isoelectric point of 6·6 (Gerba 1984; Dowd *et al.* 1998).

The inoculum used in the study was prepared by propagating the model viruses to a concentration of 10^8–10^{10} plaque-forming units (PFU) per ml in phage buffer (Reddy *et al.* 2006). The propagated virus was then enumerated and diluted to 10^5–10^6 PFU ml^{-1} using tryptic soy broth (TSB, pH of 7·2 ± 0·2) to be used as virus stock. TSB is an organic-rich media intended to act as a model for the broad range of matrices in which respiratory and gastrointestinal viruses contaminate fomites (e.g. vomitus, urine, faeces, mucus, and saliva). Use of homogeneous and well-characterized TSB was intended to reduce variability introduced by use of natural media such as faecal suspensions, mucus, or saliva. The virus stock was enumerated during every experiment to confirm titre.

Plaque assay

The double agar layer procedure was used to enumerate virus (USEPA 2001). The hosts used were *Escherichia coli* K12-3300 (ATCC #19853) for fr, *E. coli* HS(pFamp)R (ATCC #700891) for MS2, and *E. coli* CN-13 (ATCC #700609) for φX174. The double agar layer procedure was chosen to estimate the fraction of infective virus transferred on contact.

Virus transfer

To determine the amount of virus transferred on contact between a fingerpad and a nonporous glass surface, we used a protocol adapted from Ansari *et al.* (1991). Specifically, we inoculated either between 100 and 600 or between 1000 and 6000 PFU diluted in TSB on the donor surface in an aliquot of 5 μl to represent low and high titres, respectively. Borosilicate coverslips are uniform, smooth, and clean surfaces providing a proxy for nonporous fomites with consistent characteristics. All surfaces, including the fingerpads, were subsequently allowed to visibly dry while supervised by the technician before contact between surfaces was made to mimic drying after natural contamination events. All samples from which no virus could be recovered from either the donor or recipient surface following the transfer event were removed from analysis.

The volunteer placed the donor and recipient surfaces in contact for 10 ± 1 s with an average constant pressure of 25 kPa (range of 16–38 kPa) controlled by counterbalancing a triple-beam balance weighted to 500 g. The

pressure of 25 kPa is comparable to the pressure exerted by a child while gripping an object, the pressure exerted locally on the fingerpads for adults using handtools, and the pressure used in studies examining transfer of soil from surfaces to skin (Link *et al.* 1995; Hall 1997; Ferguson *et al.* 2009). We used a cotton-tipped swab applicator, wet in 500 μl of phosphate buffer saline (PBS, 1 mmol l^{-1} potassium phosphate monobasic, 155 mmol l^{-1} sodium chloride, and 3 mmol l^{-1} sodium phosphate dibasic, pH of 7·4 ± 0·05, from Invitrogen, Carlsbad, CA, USA), to remove virus from the surfaces. The applicator was wiped firmly against the surface in a sweeping, rotating, motion for 10 s before being placed back into the remaining PBS and vortexed for 10 s. We used separate swabs to remove virus from the donor and recipient surfaces. Samples were aliquoted into 100 μl of 10^0, 10^{-1} and 10^{-2} dilutions in PBS; the dilutions were assayed using the double agar layer method (USEPA 2001). The range of detection for this method is 10–200 000 PFU. If virus was unrecoverable from a surface, the lower detection limit of 10 PFU was used as an estimate for the virus recovered. The fraction transferred (f) is defined as PFU recovered from the recipient surface (R_R), relative to PFU recovered from the sum of the donor (R_D) and the recipient surfaces, as previously described (Rusin *et al.* 2002):

$$f = \frac{R_R}{(R_R + R_D)} \quad (1)$$

Dessication, or the drying of the inoculum on the surface, results in a loss of virus titre (Ansari *et al.* 1988, 1991; Rusin *et al.* 2002). Because the surface is dried prior to the transfer event, the seeded inoculum is higher than the sum of virus recovered from donor and recipient surfaces after the transfer event. We chose to calculate f using just recoverable virus from the donor and recipient surfaces (eqn 1) so that the virus inactivated by dessication is not included in the fraction of virus transferred estimated by eqn 1. We assume that the relatively short time of the contact event and subsequent hand and surface sampling does not contribute to loss in titre because of inactivation.

The experimental design varied factors including low/-high titre and direction of transfer, with blanks and replicates across the ten fingerpads. Four randomly chosen fingerpads were assigned the following four titre/direction-of-transfer factor combinations: (i) low titre/glass-to-fingerpad, (ii) low titre/fingerpad-to-glass, (iii) high titre/glass-to-fingerpad, and (iv) high titre/fingerpad-to-glass. Four additional fingerpads were assigned the same factor combinations. As all factor levels of the fingerpads of the first set were identical to the factor levels of the fingerpads on the second set, the second set of contact events are defined as replicates for the contact events from the first set. In this manner, every contact event had a corresponding replicate contact event. The remaining two fingerpads (one on each hand) were selected to act as blanks. A blank is defined as a transfer event where fingerpad or glass was inoculated with TSB that did not contain any virus. After the initial ten transfer events were completed, the volunteers washed their hands for 15 s using Softsoap® antibacterial liquid hand soap (Colgate-Palmolive, New York, NY, USA), rinsed in tap water, and dried with a Kleenex® scientific cleaning wipe (Kimberly-Clark, Irving, TX, USA) under the technician's instruction. We then used the same factor assignments for each fingerpad to measure transfer for the 'washed' hands. Twenty volunteers performed the experiment using MS2 bacteriophage, 13 of the 20 volunteers repeated the experiment using φX174 bacteriophage, and 10 of the 13 repeated a third time using fr bacteriophage. Ten volunteers completed all three experiments. Temperature and relative humidity were recorded from a thermometer and hygrometer (Springfield Precision Instruments, Wood Ridge, NJ, USA) kept at the sampling location.

Statistics

All statistics were performed using the R statistical software package (R: A Language for Statistical Computing, version 2.9.0; R Foundation for Statistical Computing, Vienna, Austria), where appropriate, descriptive statistics (mean, median, and standard deviation) are reported. Statistical significance was assessed using a significance level of $\alpha = 0{\cdot}05$. The significance of experimental factors (direction of transfer, gender, virus species, time since last handwash, and titre) on per cent of virus transferred was assessed using n-way ANOVA on untransformed data. Tukey's *post hoc* test assessed significant differences between the transfer of each phage type. Distribution parameters for normal, lognormal, and Weibull distributions are reported for the data on fraction virus transferred between surfaces (f) stratified by phage type. These distributions are used to describe microbial and/or chemical transfer (Chen *et al.* 2001; Beamer *et al.* 2002; Pérez Rodríguez *et al.* 2007). Fivefold cross-validation and Kolmogov-Smirnoff methods were used to determine distribution parameters and goodness-of-fit.

Results

Virus transfer

f was quantified for 656 transfer events. Eleven transfer events (<2% of total transfers) of the original 688 were

excluded because of a laboratory error (e.g. mislabeling and failure to add host) involving at least one of the two samples (donor or receipient surface). An additional 21 transfer events (<3% of total transfers) were excluded because virus could not be recovered from both donor and recipient surfaces after the transfer. All blanks were negative, implying fingerpads were not contaminated prior to study, and no cross-contamination occurred during inoculation. Aggregating data for all three virus species, ranged from 0·001 to >0·999 with a median, mean, and standard deviation of 0·18, 0·23 and 0·22, respectively. Median, mean and standard deviation of f were 0·32, 0·31, and 0·20, respectively, for fr; 0·18, 0·23, 0·21, respectively, for MS2; and 0·09, 0·19, 0·24 for φX174.

An n-way ANOVA investigated treatment effects on f. Gender ($P = 0{\cdot}42$) and titre ($P = 0{\cdot}79$) were not significant. Direction of transfer ($P = 0{\cdot}01$) and time since last hand wash ($P = 0{\cdot}002$) were significant, with glass-to-fingerpad and unwashed hands transferring a greater fraction than fingerpad-to-glass and washed hands, respectively. Additionally, virus species was significant ($P < 0{\cdot}001$). f was larger for fr than for both MS2 (Tukey's test $P < 0{\cdot}001$) and φX174 ($P < 0{\cdot}001$). f was not significantly different between MS2 and φX174 ($P = 0{\cdot}16$). The mean, median and standard deviation of f are presented in Table 1 grouped by significant factors (e.g. glass-to-washed finger transfer of MS2 bacteriophage, unwashed finger-to-glass transfer of fr bacteriophage).

Parameters describing the distribution of f were determined for normal, lognormal, and Weibull distributions and are available, with estimates of goodness-of-fit, separated by virus species, in Table 2. Virus species impacts not only mean f but also the best-fit distribution; MS2 and φX174 are right-skew while fr bacteriophage has a more left-skew distribution. As evidence, histograms of the data with corresponding best-fit probability density functions are provided in Fig. 1, separated by virus species and direction of transfer.

Temperature and relative humidity ranged from 20–22°C and 45–60%, respectively, over the course of the study. No correlation (using Spearman's correlation coefficient) between temperature and f was found for fr ($\rho_s = 0{\cdot}06$, $P = 0{\cdot}47$), MS2 ($\rho_s = 0{\cdot}05$, $P = 0{\cdot}65$), or φX174 ($\rho_s = -0{\cdot}02$, $P = 0{\cdot}75$) or between relative humidity and f for fr ($\rho_s = 0{\cdot}08$, $P = 0{\cdot}31$), MS2 ($\rho_s = -0{\cdot}06$, $P = 0{\cdot}57$), or φX174 ($\rho_s = 0{\cdot}04$, $P = 0{\cdot}59$).

Table 1 The number of trials (n), mean ($\hat{\mu}$), median, and standard deviation ($\hat{\sigma}$) of f for data subset by factors determined to be significant via n-way ANOVA (virus species, direction of transfer, and skin condition as determined by time since last handwash)

Phage	Direction	Handwash	n	$\hat{\mu}$	Median	$\hat{\sigma}$
MS2	Finger-to-Glass	Unwashed	75	0·24	0·18	0·24
		Washed	75	0·15	0·1	0·16
	Glass-to-Finger	Unwashed	75	0·25	0·19	0·23
		Washed	80	0·26	0·21	0·19
φ-X174	Finger-to-Glass	Unwashed	49	0·26	0·16	0·28
		Washed	50	0·17	0·14	0·17
	Glass-to-Finger	Unwashed	48	0·21	0·07	0·29
		Washed	47	0·11	0·04	0·18
fr	Finger-to-Glass	Unwashed	36	0·28	0·25	0·21
		Washed	40	0·2	0·19	0·16
	Glass-to-Finger	Unwashed	40	0·37	0·39	0·22
		Washed	40	0·39	0·4	0·11

Discussion

We demonstrate that viruses are readily transferred between skin and a model fomite surface. Aggregating 656 viral transfer events, the mean fraction of virus transferred, f, is $0{\cdot}23 \pm 0{\cdot}22$ (mean and standard deviation), consistent with previous studies on virus transfer (Ansari *et al.* 1991; Mbithi *et al.* 1992; Rusin *et al.* 2002) and may be applicable as transfer estimate for viruses of similar size and shape, such as norovirus. The relatively large sample sizes of volunteers and contact events provide robust data to estimate distributions to describe f, an important parameter needed for quantifying microbial risk (Gibson *et al.* 1999; Nicas and Sun 2006; Wein and Atkinson 2009), especially in models that utilize activity data (Julian *et al.* 2009). f is influenced by the virus species, the direction the virus is transferred (i.e. fingerpad-to-surface or surface-to-fingerpad), and the characteristics of an individual's skin, in particular whether the hands have recently been washed. Although statistically significant, the factors we identified as influential may change the fraction of virus transferred by, at most, only 5–10%. This is small relative to the effect of changing the porosity of the fomite surface which has been shown to shift f by as much as two orders of magnitude (Scott and Bloomfield 1990; Rusin *et al.* 2002). Although the contribution of fomites relative to other transmission routes in perpetuating disease burden remains uncertain, the present study suggests it is specific to the aetiological agent and ameliorated through frequent hand washing.

Virus species affects both the mean and distribution of f. Our work expands on the work of Ansari *et al.* (1991) who observed transfer differences between two human viruses using 18 total transfer events, by measuring over 600 transfer events with three different viruses. Our high number of observed transfers allowed rigorous statistical testing of treatments. Our results also demonstrate that f is influenced by the interaction of virus species and direction of transfer (Table 1). In other words, f depends on the direction of transfer, but precisely how will depend

Table 2 The parameters [mean ($\hat{\mu}$), standard deviation ($\hat{\sigma}$), shape, and scale] and goodness-of-fit for fitting normal, lognormal and Weibull distributions to the fraction of virus transferred as determined by fivefold cross validation. Parameters and goodness-of-fit are determined for each bacteriophage individually, and all bacteriophage aggregated. A *P*-value > 0·05 indicates a good fit of distribution to data

	Normal			Lognormal			Weibull		
Phage type	$\hat{\mu}$	$\hat{\sigma}$	*P*-value	$\hat{\mu}$	$\hat{\sigma}$	*P*-value	Shape	Scale	*P*-value
MS2	0·23	0·22	0·09	−2·1	1·4	0·18	0·96	0·22	0·12
φ-X174	0·19	0·24	0·03	−2·6	1·5	0·43	0·77	0·16	0·84
fr	0·31	0·2	0·45	−1·6	1·1	0·14	1·4	0·34	0·66
All phage	0·23	0·22	<0·01	−2·1	1·4	<0·01	0·94	0·23	0·09

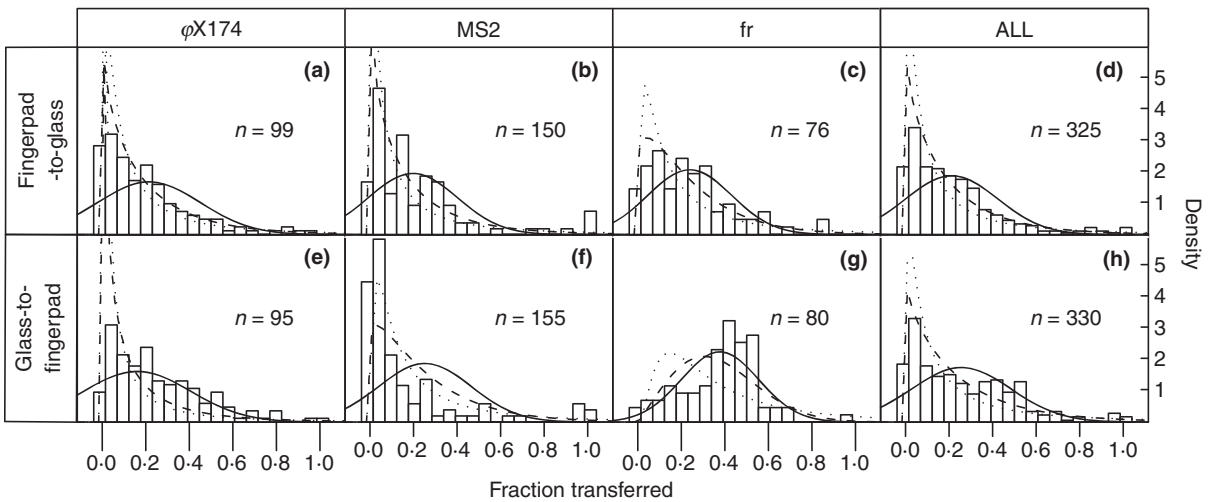

Figure 1 Cumulative distribution functions for fraction transferred by virus species and direction of transfer. Histogram of *f* for (a) φX174 fingerpad-to-glass, (b) MS2 fingerpad-to-glass, (c) fr fingerpad-to-glass, (d) all bacteriophage fingerpad-to-glass, (e) φX174 glass-to-fingerpad, (f) MS2 glass-to-fingerpad, (g) fr glass-to-fingerpad, and (h) all bacteriophage glass-to-fingerpad. The probability density function is overlaid on each histogram using the parameters reported in Table 2. (—) Normal; (- - - - -) Weibull and (·····) lognormal.

on viral species. This is consistent with observations described in the literature. Ansari *et al.* (1991) demonstrated human parainfluenza type 3 virus transfer is greater from fomite-to-fingers than fingers-to-fomite, while Mbithi *et al.* (1992), using hepatitis A virus, demonstrated the reverse: greater transfer from fingers-to-fomite than fomite-to-fingers.

Washing fingerpads prior to a virus transfer event reduces *f*. The reduction in virus transfer because of washing is greater for fingerpad-to-glass transfer than glass-to-fingerpad transfer. Changes in moisture level and pH on skin from handwashing (Gfatter *et al.* 1997), or other residual effects from the soap may contribute to this effect. To investigate the causal mechanism of reductions in *f* because of hand washing, future studies could incorporate moisture and pH measurements of the volunteers' fingerpads.

The impact of hand washing with soap and water on reduction of gastrointestinal and respiratory illness is well documented (Aiello *et al.* 2008) and is generally attributed to the reduction of pathogenic bacteria and virus on the hands (Curtis *et al.* 2000; Pickering *et al.* 2010). The results suggest that reduced viral transfer during hand-surface contacts could also contribute to the illness reduction. Further study of virus transmission may elucidate whether this finding extends to field conditions.

The influence of virus species on *f* could be because of the physicochemical properties of the virus. The surfaces, suspension media, and contact mechanics were kept constant throughout the study, and the experiments were carried out in ambient laboratory conditions such that temperature and humidity varied over small ranges. Because the viruses were the same shape (icosahedral), we attribute the observed differences in *f* between virus species to the different sizes (19–27 nm) and chemical properties of the virus capsids. In this experiment, the bacteriophage studied (MS2, φX174 and fr) have different net surface charge, as evidenced by the different

isoelectric points (3·9, 6·6 and 8·9, respectively), (Dowd *et al.* 1998; Herath *et al.* 1999) and different hydrophobicities. Specifically, φX174 was identified as the most hydrophilic and MS2 as the most hydrophobic in a study of 13 virus species by Shields and Farrah (2002); fr was not tested. Further research in this area is warranted.

Neither gender, inoculum size, temperature, nor humidity significantly influenced *f*. Significant differences in skin characteristics because of gender, such as pH, have previously been documented but the differences are small (pH of male skin was 4·7, female skin was 5·0) (van de Vijver *et al.* 2003). This difference in pH was not large enough to affect viral transfer in the present study. Inoculum size also did not significantly influence *f*, in contrast to previous work with bacteria that showed inoculum size significantly influenced bacterial *f* over multiple orders of magnitude (Montville and Schaffner 2003). Perhaps the range of titre we explored (one order of magnitude) was too low to observe an effect. Similarly, as neither temperature nor relative humidity were explicitly investigated in this study, the range in temperature (20–22°C) and relative humidity (45–60%) may have been too small to observe an effect on *f*.

There are several limitations to our study design. We minimized inter-trial variability by using glass surfaces, controlling for duration and pressure of contact, and using the same group of volunteers. In field conditions, such as when an individual contacts a virus-contaminated surface, variation may be greater as transfer events occur between a wide range of surfaces over a range of durations and contact pressures. The use of an infectivity assay (the double agar layer method) does not provide information on noninfective virus particles transferred on contact. Similarly, one PFU may be more than one infective viral particles (Galasso and Sharp 1962). Accounting for the presence of noninfective virus particles or multiple infective virus particles in one plaque may alter the fraction of infective virus transferred. Future studies could incorporate molecular methods to better understand transfer influence of noninfective particles and multiple virus per PFU on transfer.

Acknowledgement

This work was supported, in part, by the Shah Research Fellowship of Stanford University and by the United States Environmental Protection Agency (EPA) under the Science to Achieve Results (STAR) Graduate Fellowship Program. EPA has not officially endorsed this publication and the views expressed herein may not reflect the views of the EPA. The authors acknowledge the volunteers who participated in this study. Additionally, the authors thank the Boehm Lab, Robert Canales, Francisco Tamayo, and the anonymous reviewers who assisted with the work and/or provided suggestions for improving the manuscript.

References

Abbaszadegan, M., Mayer, B.K., Ryu, H. and Nwachuku, N. (2007) Efficacy of removal of CCL viruses under enhanced coagulation conditions. *Environ Sci Technol* **41**, 971–977.

Aiello, A.E., Coulborn, R.M., Perez, V. and Larson, E.L. (2008) Effect of hand hygiene on infectious disease risk in the community setting: a meta-analysis. *Am J Public Health* **98**, 1372–1381.

Ansari, S.A., Sattar, S.A., Springthorpe, V.S., Wells, G.A. and Tostowaryk, W. (1988) Rotavirus survival on human hands and transfer of infectious virus to animate and nonporous inanimate surfaces. *J Clin Microbiol* **26**, 1513–1518.

Ansari, S.A., Springthorpe, V.S., Sattar, S.A., Rivard, S. and Rahman, M. (1991) Potential role of hands in the spread of respiratory viral infections: studies with human parainuenza virus 3 and rhinovirus 14. *J Clin Microbiol* **29**, 2115–2119.

Atkinson, M.P. and Wein, L.M. (2008) Quantifying the routes of transmission for pandemic influenza. *Bull Math Biol* **70**, 820–867.

Barel, A.O., Paye, M. and Maibach, H.I. (2009) *Handbook of Cosmetic Science and Technology*. New York, NY: Informa HealthCare.

Beamer, P., Canales, R. and Leckie, J.O. (2002) Developing probability distributions for transfer efficiencies for dermal exposure. *J Expo Sci Env Epid* **19**, 274–283.

Bidawid, S., Malik, N., Adegbunrin, O., Sattar, S.A. and Farber, J.M. (2004) Norovirus cross-contamination during food handling and interruption of virus transfer by hand antisepsis:experiments with feline calicivirus as a surrogate. *J Food Protect* **67**, 103–109.

Boone, S.A. and Gerba, C.P. (2007) Significance of fomites in the spread of respiratory and enteric viral disease. *Appl Environ Microbiol* **73**, 1687–1696.

Brankston, G., Gitterman, L., Hirji, Z., Lemieux, C. and Gardam, M. (2007) Transmission of influenza A in human beings. *Lancet Infect Dis* **7**, 257–265.

Chen, Y., Jackson, K.M., Chea, F.P. and Scha_ner, D.W. (2001) Quantification and variability analysis of bacterial cross-contamination rates in common food service tasks. *J Food Protect* **64**, 72–80.

Curtis, V., Cairncross, S. and Yonli, R. (2000) Review: domestic hygiene and diarrhoea pinpointing the problem. *Trop Med Int Health* **5**, 22–32.

Dowd, S.E., Pillai, S.D., Wang, S. and Corapcioglu, M.Y. (1998) Delineating the specific influence of virus isoelectric point and size on virus adsorption and transport through sandy soils. *Appl Environ Microbiol* **64**, 405–410.

Elkhyat, A., Agache, P., Zahouani, H. and Humbert, P. (2001) A new method to measure in vivo human skin hydrophobia. *Int J Cosmet Sci* **23**, 347–352.

Ferguson, A.C., Biddle, D., Coleman, S., Bursac, Z. and Johnson, W. (2009) In-vitro soil adherence for dermal exposure using a controlled mechanical chamber. *J Appl Sci Res* **5**, 232–243.

Galasso, G.J. and Sharp, D.G. (1962) Virus particle aggregation and the plaque-forming unit. *J Immunol* **88**, 339.

Gerba, C.P. (1984) Applied and theoretical aspects of virus adsorption to surfaces. *Adv Appl Microbiol* **30**, 133–168.

Gfatter, R., Hackl, P. and Braun, F. (1997) E_ects of soap and detergents on skin surface pH, stratum corneum hydration and fat content in infants. *Dermatology* **195**, 258–262.

Gibson, L.L., Rose, J.B. and Haas, C.N. (1999) Use of quantitative microbial risk assessment for evaluation of the benefits of laundry sanitation. *Am J Infect Control* **27**, S34–S39.

Gwaltney, J.M. (1982) Transmission of experimental rhinovirus infection by contaminated surfaces. *Am J Epidemiol* **116**, 828–833.

Hall, C. (1997) External pressure at the hand during object handling and work with tools. *Int J Ind Ergon* **20**, 191–206.

Herath, G., Yamamoto, K. and Urase, T. (1999) Removal of viruses by microfiltration membranes at different solution environments. *Water Sci Technol* **40**, 331–338.

Julian, T.R., Canales, R.A., Leckie, J.O. and Boehm, A.B. (2009) A model of exposure to rotavirus from nondietary ingestion iterated by simulated intermittent contacts. *Risk Anal* **29**, 617–632.

Kownatzki, E. (2003) Hand hygiene and skin health. *J Hosp Infect* **55**, 239–245.

Liljas, L., Fridborg, K., Valegard, K., Bundule, M. and Pumpens, P. (1994) Crystal structure of bacteriophage fr capsids at 3.5 A resolution. *J Mol Biol* **244**, 279–290.

Link, L., Lukens, S. and Bush, M.A. (1995) Spherical grip strength in children 3 to 6 years of age. *Am J Occup Ther* **49**, 318.

Mbithi, J.N., Springthorpe, V.S., Boulet, J.R. and Sattar, S.A. (1992) Survival of hepatitis-A virus on human hands and its transfer on contact with animate and inanimate surfaces. *J Clin Microbiol* **30**, 757–763.

Montville, R. and Schaffner, D.W. (2003) Inoculum size inuences bacterial cross contamination between surfaces. *Appl Environ Microbiol* **69**, 7188–7193.

Nicas, M. and Jones, R.M. (2009) Relative contributions of four exposure pathways to influenza infection risk. *Risk Anal* **29**, 1292–1303.

Nicas, M. and Sun, G. (2006) An integrated model of infection risk in a health-care environment. *Risk Anal* **26**, 1085–1096.

Pérez Rodríguez, F., Valero, A., Todd, E.C.D., Carrasco, E., García-Gimeno, R.M. and Zurera, G. (2007) Modeling transfer of *Escherichia coli* O157: H7 and *Staphylococcus aureus* during slicing of a cooked meat product. *Meat Sci* **76**, 692–699.

Pickering, A.J., Davis, J., Walters, S.P., Horak, H.M., Keymer, D.P., Mushi, D., Strickfaden, R., Chynoweth, J.S. *et al.* (2010) Hands, water, and health: fecal contamination in Tanzanian communities with improved, non-networked water supplies. *Environ Sci Technol* **44**, 3267–3272.

Reddy, C.A., Beveridge, T.J., Breznak, J.A., Snyder, L., Schmidt, T.M. and Marzluf, G.A. (2006) *Methods for General and Molecular Microbiology*, 3rd edn. Washington, D.C: American Society of Microbiology Press.

Reed, S.E. (1975) An investigation of the possible transmission of rhinovirus colds through indirect contact. *J Hyg* **75**, 249–258.

Rusin, P., Maxwell, S. and Gerba, C. (2002) Comparative surface-to-hand and fingertip-to-mouth transfer efficiency of Gram-positive bacteria, Gram-negative bacteria, and phage. *J Appl Microbiol* **93**, 585–592.

Scott, E. and Bloomfield, S.F. (1990) The survival and transfer of microbial contamination via cloths, hands and utensils. *J Appl Microbiol* **68**, 271–278.

Shields, P.A. and Farrah, S.R. (2002) Characterization of virus adsorption by using DEAE-sepharose and octyl-sepharose. *Appl Environ Microbiol* **68**, 3965.

USEPA (2001) EPA method 1602: male-specific (F+) and somatic coliphage in water by Single Agar Layer (SAL) Procedure. Technical Report EPA 821-R-01-029. U.S. Environmental Protection Agency.

van de Vijver, L., Boelsma, E., Bausch-Goldbohm, R.A. and Roza, L. (2003) Subjective skin condition and its association with objective skin measurements. *Cosmet Toiletries* **118**, 45–54.

Wein, L.M. and Atkinson, M.P. (2009) Assessing infection control measures for pandemic influenza. *Risk Anal* **29**, 949–962.