ACS ENVIRONMENTAL Au
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Review

Occurrence of Human Viruses on Fomites in the Environment: A Systematic Review and Meta-analysis

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Cite This: ACS Environ. Au 2023, 3, 277−294
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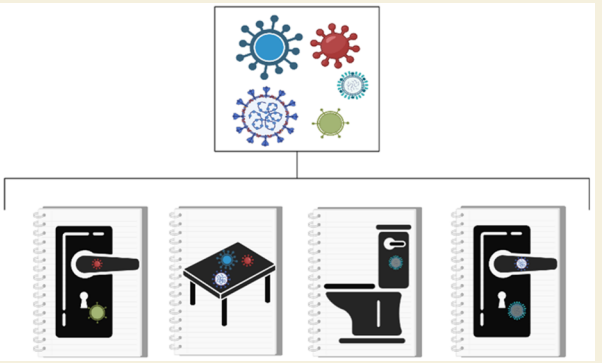
ABSTRACT: Documenting the occurrence of viruses on fomites is crucial in determining the significance of fomite-mediated transmission and the potential use of fomites for environmental disease surveillance. We conducted a systematic review and meta-analysis to compile information on the occurrence of human viruses on fomites in the environment; we identified 134 peer-reviewed papers. We compiled sampling and measurement methods, results, quality control information, and whether virus data were compared with community health data from the papers. We conducted univariate and multivariate analyses to investigate if presence of virus on fomites was associated with virus type (enveloped, nonenveloped), sampling location (healthcare setting, nonhealthcare temporary setting, nonhealthcare nontemporary setting), and area of fomite swabbed (<50, 50−100, >100 cm^2). Across 275 data sets from the 134 papers, there was the most data available for Coronaviridae and from fomites at hospitals. Positivity rates, defined as the percent positive fomite samples, were low (median = 6%). Data were available on viruses from 16 different viral families, but data on viruses from 9 families had few (n < 5) data sets. Many human virus families were not identified in this review (11 families). Less than 15% of the data sets reported virus concentrations in externally valid units (viruses per area of surface), and 16% provided a quantitative comparison between virus and health data. Virus type and area swabbed were significant predictors of virus presence on fomites, and the positivity rate of data sets collected from healthcare settings and nonhealthcare nontemporary settings (e.g., individual housing) were significantly higher than those collected in nonhealthcare temporary settings (e.g., restaurants). Data from this review indicates that viruses may be present on fomites, that fomite-mediated virus transmission may occur, and that fomites may provide information on circulation of infectious diseases in the community. However, more quantitative data on diverse viruses are needed, and method reporting needs significant improvements.



KEYWORDS: *fomite, viruses, environmental sampling, pathogens, surfaces, environmental surveillance, fomite-mediated transmission, systematic review*

INTRODUCTION

Viruses are important etiologies of infectious disease and are responsible for many recent epidemics, including those caused by SARS-CoV-2 (at present, >6 million deaths), influenza A virus H1N1 (2009, >151,000 deaths), and human immunodeficiency virus (HIV) (at present, 40.1 million deaths).[1−3] Beyond epidemics, respiratory and diarrheal diseases—often attributed to viruses—are two of the leading causes of death worldwide.[4]

Viruses are transmitted to humans via direct and indirect routes. Direct transmission occurs through direct exposure to viruses released by an infected individual. Alternatively, indirect transmission occurs through an environmental intermediary, such as inanimate objects (fomites), water, air, or food. Indirect transmission via fomites has long been understood to be important for respiratory viruses [influenza, respiratory syncytial virus (RSV), human parainfluenza virus (HPIV), severe acute respiratory syndrome coronavirus (SARS-CoV), and rhinovirus] and enteric viruses [rotavirus, adenovirus, norovirus, and hepatitis A virus (HAV)].[5] More recently, measurements of viruses in the environment, including on fomites and in wastewater, have been useful for community disease surveillance.[6−9]

Viruses are deposited in the environment when human secretions, such as mucus, saliva, urine, and feces, containing high viral titer are released from an infected individual. Once in the environment, viruses and their components (e.g., nucleic acids) can decay; decay rates are affected by parameters such as light intensity, temperature, and properties of the environ-

Received: May 22, 2023
Revised: July 12, 2023
Accepted: July 13, 2023
Published: July 25, 2023

https://doi.org/10.1021/acsenvironau.3c00025
ACS Environ. Au 2023, 3, 277−294

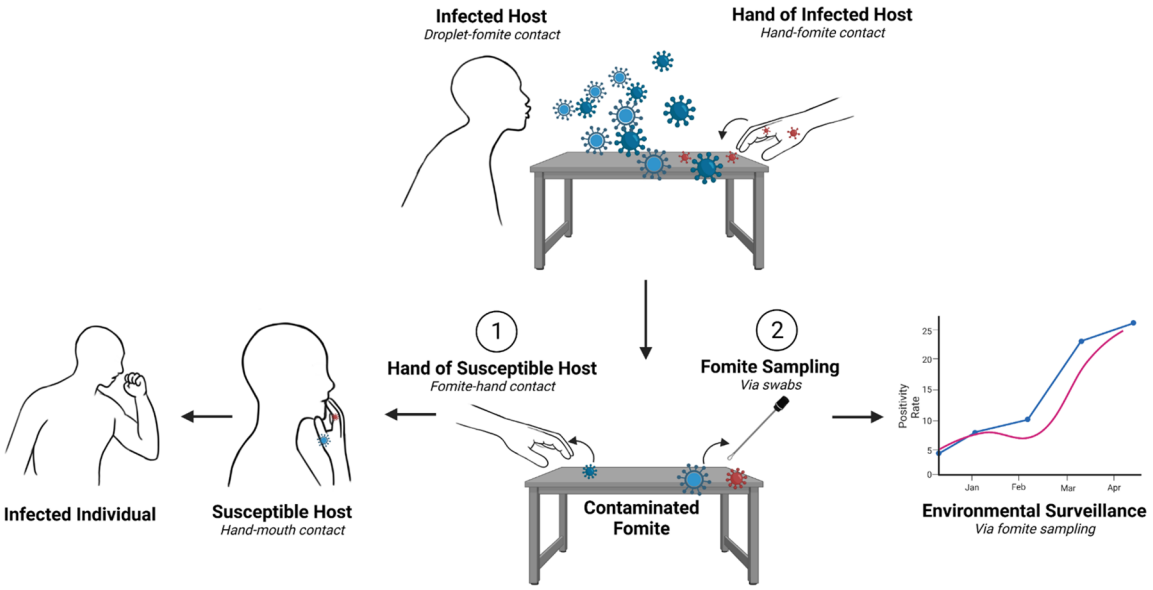


Figure 1. Example of how fomites may become contaminated by viruses and how that contamination may be relevant to human health. Fomites become contaminated when an infected host expels droplets by sneezing, coughing, or talking, which come in contact with a fomite directly or when an infected host touches a fomite using contaminated objects or hands. A susceptible host might touch the contaminated fomite (fomite−hand contact) and later touch their face and/or mucous membrane (e.g., hand−mouth contact) and become infected (labeled 1 in the diagram). Measurements of viruses on the contaminated fomite may also be used to infer the health of the population that came into contact with the fomite. The blue and red lines represent hypothetical incident cases in a population as a function of concentrations of viruses on fomite (labeled 2 in the diagram). Note: created with BioRender.com.

Table 1. Summary of the Different Categories for Datasets[a]

variable	categories
viral family	Adenoviridae, Astroviridae, Caliciviridae, Coronaviridae, Filoviridae, Flaviviridae, Hepadnaviridae, Herpesviridae, Orthomyxoviridae, Papillomaviridae, Paramyxoviridae, Parvoviridae, Picornaviridae, Pneumoviridae, Polyomaviridae, Reoviridae
location	hospital, school, residence, child care center, long-term care facility, cruise, food facility, outdoors, public building, other
implement type	cotton, foam, nylon, dacron, fiber-wrapped, rayon, flocked polypropylene, flocked polyester, other, not specified
premoisturizer/ eluent	saline solution, lysis buffer, transport medium, bovine serum albumin in normal saline (BSAS), minimal essential medium (MEM), broth medium, proteinase K solution, neutralizing solution, water, assumed dry, not specified
fomite type	door, toilet, electronic accessory, public button, public touchscreen, public handle, medical equipment, table/desk, trash can, bed, toys, sink/ sink tap, water fountain, handrail, chair, light switch, floor/floor drain, food-related item, window, air vent, personal protective equipment (PPE), disinfection equipment, wall, fabric, other, and not specified
fomite results comparison with health of community	quantitative, nonquantitative, not conducted

[a]An expanded list of items per category can be found in Tables S1−S4.

ment matrix (e.g., fomite composition,[5] air humidity,[10] and salinity of the water[11]). Viruses can infect humans through various routes, including inhalation of virus-contaminated air, ingestion of virus-contaminated water or exposure through recreational activities, consumption of contaminated foods, or contact with fomites.[12] The risk of infection via these exposures depends strongly on the concentration of viruses in the environment.[13]

This study focuses on virus-contaminated fomites both as sources of viruses that may cause infections in susceptible individuals and as sources of information regarding viral disease dynamics in a population (Figure 1). Laboratory studies have confirmed the transmission of different diseases via fomite exposure using experimental and modeling studies.[5,13−15] In general, however, it is difficult to determine the relative importance of fomite-mediated transmission on community spread of an infectious disease.[5,16,17] The occurrence and concentration of viruses on fomites are crucial in determining the significance of fomite-mediated transmission and the potential use of fomites as an environmental surveillance tool. Measurement of the concentrations of viruses on fomites can be challenging because of the low concentrations that may be present in the environment, which makes analytical sensitivity critical. Additionally, the lack of a standard method for measuring viruses on fomites presents further challenges.

The goal of this study is to compile and collate information on the occurrence of human viruses on fomites in the environment and the relationship between virus occurrence and health of the population in contact with the fomites. Using the PRISMA protocol,[18] we searched the literature for articles documenting the presence of human viruses on fomites in the environment, and collected information to allow us to investigate how their occurrence is associated with the type of virus, sampling location, and methods used for sampling. We also gathered, if available, information on how fomite data were associated with the health of people in the location where the samples were collected. The information presented herein

https://doi.org/10.1021/acsenvironau.3c00025
ACS Environ. Au 2023, 3, 277−294

provides a comprehensive summary of the occurrence of human viruses on fomites and the factors that affect their detection as a step toward understanding the role of fomites in viral transmission and their potential as an environmental surveillance tool. In addition, this work highlights key data gaps and areas for the field to improve moving forward in terms of clarity of method and results reporting and harmonization of measurement approaches.

MATERIALS AND METHODS

Following PRISMA guidelines (Figure S1), a literature search was conducted on September 23, 2022, using Scopus (search field = article title, abstract, keyword) to identify articles.[18] The search terms were (fomites OR fomite OR "high-touch surfaces" OR "high touch surfaces" OR "environmental surfaces") AND (virus* OR viral). We included "environmental surfaces" as a search term because we noticed in pilot searches that some authors referred to fomites in this way.[19] The results of the searches were imported into Covidence.[20] The program subsequently removed duplicated articles. The title and abstract of the resulting articles were screened, and if the article was deemed potentially relevant, it was advanced to full-text review. Full-text review was conducted to identify articles that met seven inclusion criteria: (1) conducted in real-world settings (not laboratory settings), (2) sampled viruses from fomites (RNA or DNA viruses), (3) measured naturally occurring (not seeded) human pathogenic viruses[21] using molecular or infectivity assays but not metagenomics methods, (4) did not only measure the effect of fomite disinfection (e.g., before-and-after effect of cleaning wipes), (5) presented primary data (e.g., not a review), (6) peer-reviewed, and (7) written in the English language. The following data were extracted from each paper: (1) study location (e.g., hospital, child care center, etc.; see Table 1); (2) virus name, as reported in the study; (3) sampling technique, including implement type (e.g., cotton swab, polyester swab, etc.), premoisturizer type, eluent type, and area sampled; (4) sample storage conditions, including temperature and length of storage; (5) list of fomites sampled; (6) selection criteria for fomites sampled; (7) description, if any, of the time of sampling in relation to cleaning or disinfection practices in the facility where the sampling took place (e.g., sampled before or after routine disinfection); (8) nucleic acid extraction kit (if applicable); (9) virus detection method [e.g., (RT-)PCR, (RT-)qPCR, culture methods]; (10) format of reported results (e.g., presence−absence, quantification); (11) reported results, including number of positive fomite samples for virus, total number of fomite samples, positivity rate (if reported), and lower limit of detection of the virus detection assay, as reported in the study; (12) list of fomites with a positive result; and (13) description, if any, of the method of comparison between fomite data and the health of the community interacting with fomites (e.g., statistical comparison between fomite data and incidence in the community). The screening of articles by title and abstract, full-text review selection, and data extraction was conducted by a single reviewer (author, W.Z.) with the support of a second reviewer (author, A.B.B.) for challenging decisions.

The data analysis was preregistered at OSF Registries.[22] Studies that met the inclusion criteria were divided into data sets per location, viral target, and analytical detection method [e.g., (RT-)qPCR, culture methods]. For example, if one paper described the occurrence of two different viruses on fomites collected in two different locations, the results were split into four separate data sets (location 1 with target 1, location 2 with target 1, location 1 with target 2, and location 2 with target 2). We found very few data sets describing concentrations of viruses in the environment using externally valid units (viruses per fomite surface area, described in more detail below), so we opted to use positivity rate as the main outcome variable.

Each data set was assigned a (1) viral family (Adenoviridae, Astroviridae, Caliciviridae, Coronaviridae, Filoviridae, Flaviviridae, Hepadnaviridae, Herpesviridae, Orthomyxoviridae, Papillomaviridae, Paramyxoviridae, Parvoviridae, Picornaviridae, Pneumoviridae, Polyomaviridae, Reoviridae) (Table 1); a location (hospital, school, residence, child care center, long-term care facility, cruise, food facility, outdoors, public building, and other) (Table 1, Table S1); a sampling implement type (cotton, foam, nylon, dacron, fiber-wrapped, rayon, flocked polypropylene, flocked polyester, other, and not specified); a premoisturizer (the liquid, if any, used to premoisten the implement prior to sampling the fomite), and an eluent (the liquid, if any, used to elute any captured virus from the implement after a fomite was swabbed) type [saline solution, lysis buffer, transport medium, bovine serum albumin in normal saline (BSAS), minimal essential medium (MEM), broth medium, proteinase K solution, neutralizing solution, water, not specified, and assumed dry (only used for premoisturizer when the study did not explicitly state the use of a "wet/prewetted/moist" swab prior to sampling)] (Table 1, Table S2); fomite types sampled [door, toilet, electronic accessory, public button, public touchscreen, public handle, medical equipment, table/desk, trash can, bed, toys, sink/sink tap, water fountain, handrail, chair, light switch, floor/floor drain, food-related item, window, air vent, personal protective equipment (PPE), disinfection equipment, wall, fabric, other, and not specified] (Table 1, Table S3); and a description, if any, of how the data obtained from fomites was compared with the health of the community interacting with fomites. The method of comparison between measurements of viruses on fomites to health observations was classified as either quantitative (if a statistical analysis was reported), nonquantitative (if no statistical analysis was reported), or not conducted (Table 1, Table S4). We defined health observations, hereafter referred to as community health data, as data related to the health status and/or characteristics of the population in contact with the fomites sampled, e.g., disease incidence and days since symptoms onset for a patient.

The primary outcome variable in this study is the percent of fomites sampled that are positive (positivity rate). However, we also recorded which fomite types were sampled within each data set and whether at least one of those fomite types was positive for the virus under study. We did this to provide an inventory of commonly sampled fomite types and a description of the probability that at least one of them was positive for viruses.

Meta-analysis

Viral family, location, and area-swabbed observations were categorized into broad groups to investigate their association with virus detection on fomites (Table 2). We hypothesized that virus structure (enveloped versus nonenveloped) impacts detection during fomite sampling. The presence/absence of a lipid envelope may affect virus decay rates on inanimate objects and hand-to-fomite transfer rates.[23,24] We, therefore, created a variable indicating whether the study detected enveloped or nonenveloped viruses. We also hypothesized that viruses may be more common on fomites in locations where sick individuals congregate (healthcare settings) than in nonhealthcare settings regardless of the length of time that individuals spend in these settings (nonhealthcare temporary settings and nonhealthcare nontemporary settings). We, therefore, created a variable indicating whether the sampling location was classified as a healthcare setting, nonhealthcare temporary settings (e.g., conven-

Table 2. Broad Categories for the Virus Type, Location, and Area of Fomite-Swabbed Variables Used for the Meta-analyses[a]

virus type (n = 275)	location (n = 275)	area swabbed (n = 169)
enveloped	healthcare setting	<50 cm^2
nonenveloped	nonhealthcare temporary setting	50−100 cm^2
	nonhealthcare nontemporary setting	>100 cm^2

[a]For the location variable, nonhealthcare residential setting includes single residential setting and congregate residential settings. An expanded list of items for the categories in the location variable can be found in Table S5.

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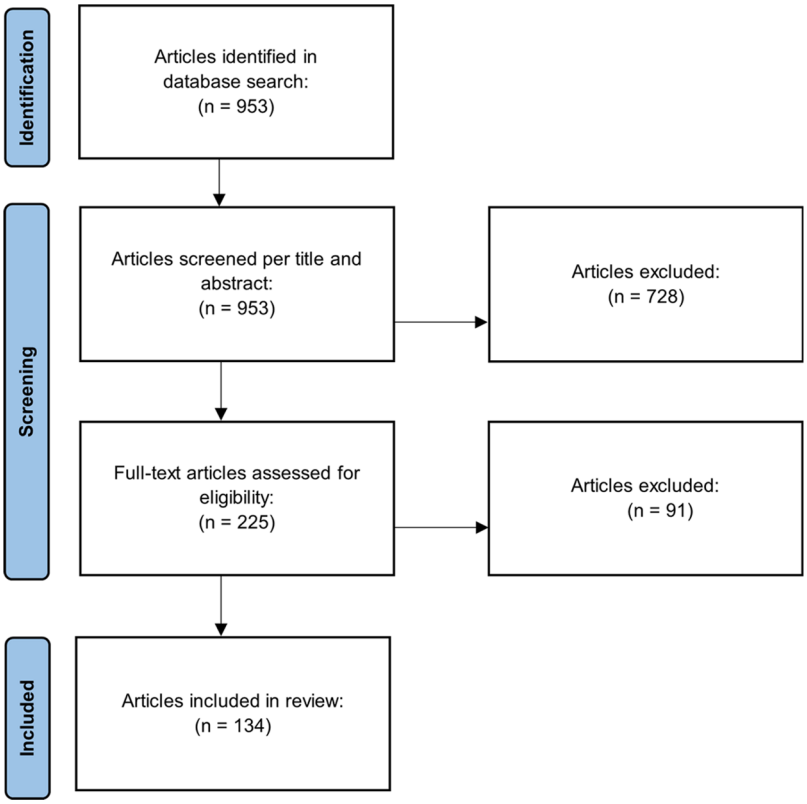

Figure 2. Results of the systematic review process in a process flow diagram as recommended by PRISMA guidelines.[18]

ience store or restaurant), or nonhealthcare nontemporary settings (e.g., individual housing, nursing homes). Lastly, we hypothesized that a larger fomite surface area swabbed would be associated with a higher positivity rate. We, therefore, created a variable indicating whether the fomite surface area sampled in the study was <50, 50−100, or >100 cm^2.

Positivity rates were combined across data sets and the resultant data was found to be not normally distributed ($W = 0.76$, $p = 2.20 \times 10^{-16}$) using a Shapiro−Wilk test, which suggests that nonparametric methods would be appropriate to test associations between positivity rates and other categorical factors.

We tested the null hypothesis that positivity rate was not associated with the virus type (enveloped versus nonenveloped), sampling location, or area of fomite swabbed using one-way nonparametric Kruskal−Wallis tests. We used Conover−Iman post hoc tests to compare positivity rates between the categories of independent variables.

We also used a multivariate model to investigate how virus type, sampling location, and area swabbed were associated with the presence of virus on fomites. We transformed the positivity rate of each data set into a binary variable. If positivity rate = 0 then the data set was assigned a 0 (virus absent), and if positivity rate > 0, then the data set was assigned a 1 (virus present). Twenty-eight percent (28%, $n = 47/169$) of the data sets were categorized with 0, and the remainder with 1. A multivariate logistic regression model was used to model the presence of virus (virus present, virus absent) as a function of virus type (enveloped, nonenveloped), location (healthcare setting, nonhealthcare residential setting, and healthcare other setting), and area swabbed (<50, 50−100, and >100 cm^2):

$$p\ (\text{presence of virus}) = 1/\left[1 + e^{-(\beta_0 + \sum_i^n \beta_i x_i)}\right]$$

Variable p (presence of virus) is the probability of a virus being present in fomite samples; β_0 is the intercept term, which represents the log odds of a virus being present in a fomite given that all the predictor variables equal zero; β_i represents the coefficients for each of the predictor variables x_i (virus type, location, and area); n represents the number of observations (data sets) included in the model; and i represents a specific observation.

We conducted a total of nine hypothesis tests. For the univariate analysis, two hypotheses were tested (two tests: Kruskal−Wallis test and post hoc test) for each independent variable [=(virus type, sampling location, or area of fomite swabbed)] for a total of six hypothesis tests. For the multivariate model, a hypothesis was tested for each independent variable [=(virus type, sampling location, or area of fomite swabbed)] for a total of three hypothesis tests. We used a p value of 0.006 (0.05/9) for $\alpha = 0.05$ to adjust for multiple comparisons (Bonferroni correction).

Although we collected data on implement type, premoisturizer type, and eluent across studies, it was not possible to test if they were associated with positivity rates or presence of virus because of the large number of missing observations, interdependence between these three variables, and the limited number of replicates between studies with respect to the combinations of methods (described further in the Results). Data analysis was completed using R version 4.1.2 within RStudio version 2021.09.1. The data used for this review and meta-analysis is available at the Stanford Data Repository.[25]

Quality Assessment

Quality Score. We evaluated the quality of each data set using 12 different criteria related to the reporting of methods (e.g., if the type of implement was reported). The full list of criteria can be found in the Supporting Information. A quality score ranging from 0 to 100% was assigned to each data set on the basis of the proportion of reported criteria items to the total number of applicable criteria items. A data set was classified as low quality if it only reported 0−33% of the applicable criteria items, as moderate quality if it reported 34−66%, and as high quality if it reported 67−100%. This follows a similar approach used by Huang et al.[26]

Publication Bias. It would be inadequate to complete standard publication bias assessment tests (e.g., funnel plot asymmetry test) with the data sets from this review given that the main outcome (positivity rate) does not measure the difference or association between two or more groups (e.g., the effect size of a treatment).

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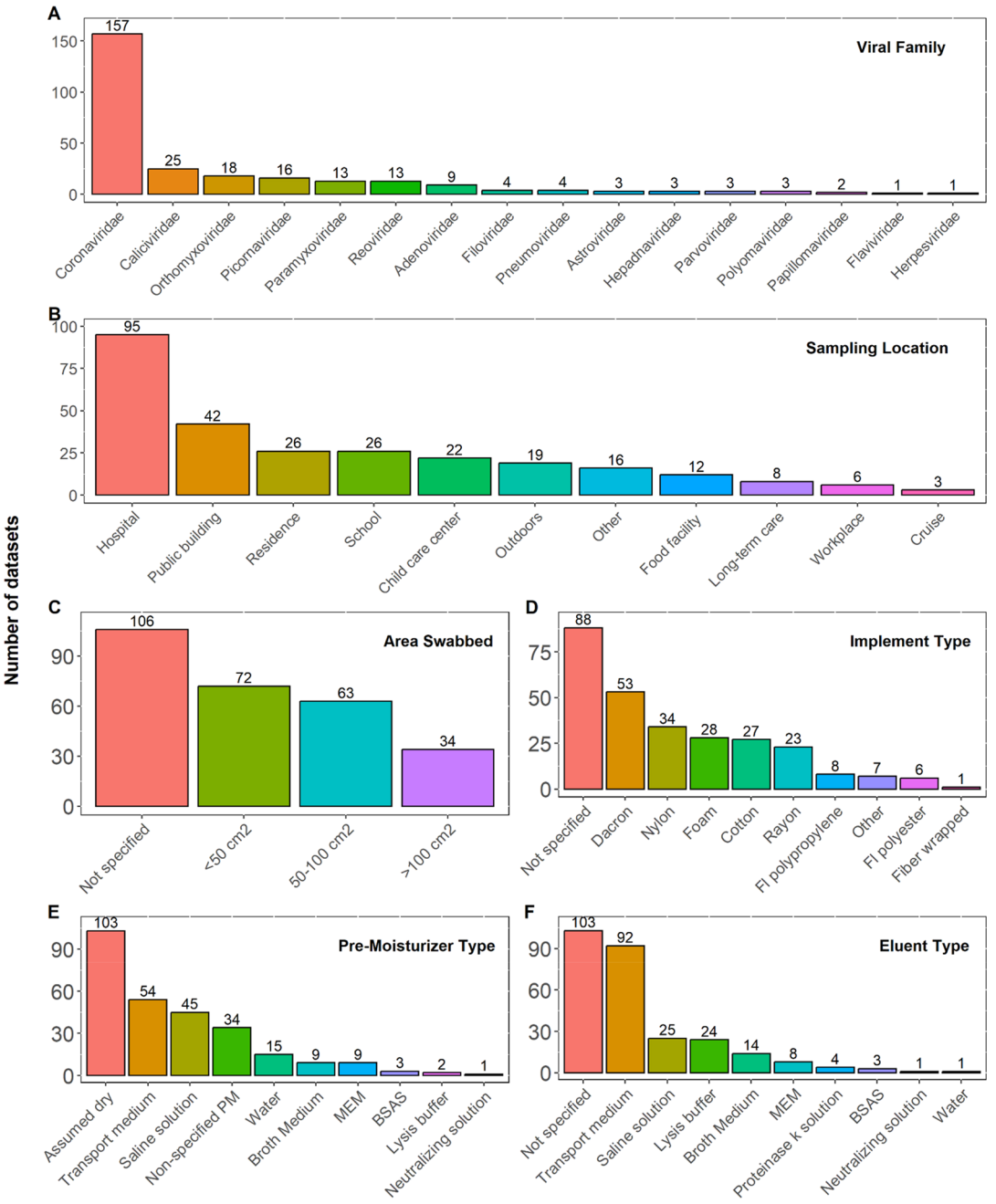

Figure 3. Number of data sets by viral target per family (A), sampling location (B), area swabbed (C), implement type (D), premoisturizer type (E), and eluent type (F). Tables S1 and S2 include the expanded list of items per category. (D) Other = Whatman paper, sponges, wipes; Fl = flocked; not specified = specified as a "swab" but did not report material. (E,F) Assumed dry = did not specify "wet/pre-wetted/moist" swab; nonspecified premoisturizer (PM) = specified "wet/pre-wetted/moist" swab but did not report the solution; MEM = minimal essential medium; BSAS = bovine serum albumin in normal saline.

Thus, to evaluate publication bias, we compared findings from studies that had multiple data sets (multiple locations, targets, or detection methods) to those that had a single data set (one location, target, and detection method). We hypothesized that if there were a tendency to publish a positive result (positivity rate > 0), we would find a greater number of negative results (positivity rate = 0) in studies that published multiple data sets than those that published a single data set. This hypothesis was evaluated by using the following equation:

$$\text{publication bias}_{\text{proportion}} = \frac{n_{\text{negative,single}}/n_{\text{single}}}{n_{\text{negative,multiple}}/n_{\text{multiple}}}$$

where $n_{\text{negative,single}}$ is the number of negative results in studies with a single data set, n_{single} is the number of studies with a single data set, $n_{\text{negative,multiple}}$ is the number of negative results in studies with multiple data sets, and n_{multiple} is the number of studies with multiple data sets. A publication bias proportion between 0 and 0.33 would indicate high bias because it would show that there is a substantially greater proportion of negative results in studies that published multiple data sets than those that published a single data set, 0.33−0.66 would indicate moderate bias, and 0.66−1 would indicate low bias because the studies that published multiple and single data sets have a similar or equal proportion of negative results.

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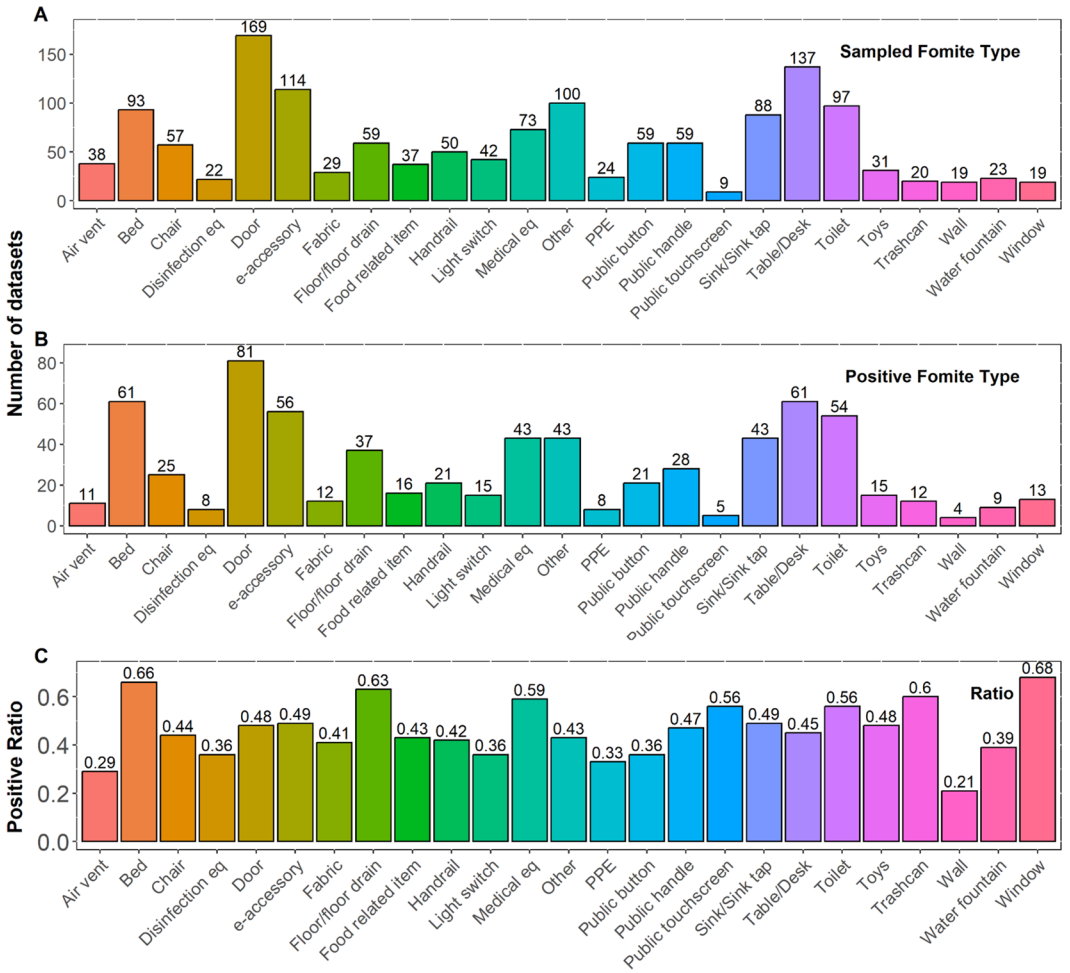

Figure 4. Number of data sets where a fomite type was sampled at least once (A), number of data sets where a fomite type was found positive at least once (B), and the positive ratio between these two numbers (C). Note: some examples of "Other" include exercise equipment, mirror, and steering wheel. Table S3 includes the expanded list of items per category, including the "Other" category. e-accessory = electronic accessory; eq = equipment; PPE = personal protective equipment.

RESULTS

Systematic Review

The review identified a total of 134 papers that met the inclusion criteria.[27−160] These papers were published between 1976 and 2022 (until the date of the literature search). The PRISMA diagram is shown in Figure 2.[18] We divided studies into data sets per location, viral family, and analytical detection method [e.g., (RT-)qPCR, culture methods], which resulted in 275 data sets. Each data set included results from 2 to 22 643 fomite samples and resultant virus positivity rates between 0 and 100% (median = 6%, interquartile range = 19%). Each data set had results from a median of six different fomite types (interquartile range = eight types).

Viral Families. The data sets described positivity rates for viruses from 16 different virus families. The largest number of data sets measured viruses in Coronaviridae (57%, n = 157), followed by Caliciviridae (9%, n = 25), Orthomyxoviridae (7%, n = 18), and Picornaviridae (6%, n = 16). The remainder of the data sets (21%, n = 59) included viruses belonging to 12 other virus families (Figure 3, Table 1). For 3 of the 16 virus families for which data sets were identified (Papillomaviridae, Flaviviridae, Herpesviridae), there were just one or two data sets per family. Seventy-three percent (73%, n = 201) of data sets came from studies that measured enveloped viruses, while 27% (n = 74) came from studies that measured nonenveloped viruses.

Of all the 275 data sets, the majority (90%, n = 248) were acquired using molecular methods, such as quantitative polymerase chain reaction (qPCR) and droplet digital PCR (ddPCR) to measure viruses, 9% (n = 24) using infectivity assays, and 1% (n = 3) using antigen assays. Sixty-two percent (62%, n = 171) of the results included in the data sets were reported exclusively in a presence/absence format; 15% (n = 41) in Ct or Cq values exclusively, which are outputs from a qPCR machine that are not externally valid as quantitative metrics, or in presence/absence and Ct or Cq values; and 23% (n = 63) in concentration formats [n = 38 in genetic material per fomite area, and n = 25 in other formats, such as gene copies/swab (gc/swab), gc/mL, log10 copies]. As only 38 of 275 data sets (less than 15%) included results reporting concentration data using externally valid units (viruses per area of surface), we opted to use positivity rate as the main outcome variable for the project.

Sampling Locations. Investigating sampling locations and their association with virus presence can help to identify high-risk areas and to understand the importance of environmental surveillance at certain sampling sites. Most data sets described the occurrence of viruses collected from fomites in hospital

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ACS Environ. Au 2023, 3, 277−294

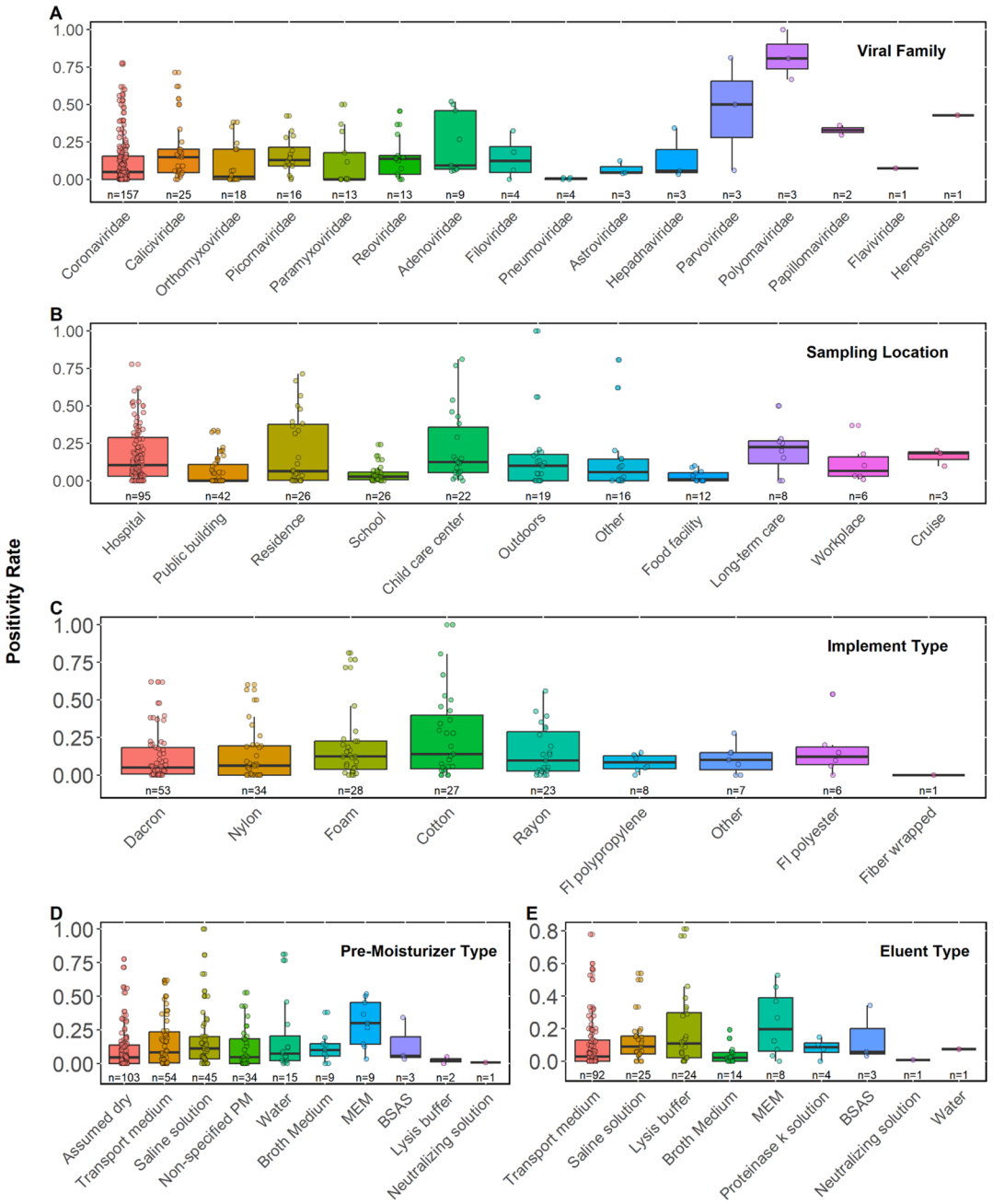

Figure 5. Positivity rate by viral family (A), sampling location (B), implement type (C), premoisturizer type (D), and eluent type (E). Each box plot is made up of the 25th quartile, median, and 75th quartile positivity rates for each category, and the length of each whisker is 1.5 times the interquartile range (IQR). Each box plot is overlaid with jittered data points from individual data sets for each category. Tables S1−S2 include the expanded list of items per category. (C) Other = Whatman paper, sponges, wipes; Fl = Flocked; not specified = specified as a "swab" but did not report material. (D,E) Assumed dry = did not specify "wet/pre-wetted/moist" swab; nonspecified premoisturizer (PM) = specified "wet/pre-wetted/moist" swab but did not report the solution; MEM = minimal essential medium; BSAS = bovine serum albumin in normal saline.

settings (35%, n = 95), followed by public building settings (15%, n = 42) and residences (9%, n = 26). The remainder of data sets (41%, n = 112) described occurrence of viruses on fomites in other locations, like long-term care facilities and outdoors (Figure 3, Table 1). We collapsed the location classification of the data sets into broader categories, including healthcare settings (35%, n = 96) from hospitals to examination rooms in clinics, nonhealthcare temporary settings (51%, n = 139) from workplaces to day care centers, and nonhealthcare nontemporary settings (15%, n = 40) from individual apartments to nursing homes.

Sampling Methods. Methods for sampling fomites for viruses typically require four steps: (1) premoisten a swablike implement with a liquid, (2) swab an area of the fomite or record the final area swabbed, (3) immerse the implement in a liquid to elute any captured virus from the implement, and (4) store the sample for future analysis. These four crucial steps during sampling can vary significantly between studies.

Premoisten Step. A large number of data sets came from studies in which there was no mention of wetting the implement prior to sampling, and we, therefore, assumed those data set methods used dry implements (37%, n = 103).

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Table 3. Total Number of Datasets Collected (N) per Location per Virus Target Family and the Median and Standard Deviation (SD) of the Positivity Rate (PR)[a]

virus family	median PR	SD PR	total N	child care	cruise	food facility	hospital	LT care	outdoors	public building	residence	school	workplace	other
Adenoviridae	9%	21%	9	2	0	1	3	0	0	0	0	2	0	1
Astroviridae	4%	5%	3	2	0	0	1	0	0	0	0	0	0	0
Caliciviridae	15%	20%	25	3	2	2	1	5	0	2	2	5	1	2
Coronaviridae	5%	16%	157	1	1	6	66	1	15	32	18	5	3	9
Filoviridae	12%	14%	4	0	0	0	4	0	0	0	0	0	0	0
Flaviviridae	7%		1	0	0	0	1	0	0	0	0	0	0	0
Hepadnaviridae	6%	17%	3	0	0	0	1	0	0	0	1	1	0	0
Herpesviridae	43%		1	1	0	0	0	0	0	0	0	0	0	0
Orthomyxoviridae	2%	15%	18	2	0	1	2	0	1	4	2	5	0	1
Papillomaviridae	33%	4%	2	0	0	0	2	0	0	0	0	0	0	0
Paramyxoviridae	0%	17%	13	1	0	1	3	0	1	2	1	1	2	1
Parvoviridae	50%	38%	3	1	0	0	1	0	0	0	0	1	0	0
Picornaviridae	13%	12%	16	4	0	1	2	1	1	2	1	3	0	1
Pneumoviridae	0%	1%	4	1	0	0	1	0	0	0	0	2	0	0
Polyomaviridae	81%	17%	3	0	0	0	0	0	1	0	1	0	0	1
Reoviridae	14%	14%	13	4	0	0	7	1	0	0	0	1	0	0

[a]LT care = long-term care facilities. Examples of "other" locations include public transportation buses and food processing plants. An expanded list of items per location can be found in Table S1.

Another group of data sets were collected by wetting the implement prior to sampling but did not report the type of premoisturizer used (12%, n = 34). The remainder of data sets were obtained using transport medium (20%, n = 54), a saline solution (16%, n = 45), and other types (14%, n = 39) as their premoisturizer (Figure 3, Table 1).

Implement. Nineteen percent (19%, n = 53) of data sets were collected using a dacron implement, 12% (n = 34) using a nylon implement, 10% (n = 28) using a foam implement, 10% (n = 27) using a cotton implement, and 16% (n = 45) using another implement type (Figure 3, Table 1). However, 32% (n = 88) of the data sets came from studies that did not report the type of implement used to sample.

Area of Fomite Swabbed. Twenty-six percent (26%, n = 72) of data sets were obtained by swabbing an area of fomite of less than 50 cm^2, 23% (n = 63) by swabbing between 50 and 100 cm^2, and 12% (n = 34) by swabbing greater than 100 cm^2 (Figure 3). Thirty-nine percent (39%, n = 106) of the data sets came from studies that did not report the area of fomite swabbed.

Eluent. The largest group of data sets, from studies that specified the type of eluent used, were collected using transport medium (33%, n = 92), followed by those using a saline solution (9%, n = 25), lysis buffer (9%, n = 24), and broth medium (5%, n = 14) as eluent. The remainder of data sets (6%, n = 17) were obtained using other eluent types (Figure 3, Table 1). However, 37% (n = 103) of data sets came from studies that did not specify the type of eluent used after sampling.

Storage Conditions. Only 40% (n = 109) of data sets did not report their sample storage conditions, but 60% (n = 166) of data sets reported storing their samples between 2 and 8 °C from 2 to 24 h during sampling and/or storing them at −20 or −80 °C until further analysis could be completed.

Fomite Types. Understanding which fomite types are most commonly sampled and found positive could help identify the fomite types that are most likely to be contaminated with viruses and guide the selection of fomites during environmental sampling. For each data set, we recorded whether or not a fomite type was sampled at least once and whether or not it was found positive at least once. Each data set included samples from a median of six different fomite types (interquartile range = eight types). Doors were sampled at least once in the majority of data sets (61%, n = 169), followed by table/desks (50%, n = 137), and electronic accessories (41%, n = 114) (Figure 4). For these most commonly sampled fomite types, at least one sample was positive for a virus in 81 of 169 (ratio = 48%) data sets that had samples from doors, 61 of 137 (ratio = 45%) data sets that had samples from tables/desks, and 56 of 114 (ratio = 49%) data sets that had samples from electronic accessories (Figure 4).

Comparison of Fomite Data with Community Health Data

Even though comparing fomite data with community health data could validate the use of fomite sampling as an environmental surveillance tool and provide insight on risk of transmission, we found that only a small percentage of data sets conducted a statistical analysis of this comparison. We found that 16% (n = 43) of data sets came from studies that performed a quantitative comparison between fomite data and the health of the community interacting with fomites, 36% (n = 100) performed a nonquantitative comparison, and 48% (n = 132) of data sets came from studies that did not conduct a comparison.

Meta-analysis

Overall, 45% of the data sets (n = 125) had a very low positivity rate (<5%), 23% (n = 63) had a low positivity rate (5−15%), 26% (n = 71) had a moderate positivity rate (15−50%), and 6% (n = 16) had a high positivity rate (>50%). We categorized the data sets on the basis of whether they measured enveloped or nonenveloped virus; whether they sampled in a healthcare setting, nonhealthcare temporary setting, or nonhealthcare nontemporary setting; and whether they sampled <50, 50−100, or >100 cm^2. Using positivity rate as the main outcome variable and the three aforementioned independent variables, we conducted univariate analyses. We found significant differences in positivity rate between the categories for each variable tested (Kruskal−Wallis, $p < 10^{-2}$;

https://doi.org/10.1021/acsenvironau.3c00025
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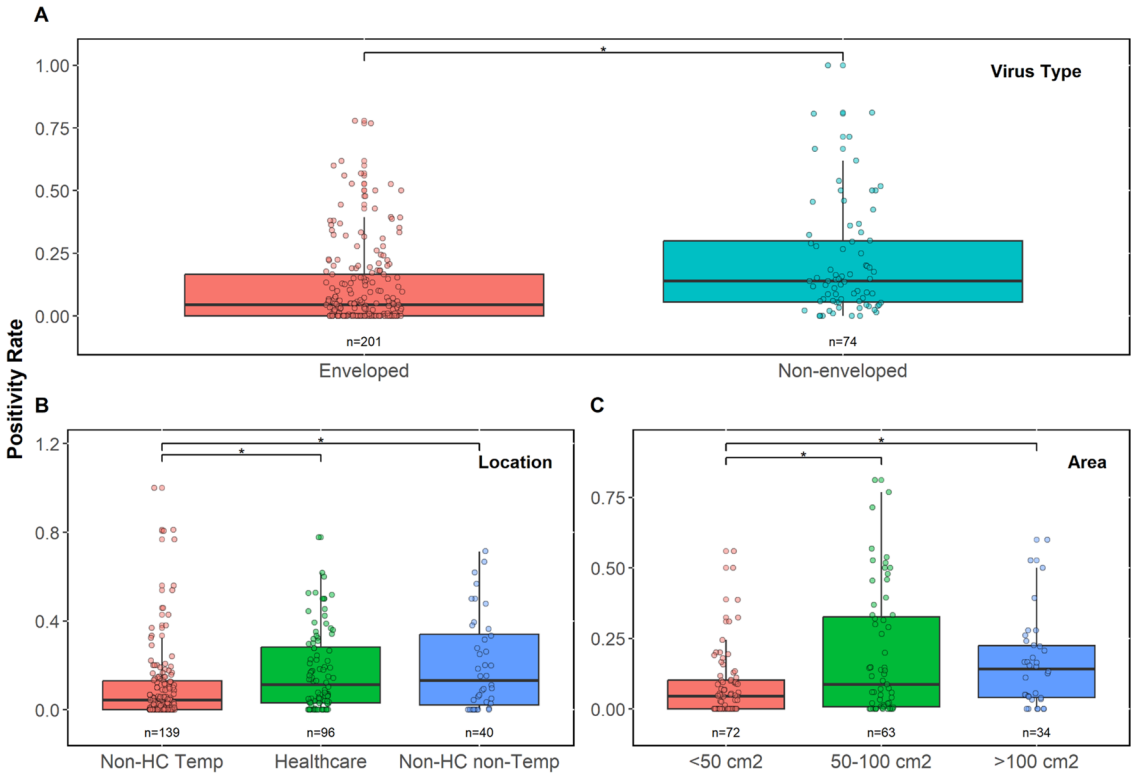

Figure 6. Positivity rate per each independent variable included in the meta-analysis: virus type (A), location (B), and area (C). Each box plot is made up of the 25th quartile, median, and 75th quartile positivity rates for each category, and the length of each whisker is 1.5 times the interquartile range (IQR). Each box plot is overlaid with jittered data points from individual data sets for each category. * = Statistically significant per the post hoc (Conover−Iman) test with a significance level of $p = 0.006$ accounting for the Bonferroni correction. Non-HC Temp = nonhealthcare temporary settings; Non-HC non-Temp = nonhealthcare nontemporary settings.

exact p-values shown in Table S6), and a post hoc test identified the significant differences between the categories of each variable (described further below).

We also converted the positivity rate of each data set into a binary variable to indicate presence or absence of a virus on fomite samples. We modeled the binary variable as a function of the three variables described previously in a multivariate logistic regression model. The model results indicated that virus type and the area of fomite swabbed are significant predictors of the presence of virus in fomite samples. Goodness of fit was confirmed using a Hosmer and Lemeshow test ($p = 0.94$), which failed to reject the null hypothesis that the model fits the data well, and a receiver operating characteristic (ROC) curve (AUC = 0.76), which indicated that the model is correctly classifying the observations into positive and negative cases with an accuracy better than random guessing (Figure S2, Table S9).

Viral Families. The highest median positivity rate was reported for Polyomaviridae (median = 80.1%, $n = 3$), while the lowest was reported for Paramyxoviridae (median = 0%, $n = 13$) (Figure 5, Table 3). Nonenveloped viruses had a higher median positivity rate (median = 13.9%, $n = 74$) than enveloped viruses (median = 4.4%, $n = 201$) (Figure 6, Table S6). A post hoc test confirmed that the positivity rate for nonenveloped viruses was significantly higher than for enveloped viruses (Conover−Iman test, $p = 3.98 \times 10^{-7}$). In agreement with the univariate analysis, the multivariate model confirmed that virus type is a significant predictor of presence of virus on fomites ($p = 3.40 \times 10^{-4}$) and indicated that the odds of finding a virus present on fomite samples increased by 10.1 times (95% CI [3.3, 44.8]) for data sets with a nonenveloped virus target compared with data sets with an enveloped virus target.

Sampling Locations. The highest median positivity rate was reported for long-term care facilities (median = 22.5%, $n = 8$), while the lowest was reported for public buildings (median = 0%, $n = 42$) (Figure 5). Once classified into broader categories, nonhealthcare nontemporary settings had the highest median positivity rate (median = 13.1%, $n = 40$), followed by healthcare settings (median = 11.3%, $n = 96$) and nonhealthcare temporary settings (median = 4.4%, $n = 139$) (Figure 6, Table S6). We found that the positivity rate in healthcare settings, as well as in nonhealthcare nontemporary settings was significantly higher than in nonhealthcare temporary settings (Conover−Iman test, exact p-values shown in Table S7), but we did not find a significant difference in positivity rate between healthcare settings and nonhealthcare nontemporary settings (Conover−Iman test, exact p-values shown in Table S7). However, the multivariate model found that sampling location is not a significant predictor of presence of virus on fomites ($p > 0.06$, exact p-values shown in Table S8).

Sampling Methods. Area of fomite swabbed was the only variable from the sampling method steps described previously that was included in the statistical analyses. A statistical analysis was not conducted for the other sampling methods steps (premoisten, implement, and eluent) because of the amount of missing information and interdependence between these variables. Therefore, our ability to interpret those results is

https://doi.org/10.1021/acsenvironau.3c00025
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limited, which highlights the need for additional research when selecting the appropriate sampling methods for a specific study.

Premoisten Step. Among the premoisturizers reported, the highest positivity rate was reported from studies using minimal essential medium (MEM) (median = 30%, n = 9), and the lowest was reported from studies using neutralizing solution as their premoisturizer (median = 1%, n = 1) (Figure 5).

Implement. Among the data sets from studies that reported the type of implement used, the highest median positivity rate was reported from studies using cotton swabs (median = 13.9%, n = 27), while the lowest was reported from studies using fiber-wrapped swabs (median = 0%, n = 1) (Figure 5).

Eluent. The highest median positivity rate was reported from studies using minimal essential medium (MEM) (median = 19.6%, n = 8), while the lowest was reported from studies using a neutralizing solution (median = 1%, n = 1) (Figure 5).

Area of Fomite Swabbed. Data sets that were obtained by swabbing an area of fomite greater than 100 cm^2 had the highest median positivity rate (median = 14.2%, n = 34), followed by those swabbing between 50 and 100 cm^2 (median = 8.7%, n = 63) and those swabbing less than 50 cm^2 (median = 4.6%, n = 72) (Figure 6, Table S6). We found that the positivity rate was significantly higher in fomite samples collected by swabbing an area of >100 and 50−100 cm^2 than those collected by swabbing an area of <50 cm^2 (Conover−Iman test, exact p-values shown on Table S7). Consistent with the univariate analysis, the multivariate model indicated that area of fomite swabbed is a significant predictor of presence of virus in fomites ($p = 4.55 \times 10^{-3}$) and that the odds of finding a virus present on fomites by swabbing an area >100 cm^2 increased by 4.5 times (95% CI [1.7, 13.7]) compared with swabbing an area of <50 cm^2.

Quality Assessment

Quality Score. Out of 275 data sets, 68% (n = 187) reported the implement type used, 63% (n = 172) reported the premoisturizer and eluent used, 61% (n = 169) reported the area of sample swabbed, 60% (n = 166) reported their sampling storage conditions, 55% (n = 150) reported the cleaning practices accounted for during sampling (e.g., sampled before routine disinfection), 25% (n = 70) reported the selection criteria for the fomites selected, 14% (n = 39) reported their sampling controls, 21% (n = 58) reported their limit of detection, 71% (n = 177/248) of those using molecular methods reported their extraction method, and 37% (n = 38/104) of those reporting their results using a format other than presence/absence used an externally valid unit (viruses per area of surface). On the basis of our quality score criteria, we classified 22% (n = 61) of the data sets as high quality, 72% (n = 197) as moderate quality, and 6% (n = 17) as low quality. The median quality score was 55% (interquartile range = 21%).

Publication Bias. We found a publication bias proportion of 0.59, which indicated that there was a larger percentage of negative results among studies with multiple data sets than in studies with a single data set. On the basis of our hypothesis, the publication bias proportion found moderate publication bias, which signals that there is a tendency to publish positive results for the data sets in this review.

DISCUSSION

We identified 275 data sets that document the presence or absence of different viruses on fomites in the environment, with most of them reporting at least one positive detection of viruses in their samples. These studies document a variety of viral targets, sampling locations, and fomite types. These data indicate that viruses are present on fomites in the environment and their presence represents a potential risk of infection and a potential to serve as a tool for environmental surveillance. It is important to note that most studies used nucleic acid amplification methods to detect viruses; these approaches do not discern between infectious and noninfectious viruses.[161] Only 9% of the data sets measured viruses using culture-based infectivity assays; more work to understand the presence of infectious viruses on fomites may be needed.

Data availability for viruses on fomites in the environment varied greatly. While there was a wealth of information available for some viral families, there was limited information for others. We identified data sets on 16 different viral families. Among those families, Coronaviridae, Caliciviridae, Orthomyxoviridae, Picornaviridae, Paramyxoviridae, Reoviridae, and Adenoviridae were well represented. Their high representation is likely because they contain viruses of high-interest, such as SARS-CoV-2 (Coronaviridae), as well as viruses for which there is experimental evidence of transmission via fomites, such as norovirus (Caliciviridae), influenza (Orthomyxoviridae), rhinovirus (Picornaviridae), parainfluenza virus (Paramyxoviridae), rotavirus (Reoviridae), and adenovirus (Adenoviridae).[5,162,163] There were only a few data sets for each of the other nine viral families. Some of those nine families also include viruses that are known to transmit through contaminated fomites [e.g., respiratory syncytial virus (RSV, Pneumoviridae)] or viruses that have been shown to persist for long periods of time on fomites [e.g., astrovirus (Astroviridae)].[5,164,165] Other viral families associated with human disease for which we did not identify data in this review include Anelloviridae, Arenaviridae, Bornaviridae, Bunyaviridae, Hepeviridae, Picobirnaviridae, Poxviridae, Retroviridae, Rhabdoviridae, Togaviridae, and Deltah.[21] Overall, our findings suggest that while progress has been made in researching viruses on fomites in the environment, there are important knowledge gaps on the presence of human viruses on fomites.

Many diverse locations were represented in the papers included in this study; however, we did note that there were some important locations for which there were no data. For example, we did not identify papers that sampled fomites in places of worship; entertainment venues (e.g., movie theaters); sport stadiums and concert venues; inside certain transportation systems, such as trains and airplanes; and nightlife venues (e.g., bars, nightclubs), even though these include important social and cultural locations where people congregate. Future studies should consider investigating fomites in these locations as they may serve as sentinel sites for environmental surveillance or important sites where fomite-mediated virus transmission could occur.

Data on viruses on fomites are critical for understanding indirect transmission of human diseases via fomites and could potentially be utilized for understanding levels of community infection. Unfortunately, we found that less than 15% of all data sets provided quantitative data on virus concentrations using externally valid units (viruses per area of surface), thereby limiting broad insights to be made regarding the importance of fomite-mediated transmission of viral diseases and the potential for fomites to inform community infectious disease surveillance. Our findings also showed a diversity in sampling methods, including a variety of materials used for

https://doi.org/10.1021/acsenvironau.3c00025
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implements, solutions used for premoisturizer and eluent, and areas of fomite swabbed. In addition, studies usually did not describe the materials that composed the surfaces sampled (e.g., metal, glass, porous vs nonporous), which is why we did not extract those data from the papers. The study highlights an opportunity to improve reporting practices, as many studies did not fully report their sampling methods. While sampling method harmonization across studies would be ideal, as a standard, future studies of viruses on fomites in the environment should provide comprehensive reporting of their sampling methods (e.g., reporting of implement type, premoisturizer type, eluent type, area of fomite swabbed, sample storage conditions) to ensure that our studies can be replicated by other researchers in the future and to understand what factors might have affected the positivity rate reported. It is also crucial for future studies to report concentration data in externally valid units (viruses per area of surface), as this is essential data for risk assessments. The environmental microbiology minimum information (EMMI) guidelines[166] can serve to guide reporting of methods and results in this area in the future.

Overall, the positivity rate found across data sets was low, with the majority of data sets having a positivity rate of less than 15%. The positivity rate of a data set can depend on the lower detection limit of the virus measurement method, including both the sampling technique and the analytical approach. A positivity rate of a data set collected using a method with a relatively high lower detection limit could be biased downward, whereas the positivity rate of a data set collected using a method with a relatively low lower detection limit could be biased upward. Only 21% of the data sets came from studies that reported lower detection limits. In our work, we showed that studies that sampled larger areas resulted in data sets with higher positivity rates than those that sampled smaller areas, thereby supporting the idea that measurement methods can influence positivity rates and highlighting that care should be taken in comparing results directly across studies.

Positivity rate is also influenced by the presence and strength of viral sources in the fomites, as well as factors that affect the persistence and removal of viruses from fomites. These in turn, depend on the specific setting and virus. We found that positivity rates of enveloped viruses were lower than nonenveloped viruses, which is consistent with studies that suggest enveloped virus persistence on fomites is reduced relative to nonenveloped viruses[24] and that nonenveloped viruses may be more readily transferred from hands to surfaces.[23] The location where the fomite was sampled also had a significant influence on positivity rate and suggested fomites in healthcare and in nonhealthcare nontemporary settings (e.g., residences of individuals) had the highest positivity rates, perhaps because of high probabilities that infected individuals are present in those environments or spend more time in contact with fomites there compared with nonhealthcare temporary settings (e.g., workplaces). Concentrations of different viruses in excretions, like mucus, saliva, and feces, also potentially influences their likelihood to be present on fomites; unfortunately, there is very limited data on concentrations of virus in different excretions.[167]

This review has a number of limitations that should be considered. First, we were only able to use the positivity rate as an outcome given the limited amount of data reporting concentration in externally valid units. Our statistical analyses, univariate and multivariate analyses, assume that the outcome of each data set is mutually independent, including those that came from the same study. One additional constraint to consider is the limited data availability for some virus families. The disproportional representation of viral families in the data sets may potentially influence some of the conclusions of the study. Another limitation is that our quality score only speaks to the level of reporting and does not indicate the proper execution of a study, as it was based on data that could be of less relevance to certain studies (e.g., reporting of area of fomite swabbed in a study looking for the presence/absence of a virus in a certain type of fomite). We were also not able to use standard publication bias assessment tools, given that our main outcome (positivity rate) was not compatible with these types of assessments. We also did not take a quantifiable approach for the fomite type variable, which makes our interpretation of those results limited. Lastly, the findings of this study are also bound by our specific search criteria terms and the inclusion of studies written in the English language, which could have excluded relevant studies.

IMPLICATIONS FOR FUTURE WORK

In this Perspective, several viral families were identified as areas for future investigation of fomites in the environment. Among those underrepresented families, Papillomaviridae, Flaviviridae, and Herpesviridae only had one or two data sets each, while Anelloviridae, Arenaviridae, Bornaviridae, Bunyaviridae, Hepeviridae, Picobirnaviridae, Poxviridae, Retroviridae, Rhabdoviridae, Togaviridae, and Deltah were not represented at all in this review. Additionally, several sampling locations were not identified in this review, including places of worship; entertainment venues (e.g., movie theaters); sport stadiums and concert venues; inside certain transportation systems, such as trains and airplanes; and nightlife venues (e.g., bars, nightclubs). To improve clarity of methods and results reporting, comprehensive reporting of sampling methods (e.g., reporting of implement type, premoisturizer type, eluent type, area of fomite swabbed, sample storage conditions) and the use of externally valid units (viruses per area of surface) when reporting concentration results are recommended. Future studies should also understand the factors that affect the positivity rate of their studies, including sampling methods (e.g., area of fomite swabbed), type of sampling location (healthcare setting, nonhealthcare temporary setting, or nonhealthcare nontemporary setting), and type of virus studied (enveloped, nonenveloped).

Future studies could explore additional factors that might affect the positivity rate, such as geographic location and climate conditions, to gain further insight into the environmental dynamics and factors influencing the occurrence of viruses. However, note that the data required for such analysis might be limited, as many authors do not provide information on climatic conditions. Additionally, viral metagenomics applied to fomite samples is likely to improve our understanding of fomite viromes,[168] so consideration of those data may be warranted in future systematic reviews. Lastly, expanding systematic reviews to include studies that conduct sampling in environments other than fomites, such as air and environmental waters, could deepen our understanding of virus occurrence in the environment.

https://doi.org/10.1021/acsenvironau.3c00025
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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsenvironau.3c00025.

Additional details about systematic review methods and meta-analysis results (Tables S1−S9 and Figures S1 and S2) (PDF)

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CRediT: **Winnie Zambrana** conceptualization (equal), data curation (lead), formal analysis (lead), investigation (equal), methodology (equal), validation (equal), visualization (lead), writing-original draft (equal), writing-review & editing (equal); **Alexandria B Boehm** conceptualization (equal), data curation (supporting), formal analysis (supporting), investigation (equal), methodology (equal), project administration (lead), resources (lead), supervision (lead), validation (equal), visualization (supporting), writing-original draft (equal), writing-review & editing (equal).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We would like to thank the Stanford Graduate Fellowship for providing funding to one of the authors (W.Z.) during the course of this research. The TOC graphic was created with BioRender.com. The views expressed in this article are solely those of the authors.

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