High time-resolution simulation of *E. coli* on hands reveals large variation in microbial exposures amongst Vietnamese farmers using human excreta for agriculture

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HIGHLIGHTS

- Videography and *E. coli* data are collected for farmers using human excreta.
- Data are used to simulate *E. coli* concentrations on hands over time.
- Farmers’ left and right hands contacted a mean 360 and 401 objects per hour.
- Microbial exposures vary substantially between farmers.
- *E. coli* in excreta and on tools, and hand-to-mouth contact frequency, most impacted risk.

GRAPHICAL ABSTRACT

Abstract

Infectious disease transmission is frequently mediated by the environment, where people’s movements through and interactions with the environment dictate risks of infection and/or illness. Capturing these interactions, and quantifying their importance, offers important insights into effective interventions. In this study, we capture high time-resolution activity data for twenty-five Vietnamese farmers during collection and land application of human excreta for agriculture. Although human excreta use improves productivity, the use increases risks of enteric infections for both farmers and end users. In our study, the activity data are integrated with environmental microbial sampling data into a stochastic-mechanistic simulation of *E. coli* contamination on hands and *E. coli* ingested. Results from the study include frequent and variable contact rates for farmers’ hands (from 34 to 208), how these contact rates differ between the left and right hands, and how these contact rates vary among farmers. Furthermore, we quantify the importance of factors such as hand-to-mouth contact frequency, *E. coli* concentration in excreta, and *E. coli* concentration in bulk materials.
1. Introduction

Transmission of many infectious diseases, such as enteric diseases, is mediated by human-environment interactions. People’s movement through, and contact with, the environment contributes to fate, transport, and transmission of infectious diseases. Quantitative microbial risk assessment (QMRA) is one example of a framework used to integrate human-environment interaction data to quantify risks associated with specific activities, understand relative contributions of various exposure pathways, and identify effective infection control strategies (Haas et al., 2014; WHO, 2016). Within this framework, exposure assessment is the process of estimating magnitude and frequency of people’s exposures to pathogens (Haas et al., 2014; WHO, 2016). The assessment is based on pathogen contamination estimates, treatment and/or intervention efficacy, and human-environment interaction data (WHO, 2016). Examples of human-environment interaction data include estimates of the amount of water and/or food ingested (intentionally or unintentionally) and frequency of hand-to-mouth contacts (Amha et al., 2015; Fuhrmann et al., 2016; Mattioli et al., 2015).

Exposure assessments, and in particular human-environment interaction data, are often based on simplified assumptions. For example, interaction data estimates (i.e., water, food, and soil intake; hand-to-mouth frequency) may be based on expert opinion, estimates from studies conducted in different contexts or study populations, or described as simplistic, linear, series of events (Amha et al., 2015; Genthe et al., 1999; Mattioli et al., 2015; Schöning et al., 2007). Increasingly, research is recognizing the need for improved human-environment interaction data (i.e., drinking water consumption, wastewater contacts), leading to more robust, evidence-based, exposure and risk estimates (Gretsch et al., 2016; Kwong et al., 2016; Teunis et al., 2016).

One approach to improving human-environment interaction data is the collection of high time-resolution (per second) activity data via videography and video translation (Ferguson et al., 2006; Julian et al., n.d.; Zartarian, 1995). In brief, study participants are observed using videography, and translators (aided by software) convert the video into a detailed (second-by-second) time series of the study participants’ environmental interactions. The resulting data is referred to as microlevel activity time series, or MLATS. The method can be used to estimate dermal, inhalation, non-dietary ingestion, and/or dietary ingestion exposures, as shown in chemical risk assessments (Beamer et al., 2009; Ferguson et al., 2013, 2006). The method has also been applied to microbial risks, primarily in the context of non-dietary ingestion exposures (Beamer et al., 2015; Julian et al., 2009; Julian and Pickering, 2015).

Here, we apply MLATS to model farmers’ risks from use of human excreta in agriculture in Vietnam. Human excreta is used extensively for agriculture and aquaculture in Southeast Asia, and Vietnam in particular (Do et al., 2007; Giang et al., 2015). Use of excreta is driven in part by financial benefits of nutrient recovery. Excreta use offsets fertilizer purchases and improves crop yield (Jensen et al., 2010). Use of human excreta in agriculture is also globally beneficial, as it captures and recycles nutrients. Nutrient capture diverts environmental pollution and offsets reliance on finite resources like phosphate rock (Cordell et al., 2011; Fuhrmeister et al., 2015; Heinonen-Tanski et al., 2005; Langergraber and Muellegger, 2005).

Despite the benefits, human excreta poses a health risk for farmers and end consumers. Excreta contains high concentrations of enteric pathogens including diarrheagenic Escherichia coli, Salmonella spp., rotavirus, norovirus, and Campylobacter spp.; hepatitis (hepatitis A and E); poliovirus; Toxoplasma gondii; and parasitic worms (Lam et al., 2015). Previous studies have identified increased risks of diarrheal disease generally, and helminth, hookworm, and Trichuris Trichuria infections specifically, for farmers reliant on human excreta (Blumenthal and Peasey, 2002; Do et al., 2007; Pham-Duc et al., 2014; Pham Duc et al., 2011).

Recommendations for reducing health risks from human excreta use are rarely met. Examples include storage of excreta for at least 6 months prior to use to allows sufficient time for inactivation of pathogens; incorporating additives (kitchen ash, waste, and/or lime) to reduce moisture, smell, increase pH, and combat flies; and using personal protective equipment (PPE), (masks, gloves, and boots) to reduce farmers’ exposure to excreta (Mackie Jensen et al., 2008; Phuc et al., 2006; Winblad, 2004). In Vietnam, excreta is rarely stored for the recommended 6 months (Jensen et al., 2010; Mackie Jensen et al., 2008). One reason is the misalignment between recommended storage times and seasonal timing or frequency of required land application (Jensen et al., 2010; Mackie Jensen et al., 2008). Personal protective equipment, although perceived to be beneficial, is also often neglected due to costs and/or perceived convenience (Knudsen et al., 2008). Another contributing factor is the prevailing belief that smell is indicative of health risks; once smell has dissipated, concerns over health decline (Knudsen et al., 2008). Therefore, recommended infection control precautions should be optimized to decrease pathogen exposures while accounting for farmers’ logistical, cultural, and behavioral concerns (Mackie Jensen et al., 2008).

In this study, we evaluate the use of high time-resolution activity data to estimate farmers’ exposures to the fecal bacteria E. coli while using human excreta in Hanoi, Vietnam. Videography is used to capture detailed high time-resolution data, which is used as the basis for a stochastic-mechanistic simulation. The simulation is parameterized using both primary data collection (measured E. coli contamination on hands, surfaces, water, and excreta) and previously published data (transfer efficiency of pathogens, surface area of contacts). Results obtained from the stochastic-mechanistic exposure simulation are compared to more traditional approaches to estimate exposure. The comparison is used to evaluate the use of high time-resolution human-environment interaction data. Finally, the simulation is used to identify factors that contribute to increased exposure, and to reaffirm infection control practices that can reduce farmers’ exposures and risks.
2. Materials and methods

2.1. Ethics

The following protocol was approved by the Ethics Commission of the Swiss Federal Institute of Technology in Zurich. Twenty-five (25) farmers were contacted through local village chiefs based on who was performing relevant agricultural activities (human excreta collection, transport, and/or land application). The farmers were provided with specific information about the research and research protocol. At the end of data collection, the farmers were also provided with general instructions on ameliorating risks from contact with human excreta. All information was provided in Vietnamese (translated from English) and was accompanied by a verbal explanation by a native Vietnamese speaker. Research proceeded only after written consent was obtained.

2.2. Study site

The study was conducted from November 2015 until February 2016 in Trai Hamlet, Van Tu commune, Phu Xuyen district in Hanoi, Vietnam. Trai Hamlet is a farming village with ~240 households that relies heavily on human and animal excreta for agriculture (Giang et al., 2012). The primary occupation for most households is domestic-scale agriculture, including animal farming. Of the village’s 56 ha land area, ~90% is devoted to paddy fields and fish ponds (Giang et al., 2012). More than half (56%) of the households use dry toilets for sanitation, and most (93%) of these households use the human excreta for agriculture (Giang et al., 2012). The study dates were chosen to coincide with the seasonal cultivation of major crops within the region, including beans, rice, and other vegetables.

2.3. Cohort

In total, 25 farmers were enrolled in the study for activity data collection (i.e., videography): 11 farmers collected human excreta from their latrine, 12 applied excreta to land, and 2 both collected and applied excreta. Of these, 15 (60% of the 25 total) also provided hand rinse samples for enumerating E. coli contamination of hands. The vast majority (96%) of farmers were female, and the median [min, max] age was 56 [38, 64].

2.4. Simulation framework

An exposure assessment simulation was developed that estimates time series of E. coli contamination on the hands and E. coli ingested due to hand-to-mouth contact events. The simulation framework is mechanistic and incorporates stochasticity of model parameters to capture variability and uncertainty, following a previously established model for estimating microbial transfer between hands and objects (Julian and Pickering, 2015). In brief, the model simulates E. coli concentrations on hands by tracking the transfer of E. coli between hands and the environment. The time series of sequential contact events (termed microlevel activity time series, or MLATS) responsible for transfer is determined using videography and video-translation (see Activity Data) (Julian et al., n.d.; Zartarian et al., 1995). We assume each contact between an object and a hand transfers bacteria to or from the hands based on: 1) bacteria contamination on the hands, 2) bacteria contamination on the object, 3) material properties of the object, and 4) the area of the hands involved in hand-to-object contact. Transfer for each contact event is modeled assuming bacterial transfer is a function of the difference (or gradient) in bacterial concentration between the hand and the object (Julian et al., 2009; Julian and Pickering, 2015). For surfaces, transfer was modeled as:

$$C_{\text{HF}} = C_{\text{HF}} + T \cdot S_{\text{OH}} (C_{\text{A}} - C_{\text{H}})$$  

where $C_{\text{HF}}$ is the final bacterial concentration on the hands (CFU/cm²), $C_{\text{HF}}$ is the initial concentration on the hands (CFU/cm²), $T$, transfer efficiency, is the proportion of bacteria that transfers between the object and the hands (unitless or g/cm²), $S_{\text{OH}}$ is the fractional surface area of the hand in contact with the object, and $C_{\text{H}}$ is the concentration on the object (CFU/cm² or CFU/g).

For bulk materials (ash, excreta, and water), transfer is modeled as:

$$C_{\text{HF}} = C_{\text{HF}} + S_{\text{OH}} (T \cdot C_{\text{A}} - C_{\text{H}})$$  

where $T_{\text{B}}$ is the transfer efficiency of bulk materials or water to hands (g/cm² or ml/cm²), and $C_{\text{B}}$ is the concentration of the bulk material or water (CFU/g or CFU/ml).

Dose of E. coli ingested by the farmers is assumed to occur due to hand-to-mouth contacts. Although object-to-mouth contacts may also increase dose, none were observed (see Activity Data). Dose is modeled using:

$$Dose = T_{\text{HM}} \cdot S_{\text{OH}} \cdot C_{\text{HF}}$$  

where $T_{\text{HM}}$ is the proportion of bacteria that transfers between the hands and the mouth (unitless), $S_{\text{OH}}$ is the surface area of the hand in contact with the mouth (cm²), and $C_{\text{HF}}$ is the bacterial concentration on the hands (CFU/cm²).

The simulation includes stochastic parameters, relying on Monte Carlo methods to incorporate variability and uncertainty. Parameter values are chosen from distributions which reflect variability and uncertainty for each parameter. Specifically, E. coli contamination of objects is randomized at the start of each simulation, whereas both transfer efficiency and surface area of the contact are randomized prior to each contact event. To instantiate the simulation, initial ($t = 0$) E. coli concentration on hands are assumed to be 0.01 CFU E. coli/cm², informed by E. coli measurements obtained at the start of videography (see Microbial contamination).

The primary outcomes from the simulation are the time series of E. coli concentration on the hands and ingested dose, defined here as the quantity of E. coli transferred to the mouth from hand-to-mouth contacts.

2.5. Parameter estimation

2.5.1. Activity data

Activity data were collected following the method of Julian and Pickering (2015). In brief, video cameras on head straps (GoPro Digital Hero 4, Woodman Labs, San Mateo, CA) were worn by farmers. Video - in first person perspective - of the range of motion of the farmers’ hands and the lower portion of their face was captured for the duration of their activity. The farmers were instructed to perform their typical activities as they usually would. Between 26 and 344 min of data were collected for each farmer.

The videos were translated using Virtual Timing Device Software for the Personal Computer (VTDP, University of Arizona), which was based on the Virtual Timing Device Software (SamaSama Consulting, Sunnyvale, CA) (Julian et al., n.d.; Zartarian et al., 1995). Translation was performed by two researchers (co-authors HSKV and MLG) as previously described (Julian and Pickering, 2015; Zartarian et al., 1995). Both researchers were trained on the use of the software, with results from initial 10 min segment validated by a third researcher (AKP). For comparison, 30 min of the data were translated by both researchers (see Activity data and Supporting information, Comparison of Translators).

2.5.2. Microbial contamination

Microbial contamination was indicated by the fecal bacteria E. coli because it is a commonly used fecal indicator bacteria, and there are
multiple, reliable, and field-portable measurement methods. *E. coli* contamination was measured on surfaces, in bulk materials, in water, and on hands following methods specified in the Supplemental information (Supplemental information: Methods). Although *E. coli* is not necessarily pathogenic, pathogenic strains (enteropathogenic, enterotoxigenic, and enterohemorrhagic) are amongst the leading causes of diarrheal disease (Kotloff et al., 2013). *E. coli* are also expected to be present in high concentrations in surfaces, bulk materials, and water in environments reliant on human excreta for agriculture.

### 2.5.2.1. Probability distribution functions

*E. coli* measurements from surfaces, bulk materials, and water were fit to probability distribution functions, defined for each object category. Probability distribution function parameters were estimated using the *fitdistr* package in R (R Core Team, 2016). When *E. coli* were detectable on 40% or more of a sample category, a normal distribution was fit to the log-transformed *E. coli* concentrations, with data below and above the limits of detection treated as left and right censored data, respectively. When $<$40% of samples had detectable *E. coli* contamination, the data were input directly into the simulation and resampled with replacement, with data below the limits of detection assumed to be uncontaminated (0 CFU/g or cm²).

### 2.5.3. Transfer

Transfer of *E. coli*, defined by the proportion transferred between the hands and objects on contact, was estimated based on a literature review (Table 1). Transfer (%) between surfaces and hands was assumed to be normally distributed, based on previous estimates of transfer rates (Julian et al., 2009; Lopez et al., 2013). Mean and standard deviation estimates are based on previously published experimental work using *E. coli*, *Acinetobacter baumannii*, or *Serratia rubidea* (Greene et al., 2015; Lopez et al., 2013).

#### 2.5.4. Surface areas

Distributions for surface areas in contact with the hands are based on the expected grip type and corresponding fractional surface area as described by AuYeung et al. (2008) (Table 2) (AuYeung et al., 2008; Beamer et al., 2015; U.S. EPA, 2011). Mean and standard deviation estimates are based on generally observed contact events are assumed to be partial finger immersions of between 2 and 3 fingers (Table 2). Surface areas of hands are assumed to be 910 cm², the center point of the recommended range for women (760–1060 cm²) as the vast majority (96%) of farmers studied were women (AuYeung et al., 2008; Beamer et al., 2015; U.S. EPA, 2011).

### 2.6. Dose assessment

Estimates of the *E. coli* ingested by the farmers based on the stochastic-mechanistic simulation are compared to models informed by assumptions of uniform exposure frequencies (Mattioli et al., 2015;
Table 2

Modeled *E. coli* contamination and surface area of contact events for the surface, bulk material, and body categories contacted during the videography. *E. coli* contamination probability distribution functions were based on microbial sample collection. Surface area probability distribution functions were based on expected grip type and corresponding fractional surface area of contact, as described by AuYeung et al. (2008). Sample size (n) used to estimate distributions. Parameters specified are log10-transformed mean and standard deviation for normal distributions (N (log10)) or minimum and maximum for uniform distributions. Resample refers to random selection with replacement of one of the observed values which are specified in the array. Grip types are defined by AuYeung et al. (2008) with associated uniform distributions specifying minimum and maximum.

<table>
<thead>
<tr>
<th>Category</th>
<th>n</th>
<th>E. coli contamination</th>
<th>Surface area</th>
<th>Parameters</th>
<th>Grip type</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Surfaces (CFU/100cm²)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bicycle</td>
<td>12</td>
<td>Resample</td>
<td>[0.0,0.0,0.0,0.360,5480]</td>
<td>Closed hand grip</td>
<td>[0.1, 0.17]</td>
<td></td>
</tr>
<tr>
<td>Cloth</td>
<td>9</td>
<td>N(log10)</td>
<td>(1.68, 0.75)</td>
<td>Front partial fingers</td>
<td>[0.04, 0.06]</td>
<td></td>
</tr>
<tr>
<td>Door/wall</td>
<td>10</td>
<td>N(log10)</td>
<td>(2.48, 1.03)</td>
<td>Partial front palm w/ fingers</td>
<td>[0.07, 0.14]</td>
<td></td>
</tr>
<tr>
<td>Footwear</td>
<td>10</td>
<td>N(log10)</td>
<td>(1.94, 1.93)</td>
<td>Front partial fingers</td>
<td>[0.04, 0.06]</td>
<td></td>
</tr>
<tr>
<td>Grass and rice seeds</td>
<td>10</td>
<td>N(log10)</td>
<td>(0.55, 1.50)</td>
<td>Partial front palm w/ fingers</td>
<td>[0.07, 0.14]</td>
<td></td>
</tr>
<tr>
<td>Handheld tools</td>
<td>10</td>
<td>N(log10)</td>
<td>(3.22, 1.21)</td>
<td>Closed hand grip</td>
<td>[0.1, 0.17]</td>
<td></td>
</tr>
<tr>
<td>Mask</td>
<td>11</td>
<td>Resample</td>
<td>[0.0,0.0,0.0,0,200,240]</td>
<td>Front partial fingers</td>
<td>[0.04, 0.06]</td>
<td></td>
</tr>
<tr>
<td>Phone</td>
<td>10</td>
<td>Resample</td>
<td>[0.0,0.0,0.0,0,720,1280]</td>
<td>Closed hand grip</td>
<td>[0.1, 0.17]</td>
<td></td>
</tr>
<tr>
<td>Paper currency</td>
<td>10</td>
<td>Point</td>
<td>0</td>
<td>Front partial fingers</td>
<td>[0.04, 0.06]</td>
<td></td>
</tr>
<tr>
<td>Bucket (plastic)</td>
<td>10</td>
<td>N(log10)</td>
<td>(1.03, 1.21)</td>
<td>Closed hand grip</td>
<td>[0.1, 0.17]</td>
<td></td>
</tr>
<tr>
<td>Polysacks bag</td>
<td>23</td>
<td>N(log10)</td>
<td>(2.03, 1.70)</td>
<td>Front partial fingers</td>
<td>[0.04, 0.06]</td>
<td></td>
</tr>
<tr>
<td>Polythene bag</td>
<td>10</td>
<td>Resample</td>
<td>[0.0,0.0,0.0,0,40,840,4000]</td>
<td>Front partial fingers</td>
<td>[0.04, 0.06]</td>
<td></td>
</tr>
<tr>
<td>Toilet paper</td>
<td>10</td>
<td>Point</td>
<td>0</td>
<td>Front partial fingers</td>
<td>[0.04, 0.06]</td>
<td></td>
</tr>
<tr>
<td>Toilet pit</td>
<td>10</td>
<td>N(log10)</td>
<td>(3.76, 1.04)</td>
<td>Open hand grip</td>
<td>[0.1,0.21]</td>
<td></td>
</tr>
<tr>
<td><strong>Bulk materials</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface water¹</td>
<td>24</td>
<td>N(log10)</td>
<td>(3.91, 0.84)</td>
<td>Partial finger immersion</td>
<td>[0.26, 0.30]</td>
<td></td>
</tr>
<tr>
<td>Domestic water¹</td>
<td>32</td>
<td>N(log10)</td>
<td>(0.12, 0.79)</td>
<td>Partial finger immersion</td>
<td>[0.26, 0.30]</td>
<td></td>
</tr>
<tr>
<td>Excreta¹</td>
<td>20</td>
<td>N(log10)</td>
<td>(4.00, 2.14)</td>
<td>Full front palm w/ fingers</td>
<td>[0.13, 0.25]</td>
<td></td>
</tr>
<tr>
<td>Mud¹</td>
<td>20</td>
<td>N(log10)</td>
<td>(2.02, 1.40)</td>
<td>Full front palm w/ fingers</td>
<td>[0.04, 0.11]</td>
<td></td>
</tr>
<tr>
<td>Ash¹</td>
<td>10</td>
<td>Resample</td>
<td>[0.0,0.0,8,9336, 657]</td>
<td>Full front palm w/ fingers</td>
<td>[0.13, 0.25]</td>
<td></td>
</tr>
<tr>
<td><strong>Body (CFU/100cm²)</strong></td>
<td>155</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hands</td>
<td></td>
<td>From model</td>
<td></td>
<td>Closed hand grip</td>
<td>[0.1, 0.17]</td>
<td></td>
</tr>
<tr>
<td>Face</td>
<td>Point</td>
<td></td>
<td>0</td>
<td>Front partial fingers</td>
<td>[0.04, 0.06]</td>
<td></td>
</tr>
<tr>
<td>Mouth</td>
<td></td>
<td>Not relevant</td>
<td></td>
<td>Partial finger immersion (2-3 fingers)</td>
<td>[0.10, 0.18]</td>
<td></td>
</tr>
</tbody>
</table>

 Özkyanak et al., 2010). The arithmetic approach estimates dose assuming:

$$Dose = \sum_{i=1}^{n} (S \times T \times C_{hi})$$  \hspace{1cm} (4)

where $C_{hi}$ is the concentration of bacteria on the hands (CFU/cm²) for the kth hand-to-mouth contact, $T$ is the transfer efficiency of bacteria on contact (unitless), $S$ is the fractional surface area of the hand (unitless), and $n$ is the number of hand-to-mouth contact events per unit time (#/hr).

Four separate sets of assumptions about *E. coli* contamination on hands and frequency of hand-to-mouth contacts are compared to the stochastic-mechanistic simulation (Table 3). The goal of the comparison was to determine the impact of using the stochastic-mechanistic simulation presented here relative to the generalizable approach used elsewhere, and to determine the impact of assumptions about model parameters. For the comparison, other values (surface area and transfer) are assumed to be the same across all models (Table 3). Specifically, *E. coli* concentrations on the hands are described by either simulated (Models 1 and 2) or measured (Models 3 and 4) *E. coli* concentrations. The simulated *E. coli* concentrations were determined by assuming a lognormal distribution describable by the *E. coli* concentrations across all farmers’ hands at the end of the simulations (see Excreta collection and Land application). The measured *E. coli* concentrations were determined by assuming a lognormal distribution describable by the *E. coli* concentrations measured on the hands of the farmers (see Hands).

Probability distributions for frequency of hand-to-mouth contacts were based on either previously published estimates for adult hand-to-mouth contacts, or fit to data obtained from videography (Table 3). Published data were obtained from Jones (2011), who fit a Weibull distribution with shape of 0.76 and scale of 7.07 to observed adult hand-to-mouth contact data amongst United States office workers (Jones, 2011; Nicas and Best, 2008). Videographic data on hand-to-mouth contact frequency for each farmer were fit to a Weibull distribution (shape = 0.93, scale = 0.98) using the fitdistcens R package (R Core Team, 2016) (Table 3), with the data left censored at the limit of detection (1 contact per length of video) for farmers with no observed contacts.

Table 3

Alternative models used to estimate ingested *E. coli* dose based on the traditional arithmetic approach using *E. coli* hand contamination data estimated from our stochastic-mechanistic simulation (Models 1 and 2, see Results) or measured *E. coli* hand contamination (Models 3 and 4) and published (Models 1 and 3) or collected (Models 2 and 4) hand-to-mouth contact frequencies as compared to the stochastic-mechanistic simulation (Model 5). Parameters specified are log10-transformed mean and log10-transformed standard deviation for normal (N) and lognormal (LNORM) distributions; shape and scale for Weibull distributions; and minimum and maximum for uniform distributions. Refers to Jones et al. (2011) reference.

<table>
<thead>
<tr>
<th>Model</th>
<th>Surface area (S, cm²)</th>
<th>Transfer (T, %)</th>
<th>E. coli on hand (C_{hi}, CFU/cm²)</th>
<th>Collection</th>
<th>Application</th>
<th>Freq. hand-mouth contacts (f_{bl, ml} \theta/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>U(0.10, 0.18)</td>
<td>N(0.34,0.25)</td>
<td>Simulated: LNORM(1.4, 1.3)</td>
<td>Published: Weibull (0.76, 7.07)¹</td>
<td>Videography: Weibull (0.93, 0.98)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td>Simulated: LNORM(0.81, 1.5)</td>
<td>Published: Weibull (0.76, 7.07)³</td>
<td>Videography: Weibull (0.93, 0.98)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td>Measured: LNORM(−1.4,1.3)</td>
<td>Published: Weibull (0.76, 7.07)¹</td>
<td>Videography: Weibull (0.93, 0.98)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
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<td>Measured: LNORM(−0.4,1.2)</td>
<td>Published: Weibull (0.76, 7.07)¹</td>
<td>Videography: Weibull (0.93, 0.98)</td>
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Dose assessment models are compared using cumulative probability distribution functions. The cumulative distribution function for the stochastic-mechanistic simulation was derived from aggregating final dose estimates for 100 simulations of each of the 13 (collection) or 15 (application) farmers (1300 and 1500 total simulations). The number of simulations was sufficient for convergence of estimates of E. coli contamination on hands (Supporting information, Fig. S7). The cumulative distribution functions for the arithmetic models are derived from the equivalent number of simulations: 1300 or 1500 for collection and application, respectively.

2.7. Sensitivity analysis

The simulation sensitivity analysis was conducted using a modified method of (Xue et al., 2006). In brief, parameter values (object contamination, transfer efficiency, and surface area) for each of the objects were set to the median (p50) point value of the probability distribution function, and the median E. coli concentrations on the hands of all farmers (exposure, in units of log10 CFU/cm²) and the summed total E. coli ingested for all farmers (dose, in units of CFU) were calculated (Supporting information Table S2). Each parameter was then individually adjusted to the 10th (p10) or 90th (p90) percentile values based on the probability distribution function, and the corresponding exposure and dose were calculated. The percentage change in exposure and dose were then calculated using the ratios of p90:p50 and p50:p10, and the parameters were rank ordered by the magnitude of the percentage change.

Because the simulation used the MLATS data directly as opposed to drawing the data from a probability distribution function, a different approach was used to estimate impact of activity data on the simulation outcomes. Specifically, we assumed the observed activity frequency for each object category was equivalent to the median (p50) point value (obs., Supporting information Table S2). We defined the 10th (p10) and 90th (p90) percentile point values based on ordering the observed frequencies for the individual farmers. The relative impact of the activity frequency was determined by estimating the percentage change in the outcomes (exposure and dose) using the ratios p90:observed frequency and observed frequency:p10 (Supporting information Table S2).

3. Results

3.1. Excreta treatment

Of the 25 farmers enrolled in the study, 1 (4%) reported storing human excreta for <2 months, 9 (36%) reported <3 months, 12 (48%) reported <4 months, and 3 (12%) did not respond. No one reported storing human excreta for >6 months, the recommended minimum storage time (Mackie Jensen et al., 2008; Phuc et al., 2006; Winblad, 2004).

3.2. Parameter estimation

3.2.1. Microbial contamination

3.2.1.1. Hands. For all 30 hand samples collected (15 farmers, samples collected before and after videography), the average [standard deviation] of E. coli concentrations measured was 2.3 [1.2] log10 MPN E. coli per hand. Four (13%) samples were below the limit of detection (<3 MPN E. coli per hand) and two (7%) samples were above the limit of detection (>10⁴ MPN E. coli per hand) (Figs. 1 and 2).

Left hand contamination was not significantly different than right hand contamination (Wilcoxon rank sum test, p = 0.83). Farmers collecting excreta had lower hand contamination than farmers applying excreta to fields (mean [standard deviation] 1.4 [1.1] log10 MPN E. coli per hand as compared to 2.6 [1.1], Wilcoxon rank sum test, p = 0.01).

3.2.1.2. Surfaces. Of the 155 surface samples collected, 81 (52%) were below the lower limit of detection (5 CFU/100 cm²). For the 14 surface categories, two (paper currency and toilet paper) had no detectable E. coli on any of the samples tested, and four (bicycle, mask, phone, and polyethylene bags) had 40% or fewer of the samples with detectable E. coli (Table 2). Contamination was modeled using sampling with replacement (Table 2). Microbial samples for the other eight surface categories (cloth, door/wall, footwear, grass and rice seeds, handheld tools, bucket (plastic), polysacks bag, and toilet pit) were fit to lognormal, base 10, distributions with means [standard deviations] ranging from 0.54 [1.54] (grass and rice seeds) to 3.76 [1.03] (toilet pit) (Table 2).

![Fig. 1. Distributions from 100 simulations for final (top) E. coli hand contamination and (bottom) E. coli dose for farmers (n = 13) collecting human excreta from latrines. Boxplots highlight median and interquartile ranges with whiskers extending up to 1.5 times the interquartile range beyond the median. Outliers are >1.5 times the interquartile range beyond the median. E. coli contamination on hands was measured for a subset (n = 6) of farmers for either the left or right hand before (red) and after (blue) videography. Error bars represent standard deviations. The use of personal protective equipment by the farmers is noted by shading the background indicating (top) gloves or (bottom) masks. The number at the bottom of each figure indicate farmers’ ID. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)](Image 138x102 to 466x330)
3.2.1.3. Bulk materials. Of the 106 bulk material samples collected, 28 (26%) were below the lower limit of detection. E. coli contamination of most materials (surface and domestic water, excreta, and mud) were fit to lognormal, base 10, distributions with means [standard deviations] ranging from 0.12 [0.79] CFU/100 ml (drinking water) to 4.00 [2.14] CFU/g (excreta) (Table 2). Because only 4/10 (40%) of ash samples contained detectable E. coli, ash contamination was modeled using sampling with replacement (Table 2).

3.2.2. Activity data

In total, 18.2 h of video were collected and translated from 25 farmers. Individual video lengths ranged from 0.17 to 1.62 h. Of the 18.2 h, 0.5 h was translated by both coders for comparison. The dual comparison showed approximately 15% deviation in frequency of the coding (see Supplemental information Table S1, Fig. S1). This is above the minimum recommended inter-observer agreement for video translation as described by Ferguson et al. (2006), but as shown by Sensitivity Analysis (see Sensitivity Analysis) likely has no meaningful impact on outcomes (Ferguson et al., 2006).

The mean [standard deviation] of a farmer’s left and right hand-object contacts were 360 [136] and 401 [284] times per hour, respectively, during collection of human excreta from dry toilets (Table 1). During land application of excreta, the left and right hands contacted an object 342 [198] and 848 [340] times per hour, respectively. The dramatically higher contact frequency of the right hand during land application is attributable to observed rapid, repeated, motions (i.e., spreading excreta, seeding).

The most common categories contacted during both excreta collection and land application were handheld tools (i.e., shovel, rakes), polysacks bag, and human excreta (Fig. 3). The toilet pit was frequently contacted only during excreta collection, while mud and surface waters were frequently contacted only during land application (Fig. 3). Contacts with body parts (hand-to-hand, hand-to-face, and hand-to-mouth) were infrequent (Fig. 3, Table 4). Notably, only 2/14 (14%)...
3.3. Exposure simulation

E. coli concentrations were simulated for both the left and right hands of 25 farmers, 11 who collected excreta, 12 who applied excreta. E. coli concentrations ranged from $10^{-4}$ to $10^{10}$ CFU/cm² over 100 simulations for all 25 farmers (Supporting information, Fig. S2). For example, the E. coli contamination on the left and right hands of Farmer ID 112 during land application varied between $10^{-2.5}$ to $10^{3}$ CFU/cm² over 100 simulations (Fig. 5). E. coli contamination on hands is dynamic, with large variation over time, as well as between different farmers.

3.3.1. Excreta collection

Overall, the simulation (100 simulations per farmer) predicted final E. coli concentrations across all farmers for both hands of mean (standard deviation) of 1.4 [1.3] log_{10} CFU/cm² during excreta collection as shown in Fig. 1. Concentrations for left and right hands ranged across farmers from median [interquartile range, IQR] of 0.2 [−0.7,0.7] and 0.6 [−0.1,1.4] log_{10} CFU, respectively, for Farmer ID 110 to 1.9 [1.2,2.4] and 1.8 [1.4,2.4] log_{10} CFU, respectively, for Farmer ID 106 (Fig. 1). The simulated median E. coli concentrations on hands were statistically significantly higher than measured E. coli concentrations by an average [standard deviation] of 2.1 [1.5] log_{10} CFU (n = 15, p = 0.0003, paired Wilcoxon rank sum test).

Only two farmers (Farmers 112 and 113) contacted their mouths (2 and 3 times, respectively), resulting in estimated E. coli ingestion of median [IQR] of 2.0 [0.5,10.0] and 0.7 [0.1, 4.2] CFU (Fig. 1).

3.3.2. Land application

The simulation of E. coli contamination on hands after land application predicted concentrations across all farmers, for both hands, of mean (standard deviation) 0.8 (1.5) log_{10} CFU/cm² as shown in Fig. 2. Concentrations for left and right hands on farmers ranged from median [IQR] of −1.1 [−1.9, −0.4] and −1.2 [−2.0, −0.4] log_{10} CFU respectively (Farmer 112) to 1.4 [0.4,2.4] and 1.3 [0.4,2.4] log_{10} CFU, respectively (Farmer 118). The estimated median E. coli concentrations on hands were statistically significantly higher than measured E. coli concentrations by an average [standard deviation] of 1.3 (1.5) log_{10} CFU (p = 0.03, paired samples t-test). Simulated E. coli concentrations following land application were statistically significantly less than simulated concentrations following excreta collections (p < 0.001, t-test), which was the reverse of what was observed for measured E. coli concentrations (see Hands).

3.4. Dose assessment

Within the stochastic-mechanistic simulation, the majority of farmers (20/25, or 80%) did not have an observed hand-to-mouth contact event, and so did not ingest E. coli during observation (Figs. 1 and 2). Of the remaining five farmers, two (Farmers 112 and 113) contacted their mouths during excreta collection, resulting in estimated E. coli ingestion of mean [95% Confidence Interval] 3.1 [1.9,4.8] and 0.9 [0.5,1.6] CFU (Fig. 1). Normalized to length of video, this corresponds to 10.4 [6.5, 16.4] and 3.2 [1.9, 5.5] CFU/hr. Four farmers (Farmers 108, 109, 112 and 117) contacted their mouths during land application, resulting in estimated E. coli ingestion of mean [95% Confidence Interval] of 1.2 [0.7,2.2], 4.2 [2.7,6.5], 3.0 [1.6,6.1], and 0.6 [0.3,1.3] CFU, respectively (Fig. 2). Normalized to video length, this corresponds to 0.7 [0.4,1.3], 2.9 [1.8,4.4], 2.2 [1.2,4.4], and 0.7 [0.3,1.5] CFU/hr. Notably, during Land Application, two of the farmers with hand-to-mouth contacts (109 and 117) wore masks for some, but not all, of the recording.

Dose estimates using distinct assumptions about parameter values highlight the impact of exposure and concentration data sources on estimated exposures (Fig. 6). The arithmetic models (Models 1–4) generally assume more frequent low dose exposures than the stochastic-mechanistic simulation (Model 5). Dose estimates are higher when simulated E. coli hand contamination (Models 1,2) is used as compared to measured E. coli hand contamination (Models 3,4), reflecting the overestimation of the stochastic-mechanistic simulation (see Exposure simulation).

3.3. Exposure simulation

3.4. Dose assessment

3.3.2. Land application

3.4. Dose assessment

Fig. 4. Cumulative probability of hand-to-mouth frequency per hour from the observed in this study (solid line) and the published by Jones (2011) (dashed line).
3.5. Sensitivity analysis

Sensitivity analysis highlighted the importance of *Escherichia coli* contamination in the environment (specifically, *E. coli* contamination of excreta, handheld tools, and the toilet pit) on both *E. coli* contamination of the hands (exposure) and *E. coli* ingested (dose) (Table 5, Figs. S3–S6). Activity data were also influential for *E. coli* contamination of hands (specifically the frequency of contacts with excreta, the toilet pit, or surface water) and *E. coli* ingested (the frequency of hand-to-mouth contacts). Neither surface area nor transfer efficiency influenced model outputs (Table 5, Figs. S3–S6).

4. Discussion

The study highlights substantial inter-individual variation in *E. coli* on hands and ingested *E. coli* for twenty-five farmers using human excreta for agriculture in Vietnam. The large variation is attributed to microbial contamination in the environment and hand-to-mouth contact frequency. Specifically, *E. coli* contamination of excreta and other frequently contacted objects (i.e., handheld tools, toilet pits) strongly influenced hand contamination. *E. coli* contamination of excreta and hand-to-mouth contact frequency influenced ingested dose. Notably, hand-to-mouth contact frequency was substantially less than previously.
observed in other settings. Our findings align with an epidemiological study showing increased risks for farmers composting excreta for <3 months (paralleling high pathogen concentrations), and not using PPE or never or rarely washing hands with soap (paralleling impact of frequent hand-to-mouth exposures) (Pham-Duc et al., 2014).

Control of human excreta through adequate treatment (i.e., addition of lime or ash, sufficient storage time) to reduce pathogen concentrations is likely the most effective intervention for excreta land application, as the sensitivity analysis of the impact of *E. coli* concentrations of human excreta on dose demonstrated. In our simulation, shifting estimated *E. coli* contamination of human excreta over the range of *E. coli* concentration values observed from microbial sampling dramatically influenced ingested *E. coli* estimates. Other potential control options such as PPE, though promising, likely have limited efficacy. PPE’s primary role is to reduce exposure and dose by impacting hand-to-mouth contacts. However, we observed that PPE did not always prevent against hand-to-mouth contact events due to imperfect compliance. Two farmers (Farmer IDs 109 and 117) used masks but still ingested *E. coli*. Furthermore, containment of excreta is also likely an effective intervention for excreta collection. Our simulation showed the highest sensitivity of toilet pit *E. coli* concentration on the dose during collection.

Surprisingly, hand contamination was higher during land application than excreta collection. This is likely due to more frequent contacts with *E. coli* from media other than human excreta, such as handheld tools, polysacks bags, mud, and surface or irrigation water. This assertion is supported in our finding that *E. coli* contamination on hands was influenced by *E. coli* contamination of many more media during application than during collection. It is possible that some of these other media may contain *E. coli* from non-human sources, such as from animals and/or growth in the environment (Ishii et al., 2010, 2006). Studies employing pathogen detection and/or source tracking assays may be better positioned to estimate the relative risks of excreta collection as compared to land application.

Exposure and risk assessments should incorporate high quality human-environment interaction data. The observed hand-to-mouth contact frequency obtained from videography for farmers was substantially lower than the hand-to-mouth contact frequency reported in the literature for adults (Jones, 2011; Nicas and Best, 2008). The study populations (Vietnamese Farmers and United States Office Workers) and target actions (excreta use and office work) are vastly different, and so it is unsurprising that exposure factors are also vastly different. As the farmers likely recognize a risk of excreta use to some degree, it is reasonable that excreta users may be more considerate of risks from hand-to-mouth contacts, resulting in the lower frequency observed. Increasingly, studies are highlighting differences in exposure factors amongst different study populations, especially when comparing high income populations to low or middle income country populations (Kwong et al., 2016; Phillips and Moya, 2013). Here, we provide evidence that context-specific, evidence-based, data for exposure assessments is needed. Risk assessment studies reliant on exposure factors data obtained from different contexts and/or expert opinion likely underestimate the uncertainty associated with assumed exposure factors.

The stochastic-mechanistic simulation of hand contamination provides insights into the potential dynamism of *E. coli* contamination on hands. Simulated *E. coli* contamination on hands frequently shifts during the observation period. The simulation dynamics mirror findings from experimental studies showing rapid temporal variability of *E. coli* and other bacteria on hands (Pickering et al., 2011; Ram et al., 2011). Additionally, the simulation suggests hand contamination is linked to microbial contamination in the environment: wide variation between simulations is largely attributable to variation in microbial contamination on objects. This finding also aligns with recent field work showing correlations between *E. coli* on hands and *E. coli* in household soils.

### Table 5

Sensitivity analysis rank and value for a subset of parameters used in the simulation of *E. coli* contamination on hands (Exposure) and *E. coli* ingested (Dose) for both collection of human excreta (Collection) and application on agricultural land (Application). The subset of parameters shown represent parameters that increased or decreased exposure or dose by >30% relative to the median (p50) simulation values (see Table S2).

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5. Conclusions

The primary finding from the study is that there is substantial inter-individual variation in E. coli hand contamination and ingested dose amongst farmers in Vietnam reliant on human excreta for agriculture. Additional findings include:

- Frequency of hand-to-mouth contacts amongst Vietnamese farmers substantially lower than the widely-used exposure factor previously reported for U.S. office workers.
- Variation in exposure and dose is driven by microbial contamination and frequency of hand-to-mouth contacts.
- Stochastic-mechanistic simulation is beneficial in that it highlights the dynamism of E. coli contamination on hands
- However, the simulation performs similar to simpler arithmetic models for estimating population-level exposures, when context-specific exposure factors are used.
- Exposure assessments should collect and integrate context-specific exposure factors to improve exposure and risk estimates.

- In Vietnam, intervention strategies should focus on reducing pathogen contamination of human excreta and handheld tools and/or prevent hand-to-mouth contacts.
- Personal protective equipment, though beneficial, is not completely protective due to imperfect use.

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Appendix A. Supporting Information

Supporting information to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.04.100.

References

Fuhrimann, S., Winkler, M.S., Stalder, M., Niwagaba, C.B., Babu, M., Kabatereine, N.B., 2017. E. coli contamination and personal protective equipment, though bene-
ficial, is not completely


( Julian et al., 2013; Navab-Daneshmand et al., n.d.). This is additionally important given frequent reports of E. coli contamination on surfaces in households throughout low and middle income countries (Ercumen et al., 2017; Julian et al., 2013; Navab-Daneshmand et al., n.d.; Pickering et al., 2012; Sinclair and Gerba, 2011).

The simulation substantially overestimates hand contamination relative to measured E. coli data. E. coli contamination of surfaces and bulk materials was measured at different times than videography and hand samples, so it is possible that the environmental surfaces sampled were more contaminated than the surfaces the farmers contacted during videography. Another potential source of bias is the simulation assumption that E. coli contamination on hands is uniformly distributed. In reality, E. coli on hands is likely heterogeneous, and areas with more frequent surface contacts (i.e., tips of fingers, front of hands) may have relatively higher local E. coli contamination.

Another potential cause for overestimation includes inaccurate curve fitting of E. coli contamination data. Collected E. coli contamination data included high rates of non-detects. When data were fit to probability distribution functions, non-detects were assumed to be censored below the limit of detection. Maximum likelihood estimation of the probability distribution functions models non-detects as contaminated at levels below the limit of detection. It is possible that these surfaces were not contaminated at all. Assuming any contamination, even low levels, would lead to overestimation relative to assumptions of no contamination. Sampling and analytical methods may need to be modified to improve data at the tails of the probability distribution functions.

Finally, the simulation assumes the proportion of E. coli transferred on contact is a function of the gradient in E. coli contamination of the two surface areas in contact. This assumption is not grounded in experimental literature, as transfer efficiency studies overwhelmingly study transfer from a contaminated to unoinfected surface (Julian et al., 2010; Lopez et al., 2013; Rusin et al., 2002). More studies are needed to determine transfer dynamics between two contaminated surfaces.

Notable study limitations include the potential for introducing bias into behaviours of the farmers, and the lack of coincident sampling, both of which may have influenced the simulation outcomes. As the farmers were asked to enroll in a study observing their behaviours, it is highly likely that their behaviours were influenced during observation (e.g., reactivity). The high proportion of personal protective equipment and relatively low frequency of hand-to-mouth contacts may be at least partially attributable to reactivity. In the context of handwashing, for example, Ram et al. (2010) demonstrate study participant reactivity in the presence of an observer (Ram et al., 2010). Another source of bias, as previously discussed, is the collection of videography and hand sampling data at different times, which may have introduced bias into the simulation.

5. Conclusions

The primary finding from the study is that there is substantial inter-individual variation in E. coli hand contamination and ingested dose amongst farmers in Vietnam reliant on human excreta for agriculture. Additional findings include:

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