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Visible Soil as an Indicator of Bacteria Concentration on Farmworkers' Hands

ABSTRACT

Visible soil on farmworker hands has been assumed to indicate microbial contamination that may lead to contamination of produce and illness in the consumer. To test this assumption, we assessed the relationship of visible soil with microbial load on farmworker hands. 78 farmworkers harvested tomatoes for 30 minutes, practiced hand hygiene (hand wash, hand sanitizers) and rinsed their hands in 0.1% peptone solution. Hand rinses were analyzed for turbidity (median Absorbance$_{600nm}$ 0.0815 ± 0.0336 IQR) and microbial load (median log$_{10}$ CFU/hand ± IQR: *E. coli* 1.27 ± 0, *Enterococcus* 3.75 ± 1.66, and coliforms 2.23 ± 1.13). After hands were photographed between hand hygiene and rinsing, they were assigned a "Visible Hand Dirtiness Score" from 0 (no visible soil) to 7 (highly visible soil) (median Score 4 ± 2 IQR). Hand score and turbidity were significantly correlated (rho = 0.549, P < 0.001). Hand score and *Enteroccocus* concentrations were weakly correlated (rho = 0.273, P = 0.015) but not coliforms (P > 0.05) or *E. coli* (P > 0.05). Our results suggest that while visible hand soil is a good proxy for hand rinse turbidity, visible soil is not a strong indicator for all microorganisms on farmworker hands.

INTRODUCTION

An estimated one in six Americans, or 48 million individuals, are infected with foodborne illness due to pathogen contamination of food each year *(4)*. Contaminated produce caused an estimated 46% of these illnesses in the past decade, more than any other food source *(25)*. Produce may be contaminated through the transfer of microorganisms from farmworkers' hands *(18)*. Farmworker hands may be contaminated with pathogens from the human host (e.g., lack of hygiene and sanitation after defecating *(27)*) or the environment (e.g., animals, other produce, equipment, water or soil *(2, 6, 17, 23, 24, 28)*). However, enteric pathogens causing foodborne illness, including those on produce and farmworker hands are rare and difficult to detect *(1, 11, 13)*. Thus, previous studies have used indicator organisms to measure the microbiological contamination of hands *(19, 21, 22)*.

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In 2013, our research group found that farmworker hands exhibited a wide range of microbial contamination as measured by indicator viruses and bacteria in the agricultural production environment *(2, 11)*. Among the multiple pathways for microorganisms to contaminate produce on the farm (e.g., hands, water, soil, tools and equipment surfaces), we found an association between microbes on farmworker's hands and produce *(19, 20)*. In a second study, performed in 2014, we found that 46% of produce had fecal contamination from a human source, further implicating hands as a vehicle of produce contamination *(27)*. In a third study, performed in 2015, our group found that farmworker hands, after hand hygiene had been performed, became re-contaminated with microbes in as little as 30 minutes after tomato harvest, and that these concentrations were equivalent to concentrations on hands of workers who had not performed hygiene *(6, 7)*.

Alhough hands are an important vehicle for microbial contamination of produce, there is no rapid measure or valid proxy of farmworker microbial load. Anecdotally, visible soil on farmworker hands is thought to indicate filth and microbial contamination that may lead to contamination of produce and illness in the consumer. To test this assumption, our goal was to take advantage of data from our previous hand hygiene study *(6)* to assess the relationship of visible soil with microbial load on farmworker hands. Using the results of this study, we can determine if farmworker hands with visible soil indicate hands with microbiological contamination.

MATERIALS AND METHODS

Design of previous hand hygiene intervention study dataset source

Farmworkers (n = 181) on two tomato farms located near Nuevo León, Mexico participated in a hand hygiene study between February and October 2014 *(6)*. This study compared the efficacy of two soap-based (traditional or pumice) products and two alcohol-based hand sanitizer (ABHS)-based hand hygiene techniques (ABHS or SaniTwice™ with ABHS) to a no-hygiene control *(6)*. For traditional soap, we used 3.5 ml of Pearl Lotion Hand Soap (a typical soap used on farms in Mexico), a non-antibacterial and nonabrasive soap. For pumice soap, we used 6 ml of GOJO Natural Orange Pumice Hand Cleaner, a gel-based surfactant formula with pumice particles. ABHS use consists of standard application of ABHS (PURELL Advanced Instant Hand Sanitizer, manufactured by GOJO) to hands and rubbing for 20 seconds, as the hands air dry. SaniTwice™ use is a two-step protocol for sanitizing hands in which the participant uses an excess of sanitizing gel, rubs for 20 seconds, wipes with a paper towel, uses sanitizing gel again, and continues rubbing the hands until dry.

All hands were standardized first by hand washing (traditional soap) and drying, after which the worker returned to work for one to two hours. Participants were then randomly assigned to one of five treatment groups: ABHS, SaniTwice™ with ABHS, traditional soap, pumice soap, and control (no hygiene treatment). After hand hygiene was performed, a photo was taken of each farmworker's hands. After the photo was taken, the farmworker provided a hand rinse sample by inserting the hands into a Whirl-Pak bag that contained 750 ml of sterile 0.1% peptone water. The hands were massaged through the bag by study staff for 20 to 30 seconds. Hands were analyzed for bacterial loads (*Enterococcus* spp., coliforms and *E. coli*) *(6, 10)* and turbidity levels of the hand rinses at 600 nm, using a spectrophotometer. The study was approved by the Emory University Institutional Review Board, Atlanta, Georgia (IRB00035460).

Design of the current study

To assess the relationship of visible soil to microbial load on farmworker hands, the 78 photos of hands from the previous study were matched to corresponding data of bacterial loads and turbidity levels in hand rinses. Additionally, we developed a novel scale that was used to assess 5 aspects of visible dirtiness, the Visible Hand Dirtiness Score.

Assessing visible hand dirtiness

Photographs *(Fig. 1)* of 78 farmworkers' hands were assessed using a numerical scale that addressed five aspects of cleanliness *(Table 1)* adapted from a previous study *(26)* in which Pickering et al. described hand cleanliness by describing soil on the palms, soil on the finger pads and soil under the fingernails *(26)*. In addition, we included multicolored soil and total soil amount in our scale to assess the visible cleanliness of the participants' hands.

A higher score corresponds to visibly dirtier hands in the photograph. One point was added to the sample's Visible Hand Dirtiness Score if there was visible dirt on the palm, one point for the fingers, and one point for the fingernails. One point was added to the score if multiple colors of soil were present on the hands, because multiple colors of soil could indicate contamination coming from different sources *(16)*. Lastly, "total dirt amount" was assessed on the total amount of dirt on the hand, using the following scale: zero points were added to the score if the hands were clean, with no visible soil on any part of the hand. One point was added if the hands had visible soil on less than 50% of the surface of the hand. Two points were added if the hands had visible soil on more than 50% of the surface of the hand. Three points were added if the hands had visible soil covering the entire surface of the hand. The scale ranges from 0 to 7, from visibly cleanest to dirtiest.

Data collection for visible hand dirtiness score

After criteria had been developed for the Visible Hand Dirtiness Score, to determine if the system was reliable, a

SCORE = 0

SCORE = 1

SCORE = 2

SCORE = 3

SCORE =4

SCORE = 5

SCORE = 6

SCORE = 7

FIGURE 1. Photographs of farmworkers' hands ranging from Visible Hand Dirtiness Scores 0–7

TABLE 1. Summary of point system for the Visible Hand Dirtiness Score

Palm	Finger Pads	Fingernails	Color	Total Dirt Amount	Visible Hand Dirtiness Score
0 or 1	0 or 1	0 or 1	0 or 1	0 or 1 or 2 or 3	Sum of all points (0–7)

TABLE 2. Characteristics of hands scored (n = 78)

Hand Score	Number of hands*	Palm	Fingerpads	Fingernails	Color	Total Dirt Amount > 1 point
0	5	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
1	2	0% (0)	0% (0)	100% (2)	0% (0)	0% (0)
2	5	0% (0)	100% (5)	0% (0)	0% (0)	0% (0)
3	20	20% (4)	95% (19)	85% (17)	0% (0)	0% (0)
4	15	100% (15)	100% (15)	87% (13)	13% (2)	0% (0)
5	16	100% (16)	100% (16)	81% (13)	31% (5)	86% (14)
6	10	100% (10)	100% (10)	100% (10)	70% (7)	100% (10)
7	5	100% (5)	100% (5)	100% (5)	100% (5)	100% (5)
1–7	**78**	**64% (50)**	**90% (70)**	**77% (60)**	**24% (19)**	**37% (29)**

*% values based on the number of hands assigned each hand score

pilot test of the scoring protocol was performed with 10 photographs that would not be used in the final database. In the pilot test, two research assistants independently scored the 10 photographs across the 5 categories (i.e., 50 entries). The two research assistants were in agreement over 90% of the time on the Visible Hand Dirtiness Score ranking of the 50 entries of the 10 photographs, confirming the reliability of the scoring system. After the scoring protocol had been improved, a second pilot test of 50 entries resulted in a higher (greater than 95%) reliability. For final data collection, 78 photographs of farmworker hands were assessed by two research assistants using the Visible Hand Dirtiness Score system independently. Again, the two research assistants were in agreement on more than 95% of the entries, and data reconciliation was performed by the two research assistants, who discussed and resolved any discrepancies. All data were entered into Microsoft Excel 2010 (Microsoft Co., Seattle, Washington).

Statistical analysis

Statistical analyses were performed using R version 3.2.2. The data were non-normal; thus $\log_{10}$ transformations and nonparametric tests were used. In using Spearman's correlation tests, rho ≤ 0.3 was considered to be a weak relationship, 0.3 < rho ≤ 0.7 a moderate relationship, and rho > 0.7 a strong relationship *(5)*.

RESULTS

Characterization of farmworkers' hands used for the study

The hands in the study *(Table 2)* represented the full range of visible dirtiness (0–7) as measured by the Visible Hand Dirtiness Score *(Table 1)*. The largest proportion of samples had a "moderate" level of soil, with Visible Hand Dirtiness Scores between 3 and 6. The parts of farmworker hands that were most often visibly dirty were the fingerpads (90% showed visible soil) and fingernails (77% showed visible soil). Only 24% of the photos showed soil in a color other than brown (yellow, green). Additionally, 91.0% of hands were positive for *Enterococcus*, 74.4% were positive for coliforms, and 5.1% were positive for *E. coli*.

Validating the visible hand dirtiness scoring system

The Visible Hand Dirtiness Score was compared to the turbidity of the hand rinses, an objective measure of visible soil on the hands, by a Spearman's correlation test. The

Visible Hand Dirtiness Score was significantly moderately associated ($P > 0.001$, rho = 0.549) with turbidity when a Spearman's correlation test was used *(Table 3)*.

Relationships among visible hand dirtiness score, turbidity, and number of indicator bacteria per hand

E. coli, Enterococcus and coliform concentrations on hands represented the full range between our lower limit of detection (1.27 $\log_{10}$ CFU/hand) and the upper limit of quantification (7.27 $\log_{10}$ CFU/hand) by our method of enumeration *(12)*. The median concentration was the highest for *Enterococcus* and lowest for *E. coli* *(Table 3)*.

To determine if there were significant relationships between bacterial load and visible soil, we performed Spearman's correlation tests *(Table 3)*. The relationship between *Enterococcus* and Visible Hand Dirtiness Score was significant ($P < 0.05$), but the rho value (rho = 0.273) was less than 0.3, suggesting that the relationship was weak *(5)*. The relationship between *Enterococcus* and turbidity was also significant ($P < 0.05$), and the rho value (rho = 0.352) between 0.3 and 0.7 indicated a moderate correlation *(5)*. There were no other significant relationships. When stratified by the five different hand hygiene groups, the relationship between *Enterococcus* or coliforms with turbidity was positively correlated for two of the five hygiene groups, and the relationship between *E. coli* and turbidity was non-significant (data not shown). When stratified by the different hand hygiene groups, the relationship between each of the bacterial indicators and the corresponding Visible Hand Dirtiness Score was non-significant (data not shown). In conclusion, our results suggest that Visible Hand Dirtiness Score and Turbidity were correlated to concentrations of some but not all indicator bacteria.

DISCUSSION

The goal of this study was to determine if, on farmworker hands, visible soil on hands was associated with bacterial load. Of the microbes tested for, *Enterococcus* had the highest concentration and *E. coli* the lowest concentration on farmworker hands, results that were similar to those of our previous study, performed between February and October 2014 *(6)*. The Visible Hand Dirtiness Score seems to be a valid measure of soil, in that it was moderately correlated with turbidity of hand rinse samples. Using this score, we found that both Visible Hand Dirtiness Score (rho = 0.273) and turbidity (rho = 0.352) were significantly associated with *Enterococcus*, but not with *E. coli* and coliforms.

The Visible Hand Dirtiness Score, a scoring system created to rank visible hand dirtiness based on photographs, was significantly moderately associated with turbidity, an objective measure of soil on hands. This correlation may be because both visible hand dirtiness and turbidity are measuring a similar characteristic (visible soil). However, it is important to note that the correlation was not strong, perhaps because of differences in measurement of other factors *(15)*. For example, all soil visible on the hand and captured by the Visible Hand Dirtiness Score may not be removed by the process of hand rinsing and therefore not measured by turbidity. Further, a turbidity reading may measure some soil types *(3)* that are not visible to the naked

TABLE 3. Farmworkers' hands (n = 78) description and relationship between Visible Hand Dirtiness Score; median *E. coli, Enterococcus*, and coliform concentrations; and Turbidity per hand

			Spearman's Rank against Hand Score		Spearman's Rank against Turbidity	
	Median	IQR‡	rho	*P*-value	rho	*P*-value
Visible Hand Dirtiness Score	4	2			0.549†	< 0.001*
Log_{10} *E. coli* CFU per hand	1.27	0	-0.025	0.826	0.094	0.413
Log_{10} *Enterococcus* CFU per hand	3.75	1.66	0.273	0.016*	0.352†	0.002*
Log_{10} Coliforms CFU per hand	2.23	1.13	-0.089	0.440	-0.095	0.407
Turbidity ($\text{Absorbance}_{600nm}$)	0.0815	0.0336	0.549†	< 0.001*		

*$P < 0.05$
†0.3 < rho < 0.7, moderate correlation (see Methods)
‡IQR: interquartile range, measure of variability

eye, such as hand-colored soil. We recommend that the Visible Hand Dirtiness Score may be a useful alternative to assess visible hand soil in the field or in settings where it is not possible to measure the turbidity of a hand rinse. Visible Hand Dirtiness Score may also be a better indicator of perception of soil on farmworker hands than turbidity, because it is based on visible soil instead of soil picked up in a hand rinse.

The Visible Hand Dirtiness Score was weakly associated with *Enterococcus* (rho = 0.273), but not significantly correlated with *E. coli* and coliforms. Because Visible Hand Dirtiness score was correlated to turbidity (rho = 0.549), we also expected a correlation between turbidity and *Enterococcus* (rho = 0.352) and a lack of a significant correlation between turbidity with *E. coli* and coliforms, which we found. One hypothesis to explain the difference in relationships between Visible Hand Dirtiness Score and turbidity with each indicator organism may be that different indicator organisms differ in their biology and their prevalence in the environment *(8)*. For example, *Enterococcus* may have been correlated with visible soil because this bacterium is commonly found in the soil environment and could be transferred to farmworker hands along with the soil during harvest *(3)*. Our previous studies on bacterial concentrations on fresh produce confirm that *Enterococcus* is highly prevalent in the agricultural environment. In one study, performed in 2000–2003, in which 923 produce samples were collected from 15 farms and 8 packing sheds *(1)*, we found that *Enterococcus* was detected on 78% of all produce samples, whereas *E. coli* was detected on 16% of produce samples *(1)*. In a second study, performed between November 2002 and December 2003, 466 produce samples were collected from 8 packing sheds, and the level of *Enterococcus* found on produce samples ranged from less than 1.0 $\log_{10}$ to 5.4 $\log_{10}$ CFU/g *(14)*.

This study has several strengths and limitations. One strength was that the Visible Hand Dirtiness Score was a valid and reproducible measure, as evidenced by two pilot tests in which two independent reviewers scored hands with > 90% agreement on each category. We also were able to perform the experiments in real life conditions, on a farm rather than using simulated conditions. One limitation of this study is the low prevalence of specific indicators (i.e., *E. coli*), suggesting that a larger sample size would have provided greater statistical power and ability to detect possible relationships. Another limitation is that we utilized indicator bacteria as a measure of contamination rather than pathogen contamination, which also would have required a larger sample size to detect the relatively low prevalence of pathogens *(5)*.

Based on these results, visible hand soil may be a moderate proxy for hand rinse turbidity, but it is not a strong indicator of bacterial load on farmworker hands. First, the Visible Hand Dirtiness Score may be a better indicator of perception of soil on farmworker hands than turbidity is, because the score is based on soil seen by the eye. Therefore, we recommend that the Visible Hand Dirtiness Score can be used as a research-based teaching tool to demonstrate to farm workers the areas commonly missed during handwashing, especially in the field, where a turbidimeter is not accessible. Second, farmworkers or managers cannot depend on visible cleanliness alone in assessing the microbiological cleanliness of hands. Therefore, regardless of whether hands look "clean," to minimize the risk of pathogen contamination on fresh produce, we recommend that growers follow Good Agricultural Practices (GAPs) as recommended in the US FDA Produce Rule *(9)* and newer, innovative hand hygiene recommendations that are proven and evidence based, such as well-formulated ABHS interventions *(6)*.

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