Chapter 4

THE EFFICACY OF WATERLESS HAND SANITIZER IN REDUCING BACTERIAL LOAD ON THE HANDS OF CHILDREN VISITING A PETTING ZOO

4.1 Abstract

Increased reports of children becoming ill after visiting petting zoos, fairs, open farms and similar attractions have brought about intensified efforts to address the transmission of zoonotic infectious agents in such settings. The Centers for Disease Control and Prevention (CDC) have released recommendations emphasizing the need to wash hands in order to reduce the transfer of infectious organisms from animals to people. The problem that presents itself is that many of these attractions, due to their traveling nature and rural settings, are unable to provide facilities that meet CDC recommendations. The CDC suggests that in instances where adequate handwashing facilities are unavailable, waterless hand sanitizers (WHS) may be of value. However, they make no distinct recommendations due to the lack of research evaluating WHS in such settings.

A study involving 102 children between the ages of 4 and 12 was conducted at the petting zoo of the 2002 Virginia State Fair located in Richmond, VA. Bacterial counts on fingers before and after WHS use were measured using a modified glove juice sampling technique. Finger length was measured from fingertip to first interphalangeal joint and widths were measured at the fingertip and knuckle to calculate sampled surface area.

Bacterial counts on the fingers of children sampled before use of WHS ranged from 40 to 75,200 CFU/ml with a geometric mean of 8,653 CFU/ml. After WHS use, counts ranged from 19 to 58,400 CFU/ml with a geometric mean of 1,727 CFU/ml. Differences (before minus after) ranged from -35,600 to 59,400 CFU/ml with a median of 8,190 CFU/ml. Transferred counts based on finger surface area before the use of the WHS ranged from 0.07 to 176.6 CFU/cm² with a mean of 31.8 CFU/cm². After WHS use, counts ranged from 0.00 to 89.1 CFU/cm² with a mean of 11.8 CFU/cm². The reduction in bacterial load before and after WHS use was 0.70 log (82.2%; P< 0.001).
It can be concluded that in situations where adequate traditional handwashing facilities are unavailable or impractical, WHS may be helpful in reducing the amount of contamination on the hands of attraction visitors.

KEYWORDS: hand hygiene, petting zoo, waterless hand sanitizer
4.2 Introduction

Increasing zoonotic infections among children attending petting zoos, open farms, fairs and other similar venues have been reported (Christie, 2002, 2003). *Escherichia coli* and *Salmonella* spp. are organisms frequently implicated in these outbreaks. Infection can result in mild clinical disease that may prompt the individual to seek medical assistance or in more severe cases result in hospitalization and in rare instances death (Belongia *et al.*, 2003; CDC, 1999; CDC, 2001a; CDC, 2001b; Crump *et al.*, 2003; Crump *et al.*, 2002; Cohen, 1998; Mead 1998; Milne *et al.*, 1999; Quinn *et al.*, 1994; Sanchez *et al.*, 2002).

The Centers for Disease Control and Prevention (CDC) reported several risks factors associated with zoonotic infections *i.e.*, *E. coli* O157:H7 and *Campylobacter*, at county fairs. These included contact with cattle, behavior promoting hand-mouth contact, such as nailbiting and purchasing food from an outdoor concession. Handwashing before eating was protective. In addition, it was also found that most individuals who acquired infection were preschool- and school-aged children (CDC, 2001a).

Due to the recent surge in infections caused by *E. coli* O157:H7 and other zoonotic agents, the CDC has released recommendations, aimed at decreasing and/or eliminating acquired infections, for attraction owners/supervisors and individuals planning on attending these venues. The need for operators of such attractions to maintain segregated facilities for animal contact and food consumption was stressed. They also discouraged hand-mouth activities such as smoking, eating, drinking, etc., in animal areas. Possibly one of the most important recommendations was to urge the attraction owners/supervisors to make adequate handwashing facilities available to their visitors. It was recommended that handwashing stations be located in both animal contact and animal-free areas, that sufficient amounts of soap, water and disposable paper towels be available and that adults supervise children during handwashing to ensure that it is properly performed. They also recommended that adequate signage be posted regarding the potential risks for enteric infection and the need for proper hand hygiene after coming in contact with the animals or their environments before eating or leaving the facilities (CDC, 2001a).
In most cases, children are not aware of the potential danger that bacterial organisms common to farm animals may present. They are also unaware of the importance of handwashing to prevent the spread of infection and they generally do not practice handwashing in an adequate manner.

Many animal attractions cannot provide the recommended handwashing stations due to their traveling nature or the rural locations where they are commonly found. Numerous researchers have commented on the effectiveness of WHS at decreasing bacterial hand contamination in human medical facilities and in controlled efficacy trials but information regarding use in agricultural settings is absent (Fendler et al., 2002; Kampf et al., 2002; Marena et al., 2002; Mody et al., 2003; Paulson et al., 1999). While the CDC suggests that “[waterless] hand sanitizers may be better that using nothing” in instances where “running water is not available,” they make no recommendations on the use of waterless hand sanitizer (WHS) due to the lack of research verifying their efficacy in these settings (CDC, 2001a).

Because animal attractions are often unable to provide adequate handwashing facilities, and because of the lack of research in this area, it is worthwhile to directly access the efficacy of WHS in environments such as petting zoos where the potential of acquiring zoonotic disease exists.

4.3 Materials and Methods

A study was conducted among the attendees of the 2002 Virginia State Fair located in Richmond, VA. A convenience sample was obtained of children between the ages of 4-12 years while they were visiting the petting zoo. They were asked to participate in the study after they had petted animals regardless of whether or not they had previously used the WHS available at the holding pens. Those that used WHS immediately before attempting to participate were excluded. An informational brochure describing the study as well as investigator contact information was made available to the children and/or supervising adults (Figure 4.1)

Sampling of children’s fingers was performed using a modification of a method described in the Annual Book of the American Society for Testing and Materials. The
technique, known as Glove Juice Sampling (GJS) is a standard methodology for determining bacterial load on the hands of healthcare personnel (Baldini et al., 2001).

The sampling solution used in this study was prepared by dissolving 0.4 g of monobasic anhydrous potassium phosphate (KH$_2$PO$_4$, Sigma Chemical Company, St. Louis, MO), 10.1 g of tribasic, dodecahydrate sodium phosphate (Na$_2$PO$_4$, Sigma Chemical Company, St. Louis, MO) and 1.0 g of isooctylphenoxy polyethoxyethanol (Triton X-100, LabChem, Inc., Pittsburgh, PA) in 1 L of distilled water. The solution was then adjusted to a pH of 7.8 and autoclaved (Baldini et al., 2001).

Both the index and middle finger on the child’s dominant hand were randomly sampled, one before the use of WHS, the other after. Prior to sampling, both of the child’s fingers were measured. The length from fingertip to first interphalangeal joint as well as fingertip and knuckle width was recorded for each of the two fingers in order to calculate the surface area. Surface area of the finger was calculated with the following equation:

$$\frac{(3.0 \text{ ml})(\# \text{ of CFU/ml})}{(\pi r^2 + 2\pi r)L} = \text{CFU/cm}^2$$

$$\pi = 3.14159$$
$$r = \text{radius}$$
$$L = \text{length}$$

The radius was calculated by dividing the average diameter measurements of the fingertip and the knuckle by 2.

Each child was asked to insert his/her finger into a sterile, 1 oz Whirl-Pak® bag (Fisher Scientific, Fair Lawn, NJ) which contained 3 ml of the sampling solution. The bag was then pulled up until the bottom of the bag met the fingertip, grasped on both sides and moved in a circular motion for 30s in an attempt to remove bacteria from the surface of the finger (Figure 4.2). The Whirl-Pak® bag was closed and placed on ice. The sampled finger was dried making every effort not to contaminate the adjacent unsampled finger. Two packs of Purell® Instant Hand Sanitizer (GOJO Industries, Inc., Akron, OH) (1.33ml/pack) were squeezed into the cupped hands of the child who was instructed to rub their hands together for 45s in a fashion mimicking handwashing. This was demonstrated by the investigator. The alternate finger was then sampled as
described previously. The participants were offered a choice of a small toy as incentive for their participation.

Within 15 hrs of sampling, ten-fold dilutions were made by serial transfers of 100 µl of the ‘glove juice’ to 900 µl of sterile phosphate buffered saline, pH 7.4 (Sigma Chemical Company, St. Louis, MO). The Miles-Misra technique (Quinn et al., 1994) was used to determine colony-forming units (CFU) per ml. Briefly, five 10 µl drops of each dilution (up to 1:10^{-6}) were plated onto both Columbia blood agar (Becton, Dickinson and Company, Sparks, MD) and MacConkey agar (Becton, Dickinson and Company, Sparks, MD). After twenty-four hours of incubation at 37º C, colony counts were performed.

Results of this experiment were analyzed with the Wilcoxon signed rank test using SAS®9 (SAS Institute, Inc., Cary, NC) statistical software program.

Children who participated in the study were also given a questionnaire administrated by the investigators. The questions were:

1. Do you use antibacterial soaps in your home? Y N
2. Do you use waterless hand sanitizers such as Purell®? Y N
3. How long has it been since you’ve washed you hands? < 1hr 1-4hrs 4-8hrs 8-12hrs >12hrs
4. Which animals did you pet here today? Calves/cows sheep/lambs goats pigs chicken ducks turkeys Other_____________________
5. What is your age?____________________________

The child’s gender was also recorded. When the child was determined to be too young to accurately answer the questions, assistance of the child’s parent(s) or supervising adult was requested.
What Are We Doing??

In the past few years there have been a number of cases of people -- often young people -- becoming ill shortly after they have visited petting fairs or farms. That's because even healthy animals can have bacteria on them that could make you sick -- especially if those bacteria get into your mouth!

Warm water and soap work very well for cleaning your hands -- but it's not always easy to remember to wash your hands before you eat or put your fingers in your mouth!! And there isn't always a tap, a bar of soap, a sink and a towel nearby... That's where the waterless hand sanitizers come in...

The Centers for Disease Control (CDC) say that using a "waterless hand sanitizer" may be better than using nothing at all - but there isn't really any research to prove that it works in a petting zoo...except for a small study done last year by researchers at

Figure 4.1. Informational brochure given to the participants, participants’ parents and/or supervising adult.
Virginia Tech that has shown some promising results.

So now we're doing a larger study to see if it really works!!!!!!

At this point, we're still recommending that you wash your hands with soap and water before eating or putting your fingers in your mouth...better safe than sorry after all!!

Thanks For Participating!!

To receive the future results of this study, send an email to the address below requesting information.

Ernest Hovingh, DVM, PhD
Virginia-Maryland Regional College of Veterinary Medicine
ehovingh@vt.edu

Figure 4.1 continued. Informational brochure given to the participants, participants' parents and/or supervising adult.
Figure 4.2. **Modified glove juice method.** a.) Participant inserts the first finger to be sampled into the Whirl-Pak® bag. b.) Bag is pulled up until the fingertip of the participant meets the bottom of the bag. c.) Bag was gripped on both sides and moved in a circular motion in an effort to remove bacteria from the finger.
4.4 Results

A total of 104 children participated in this study with usable data from 98. Their ages ranged from 4 to 12 years with a mean of 7.7 years. Forty-eight of the children were female and the remaining 48 male, 2 had no gender indicated on the data collection form. The data was analyzed in two ways. “No growth” values were replaced with 19 CFU/ml, 1 CFU/ml below the limit of detection. Bacterial counts before WHS use ranged from 40 to 75,200 CFU/ml with a geometric mean of 8,653 CFU/ml (95% CI: [(6,569; 789,176]). After WHS use, counts ranged from 19 to 58,400 CFU/ml with a geometric mean of 1,727 CFU/ml (95% CI: [1,214; 157,484]). Differences (before minus after) ranged from -35,600 to 59,400 CFU/ml with a median of 8,190 CFU/ml. In 12 (12%) instances, HBC increased after WHS application. The logarithmic reduction in bacterial load before and after WHS use was 0.70 (82.2%). (Figure 3.3).

Counts based on finger surface area before use of the WHS ranged from 0.07 to 176.6 CFU/cm² with a mean of 31.8 CFU/cm² (95% CI: [25.9; 37.7]). After WHS use, bacterial growth ranged from 0.00 to 89.1 CFU/cm² with a mean of 11.8 CFU/cm² (95% CI: [8.2; 15.4]) (Table 4.1).

Results of the questionnaire are shown in Table 4.2. Eighty-two percent (80/98) of children indicated use of antibacterial soaps in their homes, 18% (18/98) did not. Children using WHS in their homes composed 71.4% (70/98) of respondents, 27.6% (27/98) did not use WHS and 1 individual did not answer. When asked how long it had been since they last washed their hands, 39% (37/96) of individuals reported “less than a hour ago”, 46% (44/96) “between 1 to 4 hours”, 15% (14/96), “between 4 to 8 hours”, less than 1% (1/96) reported their last handwashing occurring “more than 12 hours ago” and two individuals did not answer the question. Respondents reported touching the following animals: 53% (52/98) cows/calves, 35.7% (35/98) sheep/lambs, 69.4% (68/98) goats, 23.5% (23/98) pigs, 23.2% (23/98) guinea pigs/hamsters/mice, 43.9% (43/98) rabbits, 5.1% (5/98) llamas, 4.1% (4/98) cats/dogs, 18.4% (18/98) donkeys/horses/ponies, 1% rams (1/98), snake (1/98) or did not touch any animals (1/98).
Figure 4.3.  Bacterial counts on the fingers of children sampled before and after waterless hand sanitizer use.
Table 4.1. Comparison of before and after finger bacterial counts.

<table>
<thead>
<tr>
<th></th>
<th>Mean Before</th>
<th>Mean After</th>
<th>Log Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CFU/ml</strong></td>
<td>8,653 (range 40-75,200)</td>
<td>1,727 (range 19-58,400)</td>
<td>0.70 (82.2%)</td>
</tr>
<tr>
<td><strong>CFU/cm²</strong></td>
<td>31.8 (range 0.07-176.6)</td>
<td>11.8 (range 0.00-89.1)</td>
<td>--</td>
</tr>
</tbody>
</table>
Table 4.2. Questionnaire results.

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes (%)</th>
<th>No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do you use antibacterial soaps in your home?</td>
<td>82%</td>
<td>18%</td>
</tr>
<tr>
<td>Do you use waterless hand sanitizers such as Purell?</td>
<td>71.4%</td>
<td>27.6%</td>
</tr>
<tr>
<td>How long has it been since you last washed your hands?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>“less than a hour ago”</td>
<td>39%</td>
<td>(37/96)</td>
</tr>
<tr>
<td>“between 1 to 4 hours”</td>
<td>46%</td>
<td>(44/96)</td>
</tr>
<tr>
<td>“between 4 to 8 hours”</td>
<td>15%</td>
<td>(14/96)</td>
</tr>
<tr>
<td>“more than 12 hours ago”</td>
<td>less than 1%</td>
<td>(1/96)</td>
</tr>
<tr>
<td>Which animals did you pet/touch here today?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cats/dogs</td>
<td>4.1%</td>
<td>(4/98)</td>
</tr>
<tr>
<td>cows/calves</td>
<td>53.1%</td>
<td>(52/98)</td>
</tr>
<tr>
<td>donkeys/horses/ponies</td>
<td>18.4%</td>
<td>(18/98)</td>
</tr>
<tr>
<td>goats</td>
<td>69.4%</td>
<td>(68/98)</td>
</tr>
<tr>
<td>guinea pigs/hamsters/mice</td>
<td>23.5%</td>
<td>(23/98)</td>
</tr>
<tr>
<td>llamas</td>
<td>5.1%</td>
<td>(5/98)</td>
</tr>
<tr>
<td>pigs</td>
<td>23.5%</td>
<td>(23/98)</td>
</tr>
<tr>
<td>rabbits</td>
<td>43.9%</td>
<td>(43/98)</td>
</tr>
<tr>
<td>rams</td>
<td>1%</td>
<td>(1/98)</td>
</tr>
<tr>
<td>sheep/lambs</td>
<td>35.7%</td>
<td>(35/98)</td>
</tr>
<tr>
<td>snakes</td>
<td>1%</td>
<td>(1/98)</td>
</tr>
<tr>
<td>did not pet animals</td>
<td>1%</td>
<td>(1/98)</td>
</tr>
</tbody>
</table>
4.5 Discussion and Conclusions

Waterless hand sanitizer has already been found to be effective at reducing bacterial loads on the hands of healthcare workers in human medical facilities (Fendler et al., 2002; Kampf et al., 2002; Marena et al., 2002; Mody et al., 2003, Paulson et al., 1999). The results of this study have shown reductions in bacterial loads on the fingers of children visiting a petting zoo after the use of WHS. The Food and Drug Administration’s Tentative Final Monograph for Healthcare Antiseptic Drug Products (TFM) requires at least a 2 log\textsubscript{10} reduction of bacterial load in order to meet efficacy standards. It should be noted however, that then current evaluation was not performed using the TFM protocol which calls for the intentional contamination of the hands with an indicator organism under controlled experimental conditions (McDonald et al., 2003). Instead, evaluation was performed using children’s hands naturally contaminated with a variety of organisms in the realistic setting of a petting zoo. Although TFM guidelines exist, investigators continue to use a variety of methods to evaluate efficacy of hand hygiene products. In the literature addressing WHS’ efficacy, one study used a technique similar to that used in the present study and one used a method similar to TFM protocols (Fendler et al., 2002; Kampf et al., 2002; Marena et al., 2002; Mody et al., 2003).

In several instances, HBC increased after WHS application. Occasionally, when hand rubbing/washing is performed too vigorously or for an extended time period, resident microbial flora (not contaminants) may be removed. When this occurs, bacterial counts may increase. Two applications of WHS may be necessary in order to achieve the TFM 2 log\textsubscript{10} reduction when hands are naturally contaminated and resident organisms are present.

Waterless hand sanitizer has proven itself to be useful in reducing bacterial load on the fingers of children at a petting zoo. Therefore, in other animal settings where adequate traditional handwashing facilities are unavailable or impractical, WHS may be helpful in reducing the amount of contamination on the hands.

There were several limitations inherent to this study. The investigators preferred to evaluate the efficacy of WHS on the same individual before and after use. Therefore, a modified GJS method had to be used to sample the index and middle fingers of the same individual and on the same hand. The proposed time of 1 min was modified to 30s in an
effort to decrease the time commitment of children and their parents. This may have affected the amount of bacteria collected from the fingers during sampling. The location of the petting zoo and the investigator’s laboratory were over 200 miles apart. Because of this, a delay in plating samples occurred. Although samples were placed on ice after collection, bacterial growth may have been influenced by the delay. The earliest samples taken may have been more affected by this due to the greater time between collection of the sample and plating.

Future studies may improve on this design by not modifying the GJS method used to collect bacterial contamination off of the hands. Performing collection with a sterile non-powdered glove on hands randomized between dominant and non-dominant and extending the wash time to a full 1 min, may satisfy criticism of methodology. Alternatively, if the unmodified GJS technique were used, a different child could be sampled to obtain hand bacterial counts before and after WHS use but this would likely double the sample size and samples could not be paired. Because unpaired samples have less similarity than paired, this would most likely introduce more variation.
4.6 References


