NPB FINAL RESEARCH GRANT REPORT FORMAT

Evaluation of hand hygiene procedures as interventions to decrease the risk of influenza transmission between pigs and people
(NPB project # 21-087)

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Industry Summary:

Transmission of influenza A virus (IAV) between pigs and people is one of the most significant, yet least understood, challenges facing the swine industry and public health. Proper hand hygiene protocols are important to minimize the risk of IAV transmission between pigs and people. In this study, we assessed the effectiveness of four hand sanitation protocols in decreasing or removing IAV from hands of individuals handling influenza infected pigs, including hand washing with soap and water, hand washing with water only, hand sanitizing with alcohol-based hand sanitizer, and wearing gloves. We also evaluated IAV viability on hands for up to 120 minutes. Directly after handling infected pigs, hands of all participants were contaminated with IAV (n=84) and IAV was detected up to 120 min after handling infected pigs. IAV was isolated from 7 out of 20 samples including one sample after 10 min of pig handling. The alcohol-based hand sanitizer and wearing of gloves were the most effective treatments at reducing IAV from hands, although viable IAV could not isolated after any of the treatments. Overall, our results emphasize the importance of using a hand sanitation protocol to prevent the transmission of influenza between pigs and farm workers. For more information, contact Dr. Montse Torremorell (torr0033@umn.edu).

Key Findings:

- Viable influenza was readily detected from hands of individuals handling influenza infected pigs
- Influenza was detected on hands, up to 120 minutes after handling influenza infected pigs
- Sanitizing hands with an alcohol-based sanitizer and wearing gloves appears to be most effective method at reducing influenza amounts on hands when handling IAV infected pigs
- We emphasize the need to implement effective hand sanitation protocols in swine farms to prevent the transmission of influenza between pigs and people

Keywords:

Influenza, swine, bi-directional transmission, pig human interface, farm worker, hands hygiene

Scientific Abstract:

Bidirectional transmission of influenza A virus (IAV) between pigs and people is one of the most significant, yet least understood, challenges facing the swine industry and public health. Properly sanitizing hands is crucial to prevent disease transmission. In this study, we assessed the effectiveness of four hand sanitation protocols in decreasing or removing IAV from hands in an experimentally IAV infected pig setting: a) hand washing with soap and water, b) hand washing with water only, c) hand sanitizing with alcohol-based hand sanitizer, and d) wearing gloves. We also evaluated IAV viability on hands for up to 120 minutes after handling IAV-infected pigs. Directly after handling infected pigs, hands of all participants were contaminated with IAV (n=84). Twenty hand samples, where IAV was detected, were selected for virus isolation and viable IAV was isolated from seven samples. IAV was detected in samples from hands at 10 minutes, 30 minutes, 60 minutes and 120 minutes after handling IAV infected pig. However, amount of IAV decreased overtime and viable virus was only isolated from one sample from hands at 10 minutes post IAV infected pig handling. The alcohol-based hand sanitizer and wearing of gloves were the most effective treatments at reducing a larger amount of virus from hands. Hand washing with water only and washing with soap and water reduced the amount of detectable virus but did not eliminate it.

Viable virus could not be recovered from any of the tested samples after the hand sanitation protocols. Overall, our results emphasize the importance of using a hand sanitation protocol to prevent the transmission of influenza between pigs and farm workers.

Introduction:

Bidirectional transmission of influenza A virus (IAV) between pigs and people is one of the most significant, yet least understood, challenges facing the swine industry and public health. The challenge arises from the unpredictability of swine influenza infections becoming zoonotic and, in some instances, turning into a human pandemic, as was the case in 2009 when the pandemic H1N1 influenza virus emerged. Another challenge is the frequent spillover of human-origin seasonal influenza viruses to pigs, which not only drives the diversity of IAV in pigs but also results in new strains that threaten pig health, pig productivity, and public health. Once these "new" strains are in pigs, they have the potential of causing disease when transmitted back to people. Despite these well-known risks, little is done at the pig-human interface to prevent these interspecies infections from happening in pig farms.

There are several transmission routes by which influenza transmission occurs. Both direct transmission, through contact with infected swine, and indirect transmission, through aerosols or through contaminated surfaces, facilitate the transfer of viruses between humans and pigs. For this proposal, the indirect transmission of IAV via the hands of people who have touched IAV-contaminated surfaces or handled IAV-infected animals is of particular interest. In a recent study funded by the Minnesota Pork Board, after performing chores such as processing piglets after birth, vaccination, or weaning, many chore workers' hands were IAV positive by a screening PCR test, with some hands having viable IAV cultured from them (Lopez et al, 2022). The number of workers' hand samples that tested positive was striking with 90% and 92% of hands testing positive after vaccination and performing the weaning chores, respectively. In contrast, only 9% of the hand samples were IAV positive when sampled directly after processing pigs shortly after being born. Such positivity rates of viable IAV likely contribute to the transmission of IAV between pigs within the farms and potentially serve as a source of exposure to people who touch their nose and mouth without properly washing hands.

Viable IAV has also been cultured from the hands of people infected with seasonal influenza and the Centers for Disease Control and Prevention (CDC) has recommendations on how to properly wash hands to prevent transmission of influenza between people. However, there is limited information on the duration of IAV viability on hands of swine farm workers, with hands often being contaminated with porcine nasal and oral secretions, which may protect IAV from inactivation. Furthermore, the CDC recommendation to wash hands for 20 seconds may not always be practical when performing farm chores due to lack of readily available, pen-side hand-washing equipment on farms. In addition to being impractical, the "20-second rule" may not even be helpful since farm workers' hands may also become contaminated with organic matter in addition to the aforementioned secretions that increase virus survival, thus rendering the hand washing procedures less effective.

Therefore, there is a need to further investigate the infectiousness of hands contaminated with IAV, which during the course of the workday, likely become contaminated with organic matter and pig secretions that increase virus survival. Having validated procedures to properly wash hands of farm workers will not only help mitigating the transmission of IAV but also the transmission of other pathogens including those that may be zoonotic, foodborne, and/or cause other diseases of relevance to humans and pigs.

Objectives:

The overall goal is to prevent the transmission of influenza viruses between pigs and people and more specifically to validate intervention strategies directed at preventing or minimizing indirect transmission through influenza-contaminated hands. More specifically, we propose to:

a) Evaluate the load and duration of influenza virus viability on hands of swine farm workers contaminated with body secretions of pigs.

b) Evaluate hand hygiene procedures as a feasible and effective practice to decrease the detection and viability of influenza virus on hands of swine farm workers.

Materials & Methods:

Protocols and procedures followed during the study were approved by the University of Minnesota Institutional Animal Care and Use Committee (protocol number 2110-39491A), the Institutional Biosafety Committee (protocol number 2108-39310H) and the Institutional Review Board (protocol number STUDY00014737). Participants volunteered to participate in the study and were given the option to withdraw from the study at any point in time.

Experimental design

Participant enrollment

Consenting participants were recruited amongst the University of Minnesota Veterinary Population Medicine Department undergraduate students, graduate students, and researchers, to model an assigned hand hygiene procedure and have their hands sampled before and after handling IAV-infected pigs. A subset of participants also had their hands sampled for an extended period of up to 120 minutes to assess the duration of IAV viability. Participation in the study was voluntary and participants could withdraw from the study at any point in time without consequences. Participants were required to have the current seasonal influenza vaccine, not have any underlying health conditions, and not display any influenza-like illness prior to the start of the study. Participants received a gift card at the termination of the study and compensation was proportional to $20 USD per day of participation.

To test the feasibility of using a hand sanitation procedure under farm conditions, farm workers in a 3400 head sow farm with an onsite GDU were recruited to test the hand hygiene protocols in a field setting. Participation in the study was voluntary and farm workers could decline without consequences.

Hand Sanitation Protocols

Four hand sanitation protocols were evaluated: a) hand washing with soap and water, b) hand washing with water only, c) hand sanitation with an alcohol-based hand sanitizer, and d) wearing disposable gloves. In the animal experimental setting, each of the four hand sanitation protocols was evaluated in triplicate daily for 7 days, for a total of 21 samples per protocol.

The hand washing with soap and water protocol involved rinsing hands with water for 5 seconds, lathering and scrubbing hands with approximately 2 mL of BacDown soap for 10 seconds, rinsing hands for 10 seconds with water, then drying hands with paper towel.

For the hand washing with water only protocol, participants rinsed and scrubbed hands with water for 10 seconds then dried hands with paper towel.

The hand sanitation with an alcohol-based hand sanitizer involved participants rubbing approximately 1 mL of Purell hand sanitizer on hands for 10 seconds then air drying hands.

The disposable gloves protocol involved participants wearing gloves during animal handling then removing the gloves afterwards.

Pig IAV inoculation

Inside a BSL-2 animal isolation room, four pigs, age 18-21 days old, were intranasally inoculated with 2 mL of $1x10^{5.38}$ $TCID_{50}$/mL of an H1N1 IAV. One day post IAV inoculation, 2 contact pigs were placed in the pen with the IAV inoculated pigs.

Nasal swab collection

Nasal swab samples were collected from pigs on days -1, 0, 1, 3, 5, and 7 post IAV inoculation, using rayon tip swab with liquid Stuart media (Copan Italia SpA, Brescia, Italy). Swabs were placed on ice and brought back to the lab for processing. Each swab was placed inside a 2.0 mL tube containing 2.0 mL Transport Media, then prepared into three aliquots and frozen at -80°C until testing.

Hand wipes collection

Participants donned personal protective equipment (PPE) that included coveralls, N-95 mask, goggles, face shield, hair nets and plastic boots. Disposable gloves were only worn by individuals assigned to the disposable gloves protocol. Prior to animal interaction and handling, all participants lathered their hands for 10 seconds with approximately 2 mL of soap, rinsed their hands in water for 15 seconds, then dried their hands with paper towel.

Participants had their hands sampled before and after handling IAV infected pigs, and after the randomly assigned hand hygiene protocol. Hand sampling was done by using a 3 inches x 3 inches gauze moistened with 5 mL PBS to wipe both hands, on the front and back of the hands and between fingers. The used gauze was placed in 5 mL of Transport Media, placed on ice, and brought back to the lab for processing and testing. Transport media was mixed with used gauze by squeezing the gauze several times, liquid aliquoted into three 1.7 mL microcentrifuge tubes and frozen at -80°C until testing.

To assess duration of IAV viability, a subset of participants waited 10 minutes, 30 minutes, 60 minutes, and 120 minutes after handling IAV infected pigs before having their hands sampled.

Hand wipes collection from a field setting

Farm workers' hands were sampled after their morning chores and after the hand sanitation protocol. Sampling was performed using a 3 inches x 3 inches gauze moistened with 5 mL PBS to wipe both hands, on the front and back of the hands and between fingers. Farm workers were randomly assigned one of two hand sanitation protocols: a) wash hands with soap and water for 20 seconds, and b) sanitize hands with alcohol-based hand sanitizer. The sow farm also allowed employees the choice to wear gloves. If the employee had chosen to wear gloves during chores, a hand wipe sample was collected of the gloves while they were still being worn and a second hand wipe sample was collected of the hands after the gloves were removed. Each collected gauze was placed in 5 mL of Transport Media, placed on ice, transported to the lab, processed, aliquoted into three 1.7 mL microcentrifuge tubes, then frozen at -80°C until testing.

IAV RT-qPCR

Pig nasal swabs and hand wipes were tested for the presence of IAV by extracting RNA using the MagMAX™ −96 Viral RNA Isolation kit (Applied Biosystems by Thermo Fisher Scientific, Lithuania) according to the manufacturer's instructions, on a semiautomatic MagMAX Express-96 Deepwell Magnetic Particle Processor (Applied Biosystems by Thermo Fisher Scientific). IAV RT-qPCR was performed with primers and probe targeting the IAV matrix gene as described in Slomka et al., 2010, and IAV RT-qPCR conditions as described in Nirmala et al., 2021.

IAV virus isolation

Twenty hand wipes from the experimentally infected animal study and 13 hand wipes from the swine farm sampling, with IAV RT-qPCR Ct <35, were selected for virus isolation. In the experimentally infected animal study, five hand wipe samples from each of the hand sanitation

protocols were randomly selected for virus isolation. Samples were inoculated on 18-24 hours old Madin-Darby canine kidney cells (MDKC) grown in 6-wells cell culture plates, then incubated for 5 days at 37˚C, with 5% CO_2. IAV cytopathic effect (CPE) was examined under inverted microscope after the 5 days incubation period.

Data analysis

Results from the four hand hygiene protocols comparing before and after RT-PCR Ct values were analyzed using a non-parametric ANOVA (Krustal-Wallis) by day of the study. After that, pairwise test comparisons were done post hoc. Negative Ct values were given a value of 45. Results were considered significant if $p<0.05$. A linear regression analysis was used to estimate the increase in Ct values overtime and ANOVA was used to evaluate differences overtime of the linear model.

Results:

Demographic and baseline information

Ten (n=10) participants from the University of Minnesota Veterinary Population Medicine Department signed the consent form but one participant was removed from the study due to underlying health conditions.

Experimentally infected animal hand wipes IAV RT-qPCR and virus isolation results

All challenged pigs became infected with IAV. Upon handling infected pigs, hands of all participants became readily contaminated with IAV (84/84, mean Ct 32.72, SD 1.88) and IAV was isolated from 7 out of 20 hand wipes directly after pig handling.

All hand hygiene protocols resulted in a reduction in the number of IAV RT-PCR positive samples and in the amount of viral RNA detected before and after each treatment as indicated by significant differences in Ct values ($p<0.001$) (Table 1).

Overall, there were differences among hygiene treatments with water only and soap and water having significantly lower Ct values (higher RNA load) compared to alcohol based sanitizer and using gloves (Table 2) (Figure 1). The reduction of RNA genetic material from samples from participants that washed hands with soap and water, or water only, was limited, with differences in Ct values observed on 5 out of 7 days and 1 out of 7 days, respectively. In contrast, samples from hands treated with an alcohol-based sanitizer and hands with worn gloves had a larger reduction in IAV genetic material that was observed on each of the days of the study (Figure 2). No viable virus was recovered after any of the hand hygiene procedures.

For the hand wipes collected from 10 minutes to 120 minutes directly after pig interaction, Ct values increased as time post pig handling augmented, indicating a reduction of virus amount over time (Table 3) (Figure 3). Viable virus was isolated up to 10 minutes after handling infected pigs.

Swine farm workers IAV RT-qPCR and virus isolation results

IAV was detected on swine worker hands after their morning chores in 15 out of 23 samples (mean Ct 34.78, SD 2.00). After the three hand hygiene protocols, IAV was detected in 3 out of 23 samples (mean Ct 36.47, SD 1.45). Statistical differences could not be assessed due to the limited number of samples. No viable virus was recovered from the swine farm hand wipes that were tested.

Discussion:

In order to provide recommendations to mitigate the transmission of influenza through contaminated hands, we evaluated the load and duration of IAV viability on hands of workers after handling pigs. We also evaluated four common hand hygiene protocols used in farms. The hygiene treatments included a combination of hand washing procedures with and without soap, or using an alcohol sanitizer or wearing gloves. Our results indicated that influenza virus is readily found on hands of individuals

handling infected pigs and that the load of virus on hands decreases overtime. Furthermore, we found that hand hygiene protocols consisting of using an alcohol-based hand sanitizer or wearing gloves were the most effective protocols at decreasing the load of IAV on hands.

We documented that hands of individuals become readily contaminated with IAV upon handling IAV infected animals. In the experimentally IAV infected pig study, IAV was detected on all of the hand samples directly after contact with IAV infected animals, as well as up to 120 minutes after IAV infected animal contact. Our results indicate that the concentration of IAV detected via RT-qPCR on hands decreased as time post pig contact increased. However, we only tested for the detection of IAV on hands for up to 120 minutes after only 10 minutes of IAV infected pig handling. Furthermore, viable IAV was isolated from 7 out of 20 samples directly after pig handling and one sample from the 10 minutes post IAV infected pig handling. Thus, it is important to note that viable IAV was present on hands and thus can be transmitted to other people or animals or transferred to surfaces that then become a source of infection for animals and people. Under farm conditions, it is likely that the concentration of IAV on hands is higher than the ones observed in this study and that hands remain contaminated for longer periods of time. The on-going handling of infected pigs and the constant contact with contaminated surfaces stress the importance of hands as a potential source of IAV transmission. Furthermore, differences in pig shedding exist and it is possible that hands become contaminated with higher concentrations than the ones documented in this study. Thus, interventions that mitigate IAV spread through contaminated hands must be considered.

Differences between hand hygiene protocols were observed in their ability to remove or inactivate IAV in the hands of individuals handling IAV infected pigs in both experimental and field settings. While all four hand sanitation protocols resulted in the reduction of the number of IAV RT-qPCR and viable IAV, hands that were sanitized with an alcohol-based hand sanitizer and hands covered with gloves appeared to be most effective at reducing a larger amount of IAV. Although hand washing with soap and water is recommended in many instances, our results indicated that it may not be sufficient to completely inactivate and remove the IAV from contaminated hands. Adding an alcohol-based sanitizer after hand washing may be necessary to better mitigate transmission of IAV.

In summary, our results stress the importance of contaminated hands in the transmission of IAV and that hand sanitation procedures that minimize the contamination of hands (i.e wearing disposable gloves) or that inactivate IAV (i.e alcohol-based hand sanitizer) should be used to further minimize the transmission of IAV between pigs and people.

Tables and Figures

Table 1. Summary table of the number of positive samples and average and standard deviation (SD) Ct-values before and after each treatment.

		Total			Stats		95% CI	
		No. Pos	Avg Ct	Avg sd	t-value	p-value	Lower	Higher
Soap and water	After pig interaction	21/21	33.28	1.44	-6.05	<0.0001	-7.74	-3.89
	After washing	16/21	39.14	2.13				
Water only	After pig interaction	21/21	33.39	1.51	-4.58	<0.0001	-5.70	-2.19
	After washing	18/21	37.34	2.06				
Alcohol-based sanitizer	After pig interaction	21/21	32.46	1.63	-22.94	<0.0001	-13.26	-11.11
	After washing	1/21	44.65	0.93				
Disposable gloves	After pig interaction	21/21	31.76	1.00	-23.90	<0.0001	-13.87	-11.69
	After washing	1/21	44.53	1.24				

Table 2. Summary table comparing average Ct values of influenza A virus RT-PCR after each treatment.

Hand Washing Procedure	N	Average	SD	Median
Soap and water	21	39.14[a]	3.89	37.1
Water only	21	37.34[a]	3.47	36.36
Alcohol based sanitizer	21	44.65[b]	1.61	45
Gloves	21	44.53[b]	2.15	45

Differences in superscripts are significant at $p<0.0001$

Figure 1. Individual and average Ct values of influenza A virus RT-PCR after each treatment.

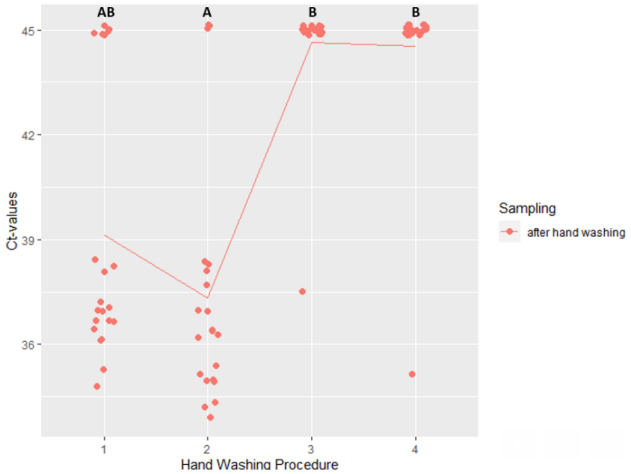

Figure 2. Daily influenza A virus RT-PCR Ct values for each treatment before and after the treatment was implemented. * indicate significant differences at $p<0.05$.

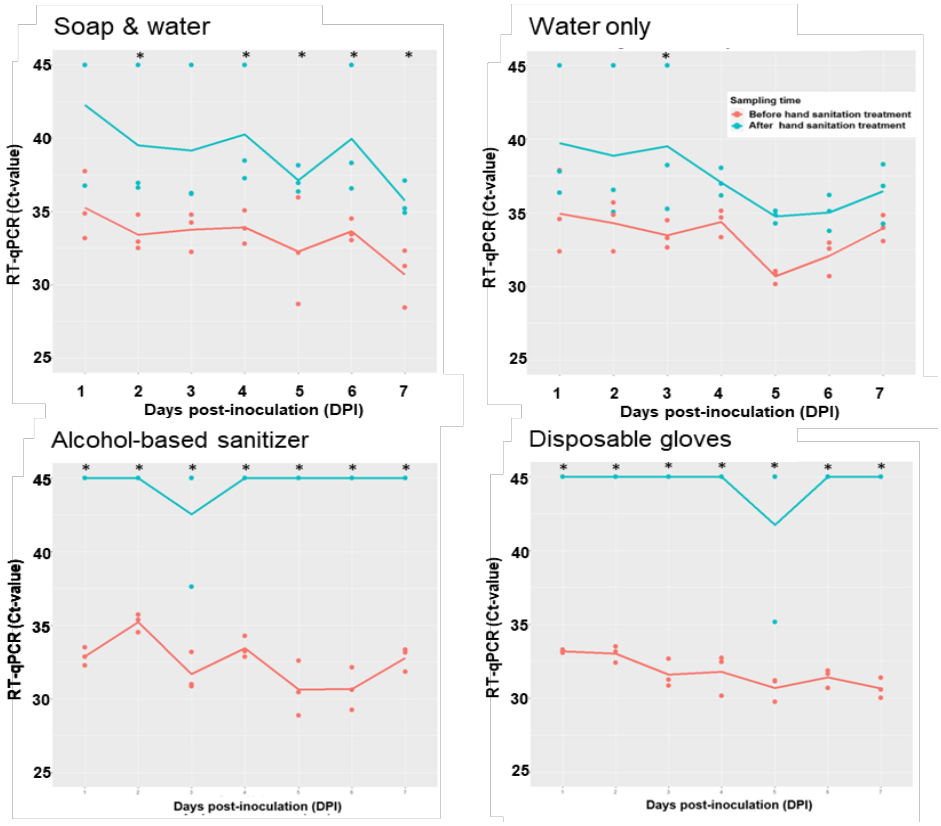

Table 3. Reduction of influenza A virus Ct values overtime evaluated by linear regression analysis.

	Estimate	SE	95% CI		t-value	p-value
			Lower	Upper		
Intercept (Time 0 after pig interaction)	31.55	0.95	29.59	33.51	33.36	<0.0001
Time (minutes)	0.05	0.01	0.03	0.08	3.97	0.0007

Figure 3. Linear regression analysis of influenza A virus RT-PCR Ct values detected on hands for up to 120 min.