

## **NPB FINAL RESEARCH GRANT**

### **Evaluation of face masks as a means to decrease the risk of influenza transmission between pigs and swine farm workers**

**(NPB project # 21-122)**

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#### **Industry Summary:**

Influenza A virus (IAV) transmission between pigs and swine farm workers poses a risk to public health. Swine workers are exposed to influenza virus by handling infected animals, touching contaminated surfaces, and/or breathing contaminated aerosols. Personal protective equipment consisting of face masks can be used to decrease the risk of aerosol exposure. In this study, we evaluated the use of three types of face masks amongst swine farm workers to potentially decrease exposure to influenza virus. We evaluated N-95 respirators, dust masks, surgical masks and compared the results to not wearing masks. Our results indicated that wearing a mask decreased the detection of IAV in the workers. Participants not wearing masks had the most influenza virus detections (24.27%), which was significantly higher ( $p < 0.01$ ) than all the detection rates for all three masks types (10.98% surgical mask, 8.33% dust mask, 5.77% N-95 mask). However, there were no statistically differences in IAV detection rates between the three masks types ( $p > 0.05$ ). Among masks, workers reported that surgical masks were the most comfortable to wear. Our results highlight the importance of using face masks to limit or prevent the transmission of influenza between pigs and farm workers. For more information, contact Dr. Montse Torremorell ([torr0033@umn.edu](mailto:torr0033@umn.edu)).

#### **Key Findings:**

- Influenza exposure to farm workers is common in endemically infected farms
- Use of face masks decreased influenza detection in swine workers
- N-95 respirators, surgical masks and dust masks offered protection against influenza aerosols with the N-95 masks providing the highest numerical protection
- Swine workers preferred wearing surgical masks over dust masks and N-95 respirators

#### **Keywords:**

Influenza, swine, bi-directional transmission, pig human interface, farm worker, face mask

#### **Scientific Abstract:**

Bidirectional transmission of influenza A virus (IAV) between pigs and farm workers poses a significant challenge to the swine industry and public health. IAV can be transmitted through infectious aerosols able to deposit on the workers' respiratory tracts and personal protective equipment consisting of face masks can be used to decrease the risk of aerosol exposure. In this study, we investigated the use of three face masks named N-95 respirators, dust masks, and surgical masks as a means to decrease the risk of IAV

exposure to farm workers and compared the results to not using the masks (control treatment). Twenty four percent (25 out of 102) of samples from workers not wearing masks tested positive while 5.77% (6/104), 8.65% (9/104) and 10.78% (11/102) tested positive for workers wearing N-95, dust mask and surgical masks, respectively. There were statistically significant differences between not wearing masks and wearing masks ( $p < 0.01$ ) with workers not wearing masks testing positive more often and having lower RT-PCR Ct values indicative of more viral RNA in the worker's nasal passages than any other face mask group. In contrast, there were no differences in the number of positive workers and their Ct values among types of masks ( $p > 0.05$ ) although N-95 masks had the highest numerical reduction. Most of the positive results were found in workers performing activities in the farrowing house and comfort scores for wearing masks were higher for the surgical masks (4.01 out of 5). Our findings add to the evidence of IAV exposure in swine is common and support using face masks as an intervention to decrease the risk of transmission of IAV between pigs and people.

### **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 IAV infections of swine becoming zoonotic and, in some instances, turning into a human pandemic, as was the case in 2009 when the H1N1 influenza pandemic virus emerged. While there is much attention paid to zoonotic diseases and transmission from pigs to people, it is the frequent spillover of human-origin seasonal influenza viruses from humans to pigs that is a main driver for IAV diversity in pig populations. These spillover events result in new reassortant viruses that threaten pig health, productivity, and public health. Once "new" reassortants 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 interspecies infections.

There are several routes by which IAV transmission occurs. Both direct transmission through contact with infected animals, and indirect transmission, through aerosols or through contaminated surfaces of fomites, facilitate the transfer of viruses between humans and pigs. Transmission of IAV via aerosols is of particular interest in swine farms because IAV can be found in the air associated with particles of multiple size ranges able to be inhaled by workers (Alonso et al., 2015) and detectable in the respiratory tract. Detectable IAV was found in the nasal passages of workers after having worked with pigs for the day. Forty one percent of workers tested IAV-RT-PCR positive during an 8-week surveillance period (Lopez-Moreno et al, 2022). At the sample level, 4.3% of samples tested positive and direct whole genome sequencing from these samples showed evidence of swine-origin IAV on the nasal passages of pig care workers. Interestingly, similar results but in lower levels were obtained from workers when first reporting to work for the day, indicative of overnight residual IAV RNA stability in the nasal passages of the workers. This study provided evidence of the risk of bi-directional transmission between pigs and workers and the need to protect workers from exposure to infectious aerosols.

Use of personal protective equipment (PPE) including disposable gloves, coveralls, goggles and face masks is recommended to mitigate exposure to IAV. Face masks are recommended for people working with pigs to decrease aerosol exposure. However, PPE use levels vary by farm and activity in part due to lack of comfort wearing them, particularly for face masks. Type N-95 respirators are considered the standard for preventing exposure to viral aerosols since 95% of airborne particles are captured in the respirator. However, a tight fit of the respirators to the face is necessary for particles to be removed and not by-pass the seal. Unfortunately, N-95 respirators are difficult to keep in place when performing

common farm chores and environmental conditions with elevated temperatures and humidity make wearing the N-95 respirators particularly uncomfortable for people. Other masks such as dust masks and surgical masks may also be used in farms. However, there is limited information available regarding their effectiveness for protecting workers from IAV at the pig farms.

In this study, we compared levels of IAV in nasal passages of workers wearing different types of face masks and compared the results to not wearing masks. We also assessed which face mask workers considered most comfortable. We used masks commonly available in farms and followed routine procedures at the farms for wearing them. Overall, there is limited information on the protective effect of the different types of facemasks against IAV exposure in pig farms. Having information about the protective effect of different types of face masks and which face mask is more likely to be used in pig farms should help mitigate the risk of IAV transmission between pigs and workers.

### **Objectives:**

Our overall goal is to prevent the transmission of influenza A viruses (IAV) between pigs and people and more specifically, to validate intervention strategies directed at preventing or minimizing indirect transmission through IAV-infectious aerosols. We proposed to:

Aim 1: Quantify the reduction in IAV positivity in nasal passages of swine workers wearing distinct types of face masks

Aim 2: Assess the feasibility of using face masks in swine farms

### **Materials & Methods:**

Protocols and procedures followed during the study were approved by the University of Minnesota Institutional Animal Care and Use Committee (protocol number 2110-39620A), the Institutional Biosafety Committee (protocol number 2108-39310H) and the Institutional Review Board (protocol number STUDY00017912). Participants volunteered to participate in the study and were given the option to withdraw from the study at any time.

#### *Experimental design*

##### *Participant recruitment and eligibility criteria*

Swine workers, age 21 or older and employed for a minimum of 30 hours per week, were recruited from five known or suspected IAV-positive farms in the Midwest between March 2023 and April 2024. Herds were identified through a network of veterinarians and producers willing to facilitate participation in the study. Each farm worker participant was invited to be part of the study and accepted through written, informed consent. Participants received a small cash incentive (gift card) and could withdraw from the study at any time. Compensation was proportional to the number of days they participated in the study with an additional bonus for completing the entire sampling scheme.

Additionally, participants were asked to complete questionnaires to gather demographic data and background information on their seasonal vaccination history and general attitudes toward comfort when

using the various face masks. Participant also reported their activities performed and in what area of the farm the activities were performed.

#### *Face masks and allocation of treatments*

Swine workers were instructed on the use of each of the face mask types. Instructions were shared in writing as part of a package the enrolled participants received that contained the various face masks and general instructions for the project. Additionally, the instructions were demonstrated visually during the enrollment process. Instructions included how to: wear the different face masks; pull and put on the straps; adjust the nosepiece; fit to cover the nose, mouth and chin; and ensure comfortable breathing and talking while wearing the face masks. However, workers were not specifically fit tested or subjected to respiratory fit-testing for any of the masks so that the use of the masks could reflect how they are commonly worn in the farms. There were three types of face masks evaluated: a) N-95 respirators, which were considered of reference to decrease IAV exposure in farm settings, b) dust masks, which were available in most farms and likely preferred due to comfort and perceived benefit against particulate matter in general, c) surgical masks, which were easier to wear and became more popular as a result of COVID-19, and often perceived as “good enough.” There were four total treatments evaluated which consisted of using one of the three masks (i.e N-95, dust mask, surgical mask), or no face mask, which was considered the control.

Workers were asked to participate in one of the treatments for at least 2 h a day for 5 days. The allocation of the treatments was done systematically by the researchers, where workers used a different treatment each day, and each day only one type of treatment was followed by the worker. Treatments were labeled sequentially to follow a set schedule. This experimental design resulted in 5 samples per participant for each of the treatments evaluated and the participants stayed in the study for a total of 20 sampling days.

#### *Nasal swab sampling*

Each participant was provided with a sampling kit containing an individually labeled package with the treatments (masks), swabs, and vials with viral transport media, and the sampling schedule with the order of treatments. Each mask was placed in an individual bag and for the days when the treatment was no mask, a bag without a mask was provided.

Swine workers were asked to self-collect a nasal swab before the treatments at the beginning of the work day, i.e., before entering the swine barns to perform the chores. After nasal swab self-collection, workers followed the treatments assigned to them for that day. After that, workers removed the face masks and self-collected another nasal swab at the completion of at least 2 hours. For the days when workers did not wear a mask, they also collected a nasal swab after 2 hours of working in the barns.

Standard human nasal swabbing methodologies were utilized (Ma et al., 2007). Study personnel received instructions, in both English and Spanish, from the researchers to self-collect the nasal swabs. Instructions were provided in face-to-face meetings during enrollment, in writing, and on a recorded video. Briefly, the procedure consisted of inserting the same swab one-inch into each nostril and rotating it twice against the inner surface of the nasal cavity. Each sample was collected using a mid-turbinate flocked swab (Copan Diagnostics Inc., Murrieta, CA) that has been previously used successfully in self-testing protocols (McGolrick et al., 2016). After collection, the swab was placed in a vial containing universal viral transport (UVT) media (Becton Dickinson & Co., Sparks, MD). The vial was placed in a Ziploc bag, then inside an envelope. The swabs were kept refrigerated until shipping by courier in insulated transport containers to the Torremorell Laboratory at the University of Minnesota.

### *IAV and RNaseP RT-qPCR*

RNA was extracted from nasal swabs 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). Influenza A virus matrix gene and human RNaseP gene were detected using a modified multiplex RT-qPCR developed and used by the CDC (Shu et al., 2021). Samples with cycle threshold (Ct) values <40 for IAV matrix gene were considered positive. Samples with Ct >37 for RNaseP gene were considered degraded and excluded from the analysis.

### *Data analysis*

Data collected from the questionnaires and RT-qPCR results were merged in a spreadsheet (Microsoft Excel, Microsoft Corporation, Redmond, Washington, USA) and organized for analysis. Differences between treatments were compared using the pairwise Wilcoxon test with correction for multiple comparisons to evaluate treatment differences before wearing the masks and working with the animals, and treatment differences after working with the animals. Differences were evaluated for the proportion of participants that tested positive and for the average RT-PCR Ct values. Data was analyzed using R-studio version 4.2.2.

## **Results:**

### *Demographic and baseline information*

A total of 48 participants from five farms were first enrolled in the study. From these, 14 participants from one farm and 6 participants from another farm were dropped from the study prior to initiate sampling because the farms either tested IAV negative or were depopulated for reasons unrelated to the study. Of the remaining 28 participants, four stopped participation and of the remaining 24, 21 completed all the sampling and three completed the majority of the sampling and remained in the study.

For the 24 participants that remained in the study, 12 were male, 10 female and two did not answer; four participants were white, 16 Hispanic/LatinX and two did not answer; and six were less than 30 years of age and 18 were 30 or more years old. Eight participants reported that they received seasonal influenza vaccine the year of the study or the year before. Of those that reported not being vaccinated, only two reported intent to vaccinate.

### *Swine farm workers IAV RT-qPCR results*

A total of 915 nasal swabs were submitted for testing; however, only 824 met the RNaseP RT-qPCR positive-inclusion criteria for the study. From these, 79 out of 824 (9.59%) tested IAV RT-qPCR positive. For samples collected before wearing the masks and working with the animals, a total of 28 out of 412 (6.80%) tested positive (Table 1). The number of positives among treatments ranged from 5.77% to 7.84% and there were no significant differences between treatments in terms of number of workers testing positive (Table 1) or in the Ct values of the positives ( $p > 0.05$ ) (Figure 1).

For samples collected after having worked with the animals, 24.51% (25 out of 102) of samples from individuals not wearing masks tested positive. For individuals that wore masks while working with the animals, 5.77% (6/104) tested positive when wearing the N-95 mask, 8.65% (9/104) for the dust mask, and 10.78% (11/102) for the surgical mask (Table 1). There were statistically significant differences between not wearing a mask (no mask) and wearing masks; in particular, workers not wearing masks

testing positive more often ( $p < 0.01$ ). In addition, the no mask group had Ct values significantly lower than any of the other groups which is indicative of more IAV RNA in these workers' nasal passages (Figure 2). In contrast, there were no differences for the different types of masks regarding the number of positive workers nor their Ct values ( $p > 0.05$ ). Pair-wise comparisons and their p-values are shown in Figure 3.

#### *Farm work area associations and face masks comfort scores*

We asked participants where in the farm they had worked while they were following the various treatments. Participants performed chores in the gilt development unit, breeding, gestation or farrowing. Most of the IAV positives were detected in participants working in the farrowing area with 63.83% positives (46 out of 51) (Table 2) while the rest (3 out of 51) worked in the gestation, breeding, or GDU. Activities taking place in the farrowing area included activities such as piglet processing, vaccination of piglets and sows, weaning of piglets, sow movement and power washing. We were not able to make specific associations for each of the activities and face mask use because workers reported being involved in multiple activities during the same day.

To assess the comfort for each treatment, we asked participants to report on a scale of 0 to 5 how comfortable wearing the mask was for that day with 0 being least comfortable and 5 most comfortable. Comfort scores for wearing masks were higher for the surgical masks (4.01 out of 5) followed by N-95 masks (3.07) and dust masks (3.01). Score for not wearing a face mask was 4.79 (Table 3).

#### **Discussion:**

In order to recommend intervention strategies to decrease the risk of bidirectional transmission of IAV-laden aerosols between pigs and humans, we investigated the use of face masks in swine workers. We evaluated three face masks - N-95 respirators, dust masks and surgical masks - and compared the results to not wearing a face mask. **Our results indicated that using face masks decreased the detection of IAV in the nasal passages of workers working with IAV-infected pigs.**

Our results also indicated that there were no statistical differences between masks although a numerical preference was observed for N-95 masks. Results from research regarding mask use for workers in human health care settings or in public are in general agreement with ours (Long et al 2020; Chen et al 2022). Typically, wearers of N-95 respirators need to be fit-tested to ensure a tight fit over the nose and mouth, but this is not commonly done with swine workers. For the purpose of this study, we provided general instructions on how to wear the masks and asked participants to wear them to the best of their abilities or as they would commonly do it so that the study represented actual farm conditions. Chores and environmental conditions at the farms also represent barriers to comfortably wear the masks which may contribute to the lack of differences between masks. Lack of differences between masks could also be due to sample size since several participants could not participate for reasons outside the study. Even with these possible limitations, we observed a significant difference between wearing masks and not wearing a mask. Specifically, workers wearing masks had significantly less IAV detected in their nasal passages than those who were not wearing masks. **Therefore, our results support the use of masks to reduce the risk of becoming IAV infected when working with pigs and decrease transmission of IAV between pigs and people.**

Responses to the surveys indicated that swine workers found surgical masks to be more comfortable than N-95 respirators or dust masks although the most comfort was reported when not wearing masks, which was not surprising. These results can be helpful in designing protocols to increase compliance in face mask use.

After all, use of PPE varies significantly among farms and having protocols that consider worker preference, although not perfect, may improve overall protection of workers.

It is important to note that a small percentage of workers tested positive to IAV by RT-PCR at the onset of the day, i.e., before beginning their daily work with pigs. Similar results have been reported previously (Lopez-Moreno et al., 2022) and are indicative of residual viral deposition in the nasal passages of workers after inhaling IAV-laden aerosols. IAV can be found in the air of infected farms and associated to particles of multiple size ranges capable to deposit in the respiratory tract of workers (Alonso et al., 2015). The IAV RNA, aka genetic material, detected in this study was most likely non-replicating although this cannot be confirmed since assays to determine virus infectivity or viability were not performed.

Our results also highlight that activities taking place in farrowing rooms, such as piglet processing, vaccination and weaning, represent a higher risk for swine workers to test IAV positive. Piglets prior to weaning are an important reservoir of IAV infections in endemically infected breeding herds (Diaz et al., 2015) and activities performed around weaning age have been reported to result in higher contamination rates of hands and clothes of workers (Lopez-Moreno et al., 2023). Therefore, results from this study support the use of face masks when performing activities taking place in piglets in the farrowing room.

In summary, our results add to the evidence that IAV exposure through aerosols is common in swine farms and supports the use of face masks as an intervention strategy to decrease the risk of IAV transmission to workers and swine. Understanding which face mask is more comfortable to swine workers should increase compliance and the likelihood of wearing masks. Understanding which stage of production is more likely to significantly result in IAV exposure to workers is helpful to target mask-use protocols during higher IAV risk periods. Collectively, results from this study should help design more effective interventions to prevent IAV transmission at the human-animal interface.

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## Tables and Figures

Table 1. Influenza A virus RT-qPCR positive results of farm workers before and after working with swine by treatment.

|                           | No mask                          | N-95                           | Dust mask                      | Surgical mask                    | Total                           |
|---------------------------|----------------------------------|--------------------------------|--------------------------------|----------------------------------|---------------------------------|
| Before working with swine | 8/102<br>(7.84 %)* <sup>a</sup>  | 7/104<br>(6.73 %) <sup>a</sup> | 6/104<br>(5.77 %) <sup>a</sup> | 7/102<br>(6.86 %) <sup>a</sup>   | 28/412<br>(6.80%)               |
| After working with swine  | 25/102<br>(24.51 %) <sup>a</sup> | 6/104<br>(5.77 %) <sup>b</sup> | 9/104<br>(8.65 %) <sup>b</sup> | 11/102<br>(10.78 %) <sup>b</sup> | 51/412<br>(12.38%) <sup>b</sup> |

\*No.positive/total samples (% of positives)

a,b Differences in superscripts within horizontal rows represent statistical differences at  $p < 0.05$ .

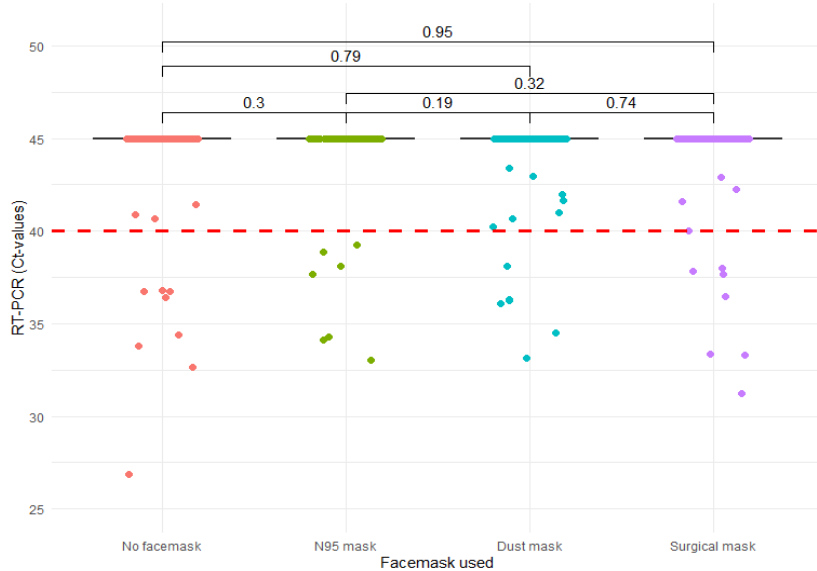
Table 2. Summary of questionnaire responses of workers using face mask by work area

| Work area              | No. responses | No. Positives (%) |
|------------------------|---------------|-------------------|
| GDU/Breeding/Gestation | 135           | 3 (2.22%)         |
| Farrowing              | 263           | 46 (63.83%)       |
| N/A                    | 14            | 2 (3.39%)         |
| Total                  | 412           | 51 (12.38%)       |

Table 3. Summary of questionnaire responses rating the comfort each face mask worn

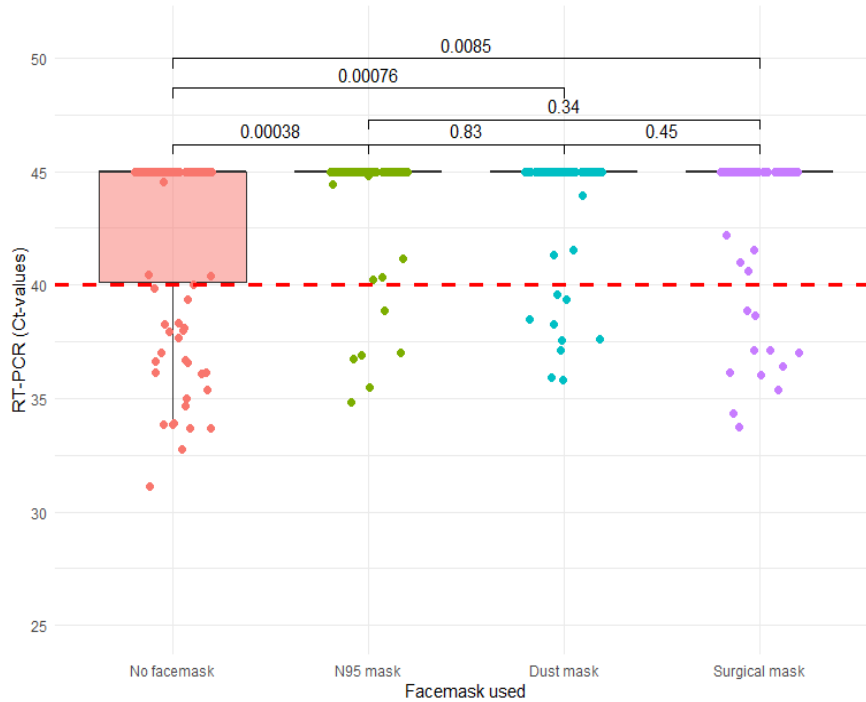
| Facemask      | Score | No. responses | %      | Average score |
|---------------|-------|---------------|--------|---------------|
| No facemask   | 0     | 0             | 0.000  | 4.79          |
|               | 1     | 0             | 0.000  |               |
|               | 2     | 0             | 0.000  |               |
|               | 3     | 6             | 5.882  |               |
|               | 4     | 3             | 2.941  |               |
|               | 5     | 63            | 61.765 |               |
|               | N/A   | 30            | 29.412 |               |
| Total         |       | 102           | 24.757 |               |
| N95 mask      | 0     | 3             | 2.885  | 3.07          |
|               | 1     | 14            | 13.462 |               |
|               | 2     | 11            | 10.577 |               |
|               | 3     | 31            | 29.808 |               |
|               | 4     | 17            | 16.346 |               |
|               | 5     | 19            | 18.269 |               |
|               | N/A   | 9             | 8.654  |               |
| Total         |       | 104           | 25.243 |               |
| Dust mask     | 0     | 1             | 0.962  | 3.01          |
|               | 1     | 14            | 13.462 |               |
|               | 2     | 13            | 12.500 |               |
|               | 3     | 42            | 40.385 |               |
|               | 4     | 19            | 18.269 |               |
|               | 5     | 13            | 12.500 |               |
|               | N/A   | 2             | 1.923  |               |
| Total         |       | 104           | 25.243 |               |
| Surgical mask | 0     | 1             | 0.980  | 4.01          |
|               | 1     | 1             | 0.980  |               |
|               | 2     | 1             | 0.980  |               |
|               | 3     | 21            | 20.588 |               |
|               | 4     | 36            | 35.294 |               |
|               | 5     | 31            | 30.392 |               |
|               | N/A   | 11            | 10.784 |               |
| Total         |       | 102           | 24.757 |               |

Figure 1. Summary plot of pair-wise Wilcoxon test comparisons of RT-PCR cycle threshold values of samples collected before wearing masks and before working with pigs, by treatment.



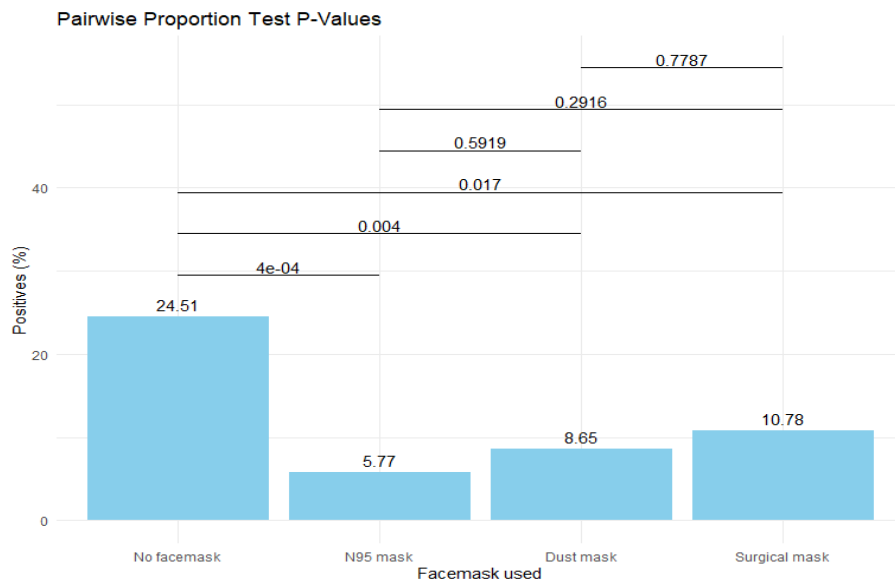
\*The number listed corresponds to the p-value of the comparison between treatments.

Figure 2. Summary plot of pair-wise Wilcoxon test comparisons of RT-PCR cycle threshold values (Ct) of samples collected after wearing the masks and having worked with pigs, by treatment.



\*The number listed corresponds to the p-value of the comparison between treatments

Figure 3. Summary plot of pairwise test comparisons of proportions of positive samples for each of the treatments after wearing the masks.



\*The number listed corresponds to the p-value of the comparison between treatments