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Methicillin-resistant Staphylococcus aureus (MRSA) in midwestern swine herds and swine workers

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METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN MIDWESTERN SWINE HERDS AND SWINE WORKERS

by

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CERTIFICATE OF APPROVAL

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MASTER'S THESIS

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This is to certify that the Master's thesis of

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has been approved by the Examining Committee
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To Barb, Kristine and Brandon, without your support, sense of humor, and understanding this would never have been completed

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ABSTRACT

Background

Recent research in the Netherlands and Canada demonstrated that swine and swine workers are colonized with methicillin-resistant *Staphylococcus aureus* (MRSA). One study in the U.S. demonstrated that the swine and swine workers on one of two farms sampled were colonized with MRSA.

Specific Aims

The specific aim of this thesis is to present a cross-sectional study of the prevalence of MRSA in swine and swine workers in multiple pork production facilities in Iowa and Illinois.

Methods

The investigators sampled the nares of nursery aged swine and the swine workers associated with 17 farms in Iowa and Illinois. A total of 404 swine and 86 workers were sampled. Both human and swine isolates were selected for molecular typing utilizing multi locus sequence typing (MLST) and the *spa* typing method.

The investigators created a brief questionnaire for swine workers about possible risk factors for carriage of MRSA. The questionnaire collected demographic data, data on potential risk factors for MRSA infection, information about contact with swine, and information on the use of personal protective equipment.

Results

MRSA was found in swine on 4 of 17 (23.53%) farms, and in swine workers on 4 of 17 (23.53%) farms. Overall prevalence in swine was 11.13% (45/404) and 31.39% (27/86) in workers. As in the Dutch and Canadian studies, ST398 was the most common strain of MRSA identified.

The primary risk factor for MRSA colonization of swine workers was working on a farm with MRSA positive pigs (odds ratio [OR] 14.4; 95% confidence interval [CI] 3.1-66.2). Workers who did not use personal protective equipment (e.g., glasses or goggles) had a significantly higher risk of carrying MRSA than workers who used personal protective equipment (OR 3.5; 95% CI 1.4-9.0). Workers who were exposed to an average of greater than 2400 swine per day had a higher risk than those who were exposed to fewer swine (OR 5.6; 95% CI 1.7-17.8).

Conclusions

This study demonstrated that some swine and their caretakers in Iowa and Illinois carry MRSA, including ST398, the livestock-associated strain first identified in the Netherlands. It demonstrated that both the swine workers and their pigs can carry identical strains of MRSA. However, to date, workers on these farms have not reported having MRSA infections.

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CHAPTER I

INTRODUCTION

In 1880 the surgeon, Sir Alexander Ogston, discovered a bacterium in pus from surgical abscesses. In 1884, Friedrich Rosenbach named two distinctly different colored colonies, one yellow and one white, *Staphylococcus aureus* and *Staphylococcus albus*. Since then *Staphylococcus aureus* has been commonly noted to occur as a commensal organism, one that exists without apparent harmful effects, on the skin or in the nasal passages of healthy humans and animals. Under certain conditions, such as stress or injury to skin surfaces, even a commensal organism may opportunistically be capable of causing infection. Clinical signs of infection in humans may range from minor skin infections (pimples, boils and impetigo), to much more serious conditions such as cellulitis, and post-operative wound infections. *S. aureus* may also cause bacteremia (bacterial infection of the blood stream), sepsis, meningitis and pneumonia.[1] Approximately 1/3 of the human population is colonized by strains of *S. aureus.*[2] Colonization of the skin or nasal cavity is not an infection and therefore, usually does not require treatment. In addition to colonizing humans, the organism can colonize many other domesticated and wild species of animals, including dogs, cats, pet birds, horses, pigs, cattle and chickens.[3] Antibiotics have been the first line of defense in treating clinical infection in both man and animals. Bacteria that are resistant to antibiotic treatment are a public health concern.

Specific Aims

The specific aim of this thesis is to present a cross-sectional study of the prevalence of methicillin-resistant *Staphylococcus aureus* in swine and swine workers in pork production facilities in Iowa and Illinois.

CHAPTER II BACKGROUND

What is Methicillin-resistant *Staphylococcus aureus* (MRSA)?

Soon after antibiotics were introduced in the 1940's, it was noted that some organisms, *S. aureus* being one of many, were becoming resistant to antibiotics such as penicillin. The resistant bacteria produced penicillinase, an enzyme that breaks down penicillin, rendering it ineffective. Methicillin, which was released in 1959, was the first antibiotic developed to resist penicillinase. By 1961 the first case of MRSA infection was reported in England. [4] Since that time, MRSA has become a worldwide problem in both human and veterinary medicine. A recent study by Klevens et al. showed that deaths from MRSA infections in the U.S. have eclipsed the number of deaths caused by HIV/AIDS on an annual basis. These investigators estimated that MRSA caused 94,000 invasive infections and over 18,000 deaths in 2005.[5] Gowitz, et al estimated that 1.5% of the U.S. population is colonized by MRSA.[6]

Transmission

Humans can acquire MRSA by contact with pus from an infected wound, by skin-to-skin contact with an infected person, or by contact with contaminated fomites, such as towels, clothing, or athletic equipment used by an infected person. For many years, MRSA was considered primarily a human pathogen, but the report of a MRSA infected dairy cow in 1972 altered that perception.[7] Investigators initially thought that the primary route of MRSA transmission between humans and animals was solely from humans to animals. This was supported by the fact that a majority of MRSA infections found in cats, dogs, pet birds and horses were caused by human strains.[8] In contrast, the most predominant strains in food animals (pigs, cattle) tended to be animal in origin.

However, a recent study demonstrated that MRSA can be transmitted in both directions, from human to animal and animal to human.[9] Once exposed to MRSA, animals can become colonized, and may serve as reservoirs from which MRSA can be transmitted to other animals and to their human handlers. A study by Sequin, et al, found that animal caretakers and veterinary personnel that contact MRSA-infected animals may become colonized by MRSA.[10] These colonized humans subsequently may transmit MRSA infection further to susceptible humans or animals.[11] Factors involved in the transmission of MRSA include: crowding, compromised skin (scratches and abrasions), contaminated items (fomites) or surfaces, and poor hygiene. [12] These factors apply to humans and animals alike. In particular, people who handle animals in their work or who have animals as pets in their homes have very close physical contact with these animals, which may facilitate transmission of MRSA. Transmission between humans and their animal contacts may be facilitated by their contaminated shared surroundings.[13]

Swine Workers and MRSA

Recently, Dutch investigators identified pigs as an important reservoir of a specific type of MRSA (ST398) which may be an emerging occupational hazard. In 2004, Voss et al. reported three infections among humans living in the Netherlands --the daughter of a pig farmer, the son of a veterinarian, and another pig farmer--caused by an unknown type of MRSA.[14] The investigators found the same "unknown" type of MRSA in the pigs of both owners. A broader survey of area pig owners subsequently found that pig owners had a 760 times greater chance of carrying MRSA than the general, non-swine exposed public. Since that initial study, studies done in France[15], The

Netherlands[16], and Canada[17] have also identified higher than normal carriage of MRSA in pig farmers. The Dutch Institute for Public Health and the Environment (RIVM) maintains a registry of MRSA isolates. Prior to 2002, no NT-MRSA ("non-typable" MRSA) isolates were registered. From 2004 to 2006 about 2% of all MRSA infections found in humans belonged to the NT-MRSA type, with the prevalence increasing to 5% in the first half of 2006, and nearly 20% in 2008. These unknown or non-typable MRSA strains have since been evaluated with other typing methods, such as MLST(multi-locus sequence typing),which distinguished the strain from other MRSA strains. The new strain is now known as ST398, a type associated with livestock, swine in particular. These molecular typing methods have allowed investigators to determine that the pig isolates and the isolates from the pig owners are identical. In fact, ST398 is the only clone in Europe that has been associated with swine. In Canada, ST398 was the predominant strain among swine, and it is also the predominant strain in the U.S.

The View From The Swine Industry

The swine industry has had little motivation to study *S. aureus* in general, or MRSA, in particular because: *S. aureus* is not a significant pathogen in swine and very few documented MRSA infections in swine workers had been reported prior to the work done in the Netherlands. Since *S. aureus* is not a major pathogen of swine there is little economic impact on swine production and thus little economic impact on the industry. In addition, only a few of the human infections caused by this clone in Europe have been severe, and only one infection has been fatal. In the U.S, only a handful of documented human infections have been caused by this clone.

In contrast to the swine industry, some researchers think that ST398 has significant implications for the swine industry. P. van der Wolf concluded in a paper presented at the Iowa State University Swine Disease Conference in 2007 that, "a new type of MRSA has established itself in livestock throughout Europe and other parts of the world forming a reservoir of infection for humans who are in close contact to these animals. This establishment has large consequences for the livestock industry and the people working in that industry. Interventions are largely unknown and research is ongoing."[18] Thomas Blaha concluded that, "Although there is no acute threat to human health due to MRSA ST398 (no steep increase of human cases reported) it is advisable to watch the occurrence and epidemiology of the pig-associated MRSA clonal line ST398 closely. Humans occupationally exposed to pigs should be educated about the potential risk for themselves and about their potential of carrying MRSA into a hospital."[19]

Prior Research

Dr. Tara Smith and her colleagues from the University of Iowa College of Public Health and the University of Iowa College of Medicine were the first to publish data documenting the presence of MLST-type 398 in a swine herd in the U.S. In this mid-western herd, the prevalence of MRSA in pigs ranged from 36% in the sows, up to 100% in nursery aged piglets.(see Figure 1.) Moreover, 64% of the swine workers associated with this herd also carried MRSA.[20] To date, no workers in this system have reported any infections caused by MRSA.

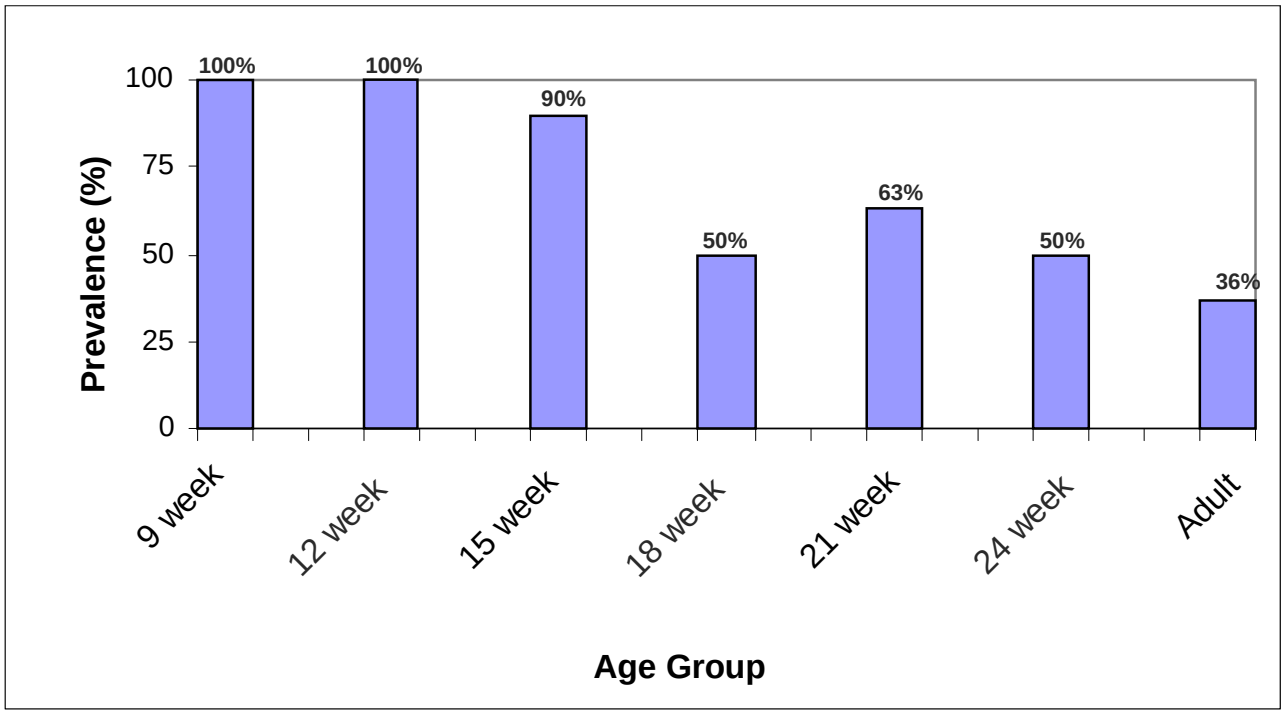

Figure 1 Prevalence of MRSA in swine from Farm1 by age group

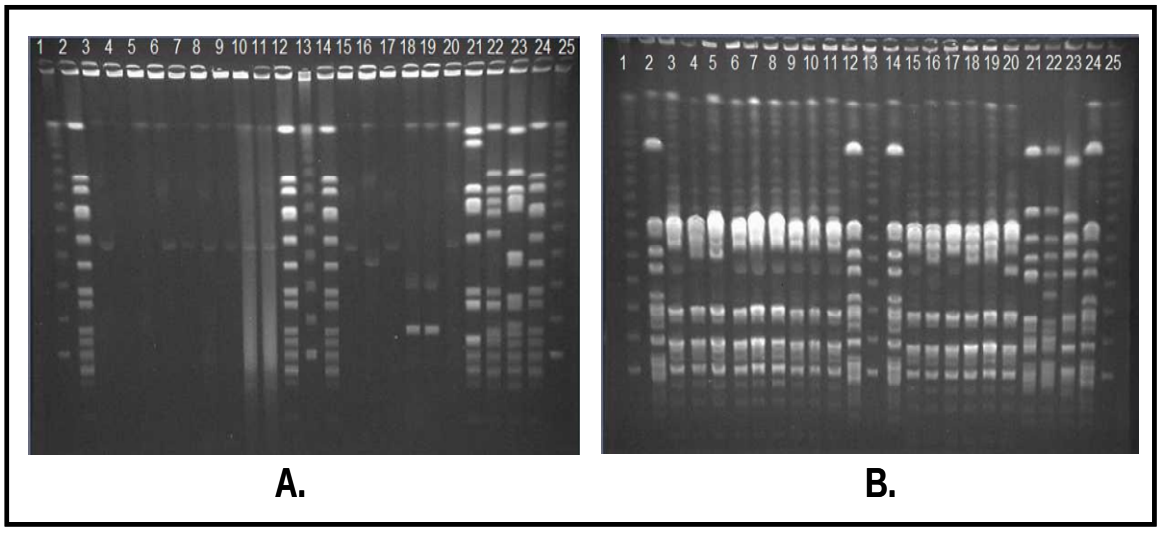

Figure 2 A: PFGE of MRSA isolates from swine; DNA digested with SmaI. B: PFGE of MRSA isolates from swine; DNA digested with EagI. Lanes 3-11, 15-20: DNA from swine isolates. Lanes 1, 13, 25: molecular weight ladder. Lanes 2, 12, 14, 24: NCTC 8325 (control strain). Lanes 21-23: USA 100, USA 300, USA 400, respectively.

CHAPTER III

METHODS

National Pork Board Sponsored Study of Swine Farms in the Midwest

The results from the initial study suggested that a larger study, encompassing more swine production systems should be done to determine more accurately the prevalence of MRSA in U.S. swine herds With funding from the Nation Pork Board, investigators from the University of Iowa College of Public Health, the University of Minnesota, and The Ohio State University did a collaborative study to assess swine production systems from Iowa, Illinois, Minnesota, Ohio, and North Carolina for the prevalence of MRSA in both the pigs and swine workers. The University of Iowa College of Public Health sampled herds from Iowa and Illinois. In addition to culturing swine and humans, the investigators created a brief questionnaire for swine workers about possible risk factors for carriage of MRSA. (see Appendix A) The questionnaire collected demographic data, data on potential risk factors for MRSA infection, information about contact with swine, and information on the use of personal protective equipment.

Farm Selection

To maximize the generalizability of the results from this study to the swine industry in Iowa and Illinois as a whole, one would perform a randomized selection of farms based on type, location, size and production segment. However, modern swine production systems, with high animal densities and variable health status between sites even within the same production system, need a high level of biosecurity. This

minimizes the possible spread of disease, but prevents free movement between sites and herds. In addition, studies of modern production systems have at times led to negative stories in the press. Thus, it is difficult to obtain permission from owners and managers to study the swine and swine workers on specific premises. Therefore, the investigators selected a convenient sample of farms based on existing veterinary relationships and through contact with various marketing cooperatives. No prior knowledge of MRSA risk of infection existed. Farms were divided into two main categories: large commercial production units producing more than 10,000 pigs per year, and smaller units producing fewer than 10,000 pigs per year. Specific inclusion criteria were: located in Iowa or Illinois, and swine population on site greater than 500 pigs (all ages included. Efforts were made to ensure that multiple farms were not selected from within any one production pyramid (i.e., no two farms have related swine). Both conventional (8) and antibiotic-free (9)farms were included in this study.

On Farm Sample Collection and Bacterial Isolation

On each farm, samples were obtained on a voluntary basis from swine workers and from animals in the nursery phase of production (animals from 6-9 weeks of age). One naris from each animal was swabbed using the BBL CultureSwab Collection and Transportation System, which contain Stuart's medium for transportation back to the laboratory. Each swine worker, who volunteered to participate in the study, had samples of both nares and a separate pharyngeal swab taken with sterile swabs as described above. Inclusion criteria for swine workers were: age 18-65, not pregnant, and employed by the swine farm. Swine worker exclusion criteria were: the existence of any immune-compromising condition, and/or current antibiotic use. Samples were kept refrigerated

and transported to the laboratory. Cultures were done as described by Khanna et al. in Canada.[21]Samples were inoculated into 2mL enrichment broth containing 10g tryptone/L, 75g NaCl/L, 10g mannitol/L and 2.5g yeast extract/L. After 24h incubation at 35°C, a loopful of broth was inoculated onto selective MRSA agar plates (BBL CHROMagar MRSA, Becton, Dickinson and Company). These plates were incubated 24-48 hours at 35ºC and examined for MRSA. Isolates were confirmed to be *S. aureus* by examining their appearance on Gram stain, and by applying the catalase test, the tube coagulase test and the *S. aureus* latex agglutination assay (Pastorex Staph-plus, Bio-Rad). Methicillin resistance was confirmed by testing for the presence of penicillin binding protein 2 (PBP2') (MRSA latex agglutination test, Oxoid Ltd., Hants, UK). MRSA isolates were stored at -80°C.

The Institutional Review Board (IRB) and the Institutional Animal Care And Use Committee (IACUC) approved the protocols. All human participants gave written informed consent prior to enrollment.

Molecular testing

Both human and swine isolates were selected for molecular typing. Pulsed field gel electrophoresis (PFGE) was performed as previously described.[22] Isolates that were non-typable after *Sma*I digestion were examined after digestion with *Eag*I. Isolates from this study were compared with the type strains for USA100, USA300, and USA400.[23] Polymerase chain reaction (PCR) assays were used for SCC*mec* typing and to detect *pvl.* Genomic DNA was extracted using the Wizard Genomic DNA preparation kit (Promega). Amplification was performed as previously described.[24] The multiplex PCR included seven primer sets: CIF2 F2, CIF2 R2, MECI P2, MECI P3, RIF5 F10, RIF5

R13, DCS F2, DCS R1, MECA P4 MECA P7, KDP F1, KDP R1, RIF4 F3, and RIF4 R9. The presence of *pvl* was determined by PCR.[25] Multi locus sequence typing (MLST) was performed on a subset of isolates that were identical by PFGE and the results were analyzed as previously described.[26] All molecular procedures included known positive and negative controls.

The *spa* typing method was also utilized. This is a method based on sequencing of the polymorphic X region of the protein A gene (*spa*), which is present in all strains of *S. aureus*. This method is further facilitated by the establishment of standardized *spa* type nomenclature and Internet shared databases.

Antimicrobial susceptibility testing

A sample of human and swine *S. aureus* isolates were tested for antimicrobial susceptibility by the broth dilution method described by the Clinical and Laboratory Standards Institute. [27] Isolates were tested for susceptibility to penicillin, oxacillin, tetracycline, erythromycin, clindamycin, trimethoprim-sulfamethoxazole, quinupristin/dalfopristin, gentamicin, levofloxacin, moxifloxacin, linezolid, daptomycin, vancomycin, and rifampin.

Survey/data analysis

Data from the questionnaire survey and culture results were linked by unique specimen number. Each individual that filled out the survey and was sampled for culture received a unique number. This number was placed on the survey and each sample. This number linked that individual, the farm, the samples, and the survey. Initially, potential risk factor associations were assessed with Fisher's exact test. Bivariable and multivariable modeling of risk factors were performed by exact logistic regression.

Multivariable modeling was performed using manual backward elimination from the initial saturated model. A trend in prevalence of MRSA in swine by age group was tested with the Cochran-Armitage trend test. The significance level was set at 0.05. Analyses were performed using SAS software version 9.1 (SAS Institute Inc., Cary, NC).

CHAPTER IV

RESULTS

MRSA Prevalence

Swine on 4 of 17 (23.53%) farms in the study carried MRSA. The prevalence of MRSA in the swine ranged from 16.67% to 100.0% on these four farms. The total number of swine sampled (all farms) was 404, with 45 samples positive for MRSA (11.13%). MRSA isolates were not obtained from the swine sampled on 13 farms. MRSA sample were obtained from swine on 4 of 8 conventional farms, and no MRSA isolates were obtained from swine on the nine farms participating in antibiotic-free marketing programs.

Similarly, swine workers from 4 of the 17 farms (23.53%) carried MRSA. The total number of swine workers sampled was 86, with 27 samples positive for MRSA (31.39%). The prevalence of MRSA carriage among workers ranged from 10.0% to 75.0% on the 4 farms with positive workers. None of the workers that were sampled on the other 13 farms carried MRSA. Of note, three farms had both swine and swine workers who carried MRSA. But one farm had colonized swine and no colonized workers and one farm had colonized workers but no colonized swine. (See Table 1).

Molecular Typing

Twenty-seven human isolates and 40 swine isolates were selected for molecular typing (See Table 2) . For Farms 1, 3, and 9, all viable isolates were sent for typing. From Farm 1, one swine isolate was not viable after storage and transport, and two human isolates were contaminated after storage and were not analyzed further. From Farm 3, one swine isolate was not viable following storage. From Farm 6, three of the

four swine isolates were selected randomly for typing. From Farm 15, the single human isolate was typed and 4 of 6 swine isolates were selected randomly.

Farm 1: Thirteen MRSA isolates from humans and 23 from swine were typed, most of which were subtypes of ST398. Eleven (84.6%) of the human isolates were ST398(t034), 1 (7.7%) was ST398(t011), and 1(7.7%) was unknown in the Ridom software. Twenty-two of the 23 (95.6%)swine isolates were ST398(t034), and 1(4.4%) was unknown in the Ridom software.

Farm 3: Eleven MRSA isolates from humans and 10 from swine were typed. The MRSA on this farm were more diverse than on other farms. Four of the 11(36.3%) human isolates were ST398(t034), 6(54.5%) were unknown, and 1(9.2%) was t330. Three of the 10(30.0%) swine isolates were ST398(t034), 1(10.0%) was ST398(t571), 1(10.0%) was ST9(t337), which is also a swine-associated type, 3 (30.0%)were New/t3446, and 2(20.0%) were unknown.

Farm 6: Three isolates from swine were typed and all were ST398(t034). None of the samples from humans working on this farm grew MRSA.

Farm 9: Two MRSA isolates were obtained from humans and none were obtained from swine. One(50.0%) of the isolates from humans working on this farm was ST398(t034). The person colonized with this organism indicated that he was a driver. Therefore, he may have had contact with pigs from other farms. The other isolate from a worker on this farm was a common human strain (21/t084).

Farm 15: One MRSA isolate from a human and 4 MRSA isolates from swine were typed and all were ST398(t034).

Table 1 Bacterial Culture Results for 17 Farms

Farm	Production Type	Human Samples	MRSA Positive	Swine Samples	MRSA Positive
Farm 1	Conventional	28	15 (53.57%)	24	24 (100.0%)
Farm 2	Ab-free	6	0	24	0
Farm 3	Conventional	12	9 (75.0%)	24	11 (45.83%)
Farm 4	Ab-free	1	0	24	0
Farm 5	Ab-free	5	0	24	0
Farm 6	Conventional	1	0	24	4 (16.67%)
Farm 7	Conventional	1	0	24	0
Farm 8	Ab-free	5	0	24	0
Farm 9	Conventional	4	2 (50.0%)	24	0
Farm 10	Conventional	3	0	24	0
Farm 11	Ab-free	2	0	24	0
Farm 12	Ab-free	1	0	24	0
Farm 13	Conventional	2	0	24	0
Farm 14	Ab-free	2	0	24	0
Farm 15	Conventional	10	1 (10.0%)	24	6 (25.0%)
Farm 16	Ab-free	1	0	24	0
Farm 17	Ab-free	2	0	20	0
Total Samples		86	27 (31.39%)	404	45 (11.13%)
Total Farms		17	4 (23.53%)		4 (23.53%)

Antibiotic Resistance

Antimicrobial susceptibility testing (AST) was only carried out on isolates from Farm 1 due to financial constraints. Isolates chosen for AST included all human isolates also sent for molecular typing, and the first two isolates taken from each age group of swine in the previously sited study by Smith et al. The age groups were 9, 12, 15, 18, 21, 24 weeks of age and adult sows.(See Figure 1).

All isolates selected for antimicrobial susceptibility testing, both human and swine, were resistant to penicillin, oxacillin and tetracycline. All were susceptible to trimethoprim-sulfamethoxazole, gentamicin, levofloxacin, moxifloxacin, linezolin, daptomycin, vancomycin, and rifampin. Of the isolates from swine that were tested, 20% were resistant to erythromycin, 13% were resistant to quinupristin-dalfopristin, and 87% were resistant to clindamycin. None of isolates from humans were resistant to erythromycin or quinupristin-dalfopristin, but one was resistant to clindamycin.

Molecular Testing of Positive Samples

Table 2 Molecular Testing Results

Farm	Isolate ID	Spa type (x /Ridom)	Motif	MLST
1	HU 01002N.1	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
1	HU 01003	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
1	HU 01007	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
1	HU 01008N	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
1	HU 01010	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
1	HU 01012	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
1	HU 01013	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
1	HU 01015	New/t011	X1-K1-A1-O1-B1-Q1-O1	398
1	HU 01017	2/unknown	T1-J1-M1-B1-M1-D1-M1-G1-M1-K1	NT
1	HU 01023*	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
1	Hu 01024	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
1	HU 01026	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
1	HU 010217	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
1	SW 01031	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
1	SW 01032	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
1	SW 01033.2	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
1	SW 01034	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
1	SW 01043.1	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
1	SW 01044#	New/unknown	T1-J1-B3-K3-K1-K1-K1-M1-K1-NEW	NT
1	SW 01045-1	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
1	SW 01046.1	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398

Farm	Isolate ID	Spa type (x /Ridom)	Motif	MLST
1	SW 01047	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
1	SW 01048	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
1	SW 01049	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
1	SW 01050	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
1	SW 01051	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
1	SW 01052	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
1	SW 01053	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
1	SW 01055	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
1	SW 01056	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
1	SW 01057	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
1	SW 01058	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
1	SW 01059	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
1	SW 01060	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
1	SW 010121	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
1	SW 010143	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
3	HU 03001	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
3	HU 03002	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
3	HU 03003T	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
3	HU 03004	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
3	HU 03005	176/unknown	T1-J1-M1-B1-M1-K1	NT
3	HU 03007	2/unknown	T1-J1-M1-B1-M1-D1-M1-G1-M1-K1	NT
3	HU 03008	2/unknown	T1-J1-M1-B1-M1-D1-M1-G1-M1-K1	NT
3	HU 03009N	203**/unknown	T1-J1-M1-B1-M1-D1-M1-G1-G1-K1	NT
3	H 03010T	136/t330	A2-A1-K1-B1-B1-M1-B1-K1-B1	NT

Farm	Isolate ID	Spa type (x /Ridom)	Motif	MLST
3	HU 03011	2/unknown	T1-J1-M1-B1-M1-D1-M1-G1-M1-K1	NT
3	HU 03012	2/unknown	T1-J1-M1-B1-M1-D1-M1-G1-M1-K1	NT
3	SW 03042	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
3	SW 03044	New/t3446	U1-J1-J1-A1-G1-J1-A1-B1	NT
3	SW 03047	New/t3446	U1-J1-J1-A1-G1-J1-A1-B1	NT
3	SW 03049	109/t571	X1-K1-A1-O1-A1-O1-B1-O1	398
3	SW 03050&	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
3	SW 03050&	New/t337	U1-K1-J1-J1-A1-G1-J1-A1-B1	9
3	SW 03056	2/unknown	T1-J1-M1-B1-M1-D1-M1-G1-M1-K1	NT
3	SW 03057	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
3	SW 03062	New/t3446	U1-J1-J1-A1-G1-J1-A1-B1	NT
3	SW 03063	2/unknown	T1-J1-M1-B1-M1-D1-M1-G1-M1-K1	NT
6	SW 0601	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
6	SW 0602	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
6	SW 0604	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
9	HU 09001 N	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
9	HU 090017	21/t084	U1-J1-G1-B1-B1-G1-G1-J1-A1-G1-J1	NT
15	HU 15009 N	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
15	SW 1501.3	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
15	SW 1505.1.2	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
15	SW 1508.2.2	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398
15	SW 15020.2	539/t034	X1-K1-A1-O1-A1-O1-B1-Q1-O1	398

Questionnaire Survey

A total of 87 swine workers from the 17 farms involved in this project volunteered to be sampled and completed a survey involving questions regarding their health history, family health history, work history, job description, exposure to pigs and pork products, and the use of personal protective equipment.

Survey results are presented in Table 3. Male swine workers predominated, with a male-to-female ratio of 3.8:1. The age groups were evenly represented. Most workers did not use tobacco products. Only 7.0% of workers reported any history of lung problems. Only 5.8% of workers reported having heart problems or chronic medical problems. No workers reported being diagnosed with clinical MRSA infection in the last 12 months.

Variables that were significantly associated with carrying MRSA were: "works with cleaning in the swine farm", "examining and treating swine", "obtaining blood or other specimens from swine", and "the average number of swine exposed to in a typical day". All four significant risk factors involved exposure to swine. However, length of employment was not significantly associated with carrying MRSA, even among respondents who had worked more than 18 years on swine farms.

Logistic regression analysis identified three independent risk factors for MRSA carriage among swine workers (See Table 4). The primary risk factor was working on a farm with MRSA positive pigs (odds ratio [OR] 14.4; 95% confidence interval [CI] 3.1-66.2). Workers who did not use personal protective equipment (e.g., glasses or goggles) had a significantly higher risk of carrying MRSA than workers who used personal protective equipment(OR 3.5; 95% CI 1.4-9.0). Workers who were exposed to an

average of greater than 2400 swine per day had a higher risk than those who were exposed to fewer swine (OR 5.6; 95% CI 1.7-17.8). In short, working with large numbers of swine, on farms with swine carriage of MRSA strains, and not wearing any personal protective equipment would present the largest risk for MRSA carriage.

Table 3 Characteristics of the Swine Worker Population and MRSA Prevalence

Variable		n	Number of MRSA positive (row %)
Gender*			
	Female	18	2 (11.1)
	Male	69	26 (37.7)
Age group			
	<29	28	11 (39.3)
	29 -44	29	6 (20.7)
	>=45	30	11 (36.7)
Tobacco			
	No	52	13 (25.0)
	Yes	35	15 (42.9)
Lung problems			
	Missing	2	0 (0.0)
	No	79	24 (30.4)
	Yes	6	4 (66.7)
Heart problems			
	Missing	1	0 (0.0)
	No	81	26 (32.1)
	Yes	5	2 (40.0)
Chronic medical problem			
	Missing	1	0 (0.0)
	No	81	26 (32.1)
	Yes	5	2 (40.0)
Respiratory illness with fever in the last 12 months			
	Missing	1	0 (0.0)
	No	61	22 (0.0)
	Yes	25	6 (0.0)
Missed work because of respiratory illness in the last 12 months			
	Missing	1	0 (0.0)
	No	78	25 (32.1)
	Yes	8	3 (37.5)
Antibiotics in the past 3 months			
	Missing	5	1 (20.0)
	No	71	25 (35.2)
	Yes	11	2 (18.2)
You or family member visited patient in the hospital in the past 12 months			
	Missing	3	0 (0.0)
	No	40	14 (35.0)
	Yes	44	14 (31.8)

Variable	n	Number of MRSA positive (row %)
You or family member visited patient in the long-term care facility in the past 12 months		
Missing	2	0 (0.0)
No	64	22 (34.4)
Yes	21	6 (28.6)
You or family member work in hospital or long-term care facility		
Missing	1	0 (0.0)
No	72	22 (30.6)
Yes	14	6 (42.9)
Diagnosed with skin of soft tissue infection in the past 12 months		
Missing	1	0 (0.0)
No	84	27 (32.1)
Yes	2	1 (50.0)
Diagnosed with MRSA in the past 12 months		
Missing	1	0 (0.0)
No	86	28 (32.6)
Yes	0	0 (0.0)
Length of employment (years)		
<=3	30	12 (40.0)
4 -18	29	8 (27.6)
>=18	28	8 (28.6)
Works with cleaning in the swine farm*		
Yes	55	12 (21.8)
No	32	16 (50.0)
Examining and treating swine*		
Yes	52	12 (23.1)
No	35	16 (45.7)
Obtains blood or other specimens from swine*		
Yes	10	0 (0.0)
No	77	28 (36.4)
Average number of swine you are exposed in a typical day*		
Missing	5	2 (40.0)
<=1000	31	6 (19.4)
1001 -2400	23	4 (17.4)
>=2400	28	16 (57.1)
Consume pork products		
Missing	4	2 (50.0)
No	4	1 (25.0)
Yes	79	25 (31.7)
Frequency that consume pork products		
Missing	5	2 (40.0)
Less than once per week	11	6 (54.6)
2-3 times per week	40	10 (25.0)
Approximately once per week	23	7 (30.4)
More than 4 times per week	8	3 (37.5)
Frequency that handles raw pork products		
Missing	8	1 (12.5)
Less than once per week	31	12 (38.7)
2-3 times per week	25	8 (32.0)
Approximately once per week	20	6 (30.0)
More than 4 times per week	3	1 (33.3)

* Significant at 95% confidence level using Chi-square or Fisher exact test

Table 4 Risk Factor Analysis with Logistic Regression

Variable		n	Unadjusted OR (95%CI)	Adjusted OR (95%CI)
Gender				
	Female	18	reference	---
	Male	69	4.8 (1-22.8)	---
Age group				
	<29	28	reference	reference
	29 -44	29	0.4 (0.1-1.3)	0.2 (0-1.1)
	>=45	30	0.9 (0.3-2.6)	2.2 (0.4-12.2)
Average number of swine you are exposed in a typical day				
	<=1000	31	reference	reference
	1001 -2400	23	0.9 (0.2-3.6)	0.5 (0.1-2.8)
	>=2400	28	**5.6 (1.7-17.8)**	**3.5 (0.7-17.6)**
In the last 12 months at work used glasses or goggles				
	Never	35	**3.5 (1.4-9)**	**5.3 (1.2-23.8)**
	Rarely, Sometimes, Most of the time or Always	52	reference	reference
Farm with positive MRSA pigs				
	Yes	54	**14.4 (3.1-66.2)**	**14.1 (2.3-87.6)**
	No	33	reference	reference

CHAPTER IV

DISCUSSION

This study has demonstrated that strains of MRSA can be found in pigs and swine workers in herds located in Iowa and Illinois, and that both can carry identical strains. Factors that increase the risk of MRSA carriage in swine workers include working with a population of swine carrying MRSA, being exposed to large numbers of swine on a daily basis, and failure to routinely use personal protective equipment. To minimize each of these risk factors one must motivate the swine workers, management and ownership to change their practices. Government guidelines or regulations may be considered to ensure that practices change.

Our survey data revealed that few swine workers use personal protective equipment. The investigator's personal experience confirms this finding. Almost every herd that the investigator has dealt with during 31years of practice as a swine veterinarian has had dust masks available in the office or break area of the farm. Farm 1 in this study, a farm that the investigator works with, provides dust masks, but the investigator has never witnessed a swine worker wearing a dust mask on this farm. The swine workers are aware that pigs on this farm carry MRSA and that a significant portion of the workers also carry MRSA. However, none of the workers wear dust masks because: "the masks are uncomfortable", "the masks are too hot in the summer", "the masks cause their eyeglasses to steam up." An anecdote illustrates how ingrained this behavior is and how difficult it will be to change practice. A 61-year-old employee of this farm was recently hospitalized with pneumonia resulting from an H1N1 influenza infection. During the course of his illness the hospital laboratory grew MRSA from his sputum. The

investigator saw him during his first week back at work and asked him why he was not wearing a dust mask, especially during his recovery from pneumonia. He said, "they are too uncomfortable." Of note, one employee of this company wears dust masks 100% of the time he spends with swine because he is allergic to endotoxin; In this study his nares did not grow MRSA.

Thus, to change practice, one will first need to change the culture at these swine farms to one that embraces dust masks or respirators as an important way workers can protect themselves from the numerous potentially toxic substances in the air on a swine farm in addition to MRSA. Moreover, workers might be more likely to wear masks that are affordable and more user friendly than they are to wear those that are currently available.

Swine workers may find it difficult to avoid exposure to large numbers of swine on a daily basis because economic challenges and technological advances have led to the development of larger and larger sized farms. Restricting farms size would be difficult, and restricting employees' exposure time would mean that owners would need to hire additional employees, raising the cost of production significantly. Restricting farm size or the number of swine to which workers are exposed would be a very hard sell. An air sampling project (unpublished) undertaken by Ferguson and Male at one of Farm 1's nursery facilities suggests that these measures might not reduce the risk of acquiring MRSA. The investigators used an N-6 Anderson Sampler to determine the presence and concentration of respirable and non-respirable MRSA in the air. They found that samples from clean and sanitized rooms sampled before the arrival of newly weaned piglets did not grow MRSA from respirable or non-respirable airborne particulates, but samples

taken from rooms after piglets had arrived grew MRSA from both respirable and non-respirable particles. These results indicate that MRSA can be collected viably from bioaerosols in confined animal feeding operations (CAFOs) if the animals are carrying MRSA. Of particular interest was the indication that viable MRSA can be deposited in the lungs after only 30 seconds of exposure in a CAFO, even in an area with a relatively small number of pigs (the nursery room housed fewer than 500 pigs, which by today's standard is a small group of pigs). Thus, the size of the population in one room or area may not be the primary risk factor for acquiring MRSA, and routine use of dust masks or respirators may be a much better intervention than trying to restrict the number of pigs to which workers are exposed.

Results of this study and other studies from across the swine producing world indicate that populations of pigs are colonized with MRSA and shedding this organism. Thus, an important question is whether MRSA can be eliminated from swine populations. The likely answer would be yes, but it would be very expensive to the herd owners. The method would involve depopulating the sow herd site and repopulating it with known MRSA negative sows. This has been accomplished over the years with several other bacterial pathogens and is very effective and very expensive. The costs include the cost of replacing maternal stock, the cost of interruption of production, and the cost of depopulating each nursery and finishing site prior to receiving the offspring from the MRSA negative sow herd. For large herds this process could cost in the millions of dollars. In addition, one would need to find breeding stock that are known to not carry MRSA. One would need to ensure that workers do not carry MRSA to prevent human-to-pig transmission. Decolonizing swine workers would add costs to this process.

Alternatively, one could attempt to decolonize the pigs, but it is likely that this would not be successful in part because food animal producers do not have access to the antibiotics required to eliminate MRSA. Moreover, the antimicrobial agents needed for decolonization would increase the cost of production and are likely to select MRSA strains that are resistant to the decolonizing agents, which would compound the problem.

This study has several strengths. This study was a novel study in that it was the first study of this size (multiple herds in multiple states) that assessed the prevalence of MRSA in both swine and swine workers in the U.S. It also compared MRSA strain types carried by swine and those carried by swine workers. This is a repeatable study. Methods used in this study are methods used in similar studies in many different countries. The sampling has been repeated and the carriage rate among swine from Farm 1 has been consistent over time. The number of farms in this study is relatively small. However, the results are generalizable to the swine industry as a whole because the study included most of the major genetic providers to the swine industry. and reflect the state of the swine industry. The results of this study are also similar to the results found in Canadian studies.

There are limitations in this study. One primary weakness is that we did not include a random sampling of swine farms in a given geographic area. Rather, the sample was drawn from herds accessed through veterinary relationships and marketing relationships. However, given the biosecurity efforts that many herds find necessary to implement to assure their high health standards are maintained, we think that it will be difficult, if not impossible to evaluate a truly random sample. Consequently, we felt it

was inappropriate to do multiple statistical tests on out data, which limit our ability to make inferences.

This cross-sectional study did not address the temporality or directionality of MRSA transmission or the incidence of new colonization. It does not address how long the carrier state or the colonized state may exist in either the human workers or the swine. In addition, we could not eliminate bias. For example, this study utilized a survey, the results of which could be affected by recall bias. This study may also have been affected by selection bias because worker participation in the survey and the biologic sampling was voluntary. Moreover, we were not able to control for confounders, such as the biosecurity of individual herds and the type and style of personal protective equipment available on the farms. Antibiotic usage on animals within and between herds may have varied over time, thereby, altering MRSA prevalence. Furthermore, language barriers and politics restricted the size of the subject pool.

This study suggests that further studies are needed. Leedom Larson et al. sampled shower facilities at several pork production sites involved with animals from Farm 1. [28] At one site positive cultures were obtained from the "dirty" or street side of the shower, where employees shower before entering the facility. These isolates were identical to those found in the pigs within the facility. These data suggest that MRSA may be transmitted several ways: by aerosol transmission throughout the unit, by colonized workers moving through the unit, or by contaminated fomites carried across the shower. At another facility, the shower facilities were in a separate building from the buildings housing swine. The investigators found no MRSA on the street side of this

shower facility, suggesting that MRSA might be transmitted by contaminated aerosols. Additional studies are needed to clarify the mode of MRSA transmissions in this setting.

The subject of MRSA colonization needs to be investigated to define time periods necessary for pigs or swine workers to clear the organism if colonization has temporal limits, or confirm that colonization is a permanent state. Most of the samples from swine workers were taken at either the midmorning break or during the lunch hour after the workers spent the morning exposed to the environment within the swine barns, not wearing personal protective equipment. Would the results change if samples were taken upon arrival at the facility, before exposure to the inside conditions? A larger question may be whether or not swine workers have the potential to carry MRSA from the farm back into their families and communities. There are currently plans in place to study this potential route of transmission.

The data in our study raises the question that within the swine industry could there exist some genetic lines of pigs that are more prone to be carriers of MRSA-ST398 than others. If this were true this would imply that there could be selection of pigs that are not carriers, and carrier animals could be culled. The use of antibiotics in animal populations is always controversial. Nevertheless, studies that critically examine the role of antimicrobial use and outcomes would be important contributions to animal husbandry and infection prevention in this setting. One will not be able to eliminate use of antibiotics in livestock production because it would be detrimental to animal welfare and it would result in loss of production. One could consider putting carrier herds in quarantine as was done in the past to control infections such as tuberculosis, brucellosis and pseudorabies. However, if public health standards require colonized swine and cattle

to be placed in quarantine, public health officials might be obliged to extend that quarantine policy to horses, dogs, cats, pet birds, etc, that carry MRSA. These would be very unpopular policies to implement. Given that *S. aureus* in general, and MRSA, in particular are ubiquitous and are harbored by many mammalian species worldwide, one must carefully consider unintended consequences when designing control measures.

Conclusion

This study demonstrated that some swine and their caretakers in Iowa and Illinois carry MRSA, including ST398, the livestock associated strain first identified in the Netherlands. It demonstrated that both the swine workers and their pigs can carry identical strains of MRSA. However, to date, workers on these farms have not reported having MRSA infections. If a MRSA strain carried by livestock evolves in its ability to not only be carried by humans, but to also infect humans, then this strain could become an important emergent pathogen. Emerging pathogens, known and as yet unknown, are critically relevant to environmental health and occupational health. The interaction between MRSA, animals, and humans is an area ripe for continued and expanding study.

APPENDIX A

Enrollment Questionnaire

Thank you for participating in this study. This questionnaire is intended to gather information on your current and past contact with swine and other potential risk factors for infection with methicillin-resistant *Staphylococcus aureus* (MRSA), a bacterium that can cause a variety of diseases, and should only take a few minutes to complete. Please answer the questions to the best of your knowledge and check the appropriate boxes. All responses will remain confidential.

DEMOGRAPHICS

1. Today's date _____/_____/_____
   (month/ day / year)

2. Your age _____ (years)

3. What is your race/ethnicity?

- ☐ American Indian/Alaska Native
- ☐ Asian
- ☐ Black or African American
- ☐ Hispanic or Latino
- ☐ Native Hawaiian or Other Pacific Islander
- ☐ White
- ☐ Other

4. What is your gender?

□ Male

□ Female

5. Do you currently use or have you ever used tobacco products – for example, cigarettes, cigars, chewing tobacco?

□ Yes Please specify what tobacco product you use:

_____

(continue to question 5a)

□ No

5a. On average, how many packs of cigarettes or other tobacco products do you use per day?_____(packs per day)

5b. If you stopped smoking, what year did you stop smoking?

MEDICAL HISTORY AND EXPOSURES

6. Do you have a medical history of any chronic lung problems such as asthma or emphysema?

□ Yes

□ No

7. Do you have a medical history of any heart disease or vascular disease?

□ Yes

□ No

8. Do you have a medical history of any other chronic medical problems such as diabetes, kidney disease, cancer, blood disease, or diseases that weaken the immune system?

□ Yes

□ No

9. Do you take medications such as anti-cancer drugs, corticosteroids like prednisone, or other drugs that weaken the immune system?

□ Yes

□ No

10. During the last 12 months have you developed a respiratory illness with fever (oral temperature ≥100.5 F), **AND** sore throat or cough for 4 or more hours?

□ Yes

□ No

11. During the last 12 months, have you missed work because of a respiratory illness?

□ Yes *(continue with question 11a)*

□ No (skip to question 12)

11a. How many days? _____ (days)

12. Have you taken antibiotics in the past three months?

□ Yes (*continue with question 12a)*

□ No (skip to question 13)

12a. List all antibiotics taken in the past three months

_____

_____

_____

13. Have you participated in team and/or contact sports in the last 3 months?

□ Yes

□ No

14. Have you spent time in a jail or other correctional facility in the previous 6 months?

□ Yes

□ No

15. Have you been hospitalized in the previous 12 months?

□ Yes

□ No

16. Have you or any family members visited a patient in the hospital in the previous 12 months?

□ Yes

□ No

17. Have you or any family members visited a patient in a long-term care facility (such as a nursing home) in the past 12 months?

□ Yes

□ No

18. Do you or any immediate family members work in a hospital or long-term care facility?

□ Yes

□ No

19. Have you been diagnosed with a skin or soft tissue infection (such as infection of the muscle) in the previous 12 months?

□ Yes

□ No

20. Have you previously been diagnosed with a methicillin-resistant *Staphylococcus aureus* infection?

□ Yes

□ No

OCCUPATIONAL AND ANIMAL EXPOSURE

21. Do you work in a swine production facility (swine farm or swine processing plant)?

□ Yes--swine farm *(continue with question 22)*

□ Yes--swine processing plant *(continue with question 22)*

□ No (*SKIP TO QUESTION 23)*

22.

What type of work do you currently perform in the plant/farm? Check all that apply.

□ Breeding
□ Farrowing

□ Nursery

□ Finishing
□ Wean to finish

□ Cleaning swine barn or trucks
□ Slaughtering and/or butchering swine
□ Packaging raw pork products
□ Cooking pork
□ Packaging cooked pork products

□ Transporting swine
□ Swine waste disposal
□ Examining and treating swine
□ Obtaining blood or other specimens from swine
□ Cleaning and disinfecting equipment and areas exposed to swine, swine products, or swine waste

- Administrative but occasionally I enter areas where swine, raw pork products or swine waste is
- Administrative, I never enter areas where swine, raw pork products or swine waste is
- Other:_____

23. How long have you been working with swine?
(Please total all years in current and previous jobs.)
_____ (years)

24. What is the average number of swine you are exposed to in a typical day?
_____ swine

25. Please write in one of the following spaces how long it has been since your last contact with swine.
_____ (days) _____ (weeks) _____ (months)

26. On average, how many days per week do you work in direct contact (meaning in the same area) with swine? _____ (days/week)

27. On days that you work with swine, how many hours (on average) do you work in direct contact with them? _____ (hours/day)

28.

20.In the last 12 months how often have you used...

	Frequency of Use	Type Most Often Used	Cleaning Practices
Eye protection	☐ Never ☐ Rarely ☐ Sometimes ☐ Most of the time ☐ Always ☐ Not Sure	☐ Goggles ☐ Glasses ☐ Other _____	☐ Wash with cleaning solution daily ☐ Wash with cleaning solution 1 - 3 times per week ☐ Wash < 1 time per week ☐ Do not wash
Mask	☐ Never ☐ Rarely ☐ Sometimes ☐ Most of the time ☐ Always ☐ Not Sure	☐ Dust mask ☐ Filtered mask ☐ Surgical mask ☐ Other _____	☐ Dispose of daily ☐ Dispose after each sick animal ☐ Dispose of after 2-7 days ☐ Reuse until unusable
Clothing	☐ Never ☐ Rarely ☐ Sometimes ☐ Most of the time ☐ Always ☐ Not Sure	☐ Aprons ☐ Coveralls ☐ Outer garments ☐ Other _____	☐ Dispose of daily ☐ Dispose after each sick animal ☐ Wash daily ☐ Wash 1-3 times per week ☐ Wash < 1 time per week ☐ Do not wash or dispose of
Footwear	☐ Never ☐ Rarely ☐ Sometimes ☐ Most of the time ☐ Always ☐ Not Sure	☐ Disposable booties ☐ Washable boots ☐ Sneakers ☐ Sandals ☐ Other _____	☐ Dispose of daily ☐ Dispose after each sick animal ☐ Wash daily ☐ Wash 1-3 times per week ☐ Wash < 1 time per week ☐ Do not wash or dispose of
Gloves	☐ Never ☐ Rarely ☐ Sometimes ☐ Most of the time ☐ Always ☐ Not Sure	☐ Disposable latex or vinyl ☐ Cloth ☐ Leather ☐ Other _____	☐ Dispose of daily ☐ Dispose after each sick animal ☐ Dispose when unusable ☐ Wash daily ☐ Wash 1-3 times per week ☐ Wash < 1 time per week ☐ Do not wash or dispose of

29. In the **past 12 months** have you worked with/been in contact with any of the following types of **live animals**? **(check *yes* or *no* for each)**

Chickens	☐ yes	☐ no
Cattle	☐ yes	☐ no

Horses	□ yes	□ no
Goats	□ yes	□ no
Sheep	□ yes	□ no

Other type of livestock, please specify _____

30. Do you have any of the following animals on your property and/or within your home?

Chickens	□ yes	□ no
Swine	□ yes	□ no
Cats	□ yes	□ no
Dogs	□ yes	□ no

Other type of animal, please specify _____

31. Do you consume pork products?

□ yes *(continue with question 32)* □ no *(end)*

32. How often do you consume pork products?

□ Less than once per week
□ Approximately once per week
□ 2-3 times per week
□ more than 4 times per week

33. How often do you handle raw/uncooked pork products?

- Less than once per week
- Approximately once per week
- 2-3 times per week
- more than 4 times per week

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