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ORIGINAL ARTICLE

Transmission through air as a possible route of exposure for MRSA

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Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) is highly prevalent in pigs and veal calves. The environment and air in pig and veal calf barns is often contaminated with LA-MRSA, and can act as a transmission source for humans. This study explores exposure–response relationships between sequence type 398 (ST398) MRSA air exposure level and nasal ST398 MRSA carriage in people working and/or living on farms. Samples and data were used from three longitudinal field studies in pig and veal calf farm populations. Samples consisted of nasal swabs from the human participants and electrostatic dust fall collectors capturing airborne settled dust in barns. In both multivariate and mutually adjusted analyses, a strong association was found between nasal ST398 MRSA carriage in people working in the barns for >20 h per week and MRSA air levels. In people working in the barns $<$ 20 h per week there was a strong association between nasal carriage and number of working hours. Exposure to ST398 MRSA in barn air seems to be an important determinant for nasal carriage, especially in the highly exposed group of farmers, next to duration of contact with animals. Intervention measures should therefore probably also target reduction of ST398 MRSA air levels.

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INTRODUCTION

Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA), commonly belonging to clonal complex (CC) 398, can be found in livestock of all species, but prevalence is especially high in pigs and veal calves.[1–5] Moreover, pig and veal calf farmers are often carriers of LA-MRSA.[3,6,7] Healthy *S. aureus* carriers rarely experience severe health effects. However, when they are hospitalized, the risk for development of infections is ~10 times higher compared with non-carriers.[8] Infections caused by resistant strains may cause serious health problems, because they are difficult to treat.

Previous research in veal calf and pig farming has shown that human carriage is positively associated with the number of working hours in the barns, which serves as a proxy for direct animal contact.[3,9,10] Nasal carriage in farmers is also associated with the prevalence of carriage among animals.[3] Moreover, contact with live pigs was the main risk factor for carriage in pig slaughterhouse workers.[11,12] This study also showed an association between nasal carriage and environmental MRSA exposure levels.[12] The environment of pig and veal calf barns (i.e., dust) was shown to be contaminated with MRSA, and could therefore act as a potential transmission source. [6,13–17] Even though the mechanism behind animal-to-human transmission is still poorly understood,[18] contact with animals is considered to be an important transmission route. Furthermore, hand–face (mouth or nose) contact is considered to be an important transmission route for *S. aureus*.[19]

Transmission through air has been suggested as another possible route for *S. aureus* and MRSA, as contaminated dust particles may circulate in the air in barns, and subsequently be inhaled by people working in these barns.[19,20] A previous study showed that on veal calf farms with at least one MRSA carrier, a higher proportion of MRSA-positive settled dust samples was found.[21] Furthermore, *S. aureus* and MRSA have been demonstrated in air samples outside of farms, but in low quantities, and risk of contamination is considered to be low for people living in the vicinity.[22–25] Airborne exposure in barns is much higher,[16,20,26] but its association with nasal MRSA carriage in humans has not yet been studied in detail. For control of occupational exposure to MRSA, it is important to determine the sources and transmission routes.

This study determines and quantifies sequence type 398 (ST398) MRSA in settled dust as a proxy for air levels in livestock barns, and is the first to explore exposure–response relationships between ST398 MRSA air exposure level and nasal ST398 MRSA carriage in people working and/or living on farms.

METHODS

Study Populations

Samples and data were used from field studies in three independent farm populations in the Netherlands. Population A came from 38 farrowing and

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farrow-to-finish farms.[27] Farmers, family members and employees on these farms were enrolled in a longitudinal study aimed at identifying intervention measures for reducing antimicrobial consumption and MRSA carriage in animals and people working and/or living on the farms. Therefore, these farms may be the so-called 'front runners', and expected to be more inclined to adapt new approaches or technologies to reduce antimicrobial consumption. In the current study, only results from the first sampling round were incorporated, as these form the baseline results when no intervention measures had yet been implemented.

Population B came from farmers, family members and employees of 49 farrowing pig farms (randomly selected from a list of farms collected during a previous study) enrolled in a longitudinal study, which aimed at determining dynamics of MRSA carriage in pig farmers and family members.[7] In general, farrowing pig farms in the Netherlands have a higher antimicrobial consumption than other farm types, such as farrow-to-finish or finisher farms. Results from the first sampling round were used in the current study.

Population C consisted of farmers, family members and employees from 49 randomly selected veal calf farms, enrolled in a longitudinal study aimed at identifying intervention measures for the reduction of MRSA prevalence in veal calves and people working and/or living on the farms.[21] Results from the second sampling round were used in the current study, which took place 12 weeks after the start of the production cycle, but before the intervention measures were introduced.

In all studies, questionnaires were filled in on general participant and farm characteristics. For the purpose of this study, and in line with previous studies, we divided participants in two groups, based on the number of working hours in the barns.[7] Persons indicating to have worked in the barns for at least 20 h on average per week were labeled 'farmer', persons working less than 20 h on average per week were labeled 'family member'. This may mean that some farmers were included in the <20 h group, and some family members, actively involved in livestock farming, may have been included in the >20 h group. Another reason is that family members usually perform different tasks in the barns, on a quantitative and qualitative level.

All participants provided written informed consent. Study protocols were approved by the Medical Ethical Committees of Utrecht University (populations A and C) and St. Elisabeth Hospital in Tilburg (population B).

Sampling Materials and Laboratory Methods

Electrostatic dust fall collectors. On every farm, 2–6 electrostatic dust fall collectors (EDCs) were placed in the barns (usually at least 1 m from the ground, out of reach of the animals) and one in the house (usually on top of a cupboard in the kitchen or living room) for ~2 weeks. EDCs consist of two sterilized electrostatic dust cloths in a polypropylene sampler, which passively capture airborne settled dust.[28] After 2 weeks, the EDCs were closed and sent by post to the laboratory, where they were stored at −80 °C or −20 °C until analyzed.

EDCs were processed as described in the study by van Cleef et al.[7] In brief, samples were suspended and mixed in saline supplemented with EDTA (final concentration 1 mM) using a Stomacher Blender (Seward, West Sussex, UK). Sample suspensions were stored at −20 °C until further processing. Two hundred microliters of the suspension were used for DNA isolation and purification using the Versant Molecular kPCR system (Siemens Healthcare Diagnostics, the Hague, the Netherlands). The elution volume was set to 50 μl. Five microliters of the DNA isolate were used in the following quantitative Real Time PCRs targeting: (i) *mec*A,[29] (ii) C01 for detection of *S. aureus* ST398,[30,31] (iii) *fem*A,[29] and (iv) *nuc* [32] for detection of *S. aureus*. Two targets were assessed for the detection of *S. aureus* because it was reported that some strains lack the *nuc* gene.[33] All targets were detected with a LightCycler 480-II (Roche Diagnostics, Almere, the Netherlands). Each reaction consisted of 10 μl LightCycler 480 Probes Master (Roche Diagnostics), 1 μl pre-mixed oligos, 4 μl molecular grade water (Roche Diagnostics) and 5 μl template.

A calibration line based on a dilution curve was normalized for run-to-run variation and used to express qPCR results in equivalent colony counts (CFUeq). Because it is not possible to quantify MRSA with a single qPCR target, an estimation of the concentration of MRSA was calculated based on the four targets. The concentration of *S. aureus* was based on the maximum concentration found by either *fem*A or *nuc*, whichever was the highest. Next, the concentration of MRSA was estimated based on the concentration of *mec*A and *S. aureus*, whichever was the lowest. Finally, the concentration of ST398 MRSA was estimated using the concentration of MRSA and the C01 target, whichever was the lowest.

Nasal swabs. Nasal swabs were collected from all participants at variable times during the day. The majority of the swabs were collected by field-workers, the rest through self-sampling. Swabs were either transported immediately to the laboratory or sent in by post, and generally processed within 24 h after arrival. Swabs were enriched in Mueller Hinton Broth supplemented with 6.5% NaCl and after overnight incubation plated on chromID *S. aureus* and chromID MRSA agar plates (BioMérieux, Craponne, France). Suspected colonies from populations A and C (mixed pig and veal calf farm populations) were subcultured on Columbia Agar with sheep blood (Oxoid, Badhoevedorp, the Netherlands) and confirmed as *S. aureus* or MRSA ST398 by the above described Real Time PCRs for the analysis of the EDCs.[27,21] For population B (farrowing pig farm population), *S. aureus* was confirmed as MRSA by a duplex PCR for *nuc* and *mec*A genes and characterized as CC398 using Staphylococcal protein A typing and multi-locus variable number of tandem repeat analysis.[7]

Statistical Analyses

Descriptive analyses were performed with SAS software version 9.2 (SAS Institute, Cary, NC, USA). Pearson's correlation coefficients were estimated to indicate the correlation between the four PCR targets (concentration per sample). Quantitative EDC results were log-transformed because they were not normally distributed. To account for clustering of data at the farm level, generalized estimating equations with an exchangeable working correlation matrix were used to estimate odds ratio (OR) and 95% confidence interval (CI).

First, associations between the outcome variable (ST398 MRSA carrier yes *vs* no) and ST398 MRSA exposure level in the air (given in log (CFUeq)) and the number of working hours were analyzed separately, but always adjusted for the potential confounders age, current smoking and gender. Subsequently, a multivariate model was used to analyze the association between ST398 MRSA carriage and air level and the number of working hours, mutually adjusted and again also adjusted for the set of potential confounders. The two exposure terms (air level and working hours) were modeled as separate independent variables, and not as a composite variable or interaction term. This was done because these variables are proxies for potentially different routes of exposure, and thus to separately explore the effect of both variables on the outcome, ST398 MRSA carriage.

Analyses were performed in the pooled study population and the populations from the three livestock farm samples separately. Furthermore, a meta-analysis was performed on the three study populations (Stata software version 10.1, StataCorp, College Station, TX, USA).

To explore the shape of the association between the probability of nasal ST398 MRSA carriage and air exposure of ST398 MRSA, we made use of penalized splines in the analysis (adjusted for potential confounders). This way, the shape of the association can be studied free from assumptions about the parameterization of the model (R open source software version 3.0.2, R foundation for Statistical Computing, Vienna, Austria). Furthermore, a principal component analysis (PCA) was carried out to analyze the clustering of the genetic targets, which were used to describe environmental MRSA ST398 exposure using the log-normalized concentration per sample of the four qPCR targets (*nuc*, *fem*A, *mec*A and C01).

RESULTS

Descriptive Characteristics

Table 1 shows descriptive characteristics from the three study populations. On all pig farms but one, ST398 MRSA was detected on the EDCs, whereas among the veal calf farms, only about a third of the farms were positive for ST398 MRSA on the EDCs. Levels were generally low on these farms, and many EDCs had non-detectable MRSA ST398 levels. However, on 80% of the veal calf farms, general MRSA (including ST398 and other types) was detected on the EDCs. The average ST398 MRSA load on the EDCs differed significantly between the three study populations (Table 1), with the highest load found in the farrowing pig farm population (average mean log(CFUeq ST398 MRSA)/farm (95% CI): 1.98 (1.80–2.15)), and the lowest in the veal calf farm population (average mean log(CFUeq ST398 MRSA)/farm (95% CI): 0.41 (0.21–0.61)). The load on 136 EDCs placed in houses was low, with no ST398 MRSA detected on 128 EDCs (94%).

Table 1. Descriptive characteristics of the farms by population.

Population	n	*MRSA prevalence (based on EDCs)*	*ST398 MRSA prevalence (based on EDCs)*	*Average mean log (CFUeq ST398 MRSA) /farm (95% CI)*
Mixed pig farms	38	38 (100%)	38 (100%)	1.11 (0.94, 1.29)
Farrowing pig farms	49	49 (100%)	48 (98%)	1.98 (1.80, 2.15)
Veal calf farms	49	39 (80%)	18 (37%)	0.41 (0.21, 0.61)

Abbreviations: CFUeq, equivalent colony counts; CI, confidence interval; EDC, electrostatic dust fall collector; MRSA, methicillin-resistant *Staphylococcus aureus*.

Table 2. Descriptive characteristics of the participants by population.

Population	*Group*	n	*Prevalence nasal MRSA/ST398 MRSA carriage*	*No. of working hours (average (range))*	*Age, years (average (range))*	*Gender (male)*	*Current smokers (yes)*
Mixed pig farms	Farmers	78	41 (53%)/41 (53%)	48.3 (20–80)	45.5 (19–70)	81%	12%
	Family members	118	10 (8%)/10 (8%)	2.3 (0–16)	23.5 (3–79)	42%	10%
	All	196	51 (26%)/51 (26%)	20.6 (0–80)	32.2 (3–79)	58%	11%
Farrowing pig farms	Farmers	104	75 (72%)/75 (72%)	45.9 (20–80)	42.4 (17–67)	82%	12%
	Family members	168	19 (11%)/19 (11%)	2.2 (0–18)	21.5 (0–83)	40%	6%
	All	272	94 (35%)/94 (35%)	18.9 (0–80)	29.5 (0–83)	56%	8%
Veal calf farms	Farmers	67	21 (31%)/18 (27%)	37.2 (20–75)	45.8 (19–68)	75%	21%
	Family members	127	13 (10%)/12 (9%)	2.8 (0–18)	22.7 (0–81)	43%	13%
	All	194	34 (18%)/30 (15%)	14.7 (0–75)	30.7 (0–81)	54%	16%
Overall	Farmers	249	137 (55%)/134 (54%)	44.3 (20–80)	44.3 (17–70)	80%	14%
	Family members	413	42 (10%)/41 (10%)	2.4 (0–18)	22.4 (0–83)	41%	9%
	All	662	179 (27%)/175 (26%)	18.2 (0–80)	30.7 (0–83)	56%	11%

Abbreviation: MRSA, methicillin-resistant *Staphylococcus aureus*.

Participants from the two pig farm populations were comparable in age, number of working hours in the barn and the percentage of current smokers and male gender (Table 2). It appeared that farmers from the veal calf farm population generally had a comparable age distribution, but had slightly less working hours in the barns, and a greater proportion of women and current smokers than the pig farmers. Family members from the veal calf farm population appeared to have a similar number of working hours, proportion of women and a slightly greater proportion of smokers than the pig farm family members. In all populations, farmers had the highest prevalence of ST398 MRSA and MRSA carriage, and in all three participant groups (farmers, family and all) the highest prevalence was found in the farrowing pig farm population. There were 247 participants who indicated to have 0 working hours in the barn, of which 7% were positive for nasal ST398 MRSA carriage.

All MRSA strains isolated from nasal swabs from participants from both pig farm populations were confirmed to belong to ST398 or CC398. In 4 out of 34 veal calf population participants positive for nasal MRSA carriage, a non-ST398 MRSA strain was found (Table 2), these were not further typed. The four participants were from different farms. On one of these farms, no ST398 MRSA was found on the EDCs but other MRSA types were found. On the other three farms both ST398 and other variants of MRSA were found on the EDCs. Of these four participants, one had received antimicrobials and had been admitted to the hospital in the 3 months prior to sampling. Of the other three participants, one had visited a hospital in the 3 months prior to sampling.

Clustering of PCR Targets

The four PCR targets (*nuc*, *fem*A, *mec*A and C01) were all positively correlated, but the strongest correlations were found between *nuc* and *fem*A (Pearson correlation coefficient (PCC): 0.98), indicating that *S. aureus* was present on the EDCs. This was followed by the correlation between *nuc* and C01 (PCC: 0.53), *fem*A and *mec*A (0.48), and C01 and *fem*A and *nuc* and *mec*A (for both PCC: 0.46). This showed that *nuc*, *fem*A and *mec*A are often found together (indicating MRSA), as are *nuc*, *fem*A and C01 (indicating ST398 *S. aureus*). The targets C01 and *mec*A had the lowest correlation coefficient (PCC: 0.29).

The PCA analysis resulted in one component on which the different markers loaded strongly and this confirmed the high likelihood for mainly ST398 MRSA to be present on the EDCs taken from pig and veal calf barns. Loadings were somewhat weaker for veal calve samples, indicative of a higher heterogeneity of MRSA strains.

Nasal Carriage, Working Hours and ST398 MRSA Air Levels

Table 3 shows the results of the analysis of associations between nasal carriage with working hours in the barns and MRSA air levels, separately, and mutually adjusted. After adjusting for potential confounders (age, gender and current smoking), air level of ST398 MRSA showed a significant positive association with nasal ST398 MRSA carriage in farmers from the farrowing pig farm population (OR (95% CI): 2.86 (1.35–6.08)) and in the farmers group in the pooled analysis (OR (95% CI): 2.34 (1.64–3.34)), as well as in the group containing all participants (OR (95% CI): 1.80 (1.38–2.34)).

The number of working hours in the barn was significantly associated with carriage in the three populations (OR ranging from 0.98 to 1.15 per hour increase). For the pooled analyses the ORs (95% CI) were 1.02 (1.00–1.04) for the farmers, 1.15 (1.07–1.24) for family members and 1.05 (1.03–1.06) for all participants. In all populations, the highest OR for nasal carriage with number of working hours in the barn were found for the family group (persons working < 20 h per week in the barns).

Multivariate pooled analyses determining the association between nasal ST398 MRSA carriage and air level of ST398 MRSA,

Table 3. Results from multivariate and mutually adjusted models: OR with 95% CI for working week and/or air level of ST398 MRSA for nasal ST398 MRSA carriage; all associations presented have been adjusted for age, gender and current smoking.

Population	*Group*	*OR (95% CI)*	*OR (95% CI)*	*Mutually adjusted ORs (95% CI)*	
		Air level (log(CFUeq))	*Working hours (1-h increase)*	*Air level (log(CFUeq))*	*Working hours (1-h increase)*
Mixed pig farms	Farmers	0.98 (0.34–2.84)	1.04 (1.01–1.08)*	1.00 (0.32–3.08)	1.04 (1.01–1.08)*
	Family	n.c.	n.c.	n.c.	n.c.
	All	0.84 (0.37–1.90)	1.05 (1.03–1.07)***	0.95 (0.37–2.44)	1.05 (1.03–1.07)***
Farrowing pig farms	Farmers	2.86 (1.35–6.08)**	1.01 (0.98–1.04)	2.82 (1.35–5.88)**	1.01 (0.97–1.05)
	Family	1.29 (0.48–3.47)	1.11 (1.02–1.21)*	1.42 (0.47–4.32)	1.12 (1.03–1.21)**
	All	1.49 (0.85–2.60)	1.05 (1.03–1.08)***	2.27 (1.22–4.24)*	1.06 (1.03–1.08)***
Veal calf farms	Farmers	1.71 (0.73–3.99)	0.98 (0.94–1.02)	1.93 (0.83–4.52)	0.97 (0.93–1.02)
	Family	1.34 (0.50–3.56)	1.15 (1.05–1.26)**	0.96 (0.36–2.58)	1.15 (1.05–1.27)**
	All	1.71 (0.82–3.54)	1.01 (0.99–1.03)	1.68 (0.79–3.55)	1.00 (0.98–1.03)
All—pooled analysis	Farmers	2.34 (1.64–3.34)***	1.02 (1.00–1.04)*	2.25 (1.57–3.22)***	1.01 (0.99–1.03)
	Family	1.27 (0.82–1.99)	1.15 (1.07–1.24)***	1.29 (0.81–2.06)	1.15 (1.07–1.23)***
	All	1.80 (1.38–2.34)***	1.05 (1.03–1.06)***	1.82 (1.35–2.46)***	1.05 (1.03–1.06)***

Abbreviations: CFUeq, equivalent colony counts; CI, confidence interval; EDC, electrostatic dust fall collector; MRSA, methicillin-resistant *Staphylococcus aureus*; n.c., not computable, these parameters could not be estimated properly with the data; OR, odds ratio. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

working hours and confounders, showed that for farmers carriage was mainly driven (based on *P*-value) by air level, after adjusting for gender, age and current smoking (OR_{pooled} (95% CI) for air level: 2.25 (1.57–3.22) and for working hours: 1.01 (0.99–1.03)). In the family group, nasal carriage was mainly driven by working hours (OR_{pooled} (95% CI) for air level: 1.29 (0.81–2.06) and for working hours: 1.15 (1.07–1.23)). For all participants grouped together, both air level and working hours were strong determinants for nasal ST398 MRSA carriage in a multiple regression model, adjusted for potential confounders (OR_{pooled} (95% CI) for air level: 1.82 (1.35–2.46) and for working hours: 1.05 (1.03–1.06)). Results from the meta-analyses showed similar results. Models based solely on general MRSA outcomes were also very similar, but the model fit for ST398 MRSA models was slightly better (results not shown).

Figure 1 shows the penalized smoothed spline plots indicating the shape of the association between nasal ST398 MRSA carriage and air level of ST398 MRSA in the barns, adjusted for working hours and potential confounders and given per participant group. The figures clearly show the steepest slopes in the farmers group.

DISCUSSION

The results reported in this study are the first presenting an exposure–response relationship between ST398 MRSA in the air of barns and nasal carriage in humans. The associations between nasal carriage and the amount of ST398 MRSA found in settled dust (as a proxy for air exposure) were very strong in both analyses with each of these variables separately with confounders and mutually adjusted with confounders, especially in people working in the barns for at least 20 h per week. In addition, an association between nasal carriage and working hours in the barn was found in those working in the barn for < 20 h per week. In other words, for people who do not enter the barn frequently, the mere presence in the barn is the main determinant to acquire MRSA. However, for those who do enter the barn frequently, the amount of MRSA in the air is the main determinant. This offers opportunities for prevention. If the amount of ST398 MRSA present in the air of the barn can be reduced, it may likely lower the risk of acquisition of ST398 MRSA by humans working there. The findings can have a large impact on predictive models for transmission, and give new insights into the importance of air exposure in the light of possible interventions.

So far, the majority of hypotheses about acquisition focused on contact with animals, approximated in this and previous studies by the number of hours worked in the barn, as the main source of exposure and the main route of transmission. The current research confirms these findings with a strong positive association between the number of working hours and the probability of nasal ST398 MRSA carriage (OR ranging from 1.0–1.2 per hour increase). The strongest association was found in the family group, that is, people working in the barn for < 20 h per week on average. Prevalence of nasal ST398 MRSA carriage in the participants with 0 working hours was 7%. This is still higher than the prevalence in a recent study among patients with non-infectious disorders recruited from Dutch general practitioners ($< 0.5\%$ for MRSA), and could indicate an elevated air level around the barn or in the farm house.[34] Sensitivity analyses showed no association ($P = 0.93$) between nasal carriage and air level for the 247 participants indicating 0 working hours in the barn. Moreover, on 94% of EDCs placed in farmhouses, no MRSA ST398 could be detected, and no measurements were done outside of the barn. The current results emphasize that more research is needed to understand the impact of (barn) air exposure, and other sources in the barns as well, on the transmission routes of ST398 MRSA to persons without direct animal contact.

Recently, it was shown that ST398 MRSA is present in the air of pig farms.[16] It can be inhaled by people present in these farms and subsequently colonize the nares. A review by Wertheim et al.[19] stated that *S. aureus* is most frequently found in the anterior nares, and that only about 20% of individuals are persistent carriers, with another 30% being intermittent carriers.[19] These figures represent the general population, but in people working with pigs these rates are much higher due to the high exposure in the barns. For example, 47% of livestock veterinarians were persistent *S. aureus* carriers, and 42% were intermittent carriers.[35] Furthermore, people who had intensive contact with pigs during a short period sporadically picked up MRSA and were negative within 24 h afterwards.[10] Also, veal calf farmers and their family members had a markedly reduced prevalence of carriage when on holiday, or during the empty barn period.[9] These findings suggest that persons continuously exposed to ST398 MRSA are frequently re-contaminated, instead of truly colonized, as proposed in a previous study.[21] Our findings that nasal carriage correlates strongly with airborne exposure (given as log(CFUeq) of ST398 MRSA) supports the theory of repeated contamination when working in the barn. When

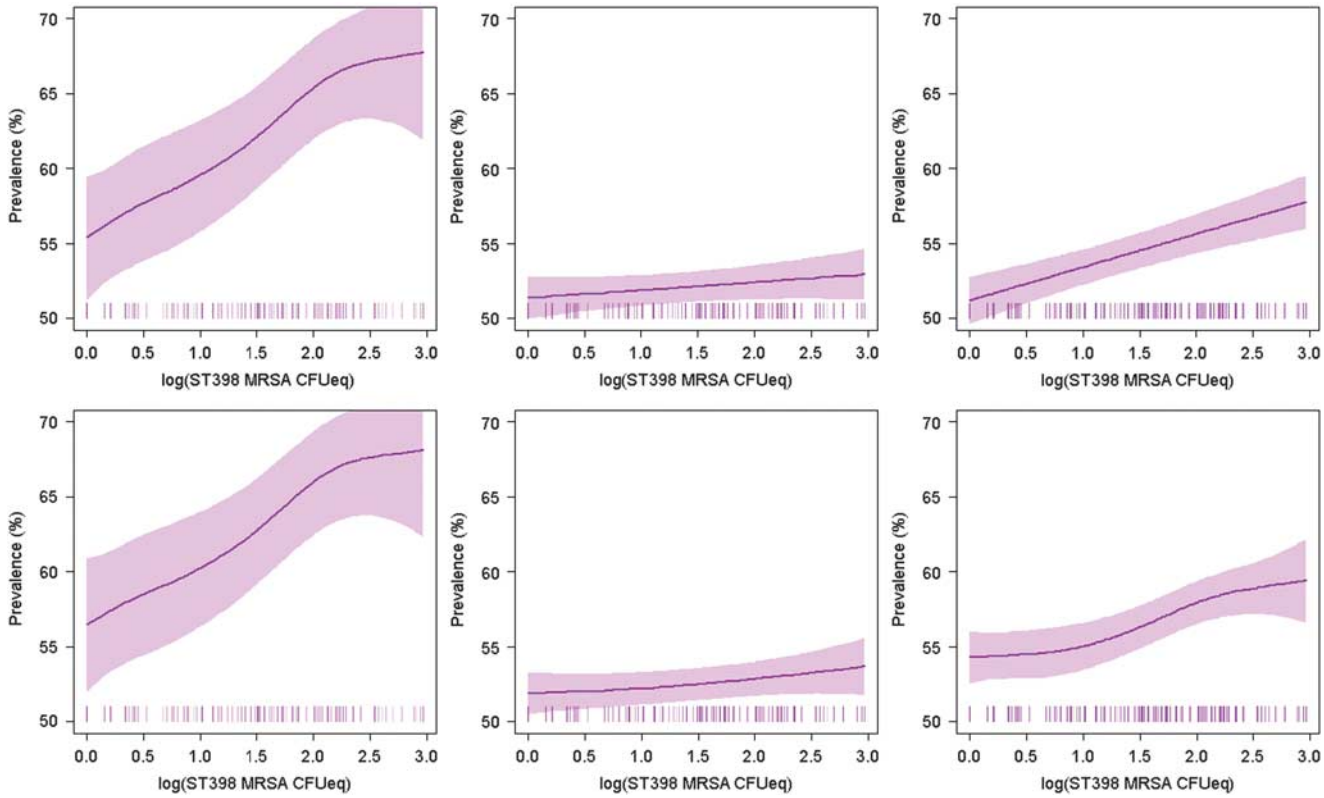

Figure 1. Association between mean air level of ST398 methicillin-resistant *Staphylococcus aureus* (MRSA) in the barn and nasal ST398 MRSA carriage adjusted for potential confounders (age, gender and current smoking) for farmers (upper left), family members (upper middle) and all participants (upper right), and mutually adjusted for working week and potential confounders for farmers (lower left), family members (lower middle) and all participants (lower right).

compared with family members, farmers constitute a highly exposed population, and in this population prevalence (54% *vs* 10%) as well as the OR for air level (2.25 *vs* 1.29) were highest. This further supports the previously mentioned hypothesis. The association between air exposure and nasal carriage could also be an ecological fallacy, resulting from the three study populations, but the meta-analysis showed this was not the case. Point estimates for the mutually adjusted multivariate meta-analyses were comparable with those of the pooled analyses, and in none of the groups significant heterogeneity between the three sample populations was shown ($P > 0.3$).

It is difficult to separate contact with animals and time exposed to MRSA in dust in the barn in the variable of working hours, but it is remarkable that the air level was so strongly associated with nasal carriage in the statistical models, even after adjustment for number of working hours in the barn. Air level is measured at a fixed static spot in the barn, which will lead to a considerable underestimation of the air exposure of farmers when exposure would have been measured using mobile portable equipment.[36,37] Air exposure is likely to be highest in the area between the animal and the nose of farmers, with the animals being the main source. Personal measurements of air exposure by means of portable gear would be preferable, as these can provide a more precise estimation of the exposure in the breathing zone and therefore less exposure misclassification, this would likely lead to stronger associations than static measurements.

In this study we determined air exposure of ST398 MRSA by performing qPCR on samples of settled dust from barn air. A drawback of performing qPCR is that it may overestimate the amount of ST398 MRSA found in the barn, as not only viable bacteria are determined, but also dead or dormant organisms. Also, the *mec*A gene can be found in other *Staphylococcus* species than MRSA. In essence, the qPCR quantifies genetic markers instead of an organism, and the presence and level of ST398 MRSA has to be estimated on four qPCR targets (*nuc*, *fem*A, *mec*A and C01) per EDC. The nasal swabs were first analyzed by culturing, and the isolates were subsequently confirmed as *S. aureus*, MRSA and/or ST398, by combining the four qPCR results. In spite of the potential overestimation of the number of organisms present, qPCR nowadays is a widely used and accepted method, as it is quick and relatively cheap.

In the current study, samples were collected from three independent populations. The two pig farm populations were similar in MRSA prevalence on farm level (based on the EDCs), but differed significantly in air exposure (average mean log(CFUeq ST398 MRSA) per farm). This was also reflected in the prevalence of nasal carriage in the human participants: the farrowing pig farm population had the highest air level and the highest prevalence, especially among the farmers. The mixed pig farms were also mainly multiplier farms,[27] and there are no clear indications as to why these two populations would differ in air level and nasal carriage of ST398 MRSA. The EDCs were analyzed in the same laboratory, and the nasal swabs in other laboratories with a slightly different method, but the results were consistent. One explanation could be that the location where the EDCs were placed differed between the two study populations. In the farrowing pig population, 45% of the EDCs were placed in the weaned piglet barn, and 50% of the EDCs were placed in the farrowing section. Several studies have shown that the highest MRSA prevalence can be found among weaned piglets,[4,14] which might result in a higher MRSA air level in those areas. In the mixed pig farm population, the EDCs were more spread over all age categories

present, which in turn could explain the lower average air level in this population. The farmers can be expected to have the same tasks in both populations, and thus have the same exposure to pigs, but the studies were performed in different years. Furthermore, the mixed pig farm population may have a higher general hygiene standard. This may explain the difference in nasal carriage between the two populations.

The lower farm and human prevalence in the veal calf farm population was expected.[3] These results confirm earlier findings that not only ST398 MRSA has spread in the veal calf farm population. The lower air level could be explained by the way Dutch veal calf farms are set up, with the barns usually open to outside air, whereas Dutch pig farms generally are closed off. Also, veal calf farms are more humid due to, for example, wetter manure. For more insight, knowledge of the exact amount of settled dust on the EDCs is necessary, but this was not measured during the current study. The greater diversity in MRSA strains found in the veal calf farms than in the pig farms was also expected based on other studies[3,38] and also on the way the veal calf sector collects veal calves from numerous (international) suppliers.[39] The lower human prevalence could be explained by the lower air level, as well as the lower exposure due to a shorter average working week for this sample population.

CONCLUSION

This study shows that the exposure to ST398 MRSA in barn air is an important determinant for nasal carriage, especially in the highly exposed group of farmers. The current study did not directly measure transmission, nor did it quantify exposure on the hands, mouth or face, and therefore it cannot distinguish between the significance of the transmission routes through air and *via* direct contact with animals. It is doubtful whether it will be possible to produce conclusive evidence on the relative importance of different transmission routes by observational studies. However, at the same time, the results are strongly suggestive of a possible role for transmission through air for nasal carriage in persons working in the barn. As family members often work in the barn as well, they are also at a higher risk for carriage or contamination. Therefore, intervention measures to reduce carriage of MRSA should not only target personal hygiene, such as washing of hands, but also the reduction of MRSA air levels.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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