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Persistence of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* in Field Workers after Short-Term Occupational Exposure to Pigs and Veal Calves[▽]

Brigitte A. G. L. van Cleef,[1,2]† Haitske Graveland,[3]† Anja P. J. Haenen,[1] Arjen W. van de Giessen,[1] Dick Heederik,[3] Jaap A. Wagenaar,[4,5] and Jan A. J. W. Kluytmans[2,6]*

Centre for Infectious Disease Control Netherlands, RIVM (National Institute for Public Health and the Environment), Bilthoven,[1] Department of Medical Microbiology, VU University Medical Centre, Amsterdam,[2] Division of Environmental Epidemiology, IRAS (Institute for Risk Assessment Sciences),[3] and Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine,[4] Utrecht University, Utrecht, Central Veterinary Institute of Wageningen UR, Lelystad,[5] and Laboratory for Microbiology and Infection Control, Amphia Hospital, Breda,[6] Netherlands

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The prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) carriage in pig and veal calf farmers in the Netherlands is estimated at 25 to 35%. However, no information is available about MRSA carriage in humans after short-term occupational exposure to pigs or veal calves. This study examines the prevalence and duration of MRSA acquisition after short-term intensive exposure to pigs or veal calves for persons not exposed to livestock on a daily basis. The study was performed with field workers who took samples from the animals or the animal houses in studies on MRSA prevalence in pig and veal farms. They were tested for MRSA by taking nasal samples before, directly after, and 24 h after they visited the farms. There were 199 sampling moments from visits to 118 MRSA-positive farms. Thirty-four of these visits (17%) resulted in the acquisition of MRSA. Thirty-one persons (94%) appeared negative again after 24 h. There were 62 visits to 34 MRSA-negative farms; none of the field workers acquired MRSA during these visits. Except for that from one person, all *spa* types found in the field workers were identical to those found in the animals or in the dust in animal houses and belonged to the livestock-associated clone. In conclusion, MRSA is frequently present after short-term occupational exposure, but in most cases the strain is lost again after 24 h.

Beginning in 2003, a specific clone of methicillin-resistant *Staphylococcus aureus* (MRSA) associated with animal husbandry has emerged (15). This livestock-associated MRSA (LA-MRSA) clone belongs to multilocus sequence typing (MLST) clonal complex (CC) 398 (7), and humans in close contact with pigs are often colonized. Humans in contact with other animals, such as veal calves and poultry, may also have a significantly higher prevalence of MRSA carriage than the general population (3, 5, 8, 9, 12, 17).

So far, the prevalence of LA-MRSA carriage is known only for persons with long-term exposure to livestock, such as persons living or working on pig or veal calf farms or livestock veterinarians (5, 12, 19). In the Netherlands, a vigorous "search-and-destroy policy" is maintained, successfully controlling MRSA in health care settings by screening persons at risk for MRSA presence (14) (www.wip.nl). As part of this policy, all persons with livestock contact are screened for the presence of MRSA upon admission to a hospital. Since it is not clear whether persons with short-term exposure to livestock acquire MRSA, the necessity of screening these persons is questionable. This study examines the prevalence and duration of MRSA acquisition after intensive short-term exposure to pigs or veal calves in persons not exposed to livestock on a daily basis.

MATERIALS AND METHODS

Study design and study population. During two cross-sectional studies investigating the prevalence of LA-MRSA on randomly selected pig and veal farms in the Netherlands (5, 12), dust samples from the animal houses and nasal swabs from pigs and veal calves were taken by field workers on the same day. These field workers (n = 40) all had short (up to a maximum of 3 h per day) but intensive contact with animals and dust on the farms and were therefore at risk of acquiring MRSA on MRSA-positive farms. Intensive contact was defined as direct physical contact with the animals during the farm visit. Acquisition was defined as a MRSA-negative initial swab, followed by a MRSA-positive swab. Standard personal protective equipment included boots and overalls provided by the farm, gloves, mouth masks, and hair nets. Hygienic procedures, including hand washing and showering, were mandatory when the field workers left the animal houses. Nasal swabs were taken from these field workers before, directly after, and 24 h after their farm visits and were tested for MRSA presence. Field workers who had livestock exposure other than that on the farms concerned were excluded from the analysis. In addition, data on farm characteristics (i.e., farm type, number of animals, other animals present, and hygiene measures) were collected by questionnaire and have been described previously (5, 12). The study protocols of both cross-sectional studies were approved by the medical ethics committees of the institutes involved as required by the law of the Netherlands (5, 12).

All farms were visited by more than one field worker. For the veal calf farms, more than one farm could be visited on one field day. One sampling moment refers to a set of three individual nasal swabs (taken before, directly after, and 24 h after the field day) pertaining to a field day on which one or more farms were visited by a field worker. Therefore, the number of field days could be different from the number of sampling moments and the number of farms visited. A farm was considered to be MRSA positive when MRSA was found in one or more animals or in dust samples taken on that particular farm. When only MRSA-negative farms were visited, the field day was considered to be MRSA

* Corresponding author. Mailing address: Laboratory for Microbiology and Infection Control, Amphia Hospital, Location Molengracht, P.O. Box 90158, 4800 RK Breda, Netherlands. Phone: 31 76 5953015. Fax: 31 76 5953820. E-mail: jankluytmans@gmail.com.
† B.A.G.L.V.C. and H.G. contributed equally to this study.
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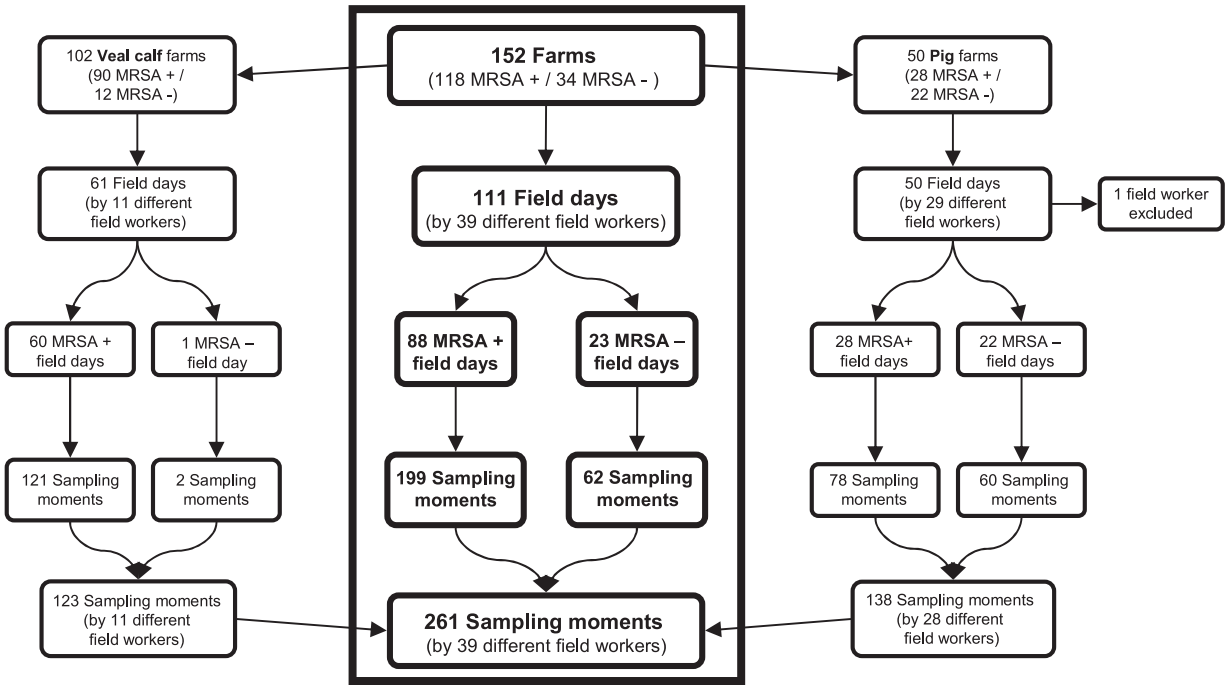

FIG. 1. Schematic overview of study design.

negative. If one or more MRSA-positive farms were visited, the field day was considered to be MRSA positive. A schematic overview of the study design is given in Fig. 1.

Laboratory analysis. Nasal swabs from the veal calf field workers were analyzed individually as published previously (4). Briefly, swabs were inoculated in a preenrichment medium containing Mueller-Hinton broth with 6.5% NaCl. After overnight aerobic incubation at 37°C, selective enrichment in phenol red mannitol broth (bioMérieux, France) with 75 mg/liter aztreonam and 5 mg/liter ceftizoxime was performed. Ten microliters of the selective enrichment broth was inoculated onto sheep blood agar (Biotrading, Netherlands) and Brilliance MRSA agar (Oxoid, Netherlands). The nasal swabs of the pig farm field workers were analyzed similarly; however, the selective enrichment step was excluded because of different protocols in the laboratories analyzing these samples. All suspected colonies were identified as *S. aureus* by using standard techniques: colony morphology and coagulase assays. The presence of the *mecA* gene was confirmed by PCR. The strains were *spa* typed by sequencing of the repetitive region of the protein A gene *spa* (6). The strains of all positive dust and pooled pig samples and a random selection of samples from three MRSA-positive veal calves per farm were *spa* typed. Data were analyzed by using Ridom Staphtype software, version 1.4.

Data analysis. Statistical analysis of complete data sets was performed using SAS software, version 9.1 (10). Descriptive analyses were undertaken, followed by logistic multivariate multilevel regression analysis (GLIMMIX and LOGISTIC procedures) to identify determinants of MRSA carriage. A P value of <0.05 was considered statistically significant.

RESULTS

In total, 152 farms (50 pig and 102 veal calf farms) were visited by 40 different field workers. One field worker was excluded because he was continuously exposed to livestock at home, and analyses were performed on data related to the remaining 39 field workers. None of them was MRSA positive on the initial swab. The 152 farms were visited in 111 field days. In total, 261 individual sampling moments were obtained. A total of 118 (78%; 28 pig and 90 veal calf farms) MRSA-positive farms were visited in 88 field days, and 34 (22%; 22 pig and 12 veal calf farms) MRSA-negative farms were visited in 23 field days. Figure 1 and Table 1 summarize the farm and field day characteristics for the pig and veal calf farms. More-extensive farm characteristics have been published in previous studies (5, 12).

In total, 199 individual sampling moments were present for 34 different field workers visiting the MRSA-positive farms (Table 2). These 34 different field workers acquired MRSA on 34 out of 199 visits (17%; 95% confidence interval [CI], 13 to 22%). Overall, 16 field workers (48%; 95% CI, 33 to 65%) acquired MRSA at least once. Five of them acquired MRSA twice (field workers 3, 7, 25, 26, and 27), and three acquired MRSA on more than two visits (field workers 4, 23, and 24). The field workers who acquired MRSA more than once visited, on average, more positive farms than field workers who acquired MRSA once (median number of positive farms visited per field worker, 10 and 1, respectively). Although the correlation between the number of sampling moments and the number of MRSA acquisitions was high, its statistical significance was only borderline (Spearman's rho, 0.87; P, 0.09).

TABLE 1. Overview of characteristics for pig and veal calf farms

Source	No. of farms visited	Avg no. of animals on farm (range)	Avg MRSA prevalence on farm [% (range)]:	
			In animals	In dust samples
Pigs	50	932 (0–3,200)[a]	33 (0–100)[b]	34 (0–100)[d]
Veal calves	102	565 (25–2,200)	28 (0–100)[c]	47 (0–100)

[a] Only sows and finisher pigs were counted.
[b] Data were obtained from pooled pig samples (10 pools of 6 pigs per farm).
[c] Data were obtained from individual veal calf samples (10 to 43 samples per farm).
[d] Five dust samples were taken per farm.

TABLE 2. Overview of sampling moments pertaining to positive field days and MRSA acquisition before, directly after, and 24 h after sampling

Farm type	Field worker(s)[a]	No. of field days (no. of farms visited)	No. of positive field days	No. of positive samples[b]:		
				Before visit	Directly after visit	24 h after visit
Pig	1	9 (9)	2	0	1 (t011)	0
	2	1 (1)	1	0	1 (t011)	0
	3	15 (15)	11	0	2 (t011 [1], t108 [1])	0
	4	18 (18)	13	0	3 (t011 [1], t108 [2])	0
	5	1 (1)	1	0	1 (t108)[c]	0
	6	1 (1)	1	0	0	1[d] (t108)
	7	7 (7)	6	0	2 (t108 [1], t567 [1])	0
	8	3 (3)	1	0	1 (t108)	0
	9	1 (1)	1	0	1 (t011)	1[e] (t011)
	10	1 (1)	1	0	1 (t011)	0
	11	1 (1)	1	0	1 (t108)	0
	12–23	74 (74)	39	0	0	0
Veal calf	24	55 (82)	54	0	8 (t011)	0
	25	39 (77)	39	0	5 (t011)	0
	26	2 (5)	2	0	2 (t011)	1[d,f]
	27	5 (10)	5	0	2 (t011)	0
	28	7 (10)	7	0	2 (t011)	0
	29–34	15 (14)	14	0	0	0
Total	34	261	199	0	33[g]	3

[a] Field workers whose visits to positive farms did not result in MRSA acquisition are grouped together.
[b] Designations in parentheses are *spa* types. Where different spa types are present for one individual, the number of samples with a particular *spa* type is given in brackets.
[c] This *spa* type is not identical to the *spa* type on the farm visited.
[d] The individual tested MRSA negative following subsequent visits to negative farms.
[e] The individual was not tested again.
[f] The sample was not *spa* typed.
[g] Excluding field worker 6, who acquired MRSA after 24 h.

After 24 h, 31 of the 33 field workers who had acquired MRSA (94% [95% CI, 83 to 98%]) were negative again. Only one field worker who was negative directly after exposure was found to be positive after 24 h; he tested negative after subsequent farm visits. The *spa* types found in field workers were t011 ($n = 25$), t108 ($n = 8$), and t567 ($n = 1$), all of which belong to CC398. All MRSA isolates except for that from one field worker (field worker 5) had *spa* types identical to those isolated either from animals or from dust on the same farms on the same visit. Persons who acquired MRSA more than once were positive for different *spa* types at different moments, depending on the farm visited.

The 34 MRSA-negative farms were visited by 19 different field workers. None of them acquired MRSA on the 62 field days.

Further statistical analysis showed that field workers acquired MRSA more often when they had visited farms where more MRSA-positive animals were present. Similar associations were found for pig and veal calf field workers (Table 3). No significant associations were found with other farm characteristics.

DISCUSSION

This study indicates that short-term occupational exposure to pigs or veal calves on MRSA-positive farms frequently results in the acquisition of MRSA. However, within 24 h after exposure, 94% of those who had acquired MRSA tested negative again; the majority of people who acquire LA-MRSA during short-term occupational exposure lose the strain within 24 h. Possibly, the high prevalence of MRSA carriage in livestock farmers and livestock veterinarians found in cross-sectional surveys is partly the result of repeated contamination instead of real colonization (5, 12, 19). Further longitudinal studies are needed to clarify these and other possible types of carriage and to determine the true dynamics and determinants of LA-MRSA carriage in humans.

It is questionable whether the nasal presence of MRSA should be considered true colonization or whether it is better described as contamination. We presume that in the animal houses on MRSA-positive farms, high concentrations of MRSA are present in the dust, and it is well known that *S. aureus* can survive in dust for long periods (2). People who

TABLE 3. MRSA acquisition in field workers in relation to MRSA prevalence among farm animals

Source[a]	Odds ratio (per 10% increase in prevalence)[b]	95% CI[c]
Pigs	2.04	1.24–3.34
Veal calves	1.28	1.06–1.53

[a] Pig samples were pooled (10 pools of 6 pigs per farm). Samples were taken from individual veal calves. The number of veal calves sampled per farm (ranging from 10 to 43 samples per farm) was equal to the square root of the total number of veal calves on that farm.
[b] Adjusted for the number of MRSA-positive dust samples on the farms.
[c] $P < 0.05$.

work in these animal houses inhale MRSA-contaminated dust particles that may persist in the nares for hours to days without truly colonizing the epithelial cells (16). Therefore, there is a risk of overestimating colonization rates, and other cross-sectional studies could overestimate colonization for the same reason. For *S. aureus*, it is confirmed that persistent colonization occurs only in 20% of persons; 60% are intermittent carriers, and 20% are noncarriers (18).

In this study, some persons acquired MRSA more frequently than others; 52% of the field workers never acquired MRSA despite their visits to MRSA-positive farms (17/33), while 24% of the field workers acquired MRSA once and another 24% acquired MRSA more than once. This could not be attributed to the number of sampling days or to sampling on specific farms, possibly due to a lack of statistical power. An explanation for this difference in acquisition may be differences in susceptibility to MRSA. Many different studies have been performed to reveal host susceptibility patterns for both *S. aureus* and MRSA, indicating that this could somehow play a role in MRSA acquisition (11). In this specific setting, hygienic behavior during work and the use of personal protective equipment may have influenced the potential for acquiring MRSA. This was not evaluated in this study and needs further investigation.

The *spa* type of one field worker (field worker 5) was different from that found on the particular farm visited (t108 versus t011); he did not report any other contact with livestock. The most plausible explanation is that more than one *spa* type was present on the farm, as found in another study (13). Due to the analytical method applied, this was not detected in the dust samples.

Part of the "search-and-destroy policy" is to screen health care workers who have been exposed to MRSA-positive patients without taking transmission-based precautions. Those who are persistently colonized are temporarily suspended from work. Samples are taken not during the work shift on which the health care worker has been exposed but during the next work shift (14) (www.wip.nl). This is done to limit the number of false-positive results due to contamination. This is consistent with our study results, which show that the presence of MRSA after short-term occupational exposure to livestock rarely persists for more than 24 h.

The small sample size was the main limitation of our study; however, significant associations between MRSA acquisition and positive animal and dust samples were found. Another limitation of this study is the difference between the analytical methods applied for the examination of the swabs from the pig and veal farms (5, 12). Studies on hospital-acquired MRSA strains in human samples suggest that selective enrichment broth with large amounts of antimicrobials can inhibit the growth of *S. aureus* in general (1). However, the detection of LA-MRSA using additional enrichment, as in this study, does not affect MRSA growth (4). Since we found only LA-MRSA strains in the nasal swabs of both pig and veal calf field workers, and all suspected strains were confirmed by *mecA* gene PCR in both protocols, it is not likely that this difference has influenced the study results.

In conclusion, LA-MRSA is frequently acquired after short-term occupational exposure. However, the majority of people who acquire LA-MRSA during occupational exposure test negative for MRSA again within 24 h. This calls into question whether these individuals are colonized or contaminated. Screening of individuals upon hospital admission within 24 h after exposure to livestock does not seem reasonable.

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