Exploratory study on the association between exposure and MRSA carriage in veal calf farmers

Kelly Bergs[1], Dick Heederik[1], Jaap Wagenaar[2], Haitske Graveland[1,]*

Samenvatting

De aanwezigheid van veegerelateerde MRSA (Lifestock Associated of LA-MRSA) in mensen is geassocieerd met intensiteit van diercontact. Er is weinig bekend over de persistentie en dynamiek van MRSA dragerschap. Daarom is een longitudinale studie uitgevoerd onder 155 kalverhouders, waarbij herhaaldelijk neus- en keelswabs zijn afgenomen voor de detectie van LA-MRSA. Hierbij zijn perioden met en zonder contact met dieren meegenomen.
De MRSA-prevalentie neemt af in afwezigheid van diercontact. Dit suggereert dat LA-MRSA een slechte persistente kolonisator is, wat relevant is voor het strikte Nederlandse MRSA-beleid gericht op beperking van de transmissie onder andere door behandeling in isolatie.

Abstract

The presence of Livestock Associated MRSA (LA-MRSA) in humans is related to intensity of animal contact. Little is known of the persistence and dynamics of LA-MRSA carriage. A longitudinal study was conducted among 155 veal farmers who were sampled with repeated nasal and throat swabs for MRSA detection. Periods with and without animal contact were covered.
The presence of LA-MRSA in farmers is strongly animal-contact related. The rapidly decreasing MRSA prevalence during absence of animal contact suggests that LA-MRSA is a poor persistent colonizer in humans. These results are of relevance for MRSA control strategies.

Introduction

Infections with Meticillin-Resistant Staphylococcus aureus (MRSA) are associated with increased morbidity and mortality, duration of hospitalization and health care costs [Marschall and Muhlemann, 2006; van Rijen and Kluytmans, 2009]. The low MRSA prevalence of below one percent in the Dutch general population and Scandinavian countries is maintained by an active "Search and Destroy" policy and restrictive antibiotic use in human healthcare on the basis of proposals made by the Dutch Working Party for Infection Prevention (www.wip.nl). Patients with increased risk for MRSA colonization are screened at hospital admission, and cared for in isolation. Furthermore, specific hospital hygiene measures have been implemented [van Rijen and Kluytmans, 2009; van Trijp et al., 2007]. This approach is costly for the health care system, but considered cost-effective.
Since 2003, MRSA belonging to Clonal Complex (CC398) has emerged in livestock and this clonal complex is by far the most prevalent livestock-associated MRSA (LA-MRSA). The emergence in livestock caused a strong increase in MRSA occurrence in humans between 2001 and 2006 in The Netherlands [van Rijen et al., 2009; van Loo et al., 2007]. Identification of risk factors and knowledge about persistence of LA-MRSA in humans is essential for successful continuation of the "Search and Destroy" strategy.
Recently, Graveland et al. [2010] observed a high prevalence of MRSA in veal farmers (~30%) and their family members (<10%). In particular, intensity of animal contact and MRSA occurrence among calves were risk factors for MRSA colonization in humans.
Understanding the dynamics of MRSA carriage in farmers occupationally exposed to MRSA is essential in designing specific control strategies. The aim of this longitudinal study was to determine the persistence and dynamics of MRSA carriage in individuals in close contact with veal calves in periods with and without animal exposure.

Material and methods

Study design an study population
The study population consisted of 155 individuals living or working on randomly selected veal farms (n=51) in The Netherlands. Participants included had no occupational contact with other animals than calves. Participants were followed for approximately two months between June and December 2008, during high and low exposed periods. During high exposure, veal calves were present on the farm. During low exposure, participants were on a holiday, or animals were absent between production cycles. The study period started three weeks prior to low exposure and ended three weeks after this period.
Participants were asked to take nasal and throat swabs in the morning (before animal contact when present) and evening (after animal contact when present). Swabs were sent to the laboratory by mail. Dry swabs were taken weekly during high exposed periods, and twice a week during low exposed periods.

[1] *Institute for Risk Assessment Sciences, Division Environmental Epidemiology, Utrecht University, P.O. Box 80.176, 3508 TD Utrecht, The Netherlands.*
[2] *Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, P.O. Box 80.176, 3508 TD Utrecht, The Netherlands.*
* *Corresponding author: Haitske Graveland, MSc, Institute for Risk Assessment Sciences (IRAS), Utrecht University, P.O. Box 80178, 3508 TD, Utrecht, The Netherlands; e-mail: h.graveland@uu.nl*

On average, participants were sampled on 10 days.
The MRSA status of a farm was determined by taking five wipe samples from stables at the beginning and end of the study using dust cloths. Questionnaires were used to register risk factors including farm characteristics, time spent on the farm, hygiene practices, and if available MRSA anamnesis, as well as potential confounders like age, gender and smoking habits. A short questionnaire was used to collect information about specific sampling days (activities, duration of animal contact) and the three days before sampling. The study protocol was approved by the Medical Ethical Committee of Utrecht Medical Centre. All participants completed the informed consent form.

The present study focuses on a small part of the entire dataset which is being collected as part of a PhD project. Results on the entire dataset with more extensive analyses will be published separately. Only two samples out of the high exposed periods and one sample of the low exposed period were included for analysis (in total three sampling days per participant).

Participants were considered MRSA positive when MRSA was isolated in at least one out of four samples per day. Persistent carriage was defined as MRSA positive results on all three sampling days. Intermittent carriage was defined as fewer than three positive days. Individuals with only MRSA negative swab samples were defined as non-carriers.

Laboratory analyses
All samples were analysed as previously described [Graveland et al., 2009]. Briefly, swabs were inoculated in a non-selective pre-enrichment containing Mueller Hinton broth with 6.5% NaCl. After overnight aerobic incubation at 37°C, 1 ml of pre-enrichment was transferred into nine ml selective enrichment broth (BioMérieux, France). Ten µl of this selective enrichment broth was inoculated onto sheep blood agar (Biotrading, The Netherlands) and MRSA Brilliance™ agar (Oxoid, The Netherlands). All suspected colonies were identified as S. aureus using standard techniques: colony morphology and coagulase assay (slide). The presence of the mecA-gene was confirmed by PCR [Fluit et al., 2001]. Randomly, a selection of strains (n=478) was confirmed by a S. aureus-specific PCR [Martineau et al., 1998].

Data analysis
Statistical analyses were conducted using SAS software 9.1 (SAS Institute, Inc., Cary, NC). Age, gender and smoking habits were included in the Regression model (Proc GENMED in SAS) as fixed effects, exposure varied over time and the exposure the three days before the nasal or throat swab was considered. A P-value of < 0.05 was considered statistically significant.

Results

MRSA prevalence
The response rate was 91%. Reasons for non-participation were no interest or lack of time. For the longitudinal study 155 persons from 51 farms were screened for presence of MRSA by nasal and throat swabs, and dust cloths. The low exposure period involved a holiday for 30 participants and a period between production cycles for 121 participants. Four participants had a combination of a period between production cycles as well as a vacation. A total of 1791 swabs was analyzed (895 nose and 896 throat swabs) together with 510 dust cloths from the stables (255 at the beginning and 255 at the end of the study).
The overall MRSA prevalence was 23% (see Table 2). Prevalence among males seemed to be higher than among females (30% vs.15%). People between 21 and 60 years of age were more frequently MRSA carrier compared to younger and older persons (28%, 14% and 10%, respectively). Veal farmers had a higher prevalence than other participants (e.g. household members) (38% vs.15-21%), and non-smokers seemed to be more frequently MRSA carriers than smokers (24% vs.21%). MRSA prevalence was calculated for different periods (see Figure 1). In addition, separate analyses were performed for persons with and without a holiday as low exposed period. All three groups (holidays, a period between production cycles, and both these groups combined) had an average prevalence of 23%. MRSA prevalence declined from 23% to 20% for persons during the low exposure period with holidays and empty stables combined. Separate analysis of the prevalence for persons without a holiday showed a declination from 22% to 21% in the low exposure period. The strongest decrease in MRSA prevalence was found in people who went on holidays during their low exposure period (from 27% to 13%). The prevalence increased during the period immediately after the low exposure period to 30% for the people who went on a holiday during their low exposure period, 27% for the people without a holiday, and 27% for both these groups combined.

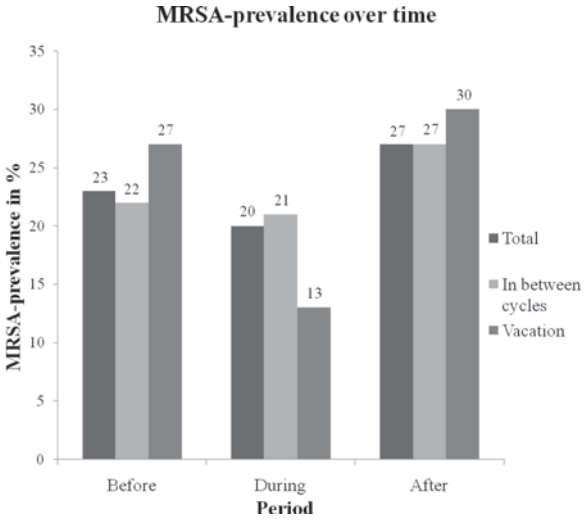

Figure 1: MRSA prevalence over time including a period between production cycles or a vacation

Risk factor analysis
Table 1 shows the results of the multiple regression analysis. Males had a more than twice as high risk to be a MRSA carrier as females (Odds Ratio (OR) = 2.137). Age was positively associated with MRSA carriage (OR = 1.019, expressed per

Table 1: Associations between MRSA carriage and determinants in a multiple regression analysis

Variable	MRSA carriership OR (95% CI)
Gender	
Female	1
Male	2.137 (1.258-3.630)[3]
Age	1.019 (1.003-1.035)[3 4]
Per 10 years	1.206 (1.030-1.411)[3]
Smoking	
Non-smoker	1
Smoker	0.496 (0.269-0.916)[3]
Number of low exposure days	0.943 (0.905-0.982)[3 4]
Per 7 days	0.662 (0.497-0.881)[3]
Hours work per observation	1.038 (1.009-1.067)[3 4]
Per 8 hours	1.346 (1.074-1.680)[3]

year). Smokers were two times less likely to be MRSA carriers compared to non-smokers (OR = 0.496). Having a longer low exposure period was negatively associated with MRSA carriage (OR = 0.943, expressed per day), while performing tasks in or near the stables was positively associated with MRSA carriage (OR = 1.038, expressed per hour).

Table 2: Persistence of MRSA carriage in farmers, family members and others

	N (% per carriertype)			
	Farmer	**Partner**	**Child**	**Other**
Non	24 (27)	24 (27)	39 (43)	3 (3)
Intermitted	12 (28)	13 (30)	17 (40)	1 (2)
Persistent	13 (76)	2 (12)	1 (6)	1 (6)

Persistence of MRSA carriage

In this study, 60% of the participants were non-carriers, 29% were intermitted carriers and 11% were persistent carriers. Persistent carriers were mainly farmers (76%) and non-carriers were mainly children of the farmers (43%) (see Table 2).

Discussion

LA-MRSA prevalence drops during a low exposure period and this is strong evidence for a relation with animal exposure. The large difference in MRSA prevalence between farmers and family members, and the observation that MRSA carriage is lower after a longer low exposed period, is in line with the hypothesis that exposure to MRSA positive stables. Therefore, presumably positive animals play a major role in MRSA carriage in farmers. Furthermore, we found that the majority of the study population was non-carrier (60%) or intermittent carrier (29%), and only 17 individuals (11%) were persistent carriers. These results indicate that carriage of MRSA in a highly-exposed population is mainly transient, or retention of MRSA-containing dust in the nasal cavities in absence of colonization occurs [Graveland et al. 2010].

Data analysis of this exploratory study does not take clustering by farm and correlations between repeated samples into account. However, earlier analyses in a cross sectional study indicated that adjustment for clustering by farm did not alter the results [Graveland et al., 2010].

Most observational studies which investigate risk factors of LA-MRSA in humans are commonly based on a single nasal swab measurement [Denis et al., 2009; Vandenbroek et al., 2009]. The present study shows considerable variability in LA-MRSA carriage over time. However, guidelines underlying the "Search and Destroy" policy have been adapted due to conclusions from observational studies based on single nasal swabs. It seems relevant to consider these results for development of a refined Search and Destroy policy for cattle farmers.

Among family members a lower MRSA prevalence is observed compared to farmers. Since family members were in most cases non or intermitted carriers, they probably lost the bacteria more easily during the low exposure period. Farmers on the other hand, who were the largest group amongst persistent carriers, had presumably a harder time to lose this pathogen during this period. The bigger decrease in MRSA prevalence during vacation compared to a period between production cycles is probably due to the fact that most persons left their farm during the vacation and therefore had no farm-related exposure. People who had a period between production cycles were probably still exposed, but to a lower extend.

Six participants from 2 different farms were intermitted carrier despite the fact that their farm was not MRSA positive during the study. MRSA status of the farm was established by applying dust cloth sampling of sedimented dust in the stables (results not shown). The MRSA carriage in these six participants might be explained by contact with other animals on the farm. Five of these participants indicated to have

[3] *P < 0.05*
[4] *OR expressed per year / day / hour difference*

such contacts. These animals could have been the MRSA source, although the kind and number of animals differed per farm, and we did not perform measurements to confirm these findings.

This study suggests that LA-MRSA is a poor persistent colonizer in humans. Further investigation on variation of MRSA carriage among calves over time is needed to see if the same is true for calves. Results from such a study might attribute to the adjustment of antibiotic usage in veal calves as part of new strategies for the control of LA-MRSA.

The results of this study may also have implications for current hospital policies. Presently, pig and veal calf farmers are defined as risk populations for MRSA carriage and are actively screened when admitted to a hospital [van Rijen and Kluytmans, 2009; WIP, 2010]. The substantial increase in health-care costs due to the presence of LA-MRSA could be reduced by introducing an exposure-free period for farmers before screening. In addition, treatment of positive farmers is not meaningful when accompanied by ongoing exposure. Together, establishing MRSA status after an exposure-free period and treatment only in absence of exposure will contribute to the restrictive antibiotic-use policy which is a key element of the "Search and Destroy" policy. The results could also have a significant impact on the development of new strategies for the control of LA-MRSA, like reducing the usage of antibiotics in veal calves.

Conclusion

This present study indicates that the prevalence of LA-MRSA in farmers is reduced when an exposure-free period is introduced. It suggests that LA-MRSA is a poor persistent colonizer in humans. Improved understanding of the role of exposure and host specificity of LA-MRSA could have a significant impact on antibiotic and infection control policies in hospitals, and more importantly, on the development of new strategies for the control of MRSA.

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